List of Publications

Promethion metabolic and behavioral phenotyping systems

Abdeen, A., et al. (2022). "The active mouse rests within: Energy management among and within individuals." Functional Ecology **n/a**(n/a).

Abstract The relationship between daily energy expenditure (DEE) and resting metabolic rate (RMR) provides insight into how organisms allocate energy to maintenance versus energetically expensive activities such as locomotor activity. Three models have been devised to describe energy management: the allocation, independent and performance models, which, respectively, predict a DEE–RMR slope of $b < 1$, $b = 1$ and $b > 1$. Here, we took paired repeated metabolic and behavioural measurements in 51 female white-footed mice to (a) evaluate which energy management models apply at the among- and within-individual levels and to (b) quantify the relationship between metabolic traits and two energetically expensive behaviours. The DEE–RMR slope was different at the among- versus within-individual levels, with values supporting the performance and allocation models at the among- and within-individual levels, respectively. Accordingly, the relationship between voluntary wheel running and RMR was positive at the among-individual level ($r = 0.40 \pm 0.21$), but negative at the within-individual level ($r = -0.23 \pm 0.10$). To our knowledge, this is the first study to simultaneously partition the relationship between RMR and behaviour at the among- versus within-individuals levels while determining which energy management models apply at each of these levels. In doing so, we have identified a mechanism through which compensation occurs at the within-individual level. A free Plain Language Summary can be found within the Supporting Information of this article.

Cai, J., et al. (2022). "Skeletal muscle provides a pro-browning microenvironment for transplanted brown adipose tissue to maintain its effect to ameliorate obesity in ob/ob mice." FASEB Journal **36**(1): e22056.

Brown adipose tissue (BAT) transplantation is a promising means of increasing whole-body energy metabolism to ameliorate obesity. However, the changes in BAT following transplantation and the effects of the microenvironment of the recipient site on graft function have yet to be fully characterized. Therefore, we aimed to determine the effects of transplanting BAT from C57BL/6 mice into the dorsal subcutaneous region or deep to the quadriceps femoris muscle of leptin-deficient ob/ob mice. Subcutaneously transplanted BAT lost features of BAT and demonstrated greater inflammatory cell infiltration and more oil cysts 16 weeks following transplantation. By contrast, the sub-muscularly transplanted BAT maintained features of BAT and was more highly vascularized. Interestingly, sub-muscular BAT transplantation led to a significant increase in oxygen consumption and less inflammation in subcutaneous fat, which was associated with long-term reductions in insulin resistance and body mass gain, whereas the subcutaneous transplants failed after 16 weeks. These results demonstrate that the

beneficial effects of BAT transplantation depend upon the microenvironment of the recipient site. Skeletal muscle may provide a microenvironment that maintains the inherent features of BAT grafts over a long period of time, which facilitates a reduction in obesity and improvements in glucose homeostasis.

Cakir, I., et al. (2022). "Histone deacetylase 6 inhibition restores leptin sensitivity and reduces obesity." Nat Metab.

The adipose tissue-derived hormone leptin can drive decreases in food intake while increasing energy expenditure. In diet-induced obesity, circulating leptin levels rise proportionally to adiposity. Despite this hyperleptinemia, rodents and humans with obesity maintain increased adiposity and are resistant to leptin's actions. Here we show that inhibitors of the cytosolic enzyme histone deacetylase 6 (HDAC6) act as potent leptin sensitizers and anti-obesity agents in diet-induced obese mice. Specifically, HDAC6 inhibitors, such as tubastatin A, reduce food intake, fat mass, hepatic steatosis and improve systemic glucose homeostasis in an HDAC6-dependent manner. Mechanistically, peripheral, but not central, inhibition of HDAC6 confers central leptin sensitivity. Additionally, the anti-obesity effect of tubastatin A is attenuated in animals with a defective central leptin-melanocortin circuitry, including db/db and MC4R knockout mice. Our results suggest the existence of an HDAC6-regulated adipokine that serves as a leptin-sensitizing agent and reveals HDAC6 as a potential target for the treatment of obesity.

Girard, R., et al. (2022). "The transcription factor hepatocyte nuclear factor 4A acts in the intestine to promote white adipose tissue energy storage." Nature

The transcription factor hepatocyte nuclear factor 4 A (HNF4A) controls the metabolic features of several endodermal epithelia. Both HNF4A and HNF4G are redundant in the intestine and it remains unclear whether HNF4A alone controls intestinal lipid metabolism. Here we show that intestinal HNF4A is not required for intestinal lipid metabolism per se, but unexpectedly influences whole-body energy expenditure in diet-induced obesity (DIO). Deletion of intestinal HNF4A caused mice to become DIO-resistant with a preference for fat as an energy substrate and energetic changes in association with white adipose tissue (WAT) beiging. Intestinal HNF4A is crucial for the fat-induced release of glucose-dependent insulinotropic polypeptide (GIP), while the reintroduction of a stabilized GIP analog rescues the DIO resistance phenotype of the mutant mice. Our study provides evidence that intestinal HNF4A plays a non-redundant role in whole-body lipid homeostasis and points to a non-cell-autonomous regulatory circuit for body-fat management.

Grebenstein, P. E., et al. (2022). "Anatabine, Nornicotine, and Anabasine Reduce Weight Gain and Body Fat through Decreases in Food Intake and Increases in Physical Activity." Journal of Clinical

Obesity is a leading cause of preventable death in the United States. Currently approved pharmacotherapies for the treatment of obesity are associated with rebound weight gain, negative side effects, and the potential for abuse. There is a need for new treatments with fewer side effects. Minor

tobacco alkaloids (MTAs) are potential candidates for novel obesity pharmacotherapies. These alkaloids are structurally related to nicotine, which can help reduce body weight, but without the same addictive potential. The purpose of the current study was to examine the effects of three MTAs (nornicotine, anatabine, and anabasine) and nicotine on weight gain, body composition, chow intake, and physical activity. We hypothesized that the MTAs and nicotine would reduce weight gain through reductions in chow intake and increases in physical activity. To test this, male Sprague Dawley rats were housed in metabolic phenotyping chambers. Following acclimation to these chambers and to (subcutaneous (sc)) injections of saline, animals received daily injections (sc) of nornicotine, anabasine, anatabine, or nicotine for one week. Compared to saline-injected animals that gained body weight and body fat during the treatment phase, injections of nornicotine and anatabine prevented additional weight gain, alongside reductions in body fat. Rats receiving anabasine and nicotine gained body weight at a slower rate relative to rats receiving saline injections, and body fat remained unchanged. All compounds reduced the intake of chow pellets. Nornicotine and nicotine produced consistent increases in physical activity 6 h post-injection, whereas anabasine's and anatabine's effects on physical activity were more transient. These results show that short-term, daily administration of nornicotine, anabasine, and anatabine has positive effects on weight loss, through reductions in body fat and food intake and increases in physical activity. Together, these findings suggest that MTAs are worthy of further investigations as anti-obesity pharmacotherapies.

Kazak, L., et al. (2022). "Combined α-and β-adrenergic receptor activation triggers thermogenesis by the futile creatine cycle."

Noradrenaline is the primary physiological regulator of adipocyte thermogenesis in response to decreased environmental temperature1. However, the molecular factors and effector pathways that lie downstream of noradrenaline-stimulated thermogenesis are still not fully understood but are purportedly driven by cAMP downstream of β-adrenergic receptor (βAR) activation. Furthermore, while the transcriptional mechanisms regulating Ucp1 are well-characterized2, the transcriptional regulation of UCP1-independent thermogenesis is largely unknown. Here, we show that brown adipose tissue (BAT) is primed to respond to environmental cold by triggering coordinated α-adrenergic receptor (αAR) and βAR signaling to induce the expression of thermogenic genes of the futile creatine cycle3,4. Using fat-specific loss-of-function models, we reveal that EBFs, ERRs, and PGC1α are required for the cold-stimulated transcriptional induction of the futile creatine cycle in vivo. Through the application of chemogenetics, we demonstrate that combined fat-selective Gαs (activated by βARs) and Gαq (activated by αARs) signaling elevates whole-body energy expenditure to a greater extent than either signaling pathway alone in a manner that is dependent on the key effector protein of the futile creatine cycle, CKB3. Moreover, genetic and pharmacological studies reveal that CKB is necessary for nearly all of the α1AR-stimulated component of brown adipocyte-intrinsic respiration and is thus critical for the full activation of noradrenaline-stimulated thermogenesis. Thus, the futile creatine cycle is integrated into facultative and adaptive thermogenesis through coordinated α1AR and β3AR signaling.

Mills, E. L., et al. (2022). "Cysteine 253 of UCP1 regulates energy expenditure and sex-dependent adipose tissue inflammation." Cell Metabolism **34**(1): 140-157 e148.

Uncoupling protein 1 (UCP1) is a major regulator of brown and beige adipocyte energy expenditure and metabolic homeostasis. However, the widely employed UCP1 loss-of-function model has recently been shown to have a severe deficiency in the entire electron transport chain of thermogenic fat. As such, the role of UCP1 in metabolic regulation in vivo remains unclear. We recently identified cysteine-253 as a regulatory site on UCP1 that elevates protein activity upon covalent modification. Here, we examine the physiological importance of this site through the generation of a UCP1 cysteine-253-null (UCP1 C253A) mouse, a precise genetic model for selective disruption of UCP1 in vivo. UCP1 C253A mice exhibit significantly compromised thermogenic responses in both males and females but display no measurable effect on fat accumulation in an obesogenic environment. Unexpectedly, we find that a lack of C253 results in adipose tissue redox stress, which drives substantial immune cell infiltration and systemic inflammatory pathology in adipose tissues and liver of male, but not female, mice. Elevation of systemic estrogen reverses this male-specific pathology, providing a basis for protection from inflammation due to loss of UCP1 C253 in females. Together, our results establish the UCP1 C253 activation site as a regulator of acute thermogenesis and sex-dependent tissue inflammation.

Nelson, M. E., et al. (2022). "Systems-level analysis of insulin action in mouse strains provides insight into tissue-and pathway-specific interactions that drive insulin resistance." Cell Metabolism.

Skeletal muscle and adipose tissue insulin resistance are major drivers of metabolic disease. To uncover pathways involved in insulin resistance, specifically in these tissues, we leveraged the metabolic diversity of different dietary exposures and discrete inbred mouse strains. This revealed that muscle insulin resistance was driven by gene-by-environment interactions and was strongly correlated with hyperinsulinemia and decreased levels of ten key glycolytic enzymes. Remarkably, there was no relationship between muscle and adipose tissue insulin action. Adipocyte size profoundly varied across strains and diets, and this was strongly correlated with adipose tissue insulin resistance. The A/J strain, in particular, exhibited marked adipocyte insulin resistance and hypertrophy despite robust muscle insulin responsiveness, challenging the role of adipocyte hypertrophy per se in systemic insulin resistance. These data demonstrate that muscle and adipose tissue insulin resistance can occur independently and underscore the need for tissue-specific interrogation to understand metabolic disease.

Wu, Q., et al. (2022). "A Protective Inter-Organ Communication Response Against Life-Threatening Malarial Anemia." bioRxiv.

Anemia is a clinical hallmark and independent risk factor of malaria mortality, the disease caused by Plasmodium spp. infection. While malarial anemia arises from parasite-induced hemolysis, whether and how host metabolic adaptation to malaria regulates anemia severity is less understood. Here we demonstrate that reprogramming of organismal iron (Fe) metabolism by the kidneys is a central component of the host metabolic response regulating the pathogenesis of life-threatening malarial anemia. Renal proximal tubule epithelial cells (RPTEC) are the main cell compartment responsible for Fe

storage and recycling during Plasmodium infection in mice. Transcriptional reprogramming of RPTEC couples immune resistance to Plasmodium infection to renal Fe export via the induction of the cellular Fe exporter SLC40A1/ferroportin 1. This integrated defense strategy is essential to deliver Fe to erythroblasts and support compensatory erythropoiesis to prevent the development of life-threatening anemia. Failure to mobilize Fe from RPTEC causes acute kidney injury (AKI) and is associated with life-threatening anemia in P. falciparum-infected individuals. These findings reveal an unexpected role of the kidneys in the control of organismal Fe metabolism and anemia severity during malaria.

Yang, X., et al. (2022). " Cytochrome P450 epoxygenase-derived EPA and DHA oxylipins 17,18-epoxyeicosatetraenoic acid and 19,20-epoxydocosapentaenoic acid promote BAT thermogenesis and WAT browning through the GPR120-AMPKα signaling pathway " Food & Function.

The mechanisms whereby fish oil rich in EPA and DHA promotes BAT thermogenesis and WAT browning are not fully understood. Thus, this study aimed to investigate the effects of cytochrome P450 (CYP) epoxygenase-derived EPA and DHA oxylipins 17,18-EpETE and 19,20-EpDPE on BAT thermogenesis and WAT browning and explore the underlying mechanism. Stromal vascular cells (SVCs) were subjected to 17,18-EpETE or 19,20-EpDPE treatment and mice were treated with the CYP epoxygenase inhibitor, the thermogenic marker genes were detected and the involvement of GPR120 and AMPKα were assessed. The in vitro results indicated that 17,18-EpETE and 19,20-EpDPE induced brown and beige adipocyte thermogenesis, with increased expression of thermogenic marker gene UCP1 in differentiated SVCs. Meanwhile, the expression of GPR120 and phosphorylation of AMPKα were increased in response to these two oxylipins. However, the inhibition of GPR120 and AMPKα inhibited the promotion of adipocyte thermogenesis. In addition, in the presence of CYP epoxygenase inhibitor MS-PPOH, EPA and DHA had no effect on increasing UCP1 expression in differentiated SVCs. Consistent with the in vitro results, the in vivo findings demonstrated that fish oil had no body fat-lowering effects and no effects on enhancing energy metabolism, iBAT thermogenesis and iWAT browning in mice fed HFD after intraperitoneal injection of CYP epoxygenase inhibitor SKF-525A. Moreover, fish oil had no effect on the elevation of GPR120 expression and activation of AMPKα in iBAT and iWAT in mice fed HFD after intraperitoneal injection of SKF-525A. In summary, our results showed that CYP epoxygenase-derived EPA and DHA oxylipins 17,18-EpETE and 19,20-EpDPE promoted BAT thermogenesis and WAT browning through the GPR120-AMPKα signaling pathway, which might contribute to the thermogenic and anti-obesity effects of fish oil.

Abu-Halaka, D., et al. (2021). "Whole body metabolism is improved by hemin added to high fat diet while counteracted by nitrite: a mouse model of processed meat consumption." Food & Function.

Nitrites and nitrates are traditional food additives used as curing agents in the food industry. They inhibit the growth of microorganisms and give a typical pink color to meat. Besides the positive effects of nitrite in foods, if present at high levels in the body, may induce hypoxia and contribute to the production of pro-carcinogenic secondary N-nitrosamines. This study investigated the whole-body metabolic effects of hemin and nitrite added to a high fat diet as red and processed red meat nutritional models. Mice were fed for 11 weeks with five different diets—(1) control diet (ND), (2) high fat diet (HFD) with 60% fat, (3) HFD with hemin (HFD + H, red meat model), (4) HFD with hemin and nitrite (HFD + HN, processed meat

model), and (5) HFD with hemin, nitrite, and secondary amine (HFD + HNN, N-nitrosamine generating model)—and several metabolic parameters were determined and respiratory measurements were performed. Mice fed with the HFD + H or HFD + HNN diet had a lower epididymal white adipose tissue (eWAT) : body ratio and lower fasting glucose level than those fed the HFD alone. In addition, our results demonstrated a relief in hepatosteatosis grade among the HFD + H and HFD + HNN diet fed mice. Nitrite added to the HFD impaired the ability to use fat for energy, opposite to the effect of hemin. This study shows that nitrite in addition to pro-carcinogenesis and hypoxia can impact metabolic disease progression when added to meat.

Alves, F. M., et al. (2021). "Iron accumulation in skeletal muscles of old mice is associated with impaired regeneration after ischaemia-reperfusion damage." J Cachexia Sarcopenia Muscle **12**(2): 476-492.

BACKGROUND: Oxidative stress is implicated in the insidious loss of muscle mass and strength that occurs with age. However, few studies have investigated the role of iron, which is elevated during ageing, in age-related muscle wasting and blunted repair after injury. We hypothesized that iron accumulation leads to membrane lipid peroxidation, muscle wasting, increased susceptibility to injury, and impaired muscle regeneration. METHODS: To examine the role of iron in age-related muscle atrophy, we compared the skeletal muscles of 3-month-old with 22- to 24-month-old 129SvEv FVBM mice. We assessed iron distribution and total elemental iron using laser ablation inductively coupled plasma mass spectrometry and Perls' stain on skeletal muscle cross-sections. In addition, old mice underwent ischaemia-reperfusion (IR) injury (90 min ischaemia), and muscle regeneration was assessed 14 days after injury. Immunoblotting was used to determine lipid peroxidation (4HNE) and iron-related proteins. To determine whether muscle iron content can be altered, old mice were treated with deferiprone (DFP) in the drinking water, and we assessed its effects on muscle regeneration after injury. RESULTS: We observed a significant increase in total elemental iron (+43%, $P < 0.05$) and lipid peroxidation (4HNE: +76%, $P < 0.05$) in tibialis anterior muscles of old mice. Iron was further increased after injury (adult: +81%, old: +135%, $P < 0.05$) and associated with increased lipid peroxidation (+41%, $P < 0.05$). Administration of DFP did not impact iron or measures of lipid peroxidation in skeletal muscle or modulate muscle mass. Increased muscle iron concentration and lipid peroxidation were associated with less efficient regeneration, evident from the smaller fibres in cross-sections of tibialis anterior muscles (-24%, $P < 0.05$) and an increased percentage of fibres with centralized nuclei (+4124%, $P < 0.05$) in muscles of old compared with adult mice. Administration of DFP lowered iron after IR injury (PRE: -32%, $P < 0.05$ and POST: -41%, $P < 0.05$), but did not translate to structural improvements. CONCLUSIONS: Muscles from old mice have increased iron levels, which are associated with increased lipid peroxidation, increased susceptibility to IR injury, and impaired muscle regeneration. Our results suggest that iron is involved in effective muscle regeneration, highlighting the importance of iron homeostasis in muscle atrophy and muscle repair.

Andersson, B., et al. (2021). "O-GlcNAc cycling mediates energy balance by regulating caloric memory." Appetite **165**: 105320.

Caloric need has long been thought a major driver of appetite. However, it is unclear whether caloric need regulates appetite in environments offered by many societies today where there is no shortage of food.

Here we observed that wildtype mice with free access to food did not match calorie intake to calorie expenditure. While the size of a meal affected subsequent intake, there was no compensation for earlier under- or over-consumption. To test how spontaneous eating is subject to caloric control, we manipulated O-linked beta-N-acetylglucosamine (O-GlcNAc), an energy signal inside cells dependent on nutrient access and metabolic hormones. Genetic and pharmacological manipulation in mice increasing or decreasing O-GlcNAcylation regulated daily intake by controlling meal size. Meal size was affected at least in part due to faster eating speed. Without affecting meal frequency, O-GlcNAc disrupted the effect of caloric consumption on future intake. Across days, energy balance was improved upon increased O-GlcNAc levels and impaired upon removal of O-GlcNAcylation. Rather than affecting a perceived need for calories, O-GlcNAc regulates how a meal affects future intake, suggesting that O-GlcNAc mediates a caloric memory and subsequently energy balance.

Balazova, L., et al. (2021). "GPR180 is a component of TGFβ signalling that promotes thermogenic adipocyte function and mediates the metabolic effects of the adipocyte-secreted factor " Nature.

Activation of thermogenic brown and beige adipocytes is considered as a strategy to improve metabolic control. Here, we identify GPR180 as a receptor regulating brown and beige adipocyte function and whole-body glucose homeostasis, whose expression in humans is associated with improved metabolic control. We demonstrate that GPR180 is not a GPCR but a component of the TGFβ signalling pathway and regulates the activity of the TGFβ receptor complex through SMAD3 phosphorylation. In addition, using genetic and pharmacological tools, we provide evidence that GPR180 is required to manifest Collagen triple helix repeat containing 1 (CTHRC1) action to regulate brown and beige adipocyte activity and glucose homeostasis. In this work, we show that CTHRC1/GPR180 signalling integrates into the TGFβ signalling as an alternative axis to fine-tune and achieve low-grade activation of the pathway to prevent pathophysiological response while contributing to control of glucose and energy metabolism.

Beppu, L. (2021). The role of Blimp-1 transcriptional regulator in adipose-resident Tregs, d-scholarship.pitt.edu.

Adipose-resident regulatory T cells are protective against local inflammation and are believed to play a critical role in preserving insulin sensitivity and glucose tolerance. Although their basic markers and roles have been studied, less is known about the transcriptional machinery regulating their differentiation and function. B lymphocyte-induced maturation protein-1 (Blimp-1) is a transcriptional regulator known to be involved in development, polarization, and maintenance of various immune cells including CD4+ T cells. Using Blimp-1 reporter mice, we discovered that Blimp-1 is constitutively expressed in a subset of visceral adipose tissue (VAT) Tregs, and that Blimp-1+ VAT Tregs are phenotypically distinct from their Blimp-1- counterparts. We also found that Treg-specific Blimp-1 deletion led to reduced ST2+ KLRG1+, IL-10-producing VAT and inguinal adipose tissue (IAT) Tregs. Surprisingly, during diet-induced obesity, Blimp-1 Treg deficient mice gained less weight, had reduced body fat percentage, and exhibited improved insulin sensitivity compared to wild-type mice. Furthermore, this was accompanied by white adipocyte beiging. It has previously been shown that IL-10 can directly induce thermogenesis. Therefore, we repeated these experiments utilizing mice with Treg-specific deletion of IL-10 and found that they phenocopied the Blimp-1 Treg deficient mice. In summary, these findings reveal that Treg-adipocyte cross-talk can occur via a

Blimp-1/IL-10 axis, and that the absence of Blimp-1+ Tregs increases white adipose tissue beiging and is metabolically protective against diet-induced obesity.

Beppu, L. Y., et al. (2021). "Tregs facilitate obesity and insulin resistance via a Blimp-1/IL-10 axis." JCI Insight **6**(3).

Interleukin-10 (IL-10) is a critical cytokine used by immune cells to suppress inflammation. Paradoxically, immune cell-derived IL-10 can drive insulin resistance in obesity by suppressing adipocyte energy expenditure and thermogenesis. However, the source of IL-10 necessary for the suppression of adipocyte thermogenesis is unknown. We show here that CD4+Foxp3+ regulatory T cells (Tregs) are a substantial source of IL-10 and that Treg-derived IL-10 can suppress adipocyte beiging. Unexpectedly, Treg-specific loss of IL-10 resulted in increased insulin sensitivity and reduced obesity in high-fat diet-fed male mice. Mechanistically, we determined that Treg-specific loss of the transcription factor Blimp-1, a driver of IL-10 expression by Tregs, phenocopied the Treg-specific IL-10-deficient mice. Loss of Blimp-1 expression in Tregs resulted in reduced ST2+KLRG1+, IL-10-secreting Tregs, particularly in the white adipose tissue. Blimp-1-deficient mice were protected from glucose intolerance, insulin resistance, and diet-induced obesity, through increased white adipose tissue browning. Taken together, our data show that Blimp-1-regulated IL-10 secretion by Tregs represses white adipose tissue beiging to maintain adipose tissue homeostasis.

Bhandarkar, N. S., et al. (2021). "Adaptation of fuel selection to acute decrease in voluntary energy expenditure is governed by dietary macronutrient composition in mice." Physiol Rep **9**(18): e15044.

In humans, exercise-induced thermogenesis is a markedly variable component of total energy expenditure, which had been acutely affected worldwide by COVID-19 pandemic-related lockdowns. We hypothesized that dietary macronutrient composition may affect metabolic adaptation/fuel selection in response to an acute decrease in voluntary activity. Using mice fed short-term high-fat diet (HFD) compared to low-fat diet (LFD)-fed mice, we evaluated whole-body fuel utilization by metabolic cages before and 3 days after omitting a voluntary running wheel in the cage. Short-term (24-48 h) HFD was sufficient to increase energy intake, fat oxidation, and decrease carbohydrate oxidation. Running wheel omission did not change energy intake, but resulted in a significant 50% decrease in total activity and a ~20% in energy expenditure in the active phase (night-time), compared to the period with wheel, irrespective of the dietary composition, resulting in significant weight gain. Yet, while in LFD wheel omission significantly decreased active phase fat oxidation, thereby trending to increase respiratory exchange ratio (RER), in HFD it diminished active phase carbohydrate oxidation. In conclusion, acute decrease in voluntary activity resulted in positive energy balance in mice on both diets, and decreased oxidation of the minor energy (macronutrient) fuel source, demonstrating that dietary macronutrient composition determines fuel utilization choices under conditions of acute changes in energetic demand.

Brierley, D. I., et al. (2021). "Central and peripheral GLP-1 systems independently suppress eating." Nat Metab **3**(2): 258-273.

The anorexigenic peptide glucagon-like peptide-1 (GLP-1) is secreted from gut enteroendocrine cells and brain preproglucagon (PPG) neurons, which, respectively, define the peripheral and central GLP-1 systems. PPG neurons in the nucleus tractus solitarii (NTS) are widely assumed to link the peripheral and central GLP-1 systems in a unified gut-brain satiation circuit. However, direct evidence for this hypothesis is lacking, and the necessary circuitry remains to be demonstrated. Here we show that PPG(NTS) neurons encode satiation in mice, consistent with vagal signalling of gastrointestinal distension. However, PPG(NTS) neurons predominantly receive vagal input from oxytocin-receptor-expressing vagal neurons, rather than those expressing GLP-1 receptors. PPG(NTS) neurons are not necessary for eating suppression by GLP-1 receptor agonists, and concurrent PPG(NTS) neuron activation suppresses eating more potently than semaglutide alone. We conclude that central and peripheral GLP-1 systems suppress eating via independent gut-brain circuits, providing a rationale for pharmacological activation of PPG(NTS) neurons in combination with GLP-1 receptor agonists as an obesity treatment strategy.

Brown, M. R., et al. (2021). "Time-restricted feeding prevents deleterious metabolic effects of circadian disruption through epigenetic control of beta cell function." Sci Adv **7**(51): eabg6856.

Circadian rhythm disruption (CD) is associated with impaired glucose homeostasis and type 2 diabetes mellitus (T2DM). While the link between CD and T2DM remains unclear, there is accumulating evidence that disruption of fasting/feeding cycles mediates metabolic dysfunction. Here, we used an approach encompassing analysis of behavioral, physiological, transcriptomic, and epigenomic effects of CD and consequences of restoring fasting/feeding cycles through time-restricted feeding (tRF) in mice. Results show that CD perturbs glucose homeostasis through disruption of pancreatic β cell function and loss of circadian transcriptional and epigenetic identity. In contrast, restoration of fasting/feeding cycle prevented CD-mediated dysfunction by reestablishing circadian regulation of glucose tolerance, β cell function, transcriptional profile, and reestablishment of proline and acidic amino acid–rich basic leucine zipper (PAR bZIP) transcription factor DBP expression/activity. This study provides mechanistic insights into circadian regulation of β cell function and corresponding beneficial effects of tRF in prevention of T2DM.

Cao, E., et al. (2021). "Mesenteric lymphatic dysfunction promotes insulin resistance and represents a potential treatment target in obesity." Nat Metab **3**(9): 1175-1188.

Visceral adipose tissue (VAT) encases mesenteric lymphatic vessels and lymph nodes through which lymph is transported from the intestine and mesentery. Whether mesenteric lymphatics contribute to adipose tissue inflammation and metabolism and insulin resistance is unclear. Here we show that obesity is associated with profound and progressive dysfunction of the mesenteric lymphatic system in mice and humans. We find that lymph from mice and humans consuming a high-fat diet (HFD) stimulates lymphatic vessel growth, leading to the formation of highly branched mesenteric lymphatic vessels that 'leak' HFD-lymph into VAT and, thereby, promote insulin resistance. Mesenteric lymphatic dysfunction is regulated by cyclooxygenase (COX)-2 and vascular endothelial growth factor (VEGF)-C-VEGF receptor (R)3 signalling. Lymph-targeted inhibition of COX-2 using a glyceride prodrug approach reverses mesenteric lymphatic dysfunction, visceral obesity and inflammation and restores glycaemic control in mice. Targeting obesity-

associated mesenteric lymphatic dysfunction thus represents a potential therapeutic option to treat metabolic disease.

Cao, Y., et al. (2021). "Therapeutic evaluation and metabolic reprograming of isosteviol sodium in a rat model of ischemic cardiomyopathy." European Journal of Pharmacology **911**: 174539.

Ischemia heart disease, one of the lethal cardiovascular diseases, irreversibly impairs cardiac function and is recognized as the primary risk factor for mortality in industrialized countries. The myocardial ischemia treatment still faces a considerable degree of increasing unmet needs. Isosteviol sodium (STVNa) and its derivatives have been proven to effectively alleviate metabolic diseases, hypertension, and heart hypertrophy. Little is known about how STVNa confers the cardioprotective effect during acute myocardial ischemia (AMI). In the present study, a rat model of acute ST-segment-elevation myocardial ischemia by left anterior descending (LAD) ligation was established. Compared to the AMI model group, STVNa administration (4 mg/kg, twice a day) well preserved left ventricle function by ejection fraction (45.10 +/- 10.39 vs. 73.64 +/- 13.15, p = 0.0013) and fractional shortening (22.94 +/- 6.28 vs. 44.00 +/- 11.05, p = 0.0017). Further analysis shows that high-dose STVNa (4 mg/kg) significantly improved the hemodynamics in AMI rats, with LVSP (88.25 +/- 12.78 vs 99.75 +/- 5.10, p = 0.018), max dP/dt (2978.45 +/- 832.46 vs 4048.56 +/- 827.23, p = 0.096), LVEDP (19.88 +/- 2.00 vs 22.26 +/- 3.21, p = 0.04) and left ventricular relaxation time constant (Tau) (0.030 +/- 0.006 vs 0.021 +/- 0.004, p = 0.021). Mechanically, STVNa administration retained the myocardial levels of phosphorylated AMPK, and CPT1b. Moreover, STVNa significantly increased the total energy expenditure, and reduced fatty acid accumulation through mitochondrial oxidative phosphorylation, which was supported by the indirect calorimetry and cellular energy analysis. Taken together, these findings suggest that STVNa is a potential cardioprotection agent for ischemic cardiomyopathy, likely through improving energy homeostasis, left ventricular hemodynamics, and heart function.

Ceddia, R. P., et al. (2021). "Increased Energy Expenditure and Protection From Diet-Induced Obesity in Mice Lacking the cGMP-Specific Phosphodiesterase PDE9." Diabetes **70**(12): 2823-2836.

Cyclic nucleotides cAMP and cGMP are important second messengers for the regulation of adaptive thermogenesis. Their levels are controlled not only by their synthesis, but also their degradation. Since pharmacological inhibitors of cGMP-specific phosphodiesterase 9 (PDE9) can increase cGMP-dependent protein kinase signaling and uncoupling protein 1 expression in adipocytes, we sought to elucidate the role of PDE9 on energy balance and glucose homeostasis in vivo. Mice with targeted disruption of the PDE9 gene, Pde9a, were fed nutrient-matched high-fat (HFD) or low-fat diets. Pde9a (-/-) mice were resistant to HFD-induced obesity, exhibiting a global increase in energy expenditure, while brown adipose tissue (AT) had increased respiratory capacity and elevated expression of Ucp1 and other thermogenic genes. Reduced adiposity of HFD-fed Pde9a (-/-) mice was associated with improvements in glucose handling and hepatic steatosis. Cold exposure or treatment with beta-adrenergic receptor agonists markedly decreased Pde9a expression in brown AT and cultured brown adipocytes, while Pde9a (-/-) mice exhibited a greater increase in AT browning, together suggesting that the PDE9-cGMP pathway augments classical cold-induced beta-adrenergic/cAMP AT browning and energy expenditure. These findings

suggest PDE9 is a previously unrecognized regulator of energy metabolism and that its inhibition may be a valuable avenue to explore for combating metabolic disease.

Centanni, S. W., et al. (2021). "The impact of intermittent exercise on mouse ethanol drinking and abstinence-associated affective behavior and physiology." Alcohol Clin Exp Res.

BACKGROUND: Negative emotional states are associated with the initiation and maintenance of alcohol use and drive relapse to drinking during withdrawal and protracted abstinence. Physical exercise is correlated with decreased negative affective symptoms, although a direct relationship between drinking patterns and exercise level has not been fully elucidated. METHODS: We incorporated intermittent running wheel access into a chronic continuous access, two-bottle choice alcohol drinking model in female C57BL/6J mice. Wheel access was granted intermittently once mice established a preference for alcohol over water. After 6 weeks, alcohol was removed (forced abstinence) and mice were given continuous access to unlocked or locked wheels. Negative affect-like behavior, home cage behavior, and metabolic activity were measured during protracted abstinence. RESULTS: Wheel access shifted drinking patterns in the mice, increasing drinking when the wheel was locked, and decreasing drinking when unlocked. Moreover, alcohol preference and consumption were strongly negatively correlated with the amount of running. An assessment of negative affect-like behavior in abstinence via the novelty suppressed feeding and saccharin preference tests (SPT) showed that unlimited wheel access mitigated abstinence-induced latency increases. Mice in abstinence also spent more time sleeping during the active dark cycle than control mice, providing additional evidence for abstinence-induced anhedonia- and depression-like behavior. Furthermore, running wheel access in abstinence decreased dark cycle sleep to comparable alcohol- and wheel-naive mice. Given the positive impact of exercise and the negative impact of alcohol on metabolic health, we compared metabolic phenotypes of alcohol-abstinent mice with and without wheel access. Wheel access increased energy expenditure, carbon dioxide production, and oxygen consumption, providing a potential metabolic mechanism through which wheel access improves affective state. CONCLUSIONS: This study suggests that including exercise in AUD treatment regimens has the potential to reduce drinking, improve affective state during abstinence and could serve as a non-pharmacological approach to prevent the development of an AUD in high-risk individuals.

Clart, L. M., et al. (2021). "Role of ERbeta in adipocyte metabolic response to wheel running following ovariectomy." Journal of Endocrinology **249**(3): 223-237.

Estrogen receptor beta (ERb), one of the two major estrogen receptors, acts via genomic and non-genomic signaling pathways to affect many metabolic functions, including mitochondrial biogenesis and respiration. This study assessed the effect of ERb classical genomic activity on adipocyte-specific and -systemic metabolic responses to wheel running exercise in a rodent model of menopause. Female mice lacking the ERb DNA-binding domain (ERbDBDKO, n = 20) and WT (n = 21) littermate controls were fed a high-fat diet (HFD), ovariectomized (OVX), and randomized to control (no running wheel) and exercise (running wheel access) groups and were followed for 8 weeks. Wheel running did not confer protection against metabolic dysfunction associated with HFD+OVX in either ERbDBDKO or WT mice, despite increased energy expenditure. Unexpectedly, in the ERbDBDKO group, wheel running increased fasting insulin and surrogate measures of insulin resistance, and modestly increased adipose tissue inflammatory

gene expression (P </= 0.05). These changes were not accompanied by significant changes in adipocyte mitochondrial respiration. It was demonstrated for the first time that female WT OVX mice do experience exercise-induced browning of white adipose tissue, indicated by a robust increase in uncoupling protein 1 (UCP1) (P </= 0.05). However, KO mice were completely resistant to this effect, indicating that full ERb genomic activity is required for exercise-induced browning. The inability to upregulate UCP1 with exercise following OVX may have resulted in the increased insulin resistance observed in KO mice, a hypothesis requiring further investigation.

Collier, J. J., et al. (2021). "Pancreatic, but not myeloid-cell, expression of interleukin-1alpha is required for maintenance of insulin secretion and whole body glucose homeostasis." Molecular Metabolism 44:101140. PMID: 33285301; PMCID: PMC7772372.

Objective: The expression of the interleukin-1 receptor type I (IL-1R) is enriched in pancreatic islet β-cells, signifying that ligands activating this pathway are important for the health and function of the insulin-secreting cell. Using isolated mouse, rat, and human islets, we identified the cytokine IL-1α as a highly inducible gene in response to IL-1R activation. In addition, IL-1α is elevated in mouse and rat models of obesity and Type 2 diabetes. Since less is known about the biology of IL-1α relative to IL-1β in pancreatic tissue, our objective was to investigate the contribution of IL-1α to pancreatic β-cell function and overall glucose homeostasis in vivo.

Methods: We generated a novel mouse line with conditional IL-1α alleles and subsequently produced mice with either pancreatic- or myeloid lineage-specific deletion of IL-1α.

Results: Using this in vivo approach, we discovered that pancreatic (IL-1αPdx1-/-), but not myeloid-cell, expression of IL-1α (IL-1αLysM-/-) was required for the maintenance of whole body glucose homeostasis in both male and female mice. Moreover, pancreatic deletion of IL-1α led to impaired glucose tolerance with no change in insulin sensitivity. This observation was consistent with our finding that glucose-stimulated insulin secretion was reduced in islets isolated from IL-1αPdx1-/- mice. Alternatively, IL-1αLysM-/- mice (male and female) did not have any detectable changes in glucose tolerance, respiratory quotient, physical activity, or food intake when compared with littermate controls.

Conclusions: Taken together, we conclude that there is an important physiological role for pancreatic IL-1α to promote glucose homeostasis by supporting glucose-stimulated insulin secretion and islet β-cell mass in vivo.

Collier, J. J., et al. (2021). " Pioglitazone Reverses Markers of Islet Beta-Cell De-Differentiation in db/db Mice While Modulating Expression of Genes Controlling Inflammation and Browning in White Adipose Tissue from Insulin-Resistant Mice and Humans." Biomedicines 10;9(9):1189.

Obesity, insulin resistance, and type 2 diabetes contribute to increased morbidity and mortality in humans. The db/db mouse is an important mouse model that displays many key features of the human

disease. Herein, we used the drug pioglitazone, a thiazolidinedione with insulin-sensitizing properties, to investigate blood glucose levels, indicators of islet β-cell health and maturity, and gene expression in adipose tissue. Oral administration of pioglitazone lowered blood glucose levels in db/db mice with a corresponding increase in respiratory quotient, which indicates improved whole-body carbohydrate utilization. In addition, white adipose tissue from db/db mice and from humans treated with pioglitazone showed increased expression of glycerol kinase. Both db/db mice and humans given pioglitazone displayed increased expression of UCP-1, a marker typically associated with brown adipose tissue. Moreover, pancreatic β-cells from db/db mice treated with pioglitazone had greater expression of insulin and Nkx6.1 as well as reduced abundance of the de-differentiation marker Aldh1a3. Collectively, these findings indicate that four weeks of pioglitazone therapy improved overall metabolic health in db/db mice. Our data are consistent with published reports of human subjects administered pioglitazone and with analysis of human adipose tissue taken from subjects treated with pioglitazone. In conclusion, the current study provides evidence that pioglitazone restores key markers of metabolic health and also showcases the utility of the db/db mouse to understand mechanisms associated with human metabolic disease and interventions that provide therapeutic benefit.

Cox, N., et al. (2021). "Diet-regulated production of PDGFcc by macrophages controls energy storage." Science **373**(6550).

The mechanisms by which macrophages regulate energy storage remain poorly understood. We identify in a genetic screen a platelet-derived growth factor (PDGF)/vascular endothelial growth factor (VEGF)-family ortholog, Pvf3, that is produced by macrophages and is required for lipid storage in fat-body cells of Drosophila larvae. Genetic and pharmacological experiments indicate that the mouse Pvf3 ortholog PDGFcc, produced by adipose tissue-resident macrophages, controls lipid storage in adipocytes in a leptin receptor- and C-C chemokine receptor type 2-independent manner. PDGFcc production is regulated by diet and acts in a paracrine manner to control lipid storage in adipose tissues of newborn and adult mice. At the organismal level upon PDGFcc blockade, excess lipids are redirected toward thermogenesis in brown fat. These data identify a macrophage-dependent mechanism, conducive to the design of pharmacological interventions, that controls energy storage in metazoans.

Dalbøge, L. S., et al. (2021). "Evaluation of VGF peptides as potential anti-obesity candidates in pre-clinical animal models." Peptides.

VGF is a peptide precursor expressed in neuroendocrine cells that is suggested to play a role in the regulation of energy homeostasis. VGF is proteolytically cleaved to yield multiple bioactive peptides. However, the specific actions of VGF-derived peptides on energy homeostasis remain unclear. The aim of the present work was to investigate the role of VGF-derived peptides in energy homeostasis and explore the pharmacological actions of VGF-derived peptides on body weight in preclinical animal models. VGF-derived peptides (NERP-1, NERP-2, PGH-NH2, PGH−OH, NERP-4, TLQP-21, TLQP-30, TLQP-62, HHPD-41, AQEE-30, and LQEQ-19) were synthesized and screened for their ability to affect neuronal activity in vitro on hypothalamic brain slices and modulate food intake and energy expenditure after acute central

administration in vivo. In addition, the effects of NERP-1, NERP-2, PGH-NH2, TLQP-21, TLQP-62, and HHPD-41 on energy homeostasis were studied after chronic central infusion. NERP-1, PGH-NH2, HHPD-41, and TLQP-62 increased the functional activity of hypothalamic neuronal networks. However, none of the peptides altered energy homeostasis after either acute or chronic ICV administration. The present data do not support the potential use of the tested VGF-derived peptides as novel anti-obesity drug candidates.

DiCarlo, G. E., et al. (2021). "Autism-Associated Variant in the SLC6A3 Gene Alters the Oral Microbiome and Metabolism in a Murine Model." Front Psychiatry **12**: 655451.

Background: Altered dopamine (DA) signaling has been associated with autism spectrum disorder (ASD), a neurodevelopmental condition estimated to impact 1 in 54 children in the United States. There is growing evidence for alterations in both gastrointestinal function and oral microbiome composition in ASD. Recent work suggests that rare variants of the SLC6A3 gene encoding the DA transporter (DAT) identified in individuals with ASD result in structural and functional changes to the DAT. One such recently identified de novo mutation is a threonine to methionine substitution at position 356 of the DAT (DAT T356M). The DAT T356M variant is associated with ASD-like phenotypes in mice homozygous for the mutation (DAT T356M(+/+)), including social deficits, hyperactivity, and impaired DA signaling. Here, we determine the impact of this altered DA signaling as it relates to altered oral microbiota, and metabolic and gastrointestinal dysfunction. Methods: In the DAT T356M(+/+) mouse, we determine the oral microbiota composition, metabolic function, and gastrointestinal (GI) function. We examined oral microbiota by 16S RNA sequencing. We measured metabolic function by examining glucose tolerance and we probed gastrointestinal parameters by measuring fecal dimensions and weight. Results: In the DAT T356M(+/+) mouse, we evaluate how altered DA signaling relates to metabolic dysfunction and altered oral microbiota. We demonstrate that male DAT T356M(+/+) mice weigh less (Wild type (WT) = 26.48 +/- 0.6405 g, DAT T356M(+/+) = 24.14 +/- 0.4083 g) and have decreased body fat (WT = 14.89 +/- 0.6206%, DAT T356M(+/+) = 12.72 +/- 0.4160%). These mice display improved glucose handling (WT = 32.60 +/- 0.3298 kcal/g, DAT T356M(+/+) = 36.97 +/- 0.4910 kcal/g), and an altered oral microbiota. We found a significant decrease in Fusobacterium abundance. The abundance of Fusobacterium was associated with improved glucose handling and decreased body fat. Conclusions: Our findings provide new insights into how DAT dysfunction may alter gastrointestinal function, composition of the oral microbiota, and metabolism. Our data suggest that impaired DA signaling in ASD is associated with a number of metabolic and gastrointestinal changes which are common in individuals with ASD.

Enriquez, R. F., et al. (2021). "AgRP signalling negatively regulates bone mass." Journal of Neuroendocrinology **33**(5): e12978.

The central nervous system is an active and major regulator of bone structure and remodelling. Specifically, signalling within the hypothalamus has been shown to be critical to ensuring that skeletal functions align with whole body metabolic supply and demand. Here, we identify agouti-related peptide

(AgRP), an orexigenic peptide exclusively co-expressed with neuropeptide Y (NPY) in the arcuate nucleus (ARC) of the hypothalamus, as another critical player in the central control of bone homeostasis. Using novel mouse models, we show that AgRP deletion leads to an increase in cortical and trabecular bone mass as a result of an increase in bone thickness despite a lean phenotype, particularly in male mice. Interestingly, male AgRP deficient mice display a significant decrease in pro-opiomelanocortin (POMC) expression in the ARC, but no change in NPY or CART expression, suggesting that the increase in bone mass in AgRP-deficient mice is unlikely to be a result of altered NPY signalling. This is consistent with the observation that bone mass is unchanged in response to the specific deletion of NPY from AgRP expressing neurones. By contrast, POMC expression in the ARC is significantly increased in female AgRP deficient mice, although AgRP deletion results in altered respiratory exchange ratio regulation in response to re-feeding after a fast in both sexes. Taken together, the present study identifies AgRP as being directly involved in the regulation of bone mass and highlights the complexity intrinsic to the neuropeptide regulation of the skeleton.

Erikson, C. M., et al. (2021). " Independent of differences in taste, B6N mice consume less alcohol than genetically similar B6J mice, and exhibit opposite polarity modulation of tonic GABA A R currents by alcohol " Neuropharmacology, Dec 20;206:108934

Genetic differences in cerebellar sensitivity to alcohol (EtOH) influence EtOH consumption phenotype in animal models and contribute to risk for developing an alcohol use disorder in humans. We previously determined that EtOH enhances cerebellar granule cell (GC) tonic GABAAR currents in low EtOH consuming rodent genotypes, but suppresses it in high EtOH consuming rodent genotypes. Moreover, pharmacologically counteracting EtOH suppression of GC tonic GABAAR currents reduces EtOH consumption in high alcohol consuming C57BL/6J (B6J) mice, suggesting a causative role. In the low EtOH consuming rodent models tested to date, EtOH enhancement of GC tonic GABAAR currents is mediated by inhibition of neuronal nitric oxide synthase (nNOS) which drives increased vesicular GABA release onto GCs and a consequent enhancement of tonic GABAAR currents. Consequently, genetic variation in nNOS expression across rodent genotypes is a key determinant of whether EtOH enhances or suppresses tonic GABAAR currents, and thus EtOH consumption. We used behavioral, electrophysiological, and immunocytochemical techniques to further explore the relationship between EtOH consumption and GC GABAAR current responses in C57BL/6N (B6N) mice. B6N mice consume significantly less EtOH and achieve significantly lower blood EtOH concentrations than B6J mice, an outcome not mediated by differences in taste. In voltage-clamped GCs, EtOH enhanced the GC tonic current in B6N mice but suppressed it in B6J mice. Immunohistochemical and electrophysiological studies revealed significantly higher nNOS expression and function in the GC layer of B6N mice compared to B6Js. Collectively, our data demonstrate that despite being genetically similar, B6N mice consume significantly less EtOH than B6J mice, a behavioral difference paralleled by increased cerebellar nNOS expression and opposite EtOH action on GC tonic GABAAR currents in each genotype.

Faber, C. L., et al. (2021). "Leptin receptor neurons in the dorsomedial hypothalamus regulate diurnal patterns of feeding, locomotion, and metabolism." Elife **10**.

The brain plays an essential role in driving daily rhythms of behavior and metabolism in harmony with environmental light-dark cycles. Within the brain, the dorsomedial hypothalamic nucleus (DMH) has been

implicated in the integrative circadian control of feeding and energy homeostasis, but the underlying cell types are unknown. Here, we identify a role for DMH leptin receptor-expressing (DMH(LepR)) neurons in this integrative control. Using a viral approach, we show that silencing neurotransmission in DMH(LepR) neurons in adult mice not only increases body weight and adiposity but also phase-advances diurnal rhythms of feeding and metabolism into the light cycle and abolishes the normal increase in dark-cycle locomotor activity characteristic of nocturnal rodents. Finally, DMH(LepR)-silenced mice fail to entrain to a restrictive change in food availability. Together, these findings identify DMH(LepR) neurons as critical determinants of the daily time of feeding and associated metabolic rhythms.

Fang, H., et al. (2021). "The Role of Reduced Methionine in Mediating the Metabolic Responses to Protein Restriction Using Different Sources of Protein." Nutrients.

Dietary protein restriction and dietary methionine restriction (MR) produce a comparable series of behavioral, physiological, biochemical, and transcriptional responses. Both dietary regimens produce a similar reduction in intake of sulfur amino acids (e.g., methionine and cystine), and both diets increase expression and release of hepatic FGF21. Given that FGF21 is an essential mediator of the metabolic phenotype produced by both diets, an important unresolved question is whether dietary protein restriction represents de facto methionine restriction. Using diets formulated from either casein or soy protein with matched reductions in sulfur amino acids, we compared the ability of the respective diets to recapitulate the metabolic phenotype produced by methionine restriction using elemental diets. Although the soy-based control diets supported faster growth compared to casein-based control diets, casein-based protein restriction and soy-based protein restriction produced comparable reductions in body weight and fat deposition, and similar increases in energy intake, energy expenditure, and water intake. In addition, the prototypical effects of dietary MR on hepatic and adipose tissue target genes were similarly regulated by casein- and soy-based protein restriction. The present findings support the feasibility of using restricted intake of diets from various protein sources to produce therapeutically effective implementation of dietary methionine restriction.

Fang, H., et al. (2021). "Hepatic Nfe2l2 Is Not an Essential Mediator of the Metabolic Phenotype Produced by Dietary Methionine Restriction." Nutrients.

The principal sensing of dietary methionine restriction (MR) occurs in the liver, where it activates multiple transcriptional programs that mediate various biological components of the response. Hepatic Fgf21 is a key target and essential endocrine mediator of the metabolic phenotype produced by dietary MR. The transcription factor, Nfe2l2, is also activated by MR and functions in tandem with hepatic Atf4 to transactivate multiple, antioxidative components of the integrated stress response. However, it is unclear whether the transcriptional responses linked to Nfe2l2 activation by dietary MR are essential to the biological efficacy of the diet. Using mice with liver-specific deletion of Nfe2l2 (Nfe2l2fl/(Alb)) and their floxed littermates (Nfe2l2fl/fl) fed either Control or MR diets, the absence of hepatic Nfe2l2 had no effect on the ability of the MR diet to increase FGF21, reduce body weight and adiposity, and increase energy expenditure. Moreover, the primary elements of the hepatic transcriptome were similarly affected by MR in both genotypes, with the only major differences occurring in induction of the P450-associated drug metabolism pathway and the pentose glucuronate interconversion pathway. The biological significance

of these pathways is uncertain but we conclude that hepatic Nfe2l2 is not essential in mediating the metabolic effects of dietary MR.

Gremminger, V. L., et al. (2021). "Skeletal muscle specific mitochondrial dysfunction and altered energy metabolism in a murine model (oim/oim) of severe osteogenesis imperfecta." Molecular Genetics and Metabolism **132**(4): 244-253.

Osteogenesis imperfecta (OI) is a heritable connective tissue disorder with patients exhibiting bone fragility and muscle weakness. The synergistic biochemical and biomechanical relationship between bone and muscle is a critical potential therapeutic target, such that muscle weakness should not be ignored. Previous studies demonstrated mitochondrial dysfunction in the skeletal muscle of oim/oim mice, which model a severe human type III OI. Here, we further characterize this mitochondrial dysfunction and evaluate several parameters of whole body and skeletal muscle metabolism. We demonstrate reduced mitochondrial respiration in female gastrocnemius muscle, but not in liver or heart mitochondria, suggesting that mitochondrial dysfunction is not global in the oim/oim mouse. Myosin heavy chain fiber type distributions were altered in the oim/oim soleus muscle with a decrease (-33 to 50%) in type I myofibers and an increase (+31%) in type IIa myofibers relative to their wildtype (WT) littermates. Additionally, altered body composition and increased energy expenditure were observed oim/oim mice relative to WT littermates. These results suggest that skeletal muscle mitochondrial dysfunction is linked to whole body metabolic alterations and to skeletal muscle weakness in the oim/oim mouse.

Hew, J. J., et al. (2021). "Low-protein diet accelerates wound healing in mice post-acute injury." Burns Trauma **9**: tkab010.

Background: Wound healing processes are influenced by macronutrient intake (protein, carbohydrate and fat). The most favourable diet for cutaneous wound healing is not known, although high-protein diets are currently favoured clinically. This experimental study investigates the optimal macronutrient balance for cutaneous wound healing using a mouse model and the Geometric Framework, a nutrient modelling method, capable of analyzing the individual and interactive effects of a wide spectrum of macronutrient intake. Methods: Two adjacent and identical full-thickness skin excisions (1 cm(2)) were surgically created on the dorsal area of male C57BL/6 mice. Mice were then allocated to one of 12 high-energy diets that varied in protein, carbohydrate and fat content. In select diets, wound healing processes, cytokine expression, energy expenditure, body composition, muscle and fat reserves were assessed. Results: Using the Geometric Framework, we show that a low-protein intake, coupled with a balanced intake of carbohydrate and fat is optimal for wound healing. Mice fed a low-protein diet progressed quickly through wound healing stages with favourable wound inflammatory cytokine expression and significantly accelerated collagen production. These local processes were associated with an increased early systemic inflammatory response and a higher overall energy expenditure, related to metabolic changes occurring in key macronutrient reserves in lean body mass and fat depots. Conclusions: The results suggest that a low-protein diet may have a greater potential to accelerate wound healing than the current clinically used high-protein diets.

Higgins, K. V., et al. (2021). "Integrative Longitudinal Analysis of Metabolic Phenotype and Microbiota Changes During the Development of Obesity." Front Cell Infect Microbiol **11**: 671926.

Obesity has increased at an alarming rate over the past two decades in the United States. In addition to increased body mass, obesity is often accompanied by comorbidities such as Type II Diabetes Mellitus and metabolic dysfunction-associated fatty liver disease, with serious impacts on public health. Our understanding of the role the intestinal microbiota in obesity has rapidly advanced in recent years, especially with respect to the bacterial constituents. However, we know little of when changes in these microbial populations occur as obesity develops. Further, we know little about how other domains of the microbiota, namely bacteriophage populations, are affected during the progression of obesity. Our goal in this study was to monitor changes in the intestinal microbiome and metabolic phenotype following western diet feeding. We accomplished this by collecting metabolic data and fecal samples for shotgun metagenomic sequencing in a mouse model of diet-induced obesity. We found that after two weeks of consuming a western diet (WD), the animals weighed significantly more and were less metabolically stable than their chow fed counterparts. The western diet induced rapid changes in the intestinal microbiome with the most pronounced dissimilarity at 12 weeks. Our study highlights the dynamic nature of microbiota composition following WD feeding and puts these events in the context of the metabolic status of the mammalian host.

Hong, H., et al. (2021). "REV-ERBα agonist SR9009 suppresses IL-1β production in macrophages through BMAL1-dependent inhibition of inflammasome." Biochemical Pharmacol. **192:114701**

The circadian clock plays an important role in adapting organisms to the daily light/dark cycling environment. Recent research findings reveal the involvement of the circadian clock not only in physiological functions but also in regulating inflammatory responses under pathological situations. Previous studies showed that the time-of-day variance of leucocyte circulation and pro-inflammatory cytokines secretion could be directly regulated by the clock-related proteins, including BMAL1 and REV-ERBα in a 24-hour oscillation pattern. To investigate the molecular mechanism behind the regulation of inflammation by the core clock components, we focus on the inflammatory responses in macrophages. Using bone marrow-derived macrophages from wild type and myeloid selective BMAL1-knockout mice, we found that the production of inflammatory cytokines, particularly IL-1β, was dependent on the timing of the lipopolysaccharide (LPS) stimulation in macrophages. Pharmacological activation of REV-ERBα with SR9009 significantly suppressed the LPS-induced inflammation in vitro and in vivo. Particularly, the effect of SR9009 on inhibiting NLRP3-mediated IL-1β and IL-18 production in macrophages was dependent on BMAL1 expression. Further analysis of the metabolic activity in LPS-treated mice showed that knockout of BMAL1 in macrophages exacerbated the hypometabolic state and delayed the recovery from LPS-induced endotoxemia even in the presence of SR9009. These results demonstrated an anti-inflammatory role of REV-ERBα in endotoxin-induced inflammation, during which the secretion of IL-1β through the NLRP3 inflammasome pathway inhibited by SR9009 was regulated by BMAL1.

Huen, S. C., et al. (2021). "Hepatic FGF21 preserves thermoregulation and cardiovascular function during bacterial inflammation." Journal of Experimental Medicine **218**(10).

Sickness behaviors, including anorexia, are evolutionarily conserved responses to acute infections. Inflammation-induced anorexia causes dramatic metabolic changes, of which components critical to survival are unique depending on the type of inflammation. Glucose supplementation during the anorectic period induced by bacterial inflammation suppresses adaptive fasting metabolic pathways, including fibroblast growth factor 21 (FGF21), and decreases survival. Consistent with this observation, FGF21-deficient mice are more susceptible to mortality from endotoxemia and polybacterial peritonitis. Here, we report that increased circulating FGF21 during bacterial inflammation is hepatic derived and required for survival through the maintenance of thermogenesis, energy expenditure, and cardiac function. FGF21 signaling downstream of its obligate coreceptor, beta-Klotho (KLB), is required in bacterial sepsis. However, FGF21 modulates thermogenesis and chronotropy independent of the adipose, forebrain, and hypothalamus, which are operative in cold adaptation, suggesting that in bacterial inflammation, either FGF21 signals through a novel, undescribed target tissue or concurrent signaling of multiple KLB-expressing tissues is required.

Imai, N., et al. (2021). "Suppression of Fatty Acid Oxidation by Thioesterase Superfamily Member 2 in Skeletal Muscle Promotes Hepatic Steatosis and Insulin Resistance." Hepatology, **75:154-169**

Thioesterase superfamily member 2 (Them2) is highly expressed in oxidative tissues where it hydrolyzes long chain fatty acyl-CoA esters to free fatty acids and CoA. Although mice globally lacking Them2 (Them2-/-) are protected against diet-induced obesity, insulin resistance and hepatic steatosis, liver-specific Them2-/- mice remain susceptible. To explore the contribution of Them2 in extrahepatic tissues, we created mice with Them2 deleted in skeletal muscle (S-Them2-/-), cardiac muscle (C-Them2-/-) or adipose tissue (A-Them2-/-). When fed a high-fat diet, S-Them2-/- but not C-Them2-/- or A-Them2-/- mice exhibited reduced weight gain. Only S-Them2-/- mice exhibited improved glucose homeostasis together with improved insulin sensitivity in skeletal muscle. Increased rates of fatty acid oxidation in skeletal muscle of S-Them2-/- mice were reflected in alterations in skeletal muscle metabolites, including short chain fatty acids, branched chain amino acids and the pentose phosphate pathway. Protection from diet-induced hepatic steatosis in S-Them2-/- mice was attributable to increased VLDL triglyceride secretion rates in support of demands of increased muscle fatty acid utilization. These results reveal a key role for skeletal muscle Them2 in the pathogenesis of diet-induced obesity, insulin resistance and hepatic steatosis.

Ip, C. K. (2021). "An optimized protocol for establishing a chronic stress model in mice." STAR Protoc **2**(2): 100448.

Chronic stress has adverse consequences on many organ systems and physiological processes. However, existing protocols show large variability in response and are not suitable for female mice. Here, we provide a step-by-step protocol for establishing a reliable chronic stress model in mice that can be used in a variety of physiological settings. This protocol has been tested to be effective to produce a consistent response to stress in several mouse strains (C57BL/6J, 129X1/SvJ, B6.V-Lepob/J) and both sexes. For complete details on the use and execution of this protocol, please refer to Ip et al. (2019).

Jeong, D. Y., et al. (2021). "Deficiency of Tristetraprolin Triggers Hyperthermia through Enhancing Hypothalamic Inflammation." Int. J. Mol. Sci. **22(7), 3328**

Tristetraprolin (TTP), an RNA-binding protein, controls the stability of RNA by capturing AU-rich elements on their target genes. It has recently been identified that TTP serves as an anti-inflammatory protein by guiding the unstable mRNAs of pro-inflammatory proteins in multiple cells. However, it has not yet been investigated whether TTP affects the inflammatory responses in the hypothalamus. Since hypothalamic inflammation is tightly coupled to the disturbance of energy homeostasis, we designed the current study to investigate whether TTP regulates hypothalamic inflammation and thereby affects energy metabolism by utilizing TTP-deficient mice. We observed that deficiency of TTP led to enhanced hypothalamic inflammation via stimulation of a variety of pro-inflammatory genes. In addition, microglial activation occurred in the hypothalamus, which was accompanied by an enhanced inflammatory response. In line with these molecular and cellular observations, we finally confirmed that deficiency of TTP results in elevated core body temperature and energy expenditure. Taken together, our findings unmask novel roles of hypothalamic TTP on energy metabolism, which is linked to inflammatory responses in hypothalamic microglial cells.

Jiang, J., et al. (2021). "Endothelial BBSome is essential for vascular, metabolic, and retinal functions." Molecular Metabolism **53:101308**

Objectives: Endothelial cells that line the entire vascular system play a pivotal role in the control of various physiological processes, including metabolism. Additionally, endothelial dysfunction is associated with many pathological conditions, including obesity. Here, we assessed the role of the BBSome, a protein complex composed of eight Bardet-Biedl syndrome (BBS) proteins in endothelial cells.

Methods: We studied the effects of BBSome disruption in endothelial cells on vascular function, body weight, glucose homeostasis, and the liver and retina. For this, we generated mice with selective BBSome disruption in endothelial cells through Bbs1 gene deletion.

Results: We found that endothelial cell-specific BBSome disruption causes endothelial dysfunction, as indicated by the impaired acetylcholine-induced vasorelaxation in both the aorta and mesenteric artery. This was associated with an increase in the contractile response to thromboxane A2 receptor agonist (U46619) in the mesenteric artery. Mechanistically, we demonstrated that mice lacking the Bbs1 gene in endothelial cells show elevated vascular angiotensinogen gene expression, implicating renin-angiotensin system activation in the vascular changes evoked by endothelial BBSome deficiency. Strikingly, our data indicate that endothelial BBSome deficiency increases body weight and fat mass and causes hepatosteatosis along with alterations in hepatic expression of lipid metabolism-related genes and metabolomics profile. In addition, electroretinogram and optical coherence tomography analyses revealed functional and structural abnormalities in the retina, evoked by absence of the endothelial BBSome.

Conclusions: Our findings demonstrate that the BBSome in endothelial cells is required for the regulation of vascular function, adiposity, hepatic lipid metabolism, and retinal function.

Jing, Y., et al. (2021). "Effect of fecal microbiota transplantation on neurological restoration in a spinal cord injury mouse model: involvement of brain-gut axis." Microbiome **9**(1): 59.

BACKGROUND: Spinal cord injury (SCI) patients display disruption of gut microbiome, and gut dysbiosis exacerbate neurological impairment in SCI models. Cumulative data support an important role of gut microbiome in SCI. Here, we investigated the hypothesis that fecal microbiota transplantation (FMT) from healthy uninjured mice into SCI mice may exert a neuroprotective effect. RESULTS: FMT facilitated functional recovery, promoted neuronal axonal regeneration, improved animal weight gain and metabolic profiling, and enhanced intestinal barrier integrity and GI motility in SCI mice. High-throughput sequencing revealed that levels of phylum Firmicutes, family Christensenellaceae, and genus Butyricimonas were reduced in fecal samples of SCI mice, and FMT remarkably reshaped gut microbiome. Also, FMT-treated SCI mice showed increased amount of fecal short-chain fatty acids (SCFAs), which correlated with alteration of intestinal permeability and locomotor recovery. Furthermore, FMT downregulated IL-1beta/NF-kappaB signaling in spinal cord and NF-kappaB signaling in gut following SCI. CONCLUSION: Our study demonstrates that reprogramming of gut microbiota by FMT improves locomotor and GI functions in SCI mice, possibly through the anti-inflammatory functions of SCFAs. Video Abstract.

Johnson, N. R., et al. (2021). "Sex-specific life extension in tauopathy mice by CSF1R inhibition causing selective microglial depletion and suppressed pathogenesis." bioRxiv.

Microglia are a fundamental component of pathogenesis in many neurological conditions and have specialized functions that vary by disease stage or specific pathology. Drugs targeting colony-stimulating factor-1 receptor (CSF1R) to block microglial proliferation in preclinical disease models have shown mixed outcomes, thus the therapeutic potential of this approach remains unclear. Here, we evaluated CSF1R inhibitors in tauopathy mice using multiple dosing schemes, drug analogs, and longitudinal measurements in the brain and plasma. In both spontaneous disease and in tau fibril inoculation models, we found a region-dependent reduction in insoluble phosphorylated tau and replication-competent tau in mice treated with CSF1R inhibitors. Surprisingly, despite greater drug exposure and microglial depletion in male mice, we observed a rescue of aberrant behavior, reduced plasma neurofilament light chain, and extended survival in female mice only. Gene expression patterns in CSF1R inhibitor-treated tauopathy mice reverted toward a normal wildtype signature, and in vivo imaging revealed suppressed astrogliosis. However, we observed drug dose-dependent upregulation of immediate early genes in male mice only, indicating excitotoxicity, which may have masked functional benefits. Drug-resilient microglia in tauopathy mice exhibited a ramified morphology similar to wildtype microglia but with greater territory occupied per cell, and their transcriptome was neither disease-associated nor homeostatic, suggesting a unique microglial subtype. Our data argue that complete or continuous microglial ablation is neither required nor desired for neuroprotection, and that selective depletion of detrimental, tauopathy-activated microglia may be achieved by precise timing and dosing of CSF1R inhibitors. Importantly, therapeutics targeting microglia must consider sex-dependent effects on functional outcomes when weighing their translational potential for neurological disease.

Jones, A. A., et al. (2021). "Photoperiod Manipulation Reveals a Light-Driven Component to Daily Patterns of Ventilation in Male C57Bl/6J Mice." Journal of Biological Rhythms **36**(4): 346-358.

Obstructive sleep apnea is a common sleep disorder that increases risk for cardiovascular disease and mortality. The severity of sleep-disordered breathing in obstructive sleep apnea patients fluctuates with the seasons, opening the possibility that seasonal changes in light duration, or photoperiod, can influence mechanisms of breathing. Photoperiod can have profound effects on internal timekeeping and can reshape metabolic rhythms in mammals. While the daily rhythm in ventilation is largely shaped by the metabolic rate, less is known about whether ventilatory rhythms are altered in accordance with metabolism under different photoperiods. Here, we investigate the relationship between ventilation and metabolism under different photoperiods using whole-body plethysmography and indirect calorimetry. We find that the daily timing of ventilation is chiefly synchronized to dark onset and that light cues are important for maintaining daily ventilatory rhythms. Moreover, changes in ventilatory patterns are not paralleled by changes in oxygen consumption, energy expenditure, or respiratory exchange rate under different photoperiods. We conclude that ventilatory patterns are not only shaped by the metabolic rate and circadian timing but are also influenced by other light-driven factors. Collectively, these findings have clinical implications for the seasonal variations in sleep-disordered breathing found in individuals with obstructive sleep apnea.

Kakall, Z. M., et al. (2021). "Dynamic regional alterations in mouse brain neuronal activity following short-term changes in energy balance." Obesity (Silver Spring) **29**(10): 1650-1663.

OBJECTIVE: Knowledge of the functional contribution to energy homeostatic control by different brain areas is limited. This study employed a systematic approach to identify brain regions specifically influenced by a positive energy balance.
METHODS: The c-fos expression was mapped throughout the mouse brain after varying durations (24 hours to up to 14 days) of high-fat diet (HFD) exposure or after reversal from a 7-day HFD to a chow diet. In parallel, the metabolic and behavioral impacts of these treatments were examined.
RESULTS: A HFD elicited rapid and pronounced compensatory responses which were, however, insufficient to overcome the impact of the positive energy balance. Rapid and dynamic responses of c-fos expression throughout the brain were seen over the course of HFD exposure, with some regions showing linear-like responses and some regions exhibiting biphasic responses. The switch from HFD to chow resulted in metabolic compensations mitigating the effects of the negative energy balance and a heightened preference for sweet taste. Interestingly, this diet switch led to a significant c-fos activation in the lateral hypothalamus, an area unresponsive to HFD intervention.
CONCLUSIONS: Plasticity exists in the extended brain networks facilitating rapid adaptations dependent on energy availability. Knowledge of these critical control points may provide novel antiobesity treatment targets.

Kakhlon, O., et al. (2021). "Alleviation of a polyglucosan storage disorder by enhancement of autophagic glycogen catabolism." EMBO Molecular Medicine **13**(10): e14554.

This work employs adult polyglucosan body disease (APBD) models to explore the efficacy and mechanism of action of the polyglucosan-reducing compound 144DG11. APBD is a glycogen storage disorder (GSD) caused by glycogen branching enzyme (GBE) deficiency causing accumulation of poorly branched glycogen inclusions called polyglucosans. 144DG11 improved survival and motor parameters in a GBE knockin

(Gbe(ys/ys)) APBD mouse model. 144DG11 reduced polyglucosan and glycogen in brain, liver, heart, and peripheral nerve. Indirect calorimetry experiments revealed that 144DG11 increases carbohydrate burn at the expense of fat burn, suggesting metabolic mobilization of pathogenic polyglucosan. At the cellular level, 144DG11 increased glycolytic, mitochondrial, and total ATP production. The molecular target of 144DG11 is the lysosomal membrane protein LAMP1, whose interaction with the compound, similar to LAMP1 knockdown, enhanced autolysosomal degradation of glycogen and lysosomal acidification. 144DG11 also enhanced mitochondrial activity and modulated lysosomal features as revealed by bioenergetic, image-based phenotyping and proteomics analyses. As an effective lysosomal targeting therapy in a GSD model, 144DG11 could be developed into a safe and efficacious glycogen and lysosomal storage disease therapy.

Kaur, S., et al. (2021). "Disruption of global hypothalamic microRNA (miR) profiles and associated behavioral changes in California mice (Peromyscus californicus) developmentally exposed …." Hormones and ….

Developmental exposure to endocrine disrupting chemicals (EDCs), e.g., bisphenol A (BPA) or genistein (GEN), causes longstanding epigenome effects. MicroRNAs (miRs) regulate which mRNAs will be translated to proteins and thereby serve as the final checkpoint in epigenetic control. Scant amount is known, however, whether EDCs affect neural miRNA (miR) patterns. We aimed to test the hypothesis that developmental exposure of California mice (Peromyscus californicus) to GEN, BPA, or both chemicals influences hypothalamic miR/small RNA profiles and ascertain the extent such biomolecular alterations correlate with behavioral and metabolic changes. California mice were developmentally exposed to GEN (250 mg/kg feed weight, FW), GEN (250 mg/kg FW)+BPA (5 mg/kg FW), low dose (LD) BPA (5 mg/kg FW), or upper dose (UD) BPA (50 mg/kg FW). Adult offspring were tested in a battery of behavioral and metabolic tests; whereupon, mice were euthanized, brains were collected and frozen, small RNAs were isolated from hypothalamic punches, and subsequently sequenced. California mice exposed to one or both EDCs engaged in one or more repetitive behaviors. GEN, LD BPA, and UD BPA altered aspects of ultrasonic and audible vocalizations. Each EDC exposure led to sex-dependent differences in differentially expressed miR/small RNAs with miR7–2, miR146, and miR148a being increased in all female and male EDC exposed groups. Current findings reveal that developmental exposure to GEN and/or BPA affects hypothalamic miR/small RNA expression patterns, and such changes correlate with EDC-induced behavioral and metabolic alterations. miR146 is likely an important mediator and biomarker of EDC exposure in mammals, including humans.

Kershaw, E. E., et al. (2021). "A murine model of the human CREBRFR457Q obesity-risk variant does not influence energy or glucose homeostasis in response to nutritional stress." PLoS One

Obesity and diabetes have strong heritable components, yet the genetic contributions to these diseases remain largely unexplained. In humans, a missense variant in Creb3 regulatory factor (CREBRF) [rs373863828 (p.Arg457Gln); CREBRFR457Q] is strongly associated with increased odds of obesity but decreased odds of diabetes. Although virtually nothing is known about CREBRF's mechanism of action,

emerging evidence implicates it in the adaptive transcriptional response to nutritional stress downstream of TORC1. The objectives of this study were to generate a murine model with knockin of the orthologous variant in mice (CREBRFR458Q) and to test the hypothesis that this CREBRF variant promotes obesity and protects against diabetes by regulating energy and glucose homeostasis downstream of TORC1. To test this hypothesis, we performed extensive phenotypic analysis of CREBRFR458Q knockin mice at baseline and in response to acute (fasting/refeeding), chronic (low- and high-fat diet feeding), and extreme (prolonged fasting) nutritional stress as well as with pharmacological TORC1 inhibition, and aging to 52 weeks. The results demonstrate that the murine CREBRFR458Q model of the human CREBRFR457Q variant does not influence energy/glucose homeostasis in response to these interventions, with the exception of possible greater loss of fat relative to lean mass with age. Alternative preclinical models and/or studies in humans will be required to decipher the mechanisms linking this variant to human health and disease.

Kesner, A. J., et al. (2021). "Sex-dependent changes in murine striatal dopamine release, sleep, and behavior during spontaneous Δ-9-tetrahydrocannabinol abstinence." bioRxiv.

Withdrawal symptoms are observed upon cessation of cannabis use in humans. Although animal studies have examined withdrawal symptoms following exposure to delta-9-tetrahydrocannabinol (THC), difficulties in obtaining objective measures of spontaneous withdrawal using paradigms that mimic cessation of use in humans have slowed research. The neuromodulator dopamine (DA) is known to be affected by chronic THC treatment and plays a role in many behaviors related to human THC withdrawal symptoms. These symptoms include sleep disturbances that often drive relapse, and emotional behaviors, e.g., irritability and anhedonia. We examined THC withdrawal-induced changes in striatal DA release and the extent to which sleep disruption and behavioral maladaptation manifest during withdrawal in a mouse chronic cannabis exposure model. Using a THC treatment regimen known to produce tolerance we measured electrically elicited DA release in acute brain slices from different striatal subregions during early and late THC abstinence. Long-term polysomnographic recordings from mice were used to assess vigilance state and sleep architecture before, during, and after THC treatment. We additionally assessed how behaviors that model human withdrawal symptoms are altered by chronic THC treatment in early and late abstinence. We detected altered striatal DA release, sleep disturbances that mimic clinical observations, and behavioral maladaptation in mice following tolerance inducing THC treatment. Sex differences were observed in nearly all metrics. Altered striatal DA release, sleep and affect-related behaviors associated with spontaneous THC abstinence were more consistently observed in male mice. To our knowledge these findings provide the first model of directly translatable non-precipitated cannabis withdrawal symptoms, in particular, sleep disruption.

Kim, S., et al. (2021). "Osteoblastic glucocorticoid signaling exacerbates high-fat-diet- induced bone loss and obesity." Bone Res **9**(1): 40.

Chronic high-fat diet (HFD) consumption not only promotes obesity and insulin resistance, but also causes bone loss through mechanisms that are not well understood. Here, we fed wild-type CD-1 mice either chow or a HFD (43% of energy from fat) for 18 weeks; HFD-fed mice exhibited decreased trabecular volume (-28%) and cortical thickness (-14%) compared to chow-fed mice. In HFD-fed mice, bone loss was due to reduced bone formation and mineral apposition, without obvious effects on bone resorption. HFD feeding also increased skeletal expression of sclerostin and caused deterioration of the osteocyte

lacunocanalicular network (LCN). In mice fed HFD, skeletal glucocorticoid signaling was activated relative to chow-fed mice, independent of serum corticosterone concentrations. We therefore examined whether skeletal glucocorticoid signaling was necessary for HFD-induced bone loss, using transgenic mice lacking glucocorticoid signaling in osteoblasts and osteocytes (HSD2(OB/OCY)-tg mice). In HSD2(OB/OCY)-tg mice, bone formation and mineral apposition rates were not suppressed by HFD, and bone loss was significantly attenuated. Interestingly, in HSD2(OB/OCY)-tg mice fed HFD, both Wnt signaling (less sclerostin induction, increased beta-catenin expression) and glucose uptake were significantly increased, relative to diet- and genotype-matched controls. The osteocyte LCN remained intact in HFD-fed HSD2(OB/OCY)-tg mice. When fed a HFD, HSD2(OB/OCY)-tg mice also increased their energy expenditure and were protected against obesity, insulin resistance, and dyslipidemia. Therefore, glucocorticoid signaling in osteoblasts and osteocytes contributes to the suppression of bone formation in HFD-fed mice. Skeletal glucocorticoid signaling is also an important determinant of glucose uptake in bone, which influences the whole-body metabolic response to HFD.

Kislal, S., et al. (2021). "Mismatch between obesogenic intrauterine environment and low-fat postnatal diet may confer offspring metabolic advantage." Obes Sci Pract **7**(4): 450-461.

Objective: Mismatch between a depleted intrauterine environment and a substrate-rich postnatal environment confers an increased risk of offspring obesity and metabolic syndrome. Maternal diet-induced obesity (MATOB) is associated with the same outcomes. These experiments tested the hypothesis that a mismatch between a nutrient-rich intrauterine environment and a low-fat postnatal environment would ameliorate offspring metabolic morbidity. Methods: C57BL6/J female mice were fed either a 60% high-fat diet (HFD) or a 10% fat control diet (CD) for 14-week pre-breeding and during pregnancy/lactation. Offspring were weaned to CD. Weight was evaluated weekly; body composition was determined using EchoMRI. Serum fasting lipids and glucose and insulin tolerance tests were performed. Metabolic rate, locomotor, and sleep behavior were evaluated with indirect calorimetry. Results: MATOB-exposed/CD-weaned offspring of both sexes had improved glucose tolerance and insulin sensitivity compared to controls. Males had improved fasting lipids. Females had significantly increased weight and body fat percentage in adulthood compared to sex-matched controls. Females also had significantly increased sleep duration and reduced locomotor activity compared to males. Conclusions: Reduced-fat dietary switch following intrauterine and lactational exposure to MATOB was associated with improved glucose handling and lipid profiles in adult offspring, more pronounced in males. A mismatch between a high-fat prenatal and low-fat postnatal environment may confer a metabolic advantage. The amelioration of deleterious metabolic programming by strict offspring adherence to a low-fat diet may have translational potential.

Korstanje, R., et al. (2021). "The Jackson Laboratory Nathan Shock Center: impact of genetic diversity on aging." Geroscience **43**(5): 2129-2137.

Healthspan is a complex trait, influenced by many genes and environmental factors that accelerate or delay aging, reduce or increase disease risk, and extend or reduce lifespan. Thus, assessing the role of genetic variation in aging requires an experimental strategy capable of modeling the genetic and biological complexity of human populations. The goal of the The Jackson Laboratory Nathan Shock Center (JAX NSC) is to provide research resources and training for geroscience investigators that seek to understand the

role of genetics and genetic diversity on the fundamental process of aging and diseases of human aging using the laboratory mouse as a model system. The JAX NSC has available novel, deeply characterized populations of aged mice, performs state-of-the-art phenotyping of age-relevant traits, provides systems genetics analysis of complex data sets, and provides all of these resources to the geroscience community. The aged animal resources, phenotyping capacity, and genetic expertise available through the JAX NSC benefit the geroscience community by fostering cutting-edge, novel lines of research that otherwise would not be possible. Over the past 15 years, the JAX NSC has transformed aging research across the geroscience community, providing aging mouse resources and tissues to researchers. All JAX NSC data and tools are publicly disseminated on the Mouse Phenome Database and the JAX NSC website, thus ensuring that the resources generated and expertise acquired through the Center are readily available to the aging research community. The JAX NSC will continue to enhance its ability to perform innovative research using a mammalian model to illuminate novel genotype-phenotype relationships and provide a rational basis for designing effective risk assessments and therapeutic interventions to boost longevity and disease-free healthspan.

Kotschi, S., et al. (2021). "NFE2L1-mediated proteasome function protects from ferroptosis." Molecular Metabolism **101436**

Objective: Ferroptosis continues to emerge as a novel modality of cell death with important therapeutic implications for a variety of diseases, most notably cancer and degenerative diseases. While susceptibility, initiation, and execution of ferroptosis have been linked to reprogramming of cellular lipid metabolism, imbalances in iron-redox homeostasis, and aberrant mitochondrial respiration, the detailed mechanisms of ferroptosis are still insufficiently well understood.

Methods and results: Here we show that diminished proteasome function is a new mechanistic feature of ferroptosis. The transcription factor nuclear factor erythroid-2, like-1 (NFE2L1) protects from ferroptosis by sustaining proteasomal activity. In cellular systems, loss of NFE2L1 reduced cellular viability after the induction of both chemically and genetically induced ferroptosis, which was linked to the regulation of proteasomal activity under these conditions. Importantly, this was reproduced in a Sedaghatian-type Spondylometaphyseal Dysplasia (SSMD) patient-derived cell line carrying mutated glutathione peroxidase-4 (GPX4), a critical regulator of ferroptosis. Also, reduced proteasomal activity was associated with ferroptosis in Gpx4-deficient mice. In a mouse model for genetic Nfe2l1 deficiency, we observed brown adipose tissue (BAT) involution, hyperubiquitination of ferroptosis regulators, including the GPX4 pathway, and other hallmarks of ferroptosis.

Conclusion: Our data highlight the relevance of the NFE2L1-proteasome pathway in ferroptosis. Manipulation of NFE2L1 activity might enhance ferroptosis-inducing cancer therapies as well as protect from aberrant ferroptosis in neurodegeneration, general metabolism, and beyond.

Krumm, C. S., et al. (2021). "Inducible hepatic expression of CREBH mitigates diet-induced obesity, insulin resistance, and hepatic steatosis in mice." Journal of Biological Chemistry **297**(1): 100815.

Cyclic AMP-responsive element-binding protein H (CREBH encoded by Creb3l3) is a transcription factor that regulates the expression of genes that control lipid and glucose metabolism as well as inflammation.

CREBH is upregulated in the liver under conditions of overnutrition, and mice globally lacking the gene (CREBH(-/-)) are highly susceptible to diet-induced obesity, insulin resistance, and hepatic steatosis. The net protective effects of CREBH have been attributed in large part to the activities of fibroblast growth factor (Fgf)-21 (Fgf21), a target gene that promotes weight loss, improves glucose homeostasis, and reduces hepatic lipid accumulation. To explore the possibility that activation of the CREBH-Fgf21 axis could ameliorate established effects of high-fat feeding, we generated an inducible transgenic hepatocyte-specific CREBH overexpression mouse model (Tg-rtTA). Acute overexpression of CREBH in livers of Tg-rtTA mice effectively reversed diet-induced obesity, insulin resistance, and hepatic steatosis. These changes were associated with increased activities of thermogenic brown and beige adipose tissues in Tg-rtTA mice, leading to reductions in fat mass, along with enhanced insulin sensitivity and glucose tolerance. Genetically silencing Fgf21 in Tg-rtTA mice abrogated the CREBH-mediated reductions in body weight loss, but only partially reversed the observed improvements in glucose metabolism. These findings reveal that the protective effects of CREBH activation may be leveraged to mitigate diet-induced obesity and associated metabolic abnormalities in both Fgf21-dependent and Fgf21-independent pathways.

Ladyman, S. R., et al. (2021). "A reduction in voluntary physical activity in early pregnancy in mice is mediated by prolactin." Elife.

As part of the maternal adaptations to pregnancy, mice show a rapid, profound reduction in voluntary running wheel activity (RWA) as soon as pregnancy is achieved. Here, we evaluate the hypothesis that prolactin, one of the first hormones to change secretion pattern following mating, is involved in driving this suppression of physical activity levels during pregnancy. We show that prolactin can acutely suppress RWA in non-pregnant female mice, and that conditional deletion of prolactin receptors (Prlr) from either most forebrain neurons or from GABA neurons prevented the early pregnancy-induced suppression of RWA. Deletion of Prlr specifically from the medial preoptic area, a brain region associated with multiple homeostatic and behavioral roles including parental behavior, completely abolished the early pregnancy-induced suppression of RWA. As pregnancy progresses, prolactin action continues to contribute to the further suppression of RWA, although it is not the only factor involved. Our data demonstrate a key role for prolactin in suppressing voluntary physical activity during early pregnancy, highlighting a novel biological basis for reduced physical activity in pregnancy.

Lee, K., et al. (2021). "The minor allele of the CREBRF rs373863828 p. R457Q coding variant is associated with reduced levels of myostatin in males: Implications for body composition." medRxiv.

The minor allele (A) of the rs373863828 variant (p.Arg457Gln) in CREBRF is restricted to indigenous peoples of the Pacific islands (including New Zealand Māori and peoples of Polynesia), with a frequency up to 25% in these populations. This allele associates with a large increase in body mass index (BMI) but with significantly lower risk of type-2 diabetes (T2D). It is unclear whether the increased BMI is driven by increased adiposity or by increased lean mass. Hence, we undertook body composition analysis using DXA in 189 young men of Māori and Pacific descent living in Aotearoa New Zealand. The rs373863828 A allele was associated with a trend toward increased relative lean mass although this was not statistically

significant (p=0.06). Notably though this allele was associated with significantly lower circulating levels of the muscle inhibitory hormone myostatin (p<0.05). This was further investigated in two Arg458Gln knockin mouse models on FVB/Nj and C57Bl/6j backgrounds. Supporting the human data, significant increases in relative lean mass were observed in male knockin mice. This was more significant in older mice (p<0.01) where it was associated with increased grip strength (p<0.01) and lower levels of myostatin (p <0.05). Overall these results provide new evidence that the rs373863828 A-allele is associated with a reduction of myostatin levels which likely contributes to increased lean muscle mass component of BMI, at least in males.

Lee, S. M., et al. (2021). "Hepatocyte-Specific Loss of PPARgamma Protects Mice From NASH and Increases the Therapeutic Effects of Rosiglitazone in the Liver." Cell Mol Gastroenterol Hepatol **11**(5): 1291-1311.

BACKGROUND & AIMS: Nonalcoholic steatohepatitis (NASH) is commonly observed in patients with type 2 diabetes, and thiazolidinediones (TZD) are considered a potential therapy for NASH. Although TZD increase insulin sensitivity and partially reduce steatosis and alanine aminotransferase, the efficacy of TZD on resolving liver pathology is limited. In fact, TZD may activate peroxisome proliferator-activated receptor gamma (PPARgamma) in hepatocytes and promote steatosis. Therefore, we assessed the role that hepatocyte-specific PPARgamma plays in the development of NASH, and how it alters the therapeutic effects of TZD on the liver of mice with diet-induced NASH. METHODS: Hepatocyte-specific PPARgamma expression was knocked out in adult mice before and after the development of NASH induced with a high fat, cholesterol, and fructose (HFCF) diet. RESULTS: HFCF diet increased PPARgamma expression in hepatocytes, and rosiglitazone further activated PPARgamma in hepatocytes of HFCF-fed mice in vivo and in vitro. Hepatocyte-specific loss of PPARgamma reduced the progression of HFCF-induced NASH in male mice and increased the benefits derived from the effects of TZD on extrahepatic tissues and non-parenchymal cells. RNAseq and metabolomics indicated that HFCF diet promoted inflammation and fibrogenesis in a hepatocyte PPARgamma-dependent manner and was associated with dysregulation of hepatic metabolism. Specifically, hepatocyte-specific loss of PPARgamma plays a positive role in the regulation of methionine metabolism, and that could reduce the progression of NASH. CONCLUSIONS: Because of the negative effect of hepatocyte PPARgamma in NASH, inhibition of mechanisms promoted by endogenous PPARgamma in hepatocytes may represent a novel strategy that increases the efficiency of therapies for NAFLD.

Lewis, J. E., et al. (2021). "Relaxin/insulin-like family peptide receptor 4 (Rxfp4) expressing hypothalamic neurons modulate food intake and preference in mice." bioRxiv.

Relaxin/insulin-like-family peptide receptor-4 (RXFP4), the cognate receptor for insulin-like peptide 5 (INSL5), has previously been implicated in feeding behaviour. To explore Rxfp4 expression and physiology, we generated Rxfp4-Cre mice. Whole body chemogenetic activation (Dq) or inhibition (Di) of Rxfp4-expressing cells using designer receptors exclusively activated by designer drugs (DREADDs) altered food

intake and preference. Potentially underlying this effect, Rxfp4-expressing neurons were identified in nodose and dorsal root ganglia and the central nervous system, including the ventromedial hypothalamus (VMH). Single-cell RNA-sequencing defined a cluster of VMH Rxfp4-labelled cells expressing Esr1, Tac1 and Oxtr. VMH-restricted activation of Rxfp4-expressing (RXFP4VMH) cells using AAV-Dq recapitulated the whole body Dq feeding phenotype. Viral tracing demonstrated RXFP4VMH neural projections to the bed nucleus of the stria terminalis, paraventricular hypothalamus, paraventricular thalamus, central nucleus of the amygdala and parabrachial nucleus. These findings identify hypothalamic RXFP4 signalling as a key regulator of food intake and preference.

Li, D., et al. (2021). "Absence of CD47 maintains brown fat thermogenic capacity and protects mice from aging-related obesity and metabolic disorder." Biochemical and Biophysical Research Communications **575**: 14-19.

Brown and beige adipocytes burn energy to produce heat and could serve as a therapeutic target to counteract metabolic diseases including obesity and type 2 diabetes. Aging is associated with reduced brown fat mass and thermogenic capacity and a risk factor for metabolic diseases. Our previous studies implicated a role for CD47 in regulating brown fat function and energy balance in young adult animals. In this study, we further determined its role in natural aging related metabolic disorders. The results demonstrated that aged CD47 deficient mice (under normal chow diet) had reduced body weight and fat mass, and improved glucose tolerance as compared to aged wild type (WT) mice. Indirect calorimetry result showed that food intake and total activity were comparable between two genotypes. However, CD47 deficient mice had increased energy expenditure and better cold tolerance, accompanied by increased white adipose tissue browning and well-maintained juvenile morphology of brown adipose tissue (BAT). Moreover, transcriptome (RNA-seq) and pathway enrichment analysis revealed that BAT from aged CD47 deficient mice had upregulated genes involving in mitochondria oxidative phosphorylation, thermogenesis, fatty acid metabolism, and valine, leucine and isoleucine (BCAA) degradation, indicating the activated BAT status in aged CD47 deficient mice. Collectively, these data suggest that blocking CD47 signaling protects mice from natural aging-associated obesity and glucose intolerance, partially though activation and expansion of the thermogenic machinery, further supporting that CD47 maybe a potential target for aging related metabolic disorder.

Li, X., et al. (2021). "Cinnamomum cassia extract promotes thermogenesis during exposure to cold via activation of brown adipose tissue." Journal of Ethnopharmacology **266**: 113413.

ETHNOPHARMACOLOGICAL RELEVANCE: Cinnamomum cassia (L.) J.Presl (Lauraceae), a widely used traditional Chinese medicine, is well known to exert hot property. It is recorded as dispelling cold drug in ancient Chinese monographs, such as Synopsis of golden chamber published in Han dynasty. According to Chinese Pharmacopoeia (2015), Cinnamomum cassia (L.) J.Presl (Cinnamon) has the functions of dispersing cold, relieving pain, warming meridians and promoting blood circulation.

AIM OF THE STUDY: The aim of this study is to evaluate the effect of Cinnamon extract (CE) on cold endurance and the mechanism of thermogenesis activity.

MATERIALS AND METHODS: The improving effect of hypothermia were evaluated with body temperature by infrared camera and multi-thermo thermometer. In vivo, the thermogenic effect was observed with energy metabolism and substrate utilization. The activation of brown adipose tissue (BAT) was evaluated with the histomorphology and expression of thermogenic protein. In vitro, the uncoupling effect on mitochondrial was evaluated with Seahorse and fluorescent staining. The mechanism of thermogenesis was explored in brown adipocyte.

RESULTS: The body temperature and energy expenditure were significantly increased by CE administration in cold environment. In morphology, lipid droplets were reduced and the number of mitochondrial was increased. CE significantly increased the non-shivering thermogenesis via upregulating the expression of thermogenic protein. In vitro, the uncoupling effect was obviously along with the decreased mitochondrial membrane potential and ATP production. It was confirmed that the thermogenesis effect was induced via lipolysis and energy metabolism. In addition, CE also alleviated myocardium injury in the morphology in cold environment. Moreover, the major constituent was identified as (1) coumarin, (2) cinnamic acid, (3) cinnamaldehyde and (4) 2-methoxy cinnamaldehyde.

CONCLUSIONS: The mechanism of improving cold tolerance was related to lipolysis and activation of BAT. Meanwhile, we provided a kind of potential prevention methods for cold injury.

Li, Y., et al. (2021). "Food reward depends on TLR4 activation in dopaminergic neurons." Pharmacological Research **169**: 105659.

The rising prevalence of obesity and being overweight is a worldwide health concern. Food reward dysregulation is the basic factor for the development of obesity. Dopamine (DA) neurons in the ventral tegmental area (VTA) play a vital role in food reward. Toll-like receptor 4 (TLR4) is a transmembrane pattern recognition receptor that can be activated by saturated fatty acids. Here, we show that the deletion of TLR4 specifically in DA neurons increases body weight, increases food intake, and decreases food reward. Conditional deletion of TLR4 also decreased the activity of DA neurons while suppressing the expression of tyrosine hydroxylase (TH) in the VTA, which regulates the concentration of DA in the nucleus accumbens (NAc) to affect food reward. Meanwhile, AAV-Cre-GFP mediated VTA-specific TLR4-deficient mice recapitulates food reward of DAT-TLR4-KO mice. Food reward could be rescued by re-expressing TLR4 in VTA DA neurons. Moreover, effects of intra-VTA infusion of lauric acid (a saturated fatty acid with 12 carbon) on food reward were abolished in mice lacking TLR4 in DA neurons. Our study demonstrates the critical role of TLR4 signaling in regulating the activity of VTA DA neurons and the normal function of the mesolimbic DA system that may contribute to food reward.

Li, Y., et al. (2021). "Thioesterase superfamily member 1 undergoes stimulus-coupled conformational reorganization to regulate metabolism in mice." Nature Communications **12**(1): 3493.

In brown adipose tissue, thermogenesis is suppressed by thioesterase superfamily member 1 (Them1), a long chain fatty acyl-CoA thioesterase. Them1 is highly upregulated by cold ambient temperature, where it reduces fatty acid availability and limits thermogenesis. Here, we show that Them1 regulates metabolism by undergoing conformational changes in response to beta-adrenergic stimulation that alter Them1 intracellular distribution. Them1 forms metabolically active puncta near lipid droplets and mitochondria. Upon stimulation, Them1 is phosphorylated at the N-terminus, inhibiting puncta formation and activity and resulting in a diffuse intracellular localization. We show by correlative light and electron microscopy that Them1 puncta are biomolecular condensates that are inhibited by phosphorylation. Thus, Them1 forms intracellular biomolecular condensates that limit fatty acid oxidation and suppress thermogenesis. During a period of energy demand, the condensates are disrupted by phosphorylation to allow for maximal thermogenesis. The stimulus-coupled reorganization of Them1 provides fine-tuning of thermogenesis and energy expenditure.

Liao, C. Y., et al. (2021). "The Autophagy Inducer Spermidine Protects Against Metabolic Dysfunction During Overnutrition." Journals of Gerontology. Series A, Biological Sciences and Medical Sciences **76**(10): 1714-1725.

Autophagy, a process catabolizing intracellular components to maintain energy homeostasis, impacts aging and metabolism. Spermidine, a natural polyamine and autophagy activator, extends life span across a variety of species, including mice. In addition to protecting cardiac and liver tissue, spermidine also affects adipose tissue through unexplored mechanisms. Here, we examined spermidine in the links between autophagy and systemic metabolism. Consistently, daily injection of spermidine delivered even at late life is sufficient to cause a trend in life-span extension in wild-type mice. We further found that spermidine has minimal metabolic effects in young and old mice under normal nutrition. However, spermidine counteracts high-fat diet (HFD)-induced obesity by increasing lipolysis in visceral fat. Mechanistically, spermidine increases the hepatokine fibroblast growth factor 21 (FGF21) expression in liver without reducing food intake. Spermidine also modulates FGF21 in adipose tissues, elevating FGF21 expression in subcutaneous fat, but reducing it in visceral fat. Despite this, FGF21 is not required for spermidine action, since Fgf21-/- mice were still protected from HFD. Furthermore, the enhanced lipolysis by spermidine was also independent of autophagy in adipose tissue, given that adipose-specific autophagy-deficient (Beclin-1flox/+Fabp4-cre) mice remained spermidine-responsive under HFD. Our results suggest that the metabolic effects of spermidine occur through systemic changes in metabolism, involving multiple mechanistic pathways.

Lin, Y., et al. (2021). "The chemerin-CMKLR1 axis limits thermogenesis by controlling a beige adipocyte/IL-33/type 2 innate immunity circuit." Sci Immunol **6**(61).

IL-33-associated type 2 innate immunity has been shown to support beige fat formation and thermogenesis in subcutaneous inguinal white adipose tissue (iWAT), but little is known about how it is regulated in iWAT. Chemerin, as a newly identified adipokine, is clinically associated with obesity and metabolic disorders. We here show that cold exposure specifically reduces chemerin and its receptor chemerin chemokine-like receptor 1 (CMKLR1) expression in iWAT. Lack of chemerin or adipocytic

CMKLR1 enhances cold-induced thermogenic beige fat via potentiating type 2 innate immune responses. Mechanistically, we identify adipocytes, particularly beige adipocytes, as the main source for cold-induced IL-33, which is restricted by the chemerin-CMKLR1 axis via dampening cAMP-PKA signaling, thereby interrupting a feed-forward circuit between beige adipocytes and type 2 innate immunity that is required for cold-induced beige fat and thermogenesis. Moreover, specific deletion of adipocytic IL-33 inhibits cold-induced beige fat and type 2 innate immune responses. Last, genetic blockade of adipocytic CMKLR1 protects against diet-induced obesity and enhances the metabolic benefits of cold stimulation in preestablished obese mice. Thus, our study identifies the chemerin-CMKLR1 axis as a physiological negative regulator of thermogenic beige fat via interrupting adipose-immune communication and suggests targeting adipose CMKLR1 as a potential therapeutic strategy for obesity-related metabolic disorders.

Liu, C., et al. (2021). "Pharmacological treatment with FGF21 strongly improves plasma cholesterol metabolism to reduce atherosclerosis." Cardiovascular Research.

Fibroblast growth factor (FGF) 21, a key regulator of energy metabolism, is currently evaluated in humans for treatment of type 2 diabetes and non-alcoholic steatohepatitis. However, the effects of FGF21 on cardiovascular benefit, particularly on lipoprotein metabolism in relation to atherogenesis, remain elusive.Here, the role of FGF21 in lipoprotein metabolism in relation to atherosclerosis development was investigated by pharmacological administration of a half-life extended recombinant FGF21 protein to hypercholesterolaemic APOE*3-Leiden.CETP mice, a well-established model mimicking atherosclerosis initiation and development in humans. FGF21 reduced plasma total cholesterol, explained by a reduction in non-HDL-cholesterol. Mechanistically, FGF21 promoted brown adipose tissue (BAT) activation and white adipose tissue (WAT) browning, thereby enhancing the selective uptake of fatty acids from triglyceride-rich lipoproteins into BAT and into browned WAT, consequently accelerating the clearance of the cholesterol-enriched remnants by the liver. In addition, FGF21 reduced body fat, ameliorated glucose tolerance and markedly reduced hepatic steatosis, related to up-regulated hepatic expression of genes involved in fatty acid oxidation and increased hepatic VLDL-triglyceride secretion. Ultimately, FGF21 largely decreased atherosclerotic lesion area, which was mainly explained by the reduction in non-HDL-cholesterol as shown by linear regression analysis, decreased lesion severity, and increased atherosclerotic plaque stability index.FGF21 improves hypercholesterolaemia by accelerating triglyceride-rich lipoprotein turnover as a result of activating BAT and browning of WAT, thereby reducing atherosclerotic lesion severity and increasing atherosclerotic lesion stability index. We have thus provided additional support for the clinical use of FGF21 in the treatment of atherosclerotic cardiovascular disease.

Loehfelm, A., et al. (2021). "A New Zealand green-lipped mussel oil-enriched high-fat diet exhibits beneficial effects on body weight and metabolism in mice." British Journal of Nutrition **125**(9): 972-982.

To induce diet-induced obesity (DIO) in rodents, diets high in saturated fat and/or carbohydrates are commonly used. In the laboratory, standardised diets evolved over time without paying particular

attention to the effect of fat composition on metabolic alterations. In the present study, customised high-fat diets (HFD) enriched with a combination of lard and different concentrations of New Zealand green-lipped mussel (Perna canaliculus) oil or MSC Hoki (Macruronus novaezelandiae, blue grenadier) liver oil, important sources of n-3 PUFA, in comparison with a solely lard-based diet, were fed to lean and DIO male C57BL/6 mice and their effects on metabolic parameters were monitored. Intriguingly, an isoenergetic HFD containing 63 % of total fat in the form of mussel oil and only 28 % in the form of lard attenuated HFD-induced body weight gain after 1 and 4 weeks, respectively. Consistently, changing a lard-enriched HFD to the mussel oil diet reduced body weight markedly even after mice had been exposed to the former diet for 10 months. The weight-reducing effect of the diet was not caused by altered energy intake or expenditure, but was associated with reduced visceral fat mass. Collectively, these data suggest a novel weight-reducing potential of green-lipped mussel oil.

Lopes, N., et al. (2021). "Distinct metabolic programs established in the thymus control effector functions of gammadelta T cell subsets in tumor microenvironments." Nature Immunology **22**(2): 179-192.

Metabolic programming controls immune cell lineages and functions, but little is known about gammadelta T cell metabolism. Here, we found that gammadelta T cell subsets making either interferon-gamma (IFN-gamma) or interleukin (IL)-17 have intrinsically distinct metabolic requirements. Whereas IFN-gamma(+) gammadelta T cells were almost exclusively dependent on glycolysis, IL-17(+) gammadelta T cells strongly engaged oxidative metabolism, with increased mitochondrial mass and activity. These distinct metabolic signatures were surprisingly imprinted early during thymic development and were stably maintained in the periphery and within tumors. Moreover, pro-tumoral IL-17(+) gammadelta T cells selectively showed high lipid uptake and intracellular lipid storage and were expanded in obesity and in tumors of obese mice. Conversely, glucose supplementation enhanced the antitumor functions of IFN-gamma(+) gammadelta T cells and reduced tumor growth upon adoptive transfer. These findings have important implications for the differentiation of effector gammadelta T cells and their manipulation in cancer immunotherapy.

Luo, L., et al. (2021). "Glucocorticoid/Adiponectin Axis Mediates Full Activation of Cold-Induced Beige Fat Thermogenesis." Biomolecules **11**(11).

Glucocorticoids (GCs), a class of corticosteroids produced by the adrenal cortex in response to stress, exert obesity-promoting effects. Although adaptive thermogenesis has been considered an effective approach to counteract obesity, whether GCs play a role in regulating cold stress-induced thermogenesis remains incompletely understood. Here, we show that the circulating levels of stress hormone corticosterone (GC in rodents) were significantly elevated, whereas the levels of adiponectin, an adipokine that was linked to cold-induced adaptive thermogenesis, were decreased 48 h post cold exposure. The administration of a glucocorticoid hydrocortisone downregulated adiponectin protein and mRNA levels in both WAT and white adipocytes, and upregulated thermogenic gene expression in inguinal fat. In contrast, mifepristone, a glucocorticoid receptor antagonist, enhanced adiponectin expression and suppressed energy expenditure in vivo. Mechanistically, hydrocortisone suppressed adiponectin expression by antagonizing PPARgamma in differentiated 3T3-L1 adipocytes. Ultimately, adiponectin deficiency restored

mifepristone-decreased oxygen consumption and suppressed the expression of thermogenic genes in inguinal fat. Taken together, our study reveals that the GCs/adiponectin axis is a key regulator of beige fat thermogenesis in response to acute cold stress.

Luo, Z., et al. (2021). "Cajanolactone A, a Stilbenoid From Cajanus canjan (L.) Millsp, Prevents High-Fat Diet-Induced Obesity via Suppressing Energy Intake." Front Pharmacol **12**: 695561.

Cajanolactone A (CLA) is a stilbenoid isolated from Cajanus canjan (L.) Millsp with the potential to prevent postmenopausal obesity. In this study, the effect of CLA on high-fat diet (HFD)-induced obesity in female C57BL/6 mice was investigated. It was found that, treatment with CLA reduced the energy intake and effectively protected the mice from HFD-induced body weight gain, fat accumulation within the adipose tissues and liver, and impairment in energy metabolism. Further investigation revealed that CLA significantly down-regulated the expression of ORX, ORXR2, pMCH, and Gal in the hypothalamus and antagonized HFD-induced changes in the expression of UCP1, Pgc-1alpha, Tfam, and Mfn1 in the inguinal white adipose tissue (iWAT); Caveolin-1, MT and UCP3 in the perigonadal white adipose tissue (pWAT); and Pdhb, IRS2, Mttp, Hadhb, and Cpt1b in the liver. CLA also protected the pWAT and liver from HFD-induced mitochondrial damage. However, neither HFD nor CLA showed an effect on the mass of brown adipose tissue (BAT) or the expression of UCP1 in the BAT. In summary, our findings suggest that CLA is a potential drug candidate for preventing diet-induced obesity, at least in females. CLA works most likely by suppressing the hypothalamic expression of orexigenic genes, which leads to reduced energy intake, and subsequently, reduced fat accumulation, thereby protecting the adipose tissues and the liver from lipid-induced mitochondrial dysfunction.

Luo, Z. H., et al. (2021). "Cajanolactone A, a stilbenoid from Cajanus cajan, inhibits energy intake and lipid synthesis/storage, and promotes energy expenditure in ovariectomized …." Biomed Pharmacother.

Background: We had reported that cajanolactone A (CLA) from Cajanus cajan dose-dependently inhibited ovariectomy-induced obesity and liver steatosis in mice, showing potential to prevent postmenopausal obesity and fatty liver. In this study, the role of CLA in the regulation of energy and lipid homeostasis was investigated.

Methods: Ovariectomized mice treated with CLA or vehicle for 12 weeks were performed a 48 h monitoring for energy metabolism and food uptake. After that, hypothalami, perigonadal (pWATs), inguinal (iWATs) and brown (BATs) adipose tissues, livers, sera, and fecal and cecal contents were collected and analyzed.

Findings: In CLA-treated mice, we observed reduced food uptake; increased energy expenditure; inhibited expression of orexigenic genes (ORX, ORXR2, pMCH and Gal) in the hypothalami, of lipogenic genes (CD36, SREBP-1c, ChREBP, PPARγ) in the livers, and of lipid storage proteins in the WATs (FSP27, MEST and caveolin-1) and livers (FSP27, Plin2 and Plin5); stimulated expression of metabolism-related proteins (pATGL and Echs1) in the adipose tissues and of thermogenic protein (UCP1) in the inguinal WATs;

increased BAT content; increased mitochondria in the pWATs and livers; inhibited angiogenesis in the pWATs; and altered gut microbiome diversity with an increased abundance of Bacteroides.

Interpretation: CLA prevents ovariectomy-induced obesity and liver steatosis via regulating energy intake and lipid synthesis/storage, promoting UCP1-dependent heat production, and protecting the mitochondrial function of hepatocytes and adipocytes. The improved gut microecology and inhibited angiogenesis may also contribute to the anti-obese activity of CLA.

MacDonald, A. J., et al. (2021). " Astrocytes in the dorsal vagal complex are not activated by systemic glucoprivation and their chemogenetic activation does not elicit homeostatic glucoregulatory responses in mice." bioRxiv.

The dorsal vagal complex (DVC) is a brainstem site regulating diverse aspects of physiology including food intake and blood glucose homeostasis. Astrocytes are purported to play an active role in regulating DVC function and, by extension, physiological parameters. Previous work has demonstrated that DVC astrocytes directly sense hormones that regulate food intake and blood glucose and are critical for their effect. In addition, DVC astrocytes in ex vivo slices respond to low tissue glucose. The response of neurons, including catecholaminergic neurons, to low glucose is conditional on intact astrocyte signalling in slice preparations suggesting astrocytes are possibly the primary sensors of glucose deprivation (glucoprivation). Based on these findings we hypothesised that if DVC astrocytes act as glucoprivation sensors in vivo they would both show a response to systemic glucoprivation and drive physiological responses to restore blood glucose. We found that 2 hours of systemic glucoprivation induced neither FOS nor glial fibrillary acidic protein (GFAP)-immunoreactivity in DVC astrocytes, specifically those in the nucleus of the solitary tract (NTS). Furthermore, we found that while chemogenetic activation of DVC astrocytes suppressed food intake by reducing both meal size and meal number, this manipulation also suppressed food intake under conditions of glucoprivation. Chemogenetic activation of DVC astrocytes did not increase basal blood glucose nor protect against insulin-induced hypoglycaemia. In male mice chemogenetic DVC astrocyte activation did not alter glucose tolerance, in female mice the initial glucose excursion was reduced, suggesting enhanced glucose absorption. Taken together this suggests that as a whole-population DVC astrocytes do not function as glucoprivation sensors in vivo in mice. Instead, we propose that DVC astrocytes play an indispensable, homeostatic role to maintain the function of glucoregulatory neuronal circuitry.

Maharjan, B. R., et al. (2021). "Exercise induces favorable metabolic changes in white adipose tissue preventing high-fat diet obesity." Physiol Rep **9**(16): e14929.

Diet and/or exercise are cost effective interventions to treat obesity. However, it is unclear if the type of exercise undertaken can prevent the onset of obesity and if it can act through different effects on fat depots. In this study we did not allow obesity to develop so we commenced the high-fat diet (HFD) and exercise programs concurrently and investigated the effect of endurance exercise (END) and high-intensity interval training (HIIT) on changes in cellular adipogenesis, thermogenesis, fibrosis, and inflammatory markers in three different fat depots, on a HFD and a chow diet. This was to assess the effectiveness of exercise to prevent the onset of obesity-induced changes. Mice fed with chow or HFD

(45% kcal fat) were trained and performed either END or HIIT for 10 weeks (3 x 40 min sessions/week). In HFD mice, both exercise programs significantly prevented the increase in body weight (END: 17%, HIIT: 20%), total body fat mass (END: 46%, HIIT: 50%), increased lean mass as a proportion of body weight (Lean mass/BW) by 14%, and improved insulin sensitivity by 22%. Further evidence of the preventative effect of exercise was seen significantly decreased markers for adipogenesis, inflammation, and extracellular matrix accumulation in both subcutaneous adipose tissue (SAT) and epididymal adipose tissue (EPI). In chow, no such marked effects were seen with both the exercise programs on all the three fat depots. This study establishes the beneficial effect of both HIIT and END exercise in preventing metabolic deterioration, collagen deposition, and inflammatory responses in fat depots, resulting in an improved whole body insulin resistance in HFD mice.

Martin, R. E., et al. (2021). "Maternal Oxycodone Treatment Results in Neurobehavioral Disruptions in Mice Offspring." eNeuro **8**(4).

Opioid drugs are increasingly being prescribed to pregnant women. Such compounds can also bind and activate opioid receptors in the fetal brain, which could lead to long-term brain and behavioral disruptions. We hypothesized that maternal treatment with oxycodone (OXY), the primary opioid at the center of the current crisis, leads to later neurobehavioral disorders and gene expression changes in the hypothalamus and hippocampus of resulting offspring. Female mice were treated daily with 5 mg OXY/kg or saline solution (control; CTL) for two weeks before breeding and then throughout gestation. Male and female offspring from both groups were tested with a battery of behavioral and metabolic tests to measure cognition, exploratory-like, anxiety-like, voluntary physical activity, and socio-communication behaviors. qPCR analyses were performed for candidate gene expression patterns in the hypothalamus and hippocampus of OXY and CTL derived offspring. Developmental exposure to OXY caused socio-communication changes that persisted from weaning through adulthood. Such offspring also showed cognitive impairments, reduced voluntary physical activity, and weighed more than CTL counterparts. In the hippocampus, prenatal exposure to OXY caused sex-dependent differences in expression of genes encoding opioid receptors and those involved in serotonin signaling. OXY exposure induced changes in neuropeptide hormone expression and the epigenetic modulator, Dnmt3a, in the hypothalamus, which could result in epigenetic changes in this brain region. The findings suggest cause for concern that consumption of OXY by pregnant mothers may result in permanent neurobehavioral changes in their offspring. Further work is needed to determine the potential underpinning epigenetic mechanisms.

Tangseefa, P., Martin, S.K., Chin, P.Y. et al. (2021). “The mTORC1 complex in pre-osteoblasts regulates whole-body energy metabolism independently of osteocalcin”, Bone Research **volume 9**

Overnutrition causes hyperactivation of mTORC1-dependent negative feedback loops leading to the downregulation of insulin signaling and development of insulin resistance. In osteoblasts (OBs), insulin signaling plays a crucial role in the control of systemic glucose homeostasis. We utilized mice with conditional deletion of Rptor to investigate how the loss of mTORC1 function in OB affects glucose

metabolism under normal and overnutrition dietary states. Compared to the controls, chow-fed Rptorob−/− mice had substantially less fat mass and exhibited adipocyte hyperplasia. Remarkably, upon feeding with high-fat diet, mice with pre- and post-natal deletion of Rptor in OBs were protected from diet-induced obesity and exhibited improved glucose metabolism with lower fasting glucose and insulin levels, increased glucose tolerance and insulin sensitivity. This leanness and resistance to weight gain was not attributable to changes in food intake, physical activity or lipid absorption but instead was due to increased energy expenditure and greater whole-body substrate flexibility. RNA-seq revealed an increase in glycolysis and skeletal insulin signaling pathways, which correlated with the potentiation of insulin signaling and increased insulin-dependent glucose uptake in Rptor-knockout osteoblasts. Collectively, these findings point to a critical role for the mTORC1 complex in the skeletal regulation of whole-body glucose metabolism and the skeletal development of insulin resistance.

Mavanji, V., et al. (2021). "Orexin in Dorsal Raphe Nucleus enhances Physical Activity and Energy Expenditure." The FASEB Journal **35**(S1).

Background Lateral hypothalamic (LH) orexin neurons modulate spontaneous physical activity (SPA), and energy expenditure (EE). Orexin deficiency results in weight gain, whereas heightened LH orexin sensitivity results in obesity resistance. Orexins act on multiple brain sites, including the serotonergic dorsal raphe nucleus (DRN), which receives excitatory projections from the orexin neurons. Our earlier studies show presence of both orexin-1 (OX1R) and orexin-2 receptors (OX2R) in DRN, and higher expression of OX1R and OX2R in DRN of obesity resistant (OR) rats. We hypothesized that orexin in DRN enhances SPA and EE. Secondarily, we hypothesized that DRN GABA (gamma-aminobutyric acid) signaling mediates the effect of orexin on SPA and EE. Methods We manipulated orexin tone in DRN either by direct injections of orexin A into DRN or by chemogenetic activation of LH orexin neurons in aged (~15 mo) orexin-cre mice. Mice were implanted with a guide cannula targeting DRN, and simultaneously prepared with the stimulatory (hM3Dq floxed) designer receptors exclusively activated by designer drugs (DREADD) virus in LH (N = 16). All mice recovered for 3 weeks to allow transfection of the DREADD virus, and randomly assigned to saline or clozapine N-oxide (CNO, DREADD activator) treatment. Mice were transferred to Promethion indirect calorimetry cages (Sable Systems International) to continuously monitor SPA and EE. Body weight and food intake were measured daily. After acclimation, mice received either IP vehicle or CNO (1 mg/kg) in a repeated measure design. Fifteen minutes prior to IP CNO or saline, animals were infused with either the GABA agonist muscimol (32.5 pmol/0.2 µl), GABA antagonist bicuculline (32.5 pmol/0.2 µl) or vehicle into DRN. In a separate experiment, either orexin A (250 pmol/0.2 µl) or saline was injected into DRN without IP CNO injection, to study the direct effect of DRN orexin on SPA and EE for 24h post-injection. Results We found that DREADD activation of LH orexin neurons increases SPA and EE. Manipulation of GABA receptors (by muscimol or bicuculline) in DRN did not affect orexin neuron activation induced enhancement of SPA. On the other hand, intra-DRN OXA enhanced SPA and EE in these mice up to 4h post-injection, without affecting food intake. Conclusions These results support the idea that OXA in DRN facilitates negative energy balance by increasing physical activity-induced energy expenditure. In addition, these data suggest that the DRN is a prominent site modulating OXA-stimulated SPA and EE, and that modulation of DRN OXA is a potential strategy to mitigate age-induced reductions in SPA and EE.

McCann, M. A., et al. (2021). "Adipose expression of CREB3L3 modulates body weight during obesity." Scientific reports.

We found the hepatic transcription factor Cyclic-AMP Responsive Element Binding Protein 3-like-3 (CREB3L3) to be expressed in adipose tissue, and selectively downregulated in the more metabolically protective subcutaneous adipose tissue in obese mice and humans. We sought to elucidate the specific role of this factor in adipose biology. CREB3L3 fat-specific knockout mice were fed a high-fat diet to induce obesity and metabolic dysfunction. Additionally, we injected a flip-excision adeno-associated virus directly into the subcutaneous inguinal adipose tissue of Adiponectin-Cre mice to create a depot-specific overexpression model for further assessment. Fat-specific ablation of CREB3L3 enhanced weight gain and insulin resistance following high-fat feeding, as fat-specific knockout mice expended less energy and possessed more inflammatory adipose tissue. Conversely, inguinal fat CREB3L3 overexpression deterred diet-induced obesity and ameliorated metabolic dysfunction. Together, this study highlights the relevance of CREB3L3 in obese adipose tissue and demonstrates its role as a powerful body weight modulator.

Mercier, C., et al. (2021). "Diabetes Impaired Ischemia-Induced PDGF (Platelet-Derived Growth Factor) Signaling Actions and Vessel Formation Through the Activation of Scr Homology 2-Containing Phosphatase-1." Arterioscler Thromb Vasc Biol **41**(9): 2469-2482.

Objective: Critical limb ischemia is a major complication of diabetes characterized by insufficient collateral vessel development and proper growth factor signaling unresponsiveness. Although mainly deactivated by hypoxia, phosphatases are important players in the deregulation of proangiogenetic pathways. Previously, SHP-1 (Scr homology 2-containing phosphatase-1) was found to be associated with the downregulation of growth factor actions in the diabetic muscle. Thus, we aimed to gain further understanding of the impact of SHP-1 on smooth muscle cell (SMC) function under hypoxic and diabetic conditions.

Approach and Results: Despite being inactivated under hypoxic conditions, high glucose level exposure sustained SHP-1 phosphatase activity in SMC and increased its interaction with PDGFR (platelet-derived growth factor receptor)-beta, thus reducing PDGF proangiogenic actions. Overexpression of an inactive form of SHP-1 fully restored PDGF-induced proliferation, migration, and signaling pathways in SMC exposed to high glucose and hypoxia. Nondiabetic and diabetic mice with deletion of SHP-1 specifically in SMC were generated. Ligation of the femoral artery was performed, and blood flow was measured for 4 weeks. Blood flow reperfusion, vascular density and maturation, and limb survival were all improved while vascular apoptosis was attenuated in diabetic SMC-specific SHP-1 null mice as compared to diabetic mice.

Conclusions: Diabetes and high glucose level exposure maintained SHP-1 activity preventing hypoxia-induced PDGF actions in SMC. Specific deletion of SHP-1 in SMC partially restored blood flow reperfusion in the diabetic ischemic limb. Therefore, local modulation of SHP-1 activity in SMC could represent a potential therapeutic avenue to improve the proangiogenic properties of SMC under ischemia and diabetes.

Mills, E. L., et al. (2021). "UCP1 governs liver extracellular succinate and inflammatory pathogenesis." Nat Metab **3**(5): 604-617.

Non-alcoholic fatty liver disease (NAFLD), the most prevalent liver pathology worldwide, is intimately linked with obesity and type 2 diabetes. Liver inflammation is a hallmark of NAFLD and is thought to

contribute to tissue fibrosis and disease pathogenesis. Uncoupling protein 1 (UCP1) is exclusively expressed in brown and beige adipocytes, and has been extensively studied for its capacity to elevate thermogenesis and reverse obesity. Here we identify an endocrine pathway regulated by UCP1 that antagonizes liver inflammation and pathology, independent of effects on obesity. We show that, without UCP1, brown and beige fat exhibit a diminished capacity to clear succinate from the circulation. Moreover, UCP1KO mice exhibit elevated extracellular succinate in liver tissue that drives inflammation through ligation of its cognate receptor succinate receptor 1 (SUCNR1) in liver-resident stellate cell and macrophage populations. Conversely, increasing brown and beige adipocyte content in mice antagonizes SUCNR1-dependent inflammatory signalling in the liver. We show that this UCP1-succinate-SUCNR1 axis is necessary to regulate liver immune cell infiltration and pathology, and systemic glucose intolerance in an obesogenic environment. As such, the therapeutic use of brown and beige adipocytes and UCP1 extends beyond thermogenesis and may be leveraged to antagonize NAFLD and SUCNR1-dependent liver inflammation.

Mishra, I., et al. (2021). "Asprosin-neutralizing antibodies as a treatment for metabolic syndrome." Elife.

Background: Recently, we discovered a new glucogenic and centrally acting orexigenic hormone - asprosin. Asprosin is elevated in metabolic syndrome (MS) patients, and its genetic loss results in reduced appetite, leanness, and blood glucose burden, leading to protection from MS.

Methods: We generated three independent monoclonal antibodies (mAbs) that recognize unique asprosin epitopes and investigated their preclinical efficacy and tolerability in the treatment of MS.

Results: Anti-asprosin mAbs from three distinct species lowered appetite and body weight, and reduced blood glucose in a dose-dependent and epitope-agnostic fashion in three independent MS mouse models, with an IC50 of ~1.5 mg/kg. The mAbs displayed a half-life of over 3days in vivo, with equilibrium dissociation-constants in picomolar to low nanomolar range.

Conclusions: We demonstrate that anti-asprosin mAbs are dual-effect pharmacologic therapy that targets two key pillars of MS - over-nutrition and hyperglycemia. This evidence paves the way for further development towards an investigational new drug application and subsequent human trials for treatment of MS, a defining physical ailment of our time.

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Montgomery, M. K., et al. (2021). "Hexosaminidase A (HEXA) regulates hepatic sphingolipid and lipoprotein metabolism in mice." FASEB Journal **35**(12): e22046.

Hexosaminidase A (HexA), a heterodimer consisting of HEXA and HEXB, converts the ganglioside sphingolipid GM2 to GM3 by removing a terminal N-acetyl-d-galactosamine. HexA enzyme deficiency in humans leads to GM2 accumulation in cells, particularly in neurons, and is associated with neurodegeneration. While HexA and sphingolipid metabolism have been extensively investigated in the context of neuronal lipid metabolism, little is known about the metabolic impact of HexA and ganglioside degradation in other tissues. Here, we focussed on the role of HexA in the liver, which is a major regulator of systemic lipid metabolism. We find that hepatic Hexa expression is induced by lipid availability and increased in the presence of hepatic steatosis, which is associated with increased hepatic GM3 content. To assess the impact of HEXA on hepatic lipid metabolism, we used an adeno-associated virus to overexpress HEXA in the livers of high-fat diet fed mice. HEXA overexpression was associated with increased hepatic GM3 content and increased expression of enzymes involved in the degradation of glycated sphingolipids, ultimately driving sphingomyelin accumulation in the liver. In addition, HEXA overexpression led to substantial proteome remodeling in cell surface lipid rafts, which was associated with increased VLDL processing and secretion, hypertriglyceridemia and ectopic lipid accumulation in peripheral tissues. This study established an important role of HEXA in modulating hepatic sphingolipid and lipoprotein metabolism.

Mooli, R. G. R., et al. (2021). "Intestinal HIF-2α Regulates GLP-1 Secretion via Lipid Sensing in L-Cells." Cellular and Molecular

Background & Aims
Compelling evidence shows that glucagon-like peptide-1 (GLP-1) has a profound effect in restoring normoglycemia in type 2 diabetic patients by increasing pancreatic insulin secretion. Although L cells are the primary source of circulating GLP-1, the current therapies do not target L cells to increase GLP-1 levels. Our study aimed to determine the molecular underpinnings of GLP-1 secretion as an impetus to identify new interventions to target endogenous L cells.

Methods
We used genetic mouse models of intestine-specific overexpression of hypoxia-inducible factor (HIF)-1α and HIF-2α (VhlΔIE), conditional overexpression of intestinal HIF-2α (Hif-2αLSL;Vilin-Cre/ERT2), and intestine-specific HIF-2α knockout mice (Hif-2αΔIE) to show that HIF signaling, especially HIF-2α, regulates GLP-1 secretion.

Results
Our data show that intestinal HIF signaling improved glucose homeostasis in a GLP-1–dependent manner. Intestinal HIF potentiated GLP-1 secretion via the lipid sensor G-protein–coupled receptor (GPR)40 enriched in L cells. We show that HIF-2α regulates GPR40 in L cells and potentiates fatty acid–induced GLP-1 secretion via ERK. Using a genetic model of intestine-specific overexpression of HIF-2α, we show that HIF-2α is sufficient to increase GLP-1 levels and attenuate diet-induced metabolic perturbations such as visceral adiposity, glucose intolerance, and hepatic steatosis. Lastly, we show that intestinal HIF-2α

signaling acts as a priming mechanism crucial for postprandial lipid-mediated GLP-1 secretion. Thus, disruption of intestinal HIF-2α decreases GLP-1 secretion, leading to glucose intolerance.

Conclusions
In summary, we show that intestinal HIF signaling, particularly HIF-2α, regulates the lipid sensor GPR40, which is crucial for the lipid-mediated GLP-1 secretion, and suggest that HIF-2α is a potential target to induce endogenous GLP-1 secretion.

Morris, E. M., et al. (2021). "Reduced Liver-Specific PGC1a Increases Susceptibility for Short-Term Diet-Induced Weight Gain in Male Mice." Nutrients **13**(8).

The central integration of peripheral neural signals is one mechanism by which systemic energy homeostasis is regulated. Previously, increased acute food intake following the chemical reduction of hepatic fatty acid oxidation and ATP levels was prevented by common hepatic branch vagotomy (HBV). However, possible offsite actions of the chemical compounds confound the precise role of liver energy metabolism. Herein, we used a hepatocyte PGC1a heterozygous (LPGC1a) mouse model, with associated reductions in mitochondrial fatty acid oxidation and respiratory capacity, to assess the role of liver energy metabolism in systemic energy homeostasis. LPGC1a male, but not female, mice had a 70% greater high-fat/high-sucrose (HFHS) diet-induced weight gain compared to wildtype (WT) mice ($p < 0.05$). The greater weight gain was associated with altered feeding behavior and lower activity energy expenditure during the HFHS diet in LPGC1a males. WT and LPGC1a mice underwent sham surgery or HBV to assess whether vagal signaling was involved in the HFHS-induced weight gain of male LPGC1a mice. HBV increased HFHS-induced weight gain (85%, $p < 0.05$) in male WT mice, but not LPGC1a mice. These data demonstrate a sex-specific role of reduced liver energy metabolism in acute diet-induced weight gain, and the need for a more nuanced assessment of the role of vagal signaling in short-term diet-induced weight gain.

Niraula, A., et al. (2021). "Prostaglandin PGE2 receptor EP4 regulates microglial phagocytosis and increases susceptibility to diet-induced obesity." bioRxiv.

Background: In rodents, susceptibility to diet-induced obesity requires microglial activation, but the molecular components of this pathway remain incompletely defined. Prostaglandin E2 (PGE2) levels increase in the mediobasal hypothalamus during high fat diet (HFD) feeding, and the PGE2 receptor EP4 regulates microglial activation state and phagocytic activity, suggesting a potential role for microglial EP4 signaling in obesity pathogenesis.

Method: Metabolic phenotyping, as assessed by body weight, energy expenditure, glucose, and insulin tolerance, was performed in microglia-specific EP4 knockout (MG-EP4 KO) mice and littermate controls on HFD. Morphological and gene expression analysis of microglia, and a histological survey of microglia-neuron interactions in the arcuate nucleus was performed. Phagocytosis was assessed using in vivo and in vitro pharmacological techniques.

Results: Microglial EP4 deletion markedly reduced weight gain and food intake in response to HFD feeding. In correspondence with this lean phenotype, insulin sensitivity was also improved in the HFD-fed MG-EP4 KO mice though glucose tolerance remained surprisingly unaffected. Mechanistically, EP4-deficient

microglia showed an attenuated phagocytic state marked by reduced CD68 expression and fewer contacts with POMC neuron soma and processes. These cellular changes observed in the microglial EP4 knockout mice corresponded with an increased density of POMC neurites extending into the paraventricular nucleus.

Conclusion: These findings reveal that microglial EP4 signaling promotes body weight gain and insulin resistance during HFD feeding. Furthermore, the data suggest that curbing microglial phagocytic function may preserve POMC cytoarchitecture and PVN input to limit overconsumption during diet-induced obesity.

Nissinen, T. A., et al. (2021). "Muscle follistatin gene delivery increases muscle protein synthesis independent of periodical physical inactivity and fasting." FASEB Journal **35**(3): e21387.

Blocking of myostatin and activins effectively counteracts muscle atrophy. However, the potential interaction with physical inactivity and fasting in the regulation of muscle protein synthesis is poorly understood. We used blockade of myostatin and activins by recombinant adeno-associated virus (rAAV)-mediated follistatin (FS288) overexpression in mouse tibialis anterior muscle. To investigate the effects on muscle protein synthesis, muscles were collected 7 days after rAAV-injection in the nighttime or in the daytime representing high and low levels of activity and feeding, respectively, or after overnight fasting, refeeding, or ad libitum feeding. Muscle protein synthesis was increased by FS288 independent of the time of the day or the feeding status. However, the activation of mTORC1 signaling by FS288 was attenuated in the daytime and by overnight fasting. FS288 also increased the amount of mTOR colocalized with lysosomes, but did not alter their localization toward the sarcolemma. This study shows that FS288 gene delivery increases muscle protein synthesis largely independent of diurnal fluctuations in physical activity and food intake or feeding status, overriding the physiological signals. This is important for eg cachectic and sarcopenic patients with reduced physical activity and appetite. The FS288-induced increase in mTORC1 signaling and protein synthesis may be in part driven by increased amount of mTOR colocalized with lysosomes, but not by their localization toward sarcolemma.

Ou, Y., et al. (2021). "Baicalin improves podocyte injury in rats with diabetic nephropathy by inhibiting PI3K/Akt/mTOR signaling pathway." Open Med (Wars) **16**(1): 1286-1298.

Objective: To investigate the effect of baicalin on diabetic nephropathy (DN) rats and podocytes and its mechanism.

Methods: The rat models with DN were established by high-fat and high-sugar diet and intraperitoneal injection of streptozotocin. The fasting blood glucose (FBG) and weight of rats in each group were measured at 0, 1, 2, 3, and 4 weeks. Their biochemical indicators, expression of inflammatory, and antioxidant factors were measured using an automatic biochemical analyzer together with ELISA. Hematoxylin-eosin staining and periodic acid-schiff staining were used to observe the morphological changes in the kidneys of rats in each group. Finally, the expressions of related molecules and PI3K/Akt/mTOR signaling pathway proteins in renal tissues and podocytes were examined by qRT-PCR and Western blot.

Results: Compared with the DN group, the FBG and weight, serum creatinine, blood urea nitrogen, total cholesterol, triacylglycerol, microalbumin, and albumin/creatinine ratio were all significantly decreased in the Baicalin treatment groups in a concentration-dependent manner. The levels of inflammatory factors in kidney tissue and podocytes were decreased. In addition, the activities of lactate dehydrogenase and malondialdehyde in tissue were decreased, while the superoxide dismutase was increased. The pathological sections showed that glomerular atrophy and glomerular basement membrane thickening caused by hyperglycemia were improved in the Baicalin treatment groups. Meanwhile, baicalin inhibited the downregulation of Nephrin and Podocin expressions and upregulation of Desmin expression caused by DN, and inhibited the expressions of p-PI3K, p-Akt, and p-mTOR proteins.

Conclusion: Baicalin slows down podocyte injury caused by DN by inhibiting the activity of PI3K/Akt/mTOR signaling pathway.

Paraiso, I. L., et al. (2021). "Xanthohumol Pyrazole Derivative Improves Diet-Induced Obesity and Induces Energy Expenditure in High-Fat Diet-Fed Mice." ACS Pharmacol Transl Sci **4**(6): 1782-1793.

The energy intake exceeding energy expenditure (EE) results in a positive energy balance, leading to storage of excess energy and weight gain. Here, we investigate the potential of a newly synthesized compound as an inducer of EE for the management of diet-induced obesity and insulin resistance. Xanthohumol (XN), a prenylated flavonoid from hops, was used as a precursor for the synthesis of a pyrazole derivative tested for its properties on high-fat diet (HFD)-induced metabolic impairments. In a comparative study with XN, we report that 4-(5-(4-hydroxyphenyl)-1-methyl-1H-pyrazol-3-yl)-5-methoxy-2-(3-methylbut-2-en-1- yl)benzene-1,3-diol (XP) uncouples oxidative phosphorylation in C2C12 cells. In HFD-fed mice, XP improved glucose tolerance and decreased weight gain by increasing EE and locomotor activity. Using an untargeted metabolomics approach, we assessed the effects of treatment on metabolites and their corresponding biochemical pathways. We found that XP and XN reduced purine metabolites and other energy metabolites in the plasma of HFD-fed mice. The induction of locomotor activity was associated with an increase in inosine monophosphate in the cortex of XP-treated mice. Together, these results suggest that XP, better than XN, affects mitochondrial respiration and cellular energy metabolism to prevent obesity in HFD-fed mice.

Park, B. S., et al. (2021). "Enhanced lipid utilization is coupled to the sickness responses triggered by lipopolysaccharide." Biochem Biophys Res Commun.

Sickness symptoms exerted via inflammatory responses occur in several infectious and chronic diseases. A growing body of evidence suggests that altered nutrient availability and metabolism are tightly coupled to inflammatory processes. However, the relationship between metabolic shifts and the development of the sickness response has not been explored fully. Therefore, we aimed to evaluate metabolic phenotypes with a mouse model showing sickness symptoms via systemic administration of lipopolysaccharide (LPS) in the present study. LPS injection elevated the lipid utilization and circulating levels of fatty acids. It also increased the levels of β-hydroxybutyric acid, a ketone body produced from fatty acids. We confirmed the functional connectivity between nutrient utilization and inflammatory responses and demonstrated enhanced lipid utilization in the hypothalamus providing insights into hypothalamic control of sickness

responses. Collectively, these findings could help develop new therapeutic strategies to treat patients with severe sickness symptoms associated with infectious and chronic human diseases.

Parker, A. M., et al. (2021). "Characterisation of the Myocardial Mitochondria Structural and Functional Phenotype in a Murine Model of Diabetic Cardiomyopathy." Front Physiol **12**: 672252.

People affected by diabetes are at an increased risk of developing heart failure than their non-diabetic counterparts, attributed in part to a distinct cardiac pathology termed diabetic cardiomyopathy. Mitochondrial dysfunction and excess reactive oxygen species (ROS) have been implicated in a range of diabetic complications and are a common feature of the diabetic heart. In this study, we sought to characterise impairments in mitochondrial structure and function in a recently described experimental mouse model of diabetic cardiomyopathy. Diabetes was induced in 6-week-old male FVB/N mice by the combination of three consecutive-daily injections of low-dose streptozotocin (STZ, each 55 mg/kg i.p.) and high-fat diet (42% fat from lipids) for 26 weeks. At study end, diabetic mice exhibited elevated blood glucose levels and impaired glucose tolerance, together with increases in both body weight gain and fat mass, replicating several aspects of human type 2 diabetes. The myocardial phenotype of diabetic mice included increased myocardial fibrosis and left ventricular (LV) diastolic dysfunction. Elevated LV superoxide levels were also evident. Diabetic mice exhibited a spectrum of LV mitochondrial changes, including decreased mitochondria area, increased levels of mitochondrial complex-III and complex-V protein abundance, and reduced complex-II oxygen consumption. In conclusion, these data suggest that the low-dose STZ-high fat experimental model replicates some of the mitochondrial changes seen in diabetes, and as such, this model may be useful to study treatments that target the mitochondria in diabetes.

Prins, K., et al. (2021). "Sex and Genotype Dependent Effects of a Restricted Access Diet in Ghrelin-Deficient Mice." Journal of the Endocrine Society

Ghrelin, a peptide hormone secreted by the stomach, stimulates both appetite and reward signalling. Its deletion in mice results in poor recovery from metabolic challenges, like starvation, but does not affect food intake or body weight. While sex differences in appetite and feeding behavior have been reported, little is known about the role of ghrelin herein. To investigate this, we used a metabolic cage system to continuously monitor responses of ghrelin-deficient (GKO) and wildtype (WT) mice to three different diets. Male and female mice (5 weeks old) were housed individually in a Promethion system (Sable Systems, USA) and provided one of three diets for 9 weeks: RA, continuous chow with restricted access to a Western-style diet (WD; 2h access, 3d/week) in the light phase; CA, continuous access to both diets; CC, continuous chow. Glucose tolerance was assessed at week 7 by IPGTT; food intake (kcal/g bodyweight), energy expenditure and locomotor activity at week 8; body weight and body composition (EchoMRI, USA) at week 9. On access days, RA mice ate up to 60% of their 24h intake during the WD access period. Following WD access GKO RA mice ate less chow than WT RA mice. Intriguingly, this compensatory reduction in food intake by GKO mice occurred at different times for males and females. GKO RA males ate 45% less chow in the dark phase immediately after WD access ($p < 0.001$). In contrast, this reduction in food intake (30% less) did not occur until the following, non-access, day in GKO RA females (genotype-sex: $p < 0.05$). Depending on diet, GKO mice showed differential regulation of energy expenditure in the

light phase. Energy expenditure was 6–17% higher in GKO than WT mice in the RA group on access days and in the CA group. On non-access days, however, GKO mice in the RA group expended 13% less energy than WT RA mice ($p < 0.005$). Regardless of diet, locomotor activity in females was greater than in males ($p < 0.001$). However, GKO females in the RA and CC groups showed a marked 30% reduction in locomotor activity compared to WTs (genotype-sex: $p < 0.05$). After nine weeks, neither sex nor genotype effects were seen in body weight gain and composition of RA animals. CA females gained 17% more body weight and had a 6.1% higher fat percentage than CA males (both $p < 0.001$). In the CC group body weight gain did not differ, but GKO females had 3.1% more fat than WT females (genotype-sex: $p < 0.01$). Glucose tolerance (AUC) was similar in all groups. In conclusion, we demonstrated that ghrelin deficiency changes the response to the three diets in a sex-dependent manner. Especially, restricted access to WD differentially affected food intake timing and locomotor activity of male and female GKO mice. These results add to the growing body of evidence that ghrelin signaling is sexually dimorphic.

Queathem, E. D., et al. (2021). "White Adipose Tissue Depots Respond to Chronic Beta-3 Adrenergic Receptor Activation in a Sexually Dimorphic and Depot Divergent Manner." Cells **10**(12).

Beta-3 adrenergic receptor activation via exercise or CL316,243 (CL) induces white adipose tissue (WAT) browning, improves glucose tolerance, and reduces visceral adiposity. Our aim was to determine if sex or adipose tissue depot differences exist in response to CL. Daily CL injections were administered to diet-induced obese male and female mice for two weeks, creating four groups: male control, male CL, female control, and female CL. These groups were compared to determine the main and interaction effects of sex (S), CL treatment (T), and WAT depot (D). Glucose tolerance, body composition, and energy intake and expenditure were assessed, along with perigonadal (PGAT) and subcutaneous (SQAT) WAT gene and protein expression. CL consistently improved glucose tolerance and body composition. Female PGAT had greater protein expression of the mitochondrial uncoupling protein 1 (UCP1), while SQAT (S, $p < 0.001$) was more responsive to CL in increasing UCP1 (SxT, $p = 0.011$) and the mitochondrial biogenesis induction protein, PPARgamma coactivator 1alpha (PGC1alpha) (SxT, $p = 0.026$). Females also displayed greater mitochondrial OXPHOS (S, $p < 0.05$) and adiponectin protein content (S, $p < 0.05$). On the other hand, male SQAT was more responsive to CL in increasing protein levels of PGC1alpha (SxT, $p = 0.046$) and adiponectin (S, $p < 0.05$). In both depots and in both sexes, CL significantly increased estrogen receptor beta (ERbeta) and glucose-related protein 75 (GRP75) protein content (T, $p < 0.05$). Thus, CL improves systemic and adipose tissue-specific metabolism in both sexes; however, sex differences exist in the WAT-specific effects of CL. Furthermore, across sexes and depots, CL affects estrogen signaling by upregulating ERbeta.

Rahbani, J. F., et al. (2021). "Creatine kinase B controls futile creatine cycling in thermogenic fat." Nature **590**(7846): 480-485.

Obesity increases the risk of mortality because of metabolic sequelae such as type 2 diabetes and cardiovascular disease(1). Thermogenesis by adipocytes can counteract obesity and metabolic diseases(2,3). In thermogenic fat, creatine liberates a molar excess of mitochondrial ADP-purportedly via a phosphorylation cycle(4)-to drive thermogenic respiration. However, the proteins that control this futile creatine cycle are unknown. Here we show that creatine kinase B (CKB) is indispensable for thermogenesis resulting from the futile creatine cycle, during which it traffics to mitochondria using an internal mitochondrial targeting sequence. CKB is powerfully induced by thermogenic stimuli in both mouse and

human adipocytes. Adipocyte-selective inactivation of Ckb in mice diminishes thermogenic capacity, increases predisposition to obesity, and disrupts glucose homeostasis. CKB is therefore a key effector of the futile creatine cycle.

Ramos, S., et al. (2021). "A HYPOMETABOLIC DEFENSE STRATEGY AGAINST PLASMODIUM INFECTION." bioRxiv.

Hypoglycemia is a clinical hallmark of severe malaria, the often-lethal presentation of Plasmodium falciparum infection of humans. Here we report that mice reduce blood glucose levels in response to Plasmodium infection via a coordinated response whereby labile heme, an alarmin produced via hemolysis, induces anorexia and represses hepatic glucose production (HGP). While protective against unfettered immune-mediated inflammation, organ damage and anemia, when sustained over time heme-driven repression of HGP can progress towards hypoglycemia, compromising host energy expenditure and thermoregulation. This hypometabolic state arrests the development of asexual stages of Plasmodium spp., which undergo pyknosis and develop mitochondrial dysfunction. In response, Plasmodium activates a transcriptional program reducing its virulence and inducing sexual differentiation towards the production of transmissible gametocytes. We infer that malaria-associated hypoglycemia represents a trade-off of an evolutionarily conserved defense strategy restricting Plasmodium spp. from accessing host-derived glucose and balancing parasite virulence and transmission.

Regmi, P., et al. (2021). "Early or delayed time-restricted feeding prevents metabolic impact of obesity in mice." Journal of Endocrinology **248**(1): 75-86.

Time-restricted feeding (TRF) initiated early during the dark phase prevents the metabolic consequences of a high-fat diet in rodent models. However, the metabolic consequences of delaying the initiation of TRF, akin to breakfast skipping in humans, is unclear. We assigned 8-week-old male C57BL/6J mice (n = 192) to chow or high-fat diet ad libitum (AL) for 4 weeks, before randomization to continue AL or 10 h of TRF, initiated at lights off (TRFe) or 4-h after lights off (TRFd) for a further 8 weeks. Oral glucose tolerance tests (1 g/kg), metabolic monitoring and body composition by echoMRI were performed, and tissues were collected at six time points. TRF reduced weight and fat mass vs AL, with a greater reduction in TRFe vs TRFd. TRF improved glucose tolerance and protected mice from high-fat diet-induced hepatosteatosis vs AL, with no difference between TRFe and TRFd. TRF increased the amplitude of Bmal1, Cry1, Per2, Nampt, and Nocturnin mRNA levels in liver. A phase delay in Bmal1, Cry1, Per2, Reverbalpha, Nampt, NAD, Sirt1, and Nocturnin was observed in TRFd. Thus, delaying TRF limited the weight benefit and induced a phase delay in the hepatic clock, but improved metabolic health. Allowing more flexibility in when TRF is initiated may increase the translational potential of this dietary approach in humans.

Richard, A. J., et al. (2021). "Loss of STAT5 in adipocytes increases subcutaneous fat mass via sex-dependent and depot-specific pathways." bioRxiv.

The STAT (Signal Transducers and Activators of Transcription) family of transcription factors contributes to adipocyte development and function. STAT5A and STAT5B are induced during adipocyte differentiation and are primarily activated by growth hormone (GH). Studies in mice lacking adipocyte GH receptor or STAT5 support their roles in lipolysis-mediated reduction of adipose tissue mass. We have generated a mouse model lacking both STAT5 genes specifically in adipocytes (STAT5AKO). Notably, both sexes of STAT5AKO mice have increased inguinal adipose tissue without any changes in gonadal fat mass. However, both depots exhibit substantial differences in fat cell size. Study of STAT5AKO mice also have revealed that GH's ability to induce insulin resistance is dependent upon STAT5 in adipocytes, but its ability to reduce adipose tissue mass is STAT5 independent. Additional observations, which were not predicted, indicate that the causes and regulation of increased fat mass in STAT5AKO mice are sex- and depot-dependent.

Ritter, M. J., et al. (2021). "Nuclear Receptor CoRepressors, NCOR1 and SMRT, are required for maintaining systemic metabolic homeostasis." Mol Metab **53**: 101315.

OBJECTIVE: The nuclear receptor corepressor 1 (NCOR1) and the silencing mediator of retinoic acid and thyroid hormone (SMRT, also known as NCOR2) play critical and specific roles in nuclear receptor action. NCOR1, both in vitro and in vivo specifically regulates thyroid hormone (TH) action in the context of individual organs such as the liver, and systemically in the context of the hypothalamic-pituitary-thyroid (HPT) axis. In contrast, selective deletion of SMRT in the liver or globally has shown that it plays very little role in TH signaling. However, both NCOR1 and SMRT have some overlapping roles in hepatic metabolism and lipogenesis. Here, we determine the roles of NCOR1 and SMRT in global physiologic function and find if SMRT could play a compensatory role in the regulation of TH action, globally.

METHODS: We used a postnatal deletion strategy to disrupt both NCOR1 and SMRT together in all tissues at 8-9 weeks of age in male and female mice. This was performed using a tamoxifen-inducible Cre recombinase (UBC-Cre-ERT2) to KO (knockout) NCOR1, SMRT, or NCOR1 and SMRT together. We used the same strategy to KO HDAC3 in male and female mice of the same age. Metabolic parameters, gene expression, and thyroid function tests were analyzed.

RESULTS: Surprisingly, adult mice that acquired NCOR1 and SMRT deletion rapidly became hypoglycemic and hypothermic and perished within ten days of deletion of both corepressors. Postnatal deletion of either NCOR1 or SMRT had no impact on mortality. NCOR1/SMRT KO mice rapidly developed hepatosteatosis and mild elevations in liver function tests. Additionally, alterations in lipogenesis, beta oxidation, along with hepatic triglyceride and glycogen levels suggested defects in hepatic metabolism. The intestinal function was intact in the NCOR1/SMRT knockout (KO) mice. The KO of HDAC3 resulted in a distinct phenotype from the NCOR1/SMRT KO mice, whereas none of the HDAC3 KO mice succumbed after tamoxifen injection.

CONCLUSIONS: The KO of NCOR1 and SMRT rapidly leads to significant metabolic abnormalities that do not survive - including hypoglycemia, hypothermia, and weight loss. Hepatosteatosis rapidly developed

along with alterations in hepatic metabolism suggesting a contribution to the dramatic phenotype from liver injury. Glucose production and absorption were intact in NCOR1/SMRT KO mice, demonstrating a multifactorial process leading to their demise. HDAC3 KO mice have a distinct phenotype from the NCOR1/SMRT KO mice-which implies that NCOR1/SMRT together regulate a critical pathway that is required for survival in adulthood and is separate from HDAC3.

Rouabhi, M., et al. (2021). "BBSome ablation in SF1 neurons causes obesity without comorbidities." Molecular Metabolism

Objectives
The hypothalamic ventromedial nucleus (VMH) plays a major role in metabolic control, but the molecular mechanisms involved remain poorly defined. We analyzed the relevance of the BBSome, a protein complex composed of 8 Bardet–Biedl syndrome (BBS) proteins including BBS1, in VMH steroidogenic factor 1 (SF1) neurons for the control of energy homeostasis and related physiological processes.

Methods
We generated mice bearing selective BBSome disruption, through Bbs1 gene deletion, in SF1 neurons (SF1Cre/Bbs1fl/fl). We analyzed the consequence on body weight, glucose homeostasis, and cardiovascular autonomic function of BBSome loss in SF1 neurons.

Results
SF1Cre/Bbs1fl/fl mice had increased body weight and adiposity under normal chow conditions. Food intake, energy absorption, and digestive efficiency were not altered by Bbs1 gene deletion in SF1 neurons. SF1Cre/Bbs1fl/fl mice exhibited lower energy expenditure, particularly during the dark cycle. Consistent with this finding, SF1Cre/Bbs1fl/fl mice displayed reduced sympathetic nerve traffic and expression of markers of thermogenesis in brown adipose tissue. SF1Cre/Bbs1fl/fl mice also had lower sympathetic nerve activity to subcutaneous white adipose tissue that was associated with a protein expression profile that promotes lipid accumulation. Notably, despite obesity and hyperinsulinemia, SF1Cre/Bbs1fl/fl mice did not exhibit significant changes in glucose metabolism, insulin sensitivity, blood pressure, and baroreflex sensitivity.

Conclusions
Our findings demonstrate that the SF1 neuron BBSome is necessary for the regulation of energy homeostasis through modulation of the activity of the sympathetic nervous system and that the SF1 neuron BBSome is required for the development of obesity-related comorbidities.

Rusu, P. M., et al. (2021). "Dietary Essential Amino Acid Restriction Promotes Hyperdipsia via Hepatic FGF21." Nutrients **13**(5).

Prior studies have reported that dietary protein dilution (DPD) or amino acid dilution promotes heightened water intake (i.e., hyperdipsia) however, the exact dietary requirements and the mechanism responsible for this effect are still unknown. Here, we show that dietary amino acid (AA) restriction is sufficient and required to drive hyperdipsia during DPD. Our studies demonstrate that particularly dietary essential AA (EAA) restriction, but not non-EAA, is responsible for the hyperdipsic effect of total dietary

AA restriction (DAR). Additionally, by using diets with varying amounts of individual EAA under constant total AA supply, we demonstrate that restriction of threonine (Thr) or tryptophan (Trp) is mandatory and sufficient for the effects of DAR on hyperdipsia and that liver-derived fibroblast growth factor 21 (FGF21) is required for this hyperdipsic effect. Strikingly, artificially introducing Thr de novo biosynthesis in hepatocytes reversed hyperdipsia during DAR. In summary, our results show that the DPD effects on hyperdipsia are induced by the deprivation of Thr and Trp, and in turn, via liver/hepatocyte-derived FGF21.

Santos, L., et al. (2021). "ScRNA-seq Reveals a Role of Mammary Luminal Epithelium in Adipocyte Adaptations." Journal of the Endocrine Society

Almost four decades of research suggest a dynamic role of ductal epithelial cells in adipocyte adaptation in mammary gland white adipose tissue (mgWAT), but factors that mediate such communication are not known. Here, we identify a complex intercellular crosstalk in mgWAT revealed by single-cell RNA-seq (scRNA-seq) and comprehensive data analysis suggest that epithelial luminal cells during cold exposure undergo major transcriptomic changes that lead to the expression of an array of genes that encode for secreted factors involved in adipose metabolism such as Adropin (Enho), neuregulin 4 (Nrg4), angiopoietin-like 4 (Angptl4), lipocalin 2 (Lcn2), milk fat globule-EGF factor 8 (Mfge8), Insulin-like growth factor-binding protein 1 (Igfbp1), and haptoglobin (Hp). To define the mammary epithelial secretome, we coin the phrase “mammokines”. We validated our cluster annotations and cluster-specific transcriptomics using eight different adipose scRNA-seq datasets including Tabula Muris and Tabula Muris Senis. In situ mRNA hybridization and ex vivo isolated mgWAT luminal cells show highly localized expression of mammokines in mammary ducts. Trajectory inference demonstrates that cold-exposed luminal cells have similar transcriptional profiles to lactation post-involution (PI), a phase defined by reappearance and maintenance of adipocytes in the mammary gland. Concomitantly, we found that under cold exposure female mgWAT maintains more adipogenic and less thermogenic potential than male scWAT and ex vivo removal of luminal epithelial cells from mgWAT markedly potentiates beige adipocyte differentiation. Conditioned media from isolated mammary epithelial cells treated with isoproterenol suppressed thermogenesis in differentiated beige/brown adipocytes and treatment of beige/brown differentiated adipocyte with mammokine LCN2 suppresses thermogenesis and increases adipogenesis. Finally, we find that mice lacking LCN2 show markedly higher cold-dependent thermogenesis in mgWAT than controls, and reconstitution of LCN2 in the mgWAT of LCN2 knockout mice promotes inhibition of thermogenesis. These results show a previously unknown role of mammary epithelium in adipocyte metabolism and suggest a potentially redundant evolutionary role of mammokines in maintaining mgWAT adiposity during cold exposure. Our data highlight mammary gland epithelium as a highly active metabolic cell type and mammokines could have broader implications in mammary gland physiology and lipid metabolism.

Sinz, S., et al. (2021). "Effects of dietary grapeseed extract on performance, energy and nitrogen balance as well as methane and nitrogen losses of lambs and goat kids." British Journal of Nutrition

The influence of phenol-rich dietary grapeseed extract on performance, energy and N balance and methane production was determined in sixteen lambs and thirteen goat kids (body weight 20·5 and 19·0 kg, 2 months of age, day 1 of study). Half of the animals received a concentrate containing grapeseed extract, and the others received concentrate without grapeseed extract (total extractable phenols analysed 27 v. 9 g/kg dietary DM; concentrate and hay 1:1). Diets were fed for 7 weeks with 1 week for determining intake, excretion and gaseous exchange in metabolism crates and respiration chambers. Overall, there was an adverse effect of the phenolic diet on apparent N digestibility and body N retention. Faecal N loss as proportion of N intake increased while urinary N loss declined. Relative to N intake, total N excretion was higher and body N retention lower in goat kids than lambs. Diets and animal species had no effect on methane emissions. The saliva of the goat kids had a higher binding capacity for condensed tannins (CT). Goat kids on the phenolic diet had higher CT concentrations in faeces and excreted more CT compared with the lambs (interaction species × diet $P < 0·001$). The lambs had overall higher ($P < 0·001$) urinary phenol concentrations than the goat kids (2·19 v. 1·48 g/l). The negative effect on body N retention and lack of effect on methane emissions make the use of the extract in the dosage applied not appealing. Species differences need to be considered in future studies.

Spitler, K. M., et al. (2021). "Regulation of plasma triglyceride partitioning by adipose-derived ANGPTL4 in mice." Sci Rep **11**(1): 7873.

Elevated plasma triglyceride levels are associated with metabolic disease. Angiopoietin-like protein 4 (ANGPTL4) regulates plasma triglyceride levels by inhibiting lipoprotein lipase (LPL). Our aim was to investigate the role of adipocyte-specific deficiency of ANGPTL4 in mice during high fat diet feeding. Adipocyte-specific ANGPTL4 deficient mice were fed a high fat diet (60% kCal from fat) for either 12 weeks or 6 months. We performed plasma metabolic measurements, triglyceride clearance and uptake assays, LPL activity assays, and assessed glucose homeostasis. Mice lacking adipocyte ANGPTL4 recapitulated the triglyceride phenotypes of whole-body ANGPTL4 deficiency, including increased adipose LPL activity, lower plasma triglyceride levels, and increased uptake of triglycerides into adipose tissue. When fed a high fat diet (HFD), these mice continued to display enhanced adipose LPL activity and initially had improved glucose and insulin sensitivity. However, after 6 months on HFD, the improvements in glucose homeostasis were largely lost. Moreover, despite higher adipose LPL activity levels, mice lacking adipocyte ANGPTL4 no longer had increased triglyceride uptake into adipose compared to littermate controls after chronic high-fat feeding. These observations suggest that after chronic high-fat feeding LPL is no longer rate-limiting for triglyceride delivery to adipocytes. We conclude that while adipocyte-derived ANGPTL4 is an important regulator of plasma triglyceride levels and triglyceride partitioning under normal diet conditions, its role is diminished after chronic high-fat feeding.

Stone, A. C., et al. (2021). "Female Mice Are Protected from Metabolic Decline Associated with Lack of Skeletal Muscle HuR." Biology (Basel) **10**(6).

Male mice lacking HuR in skeletal muscle (HuR(m-/-)) have been shown to have decreased gastrocnemius lipid oxidation and increased adiposity and insulin resistance. The same consequences have not been documented in female HuR(m-/-) mice. Here we examine this sexually dimorphic phenotype. HuR(m-/-) mice have an increased fat mass to lean mass ratio (FM/LM) relative to controls where food intake is similar. Increased body weight for male mice correlates with increased blood glucose during glucose tolerance tests (GTT), suggesting increased fat mass in male HuR(m-/-) mice as a driver of decreased glucose clearance. However, HuR(m-/-) female mice show decreased blood glucose levels during GTT relative to controls. HuR(m-/-) mice display decreased palmitate oxidation in skeletal muscle relative to controls. This difference is more robust for male HuR(m-/-) mice and more exaggerated for both sexes at high dietary fat. A high-fat diet stimulates expression of Pgc1alpha in HuR(m-/-) male skeletal muscle, but not in females. However, the lipid oxidation Pparalpha pathway remains decreased in HuR(m-/-) male mice relative to controls regardless of diet. This pathway is only decreased in female HuR(m-/-) mice fed high fat diet. A decreased capacity for lipid oxidation in skeletal muscle in the absence of HuR may thus be linked to decreased glucose clearance in male but not female mice.

Sullivan, Z. A., et al. (2021). " γδ T cells regulate the intestinal response to nutrient sensing." Science **371**(6535).

The intestine is a site of direct encounter with the external environment and must consequently balance barrier defense with nutrient uptake. To investigate how nutrient uptake is regulated in the small intestine, we tested the effect of diets with different macronutrient compositions on epithelial gene expression. We found that enzymes and transporters required for carbohydrate digestion and absorption were regulated by carbohydrate availability. The "on-demand" induction of this machinery required gammadelta T cells, which regulated this program through the suppression of interleukin-22 production by type 3 innate lymphoid cells. Nutrient availability altered the tissue localization and transcriptome of gammadelta T cells. Additionally, transcriptional responses to diet involved cellular remodeling of the epithelial compartment. Thus, this work identifies a role for gammadelta T cells in nutrient sensing.

γδ T cells link immunity to nutrition
Gamma delta (γδ) T cells are immune cells best known for host barrier defenses in epithelial tissues. Sullivan et al. discovered a previously unrecognized role for γδ T cells in sensing nutrient uptake in the small intestine (see the Perspective by Talbot and Littman). The researchers analyzed mice fed a high-carbohydrate versus a high-protein diet and observed remodeling of the small intestinal epithelium in response to dietary carbohydrates. Nutrient availability triggered an epithelial–immune cell circuit that was required for digestion and absorption of carbohydrates. Intestinal γδ T cells regulated the expression of a carbohydrate transcriptional program by limiting interleukin-22 production from type 3 innate lymphoid cells. These findings may also provide insights into how γδ T cells modulate metabolic disease.

Sun, Y., et al. (2021). "Mitochondrial TNAP controls thermogenesis by hydrolysis of phosphocreatine." Nature **593**(7860): 580-585.

Adaptive thermogenesis has attracted much attention because of its ability to increase systemic energy expenditure and to counter obesity and diabetes(1-3). Recent data have indicated that thermogenic fat cells use creatine to stimulate futile substrate cycling, dissipating chemical energy as heat(4,5). This model was based on the super-stoichiometric relationship between the amount of creatine added to mitochondria and the quantity of oxygen consumed. Here we provide direct evidence for the molecular basis of this futile creatine cycling activity in mice. Thermogenic fat cells have robust phosphocreatine phosphatase activity, which is attributed to tissue-nonspecific alkaline phosphatase (TNAP). TNAP hydrolyses phosphocreatine to initiate a futile cycle of creatine dephosphorylation and phosphorylation. Unlike in other cells, TNAP in thermogenic fat cells is localized to the mitochondria, where futile creatine cycling occurs. TNAP expression is powerfully induced when mice are exposed to cold conditions, and its inhibition in isolated mitochondria leads to a loss of futile creatine cycling. In addition, genetic ablation of TNAP in adipocytes reduces whole-body energy expenditure and leads to rapid-onset obesity in mice, with no change in movement or feeding behaviour. These data illustrate the critical role of TNAP as a phosphocreatine phosphatase in the futile creatine cycle.

Tangseefa, P., et al. (2021). "The mTORC1 complex in pre-osteoblasts regulates whole-body energy metabolism independently of osteocalcin." Bone Res **9**(1): 10.

Overnutrition causes hyperactivation of mTORC1-dependent negative feedback loops leading to the downregulation of insulin signaling and development of insulin resistance. In osteoblasts (OBs), insulin signaling plays a crucial role in the control of systemic glucose homeostasis. We utilized mice with conditional deletion of Rptor to investigate how the loss of mTORC1 function in OB affects glucose metabolism under normal and overnutrition dietary states. Compared to the controls, chow-fed Rptorob(-/-) mice had substantially less fat mass and exhibited adipocyte hyperplasia. Remarkably, upon feeding with high-fat diet, mice with pre- and post-natal deletion of Rptor in OBs were protected from diet-induced obesity and exhibited improved glucose metabolism with lower fasting glucose and insulin levels, increased glucose tolerance and insulin sensitivity. This leanness and resistance to weight gain was not attributable to changes in food intake, physical activity or lipid absorption but instead was due to increased energy expenditure and greater whole-body substrate flexibility. RNA-seq revealed an increase in glycolysis and skeletal insulin signaling pathways, which correlated with the potentiation of insulin signaling and increased insulin-dependent glucose uptake in Rptor-knockout osteoblasts. Collectively, these findings point to a critical role for the mTORC1 complex in the skeletal regulation of whole-body glucose metabolism and the skeletal development of insulin resistance.

Taylor, S. R., et al. (2021). "Dietary fructose improves intestinal cell survival and nutrient absorption." Nature **597**(7875): 263-267.

Fructose consumption is linked to the rising incidence of obesity and cancer, which are two of the leading causes of morbidity and mortality globally(1,2). Dietary fructose metabolism begins at the epithelium of

the small intestine, where fructose is transported by glucose transporter type 5 (GLUT5; encoded by SLC2A5) and phosphorylated by ketohexokinase to form fructose 1-phosphate, which accumulates to high levels in the cell(3,4). Although this pathway has been implicated in obesity and tumour promotion, the exact mechanism that drives these pathologies in the intestine remains unclear. Here we show that dietary fructose improves the survival of intestinal cells and increases intestinal villus length in several mouse models. The increase in villus length expands the surface area of the gut and increases nutrient absorption and adiposity in mice that are fed a high-fat diet. In hypoxic intestinal cells, fructose 1-phosphate inhibits the M2 isoform of pyruvate kinase to promote cell survival(5-7). Genetic ablation of ketohexokinase or stimulation of pyruvate kinase prevents villus elongation and abolishes the nutrient absorption and tumour growth that are induced by feeding mice with high-fructose corn syrup. The ability of fructose to promote cell survival through an allosteric metabolite thus provides additional insights into the excess adiposity generated by a Western diet, and a compelling explanation for the promotion of tumour growth by high-fructose corn syrup.

Terranova, M., et al. (2021). "Increasing the proportion of hazel leaves in the diet of dairy cows reduced methane yield and excretion of nitrogen in volatile form, but not milk yield." Animal Feed Science and Technology

Various feeds for ruminants have been identified that help to mitigate the greenhouse gas methane. However, even when there has been success in suppressing absolute methane emissions, intake, digestibility, and performance often decline in parallel. Ideal dietary levels of effective feeds would reduce methane production without affecting performance-related variables. Such favorable associative effects have been demonstrated in vitro by combining a high-quality forage with plants rich in phenols. In the present study, the tannin-rich leaves of hazel (Corylus avellana) gradually replaced (from 0 to 820 g/kg) a high-quality forage (dried alfalfa) in 20 types of experimental pellets fed to 20 mid-to-late lactating cows. Additionally, the cows were fed a mixed basal ration and some concentrate. The proportion of hazel in the 20 complete diets ranged from 0 to 400 g/kg dry matter. After 14 days of adaptation, 8 days were used for intensive sampling of feces (including markers for determining digesta retention time), urine, and milk. In addition, cows stayed for 2 days in open-circuit respiration chambers. Hazel leaves reduced the feed intake only slightly. Digestibility declined and mean digesta retention time was prolonged with increasing hazel proportion, likely due to the lower feeding value of the hazel leaves compared to the alfalfa. As aimed for, there were no significant effects on energy-corrected milk yield, body energy, and body N retention with increasing hazel intake, even though methane emission clearly declined in absolute term and per unit of digestible organic matter and tended to decrease per unit of energy corrected milk. In addition, increasing hazel proportions strongly shifted N excretion from urinary N (which declined from about 300 to 100 g/kg N intake) to fecal N. This could also be anticipated from the sharp decline in milk urea concentration (from about 35 to 10 mg/dL). In conclusion, hazel leaves as a feed supplement for dairy cows showed a high palatability within 3 weeks of feeding in dairy cows and great potential to mitigate emissions of methane and nitrogen in volatile form at maintained production levels. No favorable associative dosage effects seem to exist when combining tannin-rich hazel leaves with the high-quality forage alfalfa in a different proportions to a mixed basal ration. However, the present study is one of the few, where it was possible to mitigate noxious emissions of dairy cows by feeding a tannin rich feed supplement without concomitant negative impact on the animal's performance.

Thorn, T. L., et al. (2021). "Metal transporter SLC39A14/ZIP14 modulates regulation between the gut microbiome and host metabolism." bioRxiv.

> Zinc (Zn) plays a critical role in maintaining intestinal homeostasis by regulating intestinal epithelial cells, host immune cells, and gut microbiome community composition. Deletion of metal transporter Slc39a14/Zip14 causes spontaneous intestinal permeability with low-grade chronic inflammation, mild hyperinsulinemia, and greater body fat with insulin resistance in adipose, suggesting a role for ZIP14-mediated intestinal metal transport in regulating both intestinal homeostasis and systemic metabolism. Here, we showed the function of ZIP14-mediated Zn transport in the gut microbiome composition and how ZIP14-linked changes to gut microbiome community composition are correlated with changes in host metabolism. Deletion of Zip14 generated Zn-deficient epithelial cells and luminal content in the entire intestinal tract; reduced bacterial diversity and Saccharomyces cerevisiae (S. cerevisiae) overgrowth; altered host metabolome; and shifted host energy metabolism toward glucose utilization. This work provides evidence for the regulation of gut microbiome composition, host metabolome, and energy metabolism by metal transporter ZIP14.

van Beek, S. M. M., et al. (2021). "Prolonged beta2-adrenergic agonist treatment improves glucose homeostasis in diet-induced obese UCP1(-/-) mice." Am J Physiol Endocrinol Metab **320**(3): E619-E628.

> Prolonged supplementation with the beta2-agonist clenbuterol improves glucose homeostasis in diabetic rodents, likely via beta2-adrenoceptor (beta2-AR)-mediated effects in the skeletal muscle and liver. However, since rodents have, in contrast to-especially diabetic-humans, substantial quantities of brown adipose tissue (BAT) and clenbuterol has affinity to beta1- and beta3-ARs, the contribution of BAT to these improvements is unclear. Therefore, we investigated clenbuterol-mediated improvements in glucose homeostasis in uncoupling protein 1-deficient (UCP1(-/-)) mice, lacking thermogenic BAT, versus wild-type (WT) mice. Anesthetized WT and UCP1(-/-) C57Bl/6 mice were injected with saline or clenbuterol and whole body oxygen consumption was measured. Furthermore, male WT and UCP1(-/-) C57Bl/6 mice were subjected to 17-wk of chow feeding, high-fat feeding, or high-fat feeding with clenbuterol treatment between weeks 13 and 17. Body composition was measured weekly with MRI. Oral glucose tolerance and insulin tolerance tests were performed in week 15 and 17, respectively. Clenbuterol increased oxygen consumption approximately twofold in WT mice. This increase was blunted in UCP1(-/-) mice, indicating clenbuterol-mediated activation of BAT thermogenesis. High-fat feeding induced diabetogenic phenotypes in both genotypes. However, low-dose clenbuterol treatment for 2 wk significantly reduced fasting blood glucose by 12.9% in WT and 14.8% in UCP1(-/-) mice. Clenbuterol treatment improved glucose and insulin tolerance in both genotypes compared with HFD controls and normalized to chow-fed control mice independent of body mass and composition alterations. Clenbuterol improved whole body glucose homeostasis independent of UCP1. Given the low human abundancy of BAT, beta2-AR agonist treatment provides a potential novel route for glucose disposal in diabetic humans.NEW & NOTEWORTHY Improvements in whole body glucose homeostasis of rodents upon prolonged beta2-adrenergic agonist supplementation could potentially be attributed to UCP1-mediated BAT thermogenesis. Indeed, we show that acute injection with the beta2-AR agonist clenbuterol induces BAT activation in mice. However, we also demonstrate that prolonged clenbuterol supplementation robustly improves whole body glucose and insulin tolerance in a similar way in both DIO WT and UCP1(-/-) mice, indicating that beta2-AR agonist

supplementation improves whole body glucose homeostasis independent of UCP1-mediated BAT thermogenesis.

van der Zande, H. J. P., et al. (2021). "Effects of a novel polyphenol-rich plant extract on body composition, inflammation, insulin sensitivity, and glucose homeostasis in obese mice." International Journal of Obesity **45**(9): 2016-2027.

BACKGROUND/OBJECTIVES: The worldwide prevalence of obesity, metabolic syndrome and type 2 diabetes (T2D) is reaching epidemic proportions that urge the development of new management strategies. Totum-63 is a novel, plant-based polyphenol-rich active principle that has been shown to reduce body weight, fasting glycemia, glucose intolerance, and fatty liver index in obese subjects with prediabetes. Here, we investigated the effects and underlying mechanism(s) of Totum-63 on metabolic homeostasis in insulin-resistant obese mice.
METHODS: Male C57Bl6/J mice were fed a high-fat diet for 12 weeks followed by supplementation with Totum-63 for 4 weeks. The effects on whole-body energy and metabolic homeostasis, as well as on tissue-specific inflammation and insulin sensitivity were assessed using a variety of immunometabolic phenotyping tools.
RESULTS: Totum-63 decreased body weight and fat mass in obese mice, without affecting lean mass, food intake and locomotor activity, and increased fecal energy excretion and whole-body fatty acid oxidation. Totum-63 reduced fasting plasma glucose, insulin and leptin levels, and improved whole-body insulin sensitivity and peripheral glucose uptake. The expression of insulin receptor beta and the insulin-induced phosphorylation of Akt/PKB were increased in liver, skeletal muscle, white adipose tissue (WAT) and brown adipose tissue (BAT). Hepatic steatosis was also decreased by Totum-63 and associated with a lower expression of genes involved in fatty acid uptake, de novo lipogenesis, inflammation, and fibrosis. Furthermore, a significant reduction in pro-inflammatory macrophages was also observed in epididymal WAT. Finally, a potent decrease in BAT mass associated with enhanced tissue expression of thermogenic genes was found, suggesting BAT activation by Totum-63.
CONCLUSIONS: Our results show that Totum-63 reduces inflammation and improves insulin sensitivity and glucose homeostasis in obese mice through pleiotropic effects on various metabolic organs. Altogether, plant-derived Totum-63 might constitute a promising novel nutritional supplement for alleviating metabolic dysfunctions in obese people with or without T2D.

van Eenige, R., et al. (2021). "Cannabinoid type 1 receptor inverse agonism attenuates dyslipidemia and atherosclerosis in APOE *3-Leiden.CETP mice." Journal of Lipid Research **62**: 100070.

Pharmacological blockade of the cannabinoid type 1 receptor, a G protein-coupled receptor expressed in the central nervous system and various peripheral tissues, reverses diet-induced obesity and dyslipidemia through the reduction of food intake and altered nutrient partitioning. This strategy is being explored for a number of therapeutic applications; however, its potency for the treatment of atherosclerotic cardiovascular disease via improvements in lipid metabolism remains unclear. Therefore, here, we aimed to investigate whether inhibition of the endocannabinoid system can attenuate atherosclerosis development through improvement of dyslipidemia. Lean, dyslipidemic female APOE *3-Leiden.CETP transgenic mice were fed a Western-type diet supplemented with or without the cannabinoid type 1

receptor inverse agonist rimonabant (20 mg.kg body weight(-1) day(-1)) for up to 20 weeks. Plasma lipids and bile acids were determined, and atherosclerotic lesions were scored in the aortic valve region. Rimonabant lowered plasma levels of triglyceride (TG) (-56%) and non-HDL-C (-19%) and increased HDL-C (+57%). These effects were explained by decreased VLDL-TG production (-52%) and accelerated VLDL-TG turnover accompanied by pronounced browning of white adipose tissue. In addition, rimonabant attenuated reverse cholesterol transport (-30%), increased plasma bile acid levels (+160%), and increased hepatic cholesterol accumulation (+88%). Importantly, rimonabant markedly lowered atherosclerotic lesion size (-64%), which coincided with decreased lesion severity (28% vs. 56% severe lesions) and which strongly correlated with non-HDL-C exposure (R(2) = 0.60). Taken together, inhibition of the endocannabinoid system potently reverses dyslipidemia and prevents atherogenesis, even in the absence of obesity.

Wali, J. A., et al. (2021). "Impact of dietary carbohydrate type and protein-carbohydrate interaction on metabolic health." Nat Metab **3**(6): 810-828.

Reduced protein intake, through dilution with carbohydrate, extends lifespan and improves mid-life metabolic health in animal models. However, with transition to industrialised food systems, reduced dietary protein is associated with poor health outcomes in humans. Here we systematically interrogate the impact of carbohydrate quality in diets with varying carbohydrate and protein content. Studying 700 male mice on 33 isocaloric diets, we find that the type of carbohydrate and its digestibility profoundly shape the behavioural and physiological responses to protein dilution, modulate nutrient processing in the liver and alter the gut microbiota. Low (10%)-protein, high (70%)-carbohydrate diets promote the healthiest metabolic outcomes when carbohydrate comprises resistant starch (RS), yet the worst outcomes were with a 50:50 mixture of monosaccharides fructose and glucose. Our findings could explain the disparity between healthy, high-carbohydrate diets and the obesogenic impact of protein dilution by glucose-fructose mixtures associated with highly processed diets.

Wang, L., et al. (2021). "Adiponectin restrains ILC2 activation by AMPK-mediated feedback inhibition of IL-33 signaling." Journal of Experimental Medicine **218**(2).

ILC2s are present in adipose tissue and play a critical role in regulating adipose thermogenesis. However, the mechanisms underlying the activation of adipose-resident ILC2s remain poorly defined. Here, we show that IL-33, a potent ILC2 activator, stimulates phosphorylation of AMPK at Thr172 via TAK1 in primary ILC2s, which provides a feedback mechanism to inhibit IL-33-induced NF-kappaB activation and IL-13 production. Treating ILC2s with adiponectin or an adiponectin receptor agonist (AdipoRon) activated AMPK and decreased IL-33-NF-kappaB signaling. AdipoRon also suppressed cold-induced thermogenic gene expression and energy expenditure in vivo. In contrast, adiponectin deficiency increased the ILC2 fraction and activation, leading to up-regulated thermogenic gene expression in adipose tissue of cold-exposed mice. ILC2 deficiency or blocking ILC2 function by neutralization of the IL-33 receptor with anti-ST2 diminished the suppressive effect of adiponectin on cold-induced adipose thermogenesis and energy expenditure. Taken together, our study reveals that adiponectin is a negative regulator of ILC2 function in adipose tissue via AMPK-mediated negative regulation of IL-33 signaling.

Wang, Y., et al. (2021). "Mitochondrial protein IF1 is a potential regulator of glucagon-like peptide (GLP-1) secretion function of the mouse intestine." Acta Pharmaceutica Sinica B

F1 (ATPIF1) is a nuclear DNA-encoded mitochondrial protein whose activity is inhibition of the F1Fo-ATP synthase to control ATP production. IF1 activity remains unknown in the regulation of GLP-1 activity. In this study, IF1 was examined in the diet-induced obese mice using the gene knockout (If1-KO) mice. The mice gained more body weight on a high fat diet without a change in food intake. Insulin tolerance was impaired, but the oral glucose tolerance was improved through an increase in GLP-1 secretion. The KO mice exhibited an improved intestine structure, mitochondrial superstructure, enhanced mitophagy, reduced apoptosis and decreased adenine nucleotide translocase 2 (ANT2) protein in the intestinal epithelial cells together with preserved gut microbiota. The data suggest that GLP-1 secretion was enhanced in the obese If1-KO mice to preserve glucose tolerance through a signaling pathway of ANT2/mitochondria/L-cells/GLP-1/insulin. IF1 is a potential mitochondrial target for induction of GLP-1 secretion in L-cells.

Wiede, F., et al. (2021). "PTP1B is an intracellular checkpoint that limits T cell and CAR T cell anti-tumor immunity." Cancer Discovery.

Immunotherapies aimed at alleviating the inhibitory constraints on Tcells have revolutionised cancer management. To date, these have focused on the blockade of cell surface checkpoints such as PD-1. Herein we identify protein-tyrosine-phosphatase-1B (PTP1B) as an intracellular checkpoint that is upregulated in T cells in tumors. We show that the increased PTP1B limits T cell expansion and cytotoxicity to contribute to tumor growth. T cell-specific PTP1B deletion increased STAT-5 signaling and this enhanced the antigen-induced expansion and cytotoxicity of CD8+ T cells to suppress tumor growth. The pharmacological inhibition of PTP1B recapitulated the T cell-mediated repression of tumor growth and enhanced the response to PD-1 blockade. Furthermore, the deletion or inhibition of PTP1B enhanced the efficacy of adoptively-transferred chimeric-antigen-receptor (CAR) T cells against solid tumors. Our findings identify PTP1B as an intracellular checkpoint whose inhibition can alleviate the inhibitory constraints on T cells and CAR T cells to combat cancer.

Wu, D., et al. (2021). "A novel peroxisome proliferator-activated receptor gamma ligand improves insulin sensitivity and promotes browning of white adipose tissue in obese mice." Mol Metab **54**: 101363.

OBJECTIVE: Nuclear receptor Peroxisome Proliferator-Activated Receptor gamma (PPARgamma) is a promising target for the treatment of type 2 diabetes. The antidiabetic drug thiazolidinediones (TZDs) are potent insulin sensitizers as full agonists of PPARgamma, but cause unwanted side effects. Recent discoveries have shown that TZDs improve insulin sensitivity by blocking PPARgamma phosphorylation at S273, which decouples the full agonism-associated side effects. PPARgamma ligands that act through the blockage of PPARgamma phosphorylation but lack the full agonist activity would be expected to improve insulin sensitivity without TZD-associated side effects, however, chemicals that carry such traits and bind to PPARgamma with high-affinity are lacking. Moreover, TZDs are known to promote white-to-brown

adipocyte conversion and energy expenditure and appear to require their full agonism on PPARgamma for this activity. It is unknown whether a partial or non-TZD agonist of PPARgamma is capable of promoting browning effect. In this study, we developed a novel non-TZD partial agonist of PPARgamma and investigated its function on insulin sensitivity and white-to-brown conversion and energy expenditure in diet-induced obese mice.
METHODS: A novel indole-based chemical WO95E was designed via medicinal chemistry and tested for PPARgamma binding and activity and for the effect on PPARgamma phosphorylation. Diet-induced obese mice were administered with WO95E for 4 weeks. Insulin sensitivity, glucose tolerance, body weight, fat tissue weight, adipocyte size, morphology, energy expenditure, and expression levels of genes involved in PPARgamma activity, thermogenesis/browning, and TZD-related side effects were evaluated.
RESULTS: WO95E binds to PPARgamma with high affinity and acts as a PPARgamma partial agonist. WO95E inhibits PPARgamma phosphorylation and regulates PPARgamma phosphorylation-dependent genes. WO95E ameliorates insulin resistance and glucose tolerance in mice of diet-induced obesity, with minimal TZD use-associated side effects. We found that WO95E promotes white-to-brown adipocyte conversion and energy expenditure and hence protects against diet-induced obesity. WO95E decreases the size of adipocytes and suppresses adipose tissue inflammation. WO95E also suppresses obesity-associated liver steatosis.
CONCLUSIONS: WO95E improves insulin sensitivity and glucose homeostasis and promotes browning and energy expenditure by acting as a novel PPARgamma phosphorylation inhibitor/partial agonist. Our findings suggest the potential of this compound or its derivative for the therapeutic treatment of insulin resistance and obesity.

Wu, R., et al. (2021). "m6A methylation promotes white-to-beige fat transition by facilitating Hif1a translation." EMBO Reports **22**(11): e52348.

Obesity mainly results from a chronic energy imbalance. Promoting browning of white adipocytes is a promising strategy to enhance energy expenditure and combat obesity. N6-methyladenosine (m6A), the most abundant mRNA modification in eukaryotes, plays an important role in regulating adipogenesis. However, whether m6A regulates white adipocyte browning was unknown. Here, we report that adipose tissue-specific deletion of Fto, an m6A demethylase, predisposes mice to prevent high-fat diet (HFD)-induced obesity by enhancing energy expenditure. Additionally, deletion of FTO in vitro promotes thermogenesis and white-to-beige adipocyte transition. Mechanistically, FTO deficiency increases the m6A level of Hif1a mRNA, which is recognized by m6A-binding protein YTHDC2, facilitating mRNA translation and increasing HIF1A protein abundance. HIF1A activates the transcription of thermogenic genes, including Ppaggc1a, Prdm16, and Pparg, thereby promoting Ucp1 expression and the browning process. Collectively, these results unveil an epigenetic mechanism by which m6A-facilitated HIF1A expression controls browning of white adipocytes and thermogenesis, providing a potential target to counteract obesity and metabolic disease.

Xirouchaki, C. E., et al. (2021). "Skeletal muscle NOX4 is required for adaptive responses that prevent insulin resistance." Sci Adv **7**(51): eabl4988.

Reactive oxygen species (ROS) generated during exercise are considered integral for the health-promoting effects of exercise. However, the precise mechanisms by which exercise and ROS promote metabolic health remain unclear. Here, we demonstrate that skeletal muscle NADPH oxidase 4 (NOX4), which is induced after exercise, facilitates ROS-mediated adaptive responses that promote muscle function, maintain redox balance, and prevent the development of insulin resistance. Conversely, reductions in skeletal muscle NOX4 in aging and obesity contribute to the development of insulin resistance. NOX4 deletion in skeletal muscle compromised exercise capacity and antioxidant defense and promoted oxidative stress and insulin resistance in aging and obesity. The abrogated adaptive mechanisms, oxidative stress, and insulin resistance could be corrected by deleting the H2O2-detoxifying enzyme GPX-1 or by treating mice with an agonist of NFE2L2, the master regulator of antioxidant defense. These findings causally link NOX4-derived ROS in skeletal muscle with adaptive responses that promote muscle function and insulin sensitivity.

Xu, H., et al. (2021). "Pcpe2, a Novel Extracellular Matrix Protein, Regulates Adipocyte SR-BI-Mediated High-Density Lipoprotein Uptake." Arterioscler Thromb Vasc Biol 41(11): 2708-2725.

Objective: To investigate the role of adipocyte Pcpe2 (procollagen C-endopeptidase enhancer 2) in SR-BI (scavenger receptor class BI)-mediated HDL-C (high-density lipoprotein cholesterol) uptake and contributions to adipose lipid storage.
Approach and Results: Pcpe2, a glycoprotein devoid of intrinsic proteolytic activity, is believed to participate in extracellular protein-protein interactions, supporting SR-BI- mediated HDL-C uptake. In published studies, Pcpe2 deficiency increased the development of atherosclerosis by reducing SR-BI-mediated HDL-C catabolism, but the biological impact of this deficiency on adipocyte SR-BI-mediated HDL-C uptake is unknown. Differentiated cells from Ldlr-/-/Pcpe2-/- (Pcpe2-/-) mouse adipose tissue showed elevated SR-BI protein levels, but significantly reduced HDL-C uptake compared to Ldlr-/- (control) adipose tissue. SR-BI-mediated HDL-C uptake was restored by preincubation of cells with exogenous Pcpe2. In diet-fed mice lacking Pcpe2, significant reductions in visceral, subcutaneous, and brown adipose tissue mass were observed, despite elevations in plasma triglyceride and cholesterol concentrations. Significant positive correlations exist between adipose mass and Pcpe2 expression in both mice and humans.
Conclusions: Overall, these findings reveal a novel and unexpected function for Pcpe2 in modulating SR-BI expression and function as it relates to adipose tissue expansion and cholesterol balance in both mice and humans.

Xu, Y., et al. (2021). "Berberine modulates deacetylation of PPARgamma to promote adipose tissue remodeling and thermogenesis via AMPK/SIRT1 pathway." Int J Biol Sci **17**(12): 3173-3187.

Pharmacological stimulation of adipose tissue remodeling and thermogenesis to increase energy expenditure is expected to be a viable therapeutic strategy for obesity. Berberine has been reported to have pharmacological activity in adipose tissue to anti-obesity, while the mechanism remains unclear.

Here, we observed that berberine significantly reduced the body weight and insulin resistance of high-fat diet mice by promoting the distribution of brown adipose tissue and thermogenesis. We have further demonstrated that berberine activated energy metabolic sensing pathway AMPK/SIRT1 axis to increase the level of PPARgamma deacetylation, which leads to promoting adipose tissue remodeling and increasing the expression of the thermogenic protein UCP-1. These findings suggest that berberine that enhances the AMPK/SIRT1 pathway can act as a selective PPARgamma activator to promote adipose tissue remodeling and thermogenesis. This study proposes a new mechanism for the regulation of berberine in adipose tissue and offers a great prospect for berberine in obesity treatment.

Yan, C., et al. (2021). "Peripheral-specific Y1 receptor antagonism increases thermogenesis and protects against diet-induced obesity." Nature Communications **12**(1): 2622.

Obesity is caused by an imbalance between food intake and energy expenditure (EE). Here we identify a conserved pathway that links signalling through peripheral Y1 receptors (Y1R) to the control of EE. Selective antagonism of peripheral Y1R, via the non-brain penetrable antagonist BIBO3304, leads to a significant reduction in body weight gain due to enhanced EE thereby reducing fat mass. Specifically thermogenesis in brown adipose tissue (BAT) due to elevated UCP1 is enhanced accompanied by extensive browning of white adipose tissue both in mice and humans. Importantly, selective ablation of Y1R from adipocytes protects against diet-induced obesity. Furthermore, peripheral specific Y1R antagonism also improves glucose homeostasis mainly driven by dynamic changes in Akt activity in BAT. Together, these data suggest that selective peripheral only Y1R antagonism via BIBO3304, or a functional analogue, could be developed as a safer and more effective treatment option to mitigate diet-induced obesity.

Yin, C., et al. (2021). "Hypoxanthine Induces Muscular ATP Depletion and Fatigue via UCP2." Front Physiol **12**: 647743.

Hypoxanthine (Hx), an intermediate metabolite of the purine metabolism pathway which is dramatically increased in blood and skeletal muscle during muscle contraction and metabolism, is characterized as a marker of exercise exhaustion. However, the physiological effects of Hx on skeletal muscle remain unknown. Herein, we demonstrate that chronic treatment with Hx through dietary supplementation resulted in skeletal muscle fatigue and impaired the exercise performance of mice without affecting their growth and skeletal muscle development. Hx increased the uncoupling protein 2 (UCP2) expression in the skeletal muscle, which led to decreased energy substrate storage and enhanced glycolysis. These effects could also be verified in acute treatment with Hx through intraperitoneal injection. In addition, muscular specifically knockout of UCP2 through intra-muscle tissue injection of adenovirus-associated virus reversed the effects of Hx. In conclusion, we identified a novel role of Hx in the skeletal muscular fatigue mediated by UCP2-dependent mitochondrial uncoupling. This finding may shed light on the pathological mechanism of clinical muscle dysfunctions due to abnormal metabolism, such as muscle fatigue and weakness.

Ying, L., et al. (2021). "Macrophage LAMTOR1 Deficiency Prevents Dietary Obesity and Insulin Resistance Through Inflammation-Induced Energy Expenditure." Front Cell Dev Biol **9**: 672032.

> Here, we studied the metabolic function of LAMTOR1 from macrophages using LAMTOR1 macrophage-specific knockout (MKO) mice. LAMTOR1 MKO mice showed resistance to high-fat diet (HFD)-induced obesity, lipid steatosis, and glucose metabolic disorders, with elevated levels of pro-inflammatory cytokines. The energy expenditure, oxygen consumption, and CO2 production increased significantly in HFD-fed MKO vs. wild-type (WT) mice. HE and immunohistochemistry staining showed a remarkable CD68(+) Kupffer cell accumulation in the liver. Additionally, flow cytometry revealed that the proportion of macrophages and monocytes increased significantly in the liver of MKO mice. Of note, these macrophages were probably derived from the bone marrow since the proportion of CD11b(+) cells as well as the proliferative activity was also increased in the context of femoral bone marrow cells. In addition, the Kupffer cells of both WT and KO mice were double-positive for the M1 (CD86) and M2 (CD206) markers. However, the expression of both M1 and M2 macrophage-related genes was increased in the liver of HFD-fed KO mice. Murine primary hepatocytes and Kupffer cells were further isolated and incubated with oleic acid for 24 h. The glucose output of primary hepatocytes from MKO mice was not affected. However, decreased lipid tolerance was observed in LAMTOR1-deficient Kupffer cells. Overall, our results suggest that LAMTOR1 deficiency in macrophages prevents obesity and metabolic disorders via the accumulation of Kupffer cells in the liver and the consequent hyper-inflammation and increased energy expenditure. Therefore, our results provide a new perspective for macrophage-derived LAMTOR1 in the context of systemic metabolism.

Yu, F., et al. (2021). "Deficiency of intestinal Bmal1 prevents obesity induced by high-fat feeding." Nature Communications **12**(1): 5323.

> The role of intestine clock in energy homeostasis remains elusive. Here we show that mice with Bmal1 specifically deleted in the intestine (Bmal1(iKO) mice) have a normal phenotype on a chow diet. However, on a high-fat diet (HFD), Bmal1(iKO) mice are protected against development of obesity and related abnormalities such as hyperlipidemia and fatty livers. These metabolic phenotypes are attributed to impaired lipid resynthesis in the intestine and reduced fat secretion. Consistently, wild-type mice fed a HFD during nighttime (with a lower BMAL1 expression) show alleviated obesity compared to mice fed ad libitum. Mechanistic studies uncover that BMAL1 transactivates the Dgat2 gene (encoding the triacylglycerol synthesis enzyme DGAT2) via direct binding to an E-box in the promoter, thereby promoting dietary fat absorption. Supporting these findings, intestinal deficiency of Rev-erbalpha, a known BMAL1 repressor, enhances dietary fat absorption and exacerbates HFD-induced obesity and comorbidities. Moreover, small-molecule targeting of REV-ERBalpha/BMAL1 by SR9009 ameliorates HFD-induced obesity in mice. Altogether, intestine clock functions as an accelerator in dietary fat absorption and targeting intestinal BMAL1 may be a promising approach for management of metabolic diseases induced by excess fat intake.

Zhang, L., et al. (2021). "Ninjin'yoeito modulates feeding and activity under negative energy balance conditions via the NPY system." Neuropeptides **87**: 102149.

The central and peripheral neuropeptide Y (NPY) system is critically involved in feeding and energy homeostasis control. Disease conditions as well as aging can lead to reduced functionality of the NPY system and boosting it represents a promising option to improve health outcomes in these situations. Here we show that Ninjin-yoeito (NYT), a Japanese kampo medicine comprising twelve herbs, and known to be effective to treat anorexia and frailty, mediates part of its action via NPY/peptide YY (PYY) related pathways. Especially under negative energy homeostasis conditions NYT is able to promote feeding and reduces activity to conserve energy. These effects are in part mediated via signalling through the NPY system since lack of Y4 receptors or PYY leading to modification in these responses highlighting the possibility for combination treatment to improve aging related conditions on energy homeostasis control.

Zhang, L., et al. (2021). "Lack of neuropeptide FF signalling in mice leads to reduced repetitive behavior, altered drinking behavior, and fuel type selection." FASEB Journal **35**(11): e21980.

Although best known for their involvement in modulating nociception, Neuropeptide FF (NPFF) group peptides have been suggested to fulfil a variety of biological functions such as feeding, anxiety behaviors and thermogenesis. However, evidence supporting these functions of NPFF is mostly pharmacological, leaving the physiological relevance unaddressed. Here we examined the physiological impact of lack of NPFF signalling in both genders using a Npff(-/-) mouse model. NPFF expression in the mouse is restricted to the spinal cord and brainstem while its cognate receptor NPFFR2 has wider distribution throughout the brain. Both male and female Npff(-/-) mice showed reduced repetitive behaviors evidenced in the marble burying test and self-grooming test. A decrease in anxiety-related behaviors in the Npff(-/-) mice was also observe in the open field test and to a lesser degree in an elevated plus maze test. Moreover, both male and female Npff(-/-) mice exhibited increased water intake resulting from increases in drinking size, rather than number of drinking events. During a fasting-refeeding challenge, Npff(-/-) mice of both genders displayed alterations in reparatory exchange ratio that reflect a greater fuel type flexibility. Npff(-/-) mice were otherwise wild-type-like regarding body weight, body composition, feeding behaviors, locomotion or energy expenditure. Together, these findings reveal the important physiological roles of NPFF signalling in the regulation of anxiety-related and repetitive behaviors, fluid homeostasis and oxidative fuel selection, highlighting the therapeutical potential of the NPFF system in a number of behavioral and metabolic disorders.

Zhang, M., et al. (2021). "INPP4B protects from metabolic syndrome and associated disorders." Commun Biol **4**(1): 416.

A high fat diet and obesity have been linked to the development of metabolic dysfunction and the promotion of multiple cancers. The causative cellular signals are multifactorial and not yet completely understood. In this report, we show that Inositol Polyphosphate-4-Phosphatase Type II B (INPP4B) signaling protects mice from diet-induced metabolic dysfunction. INPP4B suppresses AKT and PKC signaling in the liver thereby improving insulin sensitivity. INPP4B loss results in the proteolytic cleavage

and activation of a key regulator in de novo lipogenesis and lipid storage, SREBP1. In mice fed with the high fat diet, SREBP1 increases expression and activity of PPARG and other lipogenic pathways, leading to obesity and non-alcoholic fatty liver disease (NAFLD). Inpp4b(-/-) male mice have reduced energy expenditure and respiratory exchange ratio leading to increased adiposity and insulin resistance. When treated with high fat diet, Inpp4b(-/-) males develop type II diabetes and inflammation of adipose tissue and prostate. In turn, inflammation drives the development of high-grade prostatic intraepithelial neoplasia (PIN). Thus, INPP4B plays a crucial role in maintenance of overall metabolic health and protects from prostate neoplasms associated with metabolic dysfunction.

Zhang, Y., et al. (2021). " Tetrahydroxanthohumol, a xanthohumol derivative, attenuates high-fat diet-induced hepatic steatosis by antagonizing PPARγ." Elife **10**.

We previously reported xanthohumol (XN), and its synthetic derivative tetrahydro-XN (TXN), attenuates high-fat diet (HFD)-induced obesity and metabolic syndrome in C57Bl/6J mice. The objective of the current study was to determine the effect of XN and TXN on lipid accumulation in the liver. Non-supplemented mice were unable to adapt their caloric intake to 60% HFD, resulting in obesity and hepatic steatosis; however, TXN reduced weight gain and decreased hepatic steatosis. Liver transcriptomics indicated that TXN might antagonize lipogenic PPARγ actions in vivo. XN and TXN inhibited rosiglitazone-induced 3T3-L1 cell differentiation concomitant with decreased expression of lipogenesis-related genes. A peroxisome proliferator activated receptor gamma (PPARγ) competitive binding assay showed that XN and TXN bind to PPARγ with an IC50 similar to pioglitazone and 8-10 times stronger than oleate. Molecular docking simulations demonstrated that XN and TXN bind in the PPARγ ligand-binding domain pocket. Our findings are consistent with XN and TXN acting as antagonists of PPARγ.

Zhou, B., et al. (2021). "Serum- and glucocorticoid-induced kinase drives hepatic insulin resistance by directly inhibiting AMP-activated protein kinase." Cell Rep **37**(1): 109785.

A hallmark of type 2 diabetes (T2D) is hepatic resistance to insulin's glucose-lowering effects. The serum- and glucocorticoid-regulated family of protein kinases (SGK) is activated downstream of mechanistic target of rapamycin complex 2 (mTORC2) in response to insulin in parallel to AKT. Surprisingly, despite an identical substrate recognition motif to AKT, which drives insulin sensitivity, pathological accumulation of SGK1 drives insulin resistance. Liver-specific Sgk1-knockout (Sgk1(Lko)) mice display improved glucose tolerance and insulin sensitivity and are protected from hepatic steatosis when fed a high-fat diet. Sgk1 promotes insulin resistance by inactivating AMP-activated protein kinase (AMPK) via phosphorylation on inhibitory site AMPKalpha(Ser485/491). We demonstrate that SGK1 is dominant among SGK family kinases in regulation of insulin sensitivity, as Sgk1, Sgk2, and Sgk3 triple-knockout mice have similar increases in hepatic insulin sensitivity. In aggregate, these data suggest that targeting hepatic SGK1 may have therapeutic potential in T2D.

Zhuang, A., et al. (2021). "SOD2 in skeletal muscle: New insights from an inducible deletion model." Redox Biol **47**: 102135.

Metabolic conditions such as obesity, insulin resistance and glucose intolerance are frequently associated with impairments in skeletal muscle function and metabolism. This is often linked to dysregulation of homeostatic pathways including an increase in reactive oxygen species (ROS) and oxidative stress. One of the main sites of ROS production is the mitochondria, where the flux of substrates through the electron transport chain (ETC) can result in the generation of oxygen free radicals. Fortunately, several mechanisms exist to buffer bursts of intracellular ROS and peroxide production, including the enzymes Catalase, Glutathione Peroxidase and Superoxide Dismutase (SOD). Of the latter, there are two intracellular isoforms; SOD1 which is mostly cytoplasmic, and SOD2 which is found exclusively in the mitochondria. Developmental and chronic loss of these enzymes has been linked to disease in several studies, however the temporal effects of these disturbances remain largely unexplored. Here, we induced a post-developmental (8-week old mice) deletion of SOD2 in skeletal muscle (SOD2-iMKO) and demonstrate that 16 weeks of SOD2 deletion leads to no major impairment in whole body metabolism, despite these mice displaying alterations in aspects of mitochondrial abundance and voluntary ambulatory movement. This is likely partly explained by the suggestive data that a compensatory response may exist from other redox enzymes, including catalase and glutathione peroxidases. Nevertheless, we demonstrated that inducible SOD2 deletion impacts on specific aspects of muscle lipid metabolism, including the abundance of phospholipids and phosphatidic acid (PA), the latter being a key intermediate in several cellular signaling pathways. Thus, our findings suggest that post-developmental deletion of SOD2 induces a more subtle phenotype than previous embryonic models have shown, allowing us to highlight a previously unrecognized link between SOD2, mitochondrial function and bioactive lipid species including PA.

Bazhin, A. A., et al. (2020). "A bioluminescent probe for longitudinal monitoring of mitochondrial membrane potential." Nature Chemical Biology **16**(12): 1385-1393.

Mitochondrial membrane potential (ΔΨm) is a universal selective indicator of mitochondrial function and is known to play a central role in many human pathologies, such as diabetes mellitus, cancer and Alzheimer's and Parkinson's diseases. Here, we report the design, synthesis and several applications of mitochondria-activatable luciferin (MAL), a bioluminescent probe sensitive to ΔΨm, and partially to plasma membrane potential (ΔΨp), for non-invasive, longitudinal monitoring of ΔΨm in vitro and in vivo. We applied this new technology to evaluate the aging-related change of ΔΨm in mice and showed that nicotinamide riboside (NR) reverts aging-related mitochondrial depolarization, revealing another important aspect of the mechanism of action of this potent biomolecule. In addition, we demonstrated application of the MAL probe for studies of brown adipose tissue (BAT) activation and non-invasive in vivo assessment of ΔΨm in animal cancer models, opening exciting opportunities for understanding the underlying mechanisms and for discovery of effective treatments for many human pathologies. [Figure not available: see fulltext.]

Beppu, L. Y., et al. (2020). "Tregs facilitate obesity and insulin resistance via a Blimp-1-IL-10 axis." JCI Insight.

> Interleukin-10 (IL-10) is a critical cytokine used by immune cells to suppress inflammation. Paradoxically, immune cell-derived IL-10 can drive insulin resistance in obesity by suppressing adipocyte energy expenditure and thermogenesis. However, the source of IL-10 necessary for the suppression of adipocyte thermogenesis is unknown. We show here that CD4 + Foxp3 + regulatory

Bhupana, J. N., et al. (2020). "Gas7 knockout affects PINK1 expression and mitochondrial dynamics in mouse cortical neurons." FASEB BioAdvances **2**(3): 166-181.

> Intake, locomotor activity, VO 2 consumption, and VCO 2 production)19 were measured using a four-cage Promethion-C continuous, parallel metabolic phenotyping system (Sable Systems International [SSI]) for six wild-type and six gas7-knockout male mice (36 weeks old) …

Binyamin, D., et al. (2020). "The aging mouse microbiome has obesogenic characteristics." Genome Medicine **12**(1).

> Background: During aging, there is a physiological decline, an increase of morbidity and mortality, and a natural change in the gut microbiome. In this study, we investigated the influence of the gut microbiome on different metabolic parameters in adult and aged mice. Methods: Fecal and blood samples from adult (n = 42, 100-300 days) and aging (n = 32, 550-750 days) mice were collected. Microbiome analysis was done using QIIME2. Mouse weight and body composition were measured using NMR, and insulin and leptin levels in the blood were measured with Mouse Adipokine Magnetic Bead Panel kit. Fecal microbiota transplantation experiments from adult and aged mice into young germ-free mice were carried out in order to examine the effect of the gut microbiome of adult and aging mice on weight, body composition, insulin, and leptin. Results: We demonstrate that the microbiomes from adult and aged mice are distinguishable. We also report changes in metabolic parameters as we observed significantly higher weight and fat mass and low lean mass in aged compared to adult mice along with high insulin and leptin levels in the blood. The transplanted gut microbiome from aged mice transferred part of the phenotypes seen in aged mice. Fat body mass and insulin levels were higher in the mice who received feces from aged mice than mice receiving feces from adult mice. In addition, they consumed more food and had a higher respiratory quotient compared to mice receiving adult feces. Conclusions: We conclude that aged mice have a gut microbiota with obesogenic characteristics. In addition, the gut bacterial population itself is sufficient to induce some of the manifestations of obesity.

Brierley, D. I., et al. (2020). "Central and peripheral GLP-1 systems independently and additively suppress eating." BioRxiv: 2020.2008.2003.234427-232020.234408.234403.234427.

> The anorexigenic peptide glucagon-like peptide-1 (GLP-1) is secreted from gut enteroendocrine cells and brain preproglucagon (PPG) neurons, which respectively define the peripheral and central GLP-1 systems.

As peripheral satiation signals are integrated in the nucleus tractus solitarius (NTS), PPGNTS neurons are assumed to link the peripheral and central GLP-1 systems, forming a unified GLP-1 gut-brain satiation circuit. This hypothesis, however, remains unsubstantiated. We report that PPGNTS neurons encode satiation in mice, consistent with vagal gastrointestinal distension signalling. However, PPGNTS neurons predominantly receive vagal input from oxytocin receptor-expressing vagal neurons, rather than those expressing GLP-1 receptors. Furthermore, PPGNTS neurons are not necessary for eating suppression induced by the GLP-1 receptor agonists liraglutide or semaglutide, and semaglutide and PPGNTS neuron activation additively suppress eating. Central and peripheral GLP-1 systems thus suppress eating via independent gut-brain circuits, hence PPGNTS neurons represent a rational pharmacological target for anti-obesity combination therapy with GLP-1 receptor agonists. ![Figure][1]</img> Graphical Abstract: ### Competing Interest Statement The FR + FMG laboratory receives funding from AstraZeneca, Eli Lilly and LGM for unrelated research and FMG consults for Kallyope (New York). All other authors have nothing to declare. [1]: pending:yes

Butler, M. C., et al. (2020). "Endocrine disruption of gene expression and microRNA profiles in hippocampus and hypothalamus of California mice: Association of gene expression changes with behavioural outcomes." Journal of Neuroendocrinology **32**(5).

The hypothalamus and hippocampus are sensitive to early exposure to endocrine disrupting chemicals (EDCs). Two EDCs that have raised particular concerns are bisphenol A (BPA), a widely prevalent chemical in many common household items, and genistein (GEN), a phyto-oestrogen present in soy and other plants. We hypothesised that early exposure to BPA or GEN may lead to permanent effects on gene expression profiles for both coding RNAs (mRNAs) and microRNAs (miRs), which can affect the translation of mRNAs. Such EDC-induced biomolecular changes may affect behavioural and metabolic patterns. California mice (Peromyscus californicus) male and female offspring were developmentally exposed via the maternal diet to BPA (5 mg kg-1 feed weight low dose [LD] and 50 mg kg-1 feed weight upper dose [UD]), GEN (250 mg kg-1 feed weight) or a phyto-oestrogen-free diet (AIN) control. Behavioural and metabolic tests were performed at 180 days of age. A quantitative polymerase chain reacttion analysis was performed for candidate mRNAs and miRs in the hypothalamus and hippocampus. LD BPA and GEN exposed California mice offspring showed socio-communication impairments. Hypothalamic Avp, Esr1, Kiss1 and Lepr were increased in LD BPA offspring. miR-153 was elevated but miR-181a was reduced in LD BPA offspring. miR-9 and miR-153 were increased in the hippocampi of LD BPA offspring, whereas GEN decreased hippocampal miR-7a and miR-153 expression. Correlation analyses revealed neural expression of miR-153 and miR-181a was associated with socio-communication deficits in LD BPA individuals. The findings reveal a cause for concern such that developmental exposure of BPA or GEN in California mice (and potentially by translation in humans) can lead to long standing neurobehavioural consequences.

Campelj, D. G., et al. (2020). "Sodium nitrate co-supplementation does not exacerbate low dose metronomic doxorubicin-induced cachexia in healthy mice." Scientific Reports **10**(1).

The purpose of this study was to determine whether (1) sodium nitrate (SN) treatment progressed or alleviated doxorubicin (DOX)-induced cachexia and muscle wasting; and (2) if a more-clinically relevant low-dose metronomic (LDM) DOX treatment regimen compared to the high dosage bolus commonly used

in animal research, was sufficient to induce cachexia in mice. Six-week old male Balb/C mice (n = 16) were treated with three intraperitoneal injections of either vehicle (0.9% NaCl; VEH) or DOX (4 mg/kg) over one week. To test the hypothesis that sodium nitrate treatment could protect against DOX-induced symptomology, a group of mice (n = 8) were treated with 1 mM NaNO3 in drinking water during DOX (4 mg/kg) treatment (DOX + SN). Body composition indices were assessed using echoMRI scanning, whilst physical and metabolic activity were assessed via indirect calorimetry, before and after the treatment regimen. Skeletal and cardiac muscles were excised to investigate histological and molecular parameters. LDM DOX treatment induced cachexia with significant impacts on both body and lean mass, and fatigue/malaise (i.e. it reduced voluntary wheel running and energy expenditure) that was associated with oxidative/nitrostative stress sufficient to induce the molecular cytotoxic stress regulator, nuclear factor erythroid-2-related factor 2 (NRF-2). SN co-treatment afforded no therapeutic potential, nor did it promote the wasting of lean tissue. Our data re-affirm a cardioprotective effect for SN against DOX-induced collagen deposition. In our mouse model, SN protected against LDM DOX-induced cardiac fibrosis but had no effect on cachexia at the conclusion of the regimen.

Campelj, D. G., et al. (2020). "The paradoxical effect of parp inhibitor bgp-15 on irinotecan-induced cachexia and skeletal muscle dysfunction." Cancers **12**(12): 1-28.

Chemotherapy-induced muscle wasting and dysfunction is a contributing factor to cachexia alongside cancer and increases the risk of morbidity and mortality. Here, we investigate the effects of the chemotherapeutic agent irinotecan (IRI) on skeletal muscle mass and function and whether BGP-15 (a poly-(ADP-ribose) polymerase-1 (PARP-1) inhibitor and heat shock protein co-inducer) adjuvant therapy could protect against IRI-induced skeletal myopathy. Healthy 6-week-old male Balb/C mice (n = 24; 8/group) were treated with six intraperitoneal injections of either vehicle, IRI (30 mg/kg) or BGP-15 adjuvant therapy (IRI+BGP; 15 mg/kg) over two weeks. IRI reduced lean and tibialis anterior mass, which were attenuated by IRI+BGP treatment. Remarkably, IRI reduced muscle protein synthesis, while IRI+BGP reduced protein synthesis further. These changes occurred in the absence of a change in crude markers of mammalian/mechanistic target of rapamycin (mTOR) Complex 1 (mTORC1) signaling and protein degradation. Interestingly, the cytoskeletal protein dystrophin was reduced in both IRI-and IRI+BGP-treated mice, while IRI+BGP treatment also decreased β-dystroglycan, suggesting significant remodeling of the cytoskeleton. IRI reduced absolute force production of the soleus and extensor digitorum longus (EDL) muscles, while IRI+BGP rescued absolute force production of the soleus and strongly trended to rescue force output of the EDL (p = 0.06), which was associated with improvements in mass. During the fatiguing stimulation, IRI+BGP-treated EDL muscles were somewhat susceptible to rupture at the musculotendinous junction, likely due to BGP-15's capacity to maintain the rate of force development within a weakened environment characterized by significant structural remodeling. Our paradoxical data highlight that BGP-15 has some therapeutic advantage by attenuating IRI-induced skeletal myopathy; however, its effects on the remodeling of the cytoskeleton and extracellular matrix, which appear to make fast-twitch muscles more prone to tearing during contraction, could suggest the induction of muscular dystrophy and, thus, require further characterization.

Carreau, A. M., et al. (2020). "Bariatric surgery rapidly decreases cardiac dietary fatty acid partitioning and hepatic insulin resistance through increased intra-abdominal adipose tissue storage and reduced spillover in type 2 diabetes." Diabetes **69**(4): 567-577.

> Reduced storage of dietary fatty acids (DFAs) in abdominal adipose tissues with enhanced cardiac partitioning has been shown in subjects with type 2 diabetes (T2D) and prediabetes. We measured DFA metabolism and organ partitioning using positron emission tomography with oral and intravenous long-chain fatty acid and glucose tracers during a standard liquid meal in 12 obese subjects with T2D before and 8–12 days after bariatric surgery (sleeve gastrectomy or sleeve gastrectomy and biliopancreatic diversion with duodenal switch). Bariatric surgery reduced cardiac DFA uptake from a median (standard uptake value [SUV]) 1.75 (interquartile range 1.39–2.57) before to 1.09 (1.04–1.53) after surgery (P 5 0.01) and systemic DFA spillover from 56.7 mmol before to 24.7 mmol over 6 h after meal intake after surgery (P 5 0.01), with a significant increase in intra-abdominal adipose tissue DFA uptake from 0.15 (0.04–0.31] before to 0.49 (0.20–0.59) SUV after surgery (P 5 0.008). Hepatic insulin resistance was significantly reduced in close association with increased DFA storage in intra-abdominal adipose tissues (r 5 20.79, P 5 0.05) and reduced DFA spillover (r 5 0.76, P 5 0.01). We conclude that bariatric surgery in subjects with T2D rapidly reduces cardiac DFA partitioning and hepatic insulin resistance at least in part through increased intra-abdominal DFA storage and reduced spillover.

Ceddia, R., et al. (2020). "Impairment of the Gβγ-SNAP25 brake on exocytosis enhances insulin action, protects against diet-induced obesity, and promotes adipocyte browning." bioRxiv.

> The Gβγ complex inhibits vesicle exocytosis by two mechanisms: inhibiting calcium entry by binding to voltage gated calcium channels, and binding to SNAP25 in the SNAP Receptor (SNARE) complex. To de-convolute the role of each of these mechanisms in vivo, we have made a mouse with the second mechanism disabled. The SNAP25Δ3 mutation renders the SNARE complex deficient in binding to Gβγ and was used to investigate the importance of the Gβγ-SNAP25 interaction in glucose stimulated insulin secretion (GSIS) and global metabolic homeostasis. GSIS and α2A adrenergic receptor-mediated inhibition of GSIS were not altered in SNAP25Δ3/Δ3 mice. Nevertheless, SNAP25Δ3/Δ3 mice exhibited a marked improvement in insulin sensitivity and were resistant to weight gain when challenged with a high fat diet (HFD). Reduced food consumption in the early stages of HFD feeding were partly responsible for the inability of SNAP25Δ3/Δ3 mice to gain weight on HFD. Additionally, improved insulin-mediated glucose uptake into white adipose tissue and increased 'browning' were observed in SNAP25Δ3/Δ3 mice, which is consistent with an impaired ability to retain energy stores. These phenotypic changes in SNAP25Δ3/Δ3 mice are all metabolically protective, indicating that pharmacological targeting of the Gβγ-SNAP25 interaction may have a metabolic benefit.

Chang, Y.-C., et al. (2020). A common East Asian-specic ALDH2 mutation causes obesity and insulin resistance: therapeutic effect of reducing toxic aldehydes by ALDH2 activation, researchsquare.com.

> Obesity and type 2 diabetes have reached pandemic proportion. In particular, the population with diabetes is expected to rise rapidly in East and South Asia. ALDH2 (acetaldehyde dehydrogenase 2, mitochondrial) is the key metabolizing enzyme of acetaldehyde and other toxic aldehydes, such as 4-

hydroxynonenal (4- HNE). A missense mutation, Glu504Lys of ALDH2 (denoted as the ALDH2*2 allele) is prevalent in 560 million East Asians, resulting in reduced ALDH2 enzymatic activity. We found that Aldh2*2/*2 homozygous knock-in (KI) mice mimicking human Glu504Lys mutation were prone to develop diet-induced obesity, glucose intolerance, insulin resistance, and fatty liver on a high-fat high-sucrose diet compared with controls. The Aldh2 KI mice demonstrated reduced energy expenditure and thermogenesis. Proteomic analyses of the brown adipose tissue (BAT) of the Aldh2 KI mice identied increased 4-HNE-adducted proteins involved in fatty acid oxidation and electron transport chain. Fatty acid oxidation rate and mitochondrial electron transport activity were reduced in the BAT of the Aldh2 KI mice, which explained the decrease in thermogenesis and energy expenditure.

Chelsea L. Faber, J. D. (2020). "Leptin-receptor neurons in the dorsomedial hypothalamus regulate the timing of circadian rhythms in feeding and metabolism in mice." bioRxiv **2507**(1): 1-9.

… Energy expenditure 195 measurements were obtained by a computer-controlled indirect calorimeter System 196 (Promethion, Sable Systems, Las Vegas NV) with support from the Energy Balance Core of the 197 NORC at the University of Washington, as previously described …

Cooke, D., et al. (2020). "Weight Loss and Concomitant Adipose Autophagy in Methionine-Restricted Obese Mice is Not Dependent on Adiponectin or FGF21." Obesity **28**(6): 1075-1085.

Objective: Identifying novel approaches to combat obesity is important to improve health span. It was hypothesized that methionine restriction (MR) will induce weight loss in obese mice by reducing adipose tissue mass caused by increased energy expenditure and reprogramming of adipose tissue homeostasis. The roles of adiponectin (ADIPOQ) and fibroblast growth factor 21 (FGF21) during weight loss in MR mice were also tested. Methods: Diet-induced obese (DIO) male C57BL/6J (wild type), Adipoq-deficient (Adipoq knockout [KO]), Fgf21-KO, and Adipoq-Fgf21 double-KO mice were used. Following a switch to high-fat control (DIO-CF, 60% fat/0.86% methionine) or MR (DIO-MR, 60% fat/0.12% methionine) diet, physiological parameters were measured, and inguinal and perigonadal adipose tissues were examined. Results: Obese mice subjected to MR showed loss of body weight and adiposity, increased energy expenditure, and improved glucose tolerance that were independent of the actions of ADIPOQ and FGF21. MR induced reduction of circulating lipids, glucose, insulin, leptin, and insulin like growth factor 1 and increased β-hydroxybutyrate, ADIPOQ, and FGF21 concentrations. In fat, MR upregulated protein levels of adipose triglyceride lipase, apoptosis-inducing factor, lysosomal-associated membrane proteins 1 and 2, autophagy-related protein 5, beclin-1, and light chain 3B I and II. Conclusions: MR reduction of adipose tissue mass in obese mice is associated with elevated lipolysis, apoptosis, and autophagy and occurs independently of the actions of ADIPOQ and FGF21.

Debruin, D. A., et al. (2020). "Exercise May Ameliorate the Detrimental Side Effects of High Vitamin D Supplementation on Muscle Function in Mice." Journal of Bone and Mineral Research **35**(6): 1092-1106.

Vitamin D is commonly prescribed to normalize deficiencies and to treat osteoporosis. However, the effect vitamin D supplements have on skeletal muscle health is equivocal. Although vitamin D is known to play a role in the various processes that maintain muscle integrity and function, recent studies utilizing high bolus dose vitamin D supplementation has demonstrated an increased risk of falls. Thus, the aim of this study was to investigate the effects of high vitamin D supplementation on skeletal muscle function with and without exercise enrichment. Four-week old C57BL/10 mice (n = 48) were separated into either normal vitamin D (1500 IU/kg diet; unsupplemented) or high vitamin D (20,000 IU/kg diet; supplemented) treatment groups. Each dietary group was further separated into interventional subgroups where mice either remained sedentary or received exercise-enrichment for 8 weeks in the form of voluntary running. Following the intervention period, whole body in vivo and ex vivo contractile analysis were performed. High vitamin D supplementation decreased force production in the slow-twitch soleus muscles of sedentary mice ($p < .01$); however, exercise normalized this effect. Eight weeks of exercise did not improve fatigue resistance of the extensor digitorum longus (EDL) or soleus muscles in unsupplemented mice, likely due to low levels of activation in these muscles. In contrast, fatigability was improved in the EDL ($p < .01$) and even more so in the soleus ($p < .001$) in the supplemented exercise-enriched group. Our data highlights that increasing vitamin D levels above normal reduces postural muscle force as seen in the soleus. Thus, unnecessary vitamin D supplementation may contribute to the increased risk of falls observed in some studies. Interestingly, when vitamin D supplementation was combined with exercise, force production was effectively restored, and fatigue resistance improved, even in muscles lowly activated. Regular exercise may modulate the effects of vitamin D on skeletal muscle, and be recommended for individuals receiving vitamin D supplements. © 2020 The Authors. Journal of Bone and Mineral Research published by American Society for Bone and Mineral Research.

Deem, J., et al. (2020). "Cold-induced hyperphagia requires AgRP-neuron activation in mice." eLife **9**.

To maintain energy homeostasis during cold exposure, the increased energy demands of thermogenesis must be counterbalanced by increased energy intake. To investigate the neurobiological mechanisms underlying this cold-induced hyperphagia, we asked whether agouti-related peptide (AgRP) neurons are activated when animals are placed in a cold environment and, if so, whether this response is required for the associated hyperphagia. We report that AgRP-neuron activation occurs rapidly upon acute cold exposure, as do increases of both energy expenditure and energy intake, suggesting the mere perception of cold is sufficient to engage each of these responses. We further report that silencing of AgRP neurons selectively blocks the effect of cold exposure to increase food intake but has no effect on energy expenditure. Together, these findings establish a physiologically important role for AgRP neurons in the hyperphagic response to cold exposure.

DiTacchio, K. A., et al. (2020). "JARID1a Ablation in the Liver Alters Systemic Metabolism and Adaptation to Feeding." Cell Reports **31**(8).

The liver is a key regulator of systemic energy homeostasis whose proper function is dependent on the circadian clock. Here, we show that livers deficient in the oscillator component JARID1a exhibit a dysregulation of genes involved in energy metabolism. Importantly, we find that mice that lack hepatic JARID1a have decreased lean body mass, decreased respiratory exchange ratios, faster production of ketones, and increased glucose production in response to fasting. Finally, we find that JARID1a loss compromises the response of the hepatic transcriptome to nutrient availability. In all, ablation of hepatic JARID1a disrupts the coordination of hepatic metabolic programs with whole-body consequences.

Dong, T. S., et al. (2020). "Intraperitoneal Treatment of Kisspeptin Suppresses Appetite and Energy Expenditure and Alters Gastrointestinal Hormones in Mice." Digestive Diseases and Sciences **65**(8): 2254-2263.

Background: Kisspeptin is a neuropeptide that plays an integral role in the regulation of energy intake and reproduction by acting centrally on the hypothalamus–pituitary–gonadal axis. Our current study explores for the first time the effects of a pharmacological treatment of intraperitoneal kisspeptin-10 on murine feeding behavior, respirometry parameters, energy balance, and metabolic hormones. Methods: Two groups (n = 16) of age- and sex-matched C57BL/6 wild-type adult mice were individually housed in metabolic cages and intraperitoneally injected with either kisspeptin-10 (2 nmol in 200 µl of saline) (10 µM) or vehicle before the beginning of a dark-phase cycle. Microstructure of feeding and drinking behavior, respirometry gases, respiratory quotient (RQ), total energy expenditure (TEE), metabolic hormones, oral glucose tolerance, and lipid profiles were measured. Results: Intraperitoneal treatment with kisspeptin-10 caused a significant reduction in food intake, meal frequency, meal size, and eating rate. Kisspeptin-10 significantly decreased TEE during both the dark and light phase cycles, while also increasing the RQ during the dark-phase cycle. In addition, mice injected with kisspeptin-10 had significantly higher plasma levels of insulin (343.8 pg/ml vs. 106.4 pg/ml; p = 0.005), leptin (855.5 pg/ml vs. 173.1 pg/ml; p = 0.02), resistin (9411.1 pg/ml vs. 4116.5 pg/ml; p = 0.001), and HDL (147.6 mg/dl vs 97.1 mg/dl; p = 0.04). Conclusion: A pharmacological dose of kisspeptin-10 significantly altered metabolism by suppressing food intake, meal size, eating rate, and TEE while increasing the RQ. These changes were linked to increased levels of insulin, leptin, resistin, and HDL. The current results suggest that a peripheral kisspeptin treatment could alter metabolism and energy homeostasis by suppressing appetite, food intake, and fat accumulation.

Ehrlicher, S. E., et al. (2020). "Mitochondrial adaptations to exercise do not require Bcl2-mediated autophagy but occur with BNIP3/Parkin activation." FASEB Journal **34**(3): 4602-4618.

Understanding the mechanisms regulating mitochondrial respiratory function and adaptations to metabolic challenges, such as exercise and high dietary fat, is necessary to promote skeletal muscle health and attenuate metabolic disease. Autophagy is a constitutively active degradation pathway that promotes mitochondrial turnover and transiently increases postexercise. Recent evidence indicates Bcl2 mediates

exercise-induced autophagy and skeletal muscle adaptions to training during high-fat diet. We determined if improvements in mitochondrial respiration due to exercise training required Bcl2-mediated autophagy using a transgenic mouse model of impaired inducible autophagy (Bcl2AAA). Mitochondrial adaptations to a treadmill exercise training protocol, in either low-fat or high-fat diet fed mice, did not require Bcl2-mediated autophagy activation. Instead, training increased protein synthesis rates and basal autophagy in the Bcl2AAA mice, while acute exercise activated BNIP3 and Parkin autophagy. High-fat diet stimulated lipid-specific mitochondrial adaptations. These data demonstrate increases in basal mitochondrial turnover, not transient activation with exercise, mediate adaptations to exercise and high-fat diet.

Ewbank, S. N., et al. (2020). "Chronic Gq signaling in AgRP neurons does not cause obesity." Proceedings of the National Academy of Sciences of the United States of America **117**(34): 20874-20880.

Maintaining energy homeostasis requires coordinating physiology and behavior both on an acute timescale to adapt to rapid fluctuations in caloric intake and on a chronic timescale to regulate body composition. Hypothalamic agouti-related peptide (AgRP)-expressing neurons are acutely activated by caloric need, and this acute activation promotes increased food intake and decreased energy expenditure. On a longer timescale, AgRP neurons exhibit chronic hyperactivity under conditions of obesity and high dietary fat consumption, likely due to leptin resistance; however, the behavioral and metabolic effects of chronic AgRP neuronal hyperactivity remain unexplored. Here, we use chemogenetics to manipulate Gq signaling in AgRP neurons in mice to explore the hypothesis that chronic activation of AgRP neurons promotes obesity. Inducing chronic Gq signaling in AgRP neurons initially increased food intake and caused dramatic weight gain, in agreement with published data; however, food intake returned to baseline levels within 1 wk, and body weight returned to baseline levels within 60 d. Additionally, we found that, when mice had elevated body weight due to chronic Gq signaling in AgRP neurons, energy expenditure was not altered but adiposity and lipid metabolism were both increased, even under caloric restriction. These findings reveal that the metabolic and behavioral effects of chronic Gq signaling in AgRP neurons are distinct from the previously reported effects of acute Gq signaling and also of leptin insensitivity.

Flippo, K. H., et al. (2020). "FGF21 signaling in glutamatergic neurons is required for weight loss associated with dietary protein dilution." Scientific Reports **10**(1).

Alterations in macronutrient intake can have profound effects on energy intake and whole-body metabolism. For example, reducing protein intake increases energy expenditure, increases insulin sensitivity and decreases body weight in rodents. Fibroblast growth factor 21 (FGF21) signaling in the brain is necessary for the metabolic effects of dietary protein restriction and has more recently been proposed to promote protein preference. However, the neuron populations through which FGF21 elicits these effects are unknown. Here, we demonstrate that deletion of β-klotho in glutamatergic, but not GABAergic, neurons abrogated the effects of dietary protein restriction on reducing body weight, but not on improving insulin sensitivity in both diet-induced obese and lean mice. Specifically, FGF21 signaling in glutamatergic neurons is necessary for protection against body weight gain and induction of UCP1 in

adipose tissues associated with dietary protein restriction. However, β-klotho expression in glutamatergic neurons was dispensable for the effects of dietary protein restriction to increase insulin sensitivity. In addition, we report that FGF21 administration does not alter protein preference, but instead promotes the foraging of other macronutrients primarily by suppressing simple sugar consumption. This work provides important new insights into the neural substrates and mechanisms behind the endocrine control of metabolism during dietary protein dilution.

Forney, L. A., et al. (2020). "Dietary Methionine Restriction Signals to the Brain Through Fibroblast Growth Factor 21 to Regulate Energy Balance and Remodeling of Adipose Tissue." Obesity **28**(10): 1912-1921.

Objective: Restricting dietary methionine to 0.17% in mice increases energy expenditure (EE), reduces fat deposition, and improves metabolic health by increasing hepatic fibroblast growth factor 21 (FGF21). The goal of this study was to compare each of these responses in mice with the coreceptor for FGF21 deleted in either adipose tissue or the brain. Methods: Methionine-restriction (MR) diets were fed to age-matched cohorts of mice with the coreceptor for FGF21 deleted in either adipose tissue or the brain. The physiological and transcriptional responses to MR were compared in the respective cohorts. Results: Tissue-specific deletion of the FGF21 coreceptor in adipose tissue did not abrogate the ability of dietary MR to increase EE and reduce fat deposition. Tissue-specific deletion of the FGF21 coreceptor from the brain produced mice that were unable to respond to the effects of MR on EE or the remodeling of adipose tissue. Conclusions: The increase in FGF21 produced by dietary MR acts primarily in the brain to produce its physiological effects on energy balance. In contrast, the effects of MR on hepatic gene expression were intact in both models, supporting a mechanism that directly links detection of reduced methionine in the liver to transcriptional mechanisms that alter gene expression in the liver.

Forney, L. A., et al. (2020). "Sexually Dimorphic Effects of Dietary Methionine Restriction are Dependent on Age when the Diet is Introduced." Obesity **28**(3): 581-589.

Objective: Restricting dietary methionine to 0.17% in male mice increases energy expenditure, reduces fat deposition, and improves metabolic health. The goal of this work was to compare each of these responses in postweaning male and female mice and in physically mature male and female mice. Methods: Methionine-restricted (MR) diets were fed to age-matched cohorts of male and female mice for 8 to 10 weeks beginning at 8 weeks of age or beginning at 4 months of age. The physiological and transcriptional responses to MR were compared in the respective cohorts. Results: Dietary MR produced sexually dimorphic changes in body composition in young growing animals, with males preserving lean at the expense of fat and females preserving fat at the expense of lean. The effects of MR on energy balance were comparable between sexes when the diet was initiated after attainment of physical maturity (4 months), and metabolic and endocrine responses were also comparable between males and females after 8 weeks on the MR diet. Conclusions: The sexually dimorphic effects of MR are limited to nutrient partitioning between lean and fat tissue deposition in young, growing mice. Introduction of the diet after physical maturity produced comparable effects on growth and metabolic responses in male and female mice.

Henneicke, H., et al. (2020). "Skeletal glucocorticoid signalling determines leptin resistance and obesity in aging mice." Molecular Metabolism **42**.

Objective: Aging and chronic glucocorticoid excess share a number of critical features, including the development of central obesity, insulin resistance and osteoporosis. Previous studies have shown that skeletal glucocorticoid signalling increases with aging and that osteoblasts mediate the detrimental skeletal and metabolic effects of chronic glucocorticoid excess. Here, we investigated whether endogenous glucocorticoid action in the skeleton contributes to metabolic dysfunction during normal aging. Methods: Mice lacking glucocorticoid signalling in osteoblasts and osteocytes (HSD2OB/OCY-tg mice) and their wild-type littermates were studied until 3, 6, 12 and 18 months of age. Body composition, adipose tissue morphology, skeletal gene expression and glucose/insulin tolerance were assessed at each timepoint. Leptin sensitivity was assessed by arcuate nucleus STAT3 phosphorylation and inhibition of feeding following leptin administration. Tissue-specific glucose uptake and adipose tissue oxygen consumption rate were also measured. Results: As they aged, wild-type mice became obese and insulin-resistant. In contrast, HSD2OB/OCY-tg mice remained lean and insulin-sensitive during aging. Obesity in wild-type mice was due to leptin resistance, evidenced by an impaired ability of exogenous leptin to suppress food intake and phosphorylate hypothalamic STAT3, from 6 months of age onwards. In contrast, HSD2OB/OCY-tg mice remained leptin-sensitive throughout the study. Compared to HSD2OB/OCY-tg mice, leptin-resistant wild-type mice displayed attenuated sympathetic outflow, with reduced tyrosine hydroxylase expression in both the hypothalamus and thermogenic adipose tissues. Adipose tissue oxygen consumption rate declined progressively in aging wild-type mice but was maintained in HSD2OB/OCY-tg mice. At 18 months of age, adipose tissue glucose uptake was increased 3.7-fold in HSD2OB/OCY-tg mice, compared to wild-type mice. Conclusions: Skeletal glucocorticoid signalling is critical for the development of leptin resistance, obesity and insulin resistance during aging. These findings underscore the skeleton's importance in the regulation of body weight and implicate osteoblastic/osteocytic glucocorticoid signalling in the aetiology of aging-related obesity and metabolic disease.

Her, T. K., et al. (2020). "Dietary carbohydrates modulate metabolic and β-cell adaptation to high-fat diet-induced obesity." American Journal of Physiology - Endocrinology and Metabolism **318**(6): E856-E865.

Dietary carbohydrates modulate metabolic and cell adaptation to high-fat diet-induced obesity. Am J Physiol Endocrinol Metab 318: E856-E865, 2020. First published April 21, 2020; doi:10.1152/ajpendo.00539.2019.-Obesity is associated with several chronic comorbidities, one of which is type 2 diabetes mellitus (T2DM). The pathogenesis of obesity and T2DM is influenced by alterations in diet macronutrient composition, which regulate energy expenditure, metabolic function, glucose homeostasis, and pancreatic islet cell biology. Recent studies suggest that increased intake of dietary carbohydrates plays a previously underappreciated role in the promotion of obesity and consequent metabolic dysfunction. Thus, in this study, we utilized mouse models to test the hypothesis that dietary carbohydrates modulate energetic, metabolic, and islet adaptions to high-fat diets. To address this, we exposed C57BL/6J mice to 12 wk of 3 eucaloric high-fat diets (60% calories from fat) with varying total carbohydrate (1-20%) and sucrose (0-20%) content. Our results show that severe restriction of dietary carbohydrates characteristic of ketogenic diets reduces body fat accumulation, enhances energy expenditure, and reduces prevailing glycemia and insulin resistance compared with carbohydrate-rich,

high-fat diets. Moreover, severe restriction of dietary carbohydrates also results in functional, morphological, and molecular changes in pancreatic islets highlighted by restricted capacity for cell mass expansion and alterations in insulin secretory response. These studies support the hypothesis that lowcarbohydrate/ high-fat diets provide antiobesogenic benefits and suggest further evaluation of the effects of these diets on cell biology in humans.

Hew, J. J., et al. (2020). "Mouse models in burns research: Characterisation of the hypermetabolic response to burn injury." Burns **46**(3): 663-674.

Objective: The aim of the study is to characterise burn induced hypermetabolism in a mouse model. Summary Background Data: There are many mouse models of burn injury currently available however, their use in burns research is limited by the general assumption that post-burn hypermetabolism is difficult to study in these models. Methods: Male Balb/c mice were subjected to either a small (1 cm2) or large (4 cm2) contact burn. The hypermetabolic response to burn injury was determined by measuring changes in basal energy expenditure. The hormonal and inflammatory mediators of hypermetabolism, and the catabolic alterations secondary to hypermetabolism were also examined. Results: Post-burn hypermetabolism was induced in both models of small and large burn. However, large burns resulted in prolonged wound healing, a more pronounced and sustained increase in basal energy expenditure, and a greater stress and systemic inflammatory response with profound catabolic consequences. Conclusions: In the present study, we have successfully characterised the burn induced systemic hypermetabolic response in a mouse model of small and large burn. These models may prove useful for researchers studying the complex aetiology of hypermetabolism and interventions.

Holter, M. M., et al. (2020). "Hepatocyte p53 ablation induces metabolic dysregulation that is corrected by food restriction and vertical sleeve gastrectomy in mice." FASEB Journal **34**(1): 1846-1858.

P53 has been implicated in the pathogenesis of obesity and diabetes; however, the mechanisms and tissue sites of action are incompletely defined. Therefore, we investigated the role of hepatocyte p53 in metabolic homeostasis using a hepatocyte-specific p53 knockout mouse model. To gain further mechanistic insight, we studied mice under two complementary conditions of restricted weight gain: vertical sleeve gastrectomy (VSG) or food restriction. VSG or sham surgery was performed in high-fat diet-fed male hepatocyte-specific p53 wild-type and knockout littermates. Sham-operated mice were fed ad libitum or food restricted to match their body weight to VSG-operated mice. Hepatocyte-specific p53 ablation in sham-operated ad libitum-fed mice impaired glucose homeostasis, increased body weight, and decreased energy expenditure without changing food intake. The metabolic deficits induced by hepatocyte-specific p53 ablation were corrected, in part by food restriction, and completely by VSG. Unlike food restriction, VSG corrected the effect of hepatocyte p53 ablation to lower energy expenditure, resulting in a greater improvement in glucose homeostasis compared with food restricted mice. These data reveal an important new role for hepatocyte p53 in the regulation of energy expenditure and body weight and suggest that VSG can improve alterations in energetics associated with p53 dysregulation.

Jais, A., et al. (2020). "PNOCARC Neurons Promote Hyperphagia and Obesity upon High-Fat-Diet Feeding." Neuron **106**(6): 1009-1025.e1010.

Calorie-rich diets induce hyperphagia and promote obesity, although the underlying mechanisms remain poorly defined. We find that short-term high-fat-diet (HFD) feeding of mice activates prepronociceptin (PNOC)-expressing neurons in the arcuate nucleus of the hypothalamus (ARC). PNOCARC neurons represent a previously unrecognized GABAergic population of ARC neurons distinct from well-defined feeding regulatory AgRP or POMC neurons. PNOCARC neurons arborize densely in the ARC and provide inhibitory synaptic input to nearby anorexigenic POMC neurons. Optogenetic activation of PNOCARC neurons in the ARC and their projections to the bed nucleus of the stria terminalis promotes feeding. Selective ablation of these cells promotes the activation of POMC neurons upon HFD exposure, reduces feeding, and protects from obesity, but it does not affect food intake or body weight under normal chow consumption. We characterize PNOCARC neurons as a novel ARC neuron population activated upon palatable food consumption to promote hyperphagia.

Jian, C., et al. (2020). "Low-Dose Sorafenib Acts as a Mitochondrial Uncoupler and Ameliorates Nonalcoholic Steatohepatitis." Cell Metabolism **31**(5): 892-908.e811.

Nonalcoholic steatohepatitis (NASH) is becoming one of the leading causes of hepatocellular carcinoma (HCC). Sorafenib is the only first-line therapy for advanced HCC despite its serious adverse effects. Here, we report that at an equivalent of approximately one-tenth the clinical dose for HCC, sorafenib treatment effectively prevents the progression of NASH in both mice and monkeys without any observed significant adverse events. Mechanistically, sorafenib's benefit in NASH is independent of its canonical kinase targets in HCC, but involves the induction of mild mitochondrial uncoupling and subsequent activation of AMP–activated protein kinase (AMPK). Collectively, our findings demonstrate a previously unappreciated therapeutic effect and signaling mechanism of low-dose sorafenib treatment in NASH. We envision that this new therapeutic strategy for NASH has the potential to translate into a beneficial anti-NASH therapy with fewer adverse events than is observed in the drug's current use in HCC.

Jin, S., et al. (2020). "Function of astrocyte MyD88 in high-fat-diet-induced hypothalamic inflammation." Journal of Neuroinflammation **17**(1).

Background: A growing body of evidence shows that hypothalamic inflammation is an important factor in the initiation of obesity. In particular, reactive gliosis accompanied by inflammatory responses in the hypothalamus are pivotal cellular events that elicit metabolic abnormalities. In this study, we examined whether MyD88 signaling in hypothalamic astrocytes controls reactive gliosis and inflammatory responses, thereby contributing to the pathogenesis of obesity. Methods: To analyze the role of astrocyte MyD88 in obesity pathogenesis, we used astrocyte-specific Myd88 knockout (KO) mice fed a high-fat diet (HFD) for 16 weeks or injected with saturated free fatty acids. Astrocyte-specific gene expression in the hypothalamus was determined using real-time PCR with mRNA purified by the Ribo-Tag system. Immunohistochemistry was used to detect the expression of glial fibrillary acidic protein, ionized calcium-binding adaptor molecule 1, phosphorylated signal transducer and activator of transcription 3, and α-

melanocyte-stimulating hormone in the hypothalamus. Animals' energy expenditure was measured using an indirect calorimetry system. Results: The astrocyte-specific Myd88 KO mice displayed ameliorated hypothalamic reactive gliosis and inflammation induced by injections of saturated free fatty acids and a long-term HFD. Accordingly, the KO mice were resistant to long-term HFD-induced obesity and showed an improvement in HFD-induced leptin resistance. Conclusions: These results suggest that MyD88 in hypothalamic astrocytes is a critical molecular unit for obesity pathogenesis that acts by mediating HFD signals for reactive gliosis and inflammation.

Jing, Y., et al. (2020). Effect of fecal microbiota transplantation on neurological restoration in a spinal cord injury mouse model: involvement of brain-gut axis, researchsquare.com.

Background: Spinal cord injury (SCI) patients display disruption of gut microbiome and gut dysbiosis exacerbate neurological impairment in SCI models. Cumulative data support an important role of gut microbiome in SCI. Here, we investigated the hypothesis that fecal microbiota transplantation (FMT) may exert a neuroprotective effect on SCI mice. Results: We found that FMT facilitated functional recovery, promoted neural axonal regeneration, improved animal weight gain and metabolic profiling, and enhanced intestinal barrier integrity and GI motility. High-throughput sequencing revealed that levels of phylum Firmicutes, genus Blautia, Anaerostipes and Lactobacillus were reduced in fecal samples of SCI mice, and FMT remarkably reshaped gut microbiome. Also, FMT-treated SCI mice showed increased amount of fecal short-chain fatty acids (SCFAs), which correlated with alteration of intestinal permeability and locomotor recovery. Furthermore, FMT down-regulated IL-1β/NF-κB signaling in spinal cord and NF-κB signaling in gut. Conclusion: Our study demonstrates that reprogramming of gut microbiota by FMT improves locomotor and GI functions in SCI mice, possibly through the anti-inflammatory functions of SCFAs.

Kelly, K. P. (2020). Meal timing alone alters lipid oxidation rate without affecting corticosterone in mice and humans, ir.vanderbilt.edu.

Page 1. Meal timing alone alters lipid oxidation rate without affecting corticosterone in mice and humans By Kevin Parsons Kelly Dissertation Submitted to the Faculty of the Graduate School of Vanderbilt University in partial fulfillment of the requirements for the degree of …

Krisko, T. I., et al. (2020). "Dissociation of Adaptive Thermogenesis from Glucose Homeostasis in Microbiome-Deficient Mice." Cell Metabolism **31**(3): 592-604.e599.

The gut microbiome contributes to metabolic health and disease in the host. Krisko and colleagues demonstrate that the gut microbiome supports hepatic gluconeogenesis to maintain euglycemia, but that this is independent from the regulation of energy expenditure and the adaptive thermogenic response to cold or high-fat feeding.

Lee, N. J., et al. (2020). "Lack of NPY in neurotensin neurons leads to a lean phenotype." Neuropeptides **80**.

> Neuropeptide Y (NPY) producing neurons in the arcuate nucleus (Arc) of the hypothalamus are essential to the regulation of food intake and energy homeostasis. Whilst they have classically been thought to co-express agouti-related peptide (AgRP), it is now clear that there is a sub-population of NPY neurons in the Arc that do not. Here, we show that a subset of AgRP-negative, NPY-positive neurons in the Arc also express neurotensin (NTS) and we use an NTS-Cre line to investigate the function of this sub-population of NPY neurons. The lack of NPY in NTS-positive neurons led to a marked reduction in fat mass and bodyweight as well as a significant reduction in food intake in male NPYlox/lox; NTScre/+ mice compared to controls. Despite the reduction in food intake, overall energy expenditure was similar between genotypes due to concomitant reduction in activity in NPYlox/lox; NTScre/+ mice. Furthermore, cortical bone mass was significantly reduced in NPYlox/lox;NTScre/+ mice with no evident alterations in the cancellous bone compartment, likely due to reduced leptin levels as a result of their reduced adiposity. Taken together, these data suggest that the sub-population of Arc NPY neurons expressing NTS are critical for regulating food intake, activity and fat mass but are not directly involved in the control of bone mass.

Lensu, S., et al. (2020). Prebiotic Faecalibacterium prausnitzii Prevent Diet-induced Hepatic Steatosis in Rats Targeting High Fat, preprints.org.

> … 2.3. Indirect metabolic measurements The indirect measures of metabolism were analyzed from respiratory gases with oxygen, CO2 and capacitive water vapor partial pressure analyzer (Promethion®GA3, Sable Systems, Las Vegas, NV, USA) …

Lensu, S., et al. (2020). "Prebiotic xylo-oligosaccharides ameliorate high-fat-diet-induced hepatic steatosis in rats." Nutrients **12**(11): 1-23.

> Understanding the importance of the gut microbiota (GM) in non-alcoholic fatty liver disease (NAFLD) has raised the hope for therapeutic microbes. We have shown that high hepatic fat content associated with low abundance of Faecalibacterium prausnitzii in humans and, further, the administration of F. prausnitzii prevented NAFLD in mice. Here, we aimed at targeting F. prausnitzii by prebiotic xylo-oligosaccharides (XOS) to treat NAFLD. First, the effect of XOS on F. prausnitzii growth was assessed in vitro. Then, XOS was supplemented or not with high (HFD, 60% of energy from fat) or low (LFD) fat diet for 12 weeks in Wistar rats (n = 10/group). XOS increased F. prausnitzii growth, having only a minor impact on the GM composition. When supplemented with HFD, XOS ameliorated hepatic steatosis. The underlying mechanisms involved enhanced hepatic β-oxidation and mitochondrial respiration. Nuclear magnetic resonance (1 H-NMR) analysis of cecal metabolites showed that, compared to the HFD, the LFD group had a healthier cecal short-chain fatty acid profile and on the HFD, XOS reduced cecal isovalerate and tyrosine, metabolites previously linked to NAFLD. Cecal branched-chain fatty acids associated positively and butyrate negatively with hepatic triglycerides. In conclusion, XOS supplementation can ameliorate NAFLD by improving hepatic oxidative metabolism and affecting GM.

Li, A. J., et al. (2020). "Repeated pharmacogenetic catecholamine neuron activation in the ventrolateral medulla attenuates subsequent glucoregulatory responses." Diabetes **69**(12): 2747-2755.

> Hindbrain catecholamine (CA) neurons are essential for elicitation of protective counterregulatory responses (CRRs) to glucose deficit, including increased feeding and elevation of circulating corticosterone, epinephrine, and glucose. Severe or repeated antecedent glucopri-vation results in attenuation of these CRRs and failure to correct glucose deficit, constituting a potentially lethal condition known as hypoglycemia-associated auto-nomic failure (HAAF) that may occur in patients with diabetes on insulin therapy. Recently, we demonstrated that selective pharmacogenetic activation of CA neuron subpopulations in the ventrolateral medulla during nor-moglycemia elicits these CRRs in a site-specific manner. In the present experiment, we examined the effect of repeated pharmacogenetic activation of CA neurons in the A1/C1 cell group on subsequent elicitation of feed-ing, corticosterone secretion, and respiratory quotient. We found that this prior treatment attenuated these responses to subsequent pharmacogenetic stimulation, similar to attenuation of these CRRs following repeated antecedent glucoprivation. This suggests that functional impairment of A1/C1 CA neurons resulting from antecedent glucoprivation may account, at least in part, for impairment of specific CRRs critical for restoration of normoglycemia in response to glucose deficit. Thus, a pharmacogenetic approach to selective activation of key neural circuits could provide a means of identifying neuropathogenic mechanisms contributing to HAAF.

Li, Y., et al. (2020). "Thioesterase Superfamily Member 1 Undergoes Stimulus-coupled Reorganization to Regulate Metabolic Activity in Brown Adipose Tissue." The FASEB Journal **34**(S1): 1-1.

> Page 1. 1 Thioesterase Superfamily Member 1 Undergoes Stimulus-coupled Reorganization to Regulate Metabolism Yue Li 1,2 , Norihiro Imai 3 , Samaksh Goyal 1,2 , Hayley T. Nicholls 3 , Tibor I. Krisko 3 , Mahnoor Baqai 1 …

Lim, S. M., et al. (2020). "Sexually dimorphic response of increasing dietary intake of high amylose wheat on metabolic and reproductive outcomes in male and female mice." Nutrients **12**(1).

> High amylose wheat (HAW) has a higher resistant starch content and lower glycaemic index than standard amylose wheat (SAW), which may be associated with health benefits. This study aimed to determine the effects of replacing SAW with HAW on metabolic and reproductive parameters in male and female mice. Male and female C57BL/6 mice were randomly divided into groups (n = 8/group/sex) and fed either a SAW65 (65% SAW w/w; control), HAW35 (35% HAW w/w), HAW50 (50% HAW w/w) or HAW65 (65% HAW w/w) diet for eight weeks. In male but not female, the HAW65 group had a lower abdominal circumference, relative total fat mass, relative gonadal fat mass and plasma leptin concentration compared to the HAW35 group. There were no differences in fasting blood glucose concentrations or plasma concentrations of cholesterol, triglycerides or non-esterified fatty acids between groups in either males or females. The HAW-fed males had a higher testicular weight and HAW-fed females spent less time in diestrus and a longer time in metestrus compared to the SAW-fed mice. Higher dietary intake of HAW

appears to reduce abdominal fat deposition compared to the lower level of HAW in a sexually dimorphic manner. The impacts on reproductive parameters in the HAW-fed mice require further investigation.

Loehfelm, A., et al. (2020). "A New Zealand green-lipped mussel oil-enriched high-fat diet exhibits beneficial effects on body weight and metabolism in mice." British Journal of Nutrition.

To induce diet-induced obesity (DIO) in rodents, diets high in saturated fat and/or carbohydrates are commonly used. In the laboratory, standardized diets evolved over time without having paid particular attention to the effect of fat composition on metabolic alterations. In the present study, customized high-fat diets (HFDs) enriched in a combination of lard and different concentrations of New Zealand green-lipped mussel (Perna canaliculus) oil or Hoki (Macruronus novaezelandiae, blue grenadier) liver oil, important sources of ω3-polyunsaturated fatty acids, in comparison to a solely lard based diet were fed to lean and DIO male C57BL/6 mice and their effects on metabolic parameters were monitored. Intriguingly, an isocaloric HFD containing 63% of the total fat in the form of mussel oil and only 28% in the form of lard, attenuated the HFD-induced body weight gain after 1 and 4 weeks, respectively. Consistently, changing a lard enriched HFD to the mussel oil diet reduced body weight markedly even after mice had been exposed to the former diet for 10 months. The weight-reducing effect of the diet was not caused by altered energy intake or expenditure but was associated with reduced visceral fat mass. Collectively, these data suggest a novel weight-reducing potential of green-lipped mussel oil.

Masson, S. W. C., et al. (2020). "β-catenin regulates muscle glucose transport via actin remodelling and M-cadherin binding." Molecular Metabolism **42**.

Objective: Skeletal muscle glucose disposal following a meal is mediated through insulin-stimulated movement of the GLUT4-containing vesicles to the cell surface. The highly conserved scaffold-protein β-catenin is an emerging regulator of vesicle trafficking in other tissues. Here, we investigated the involvement of β-catenin in skeletal muscle insulin-stimulated glucose transport. Methods: Glucose homeostasis and transport was investigated in inducible muscle specific β-catenin knockout (BCAT-mKO) mice. The effect of β-catenin deletion and mutation of β-catenin serine 552 on signal transduction, glucose uptake and protein–protein interactions were determined in L6-G4-myc cells, and β-catenin insulin-responsive binding partners were identified via immunoprecipitation coupled to label-free proteomics. Results: Skeletal muscle specific deletion of β-catenin impaired whole-body insulin sensitivity and insulin-stimulated glucose uptake into muscle independent of canonical Wnt signalling. In response to insulin, β-catenin was phosphorylated at serine 552 in an Akt-dependent manner, and in L6-G4-myc cells, mutation of β-cateninS552 impaired insulin-induced actin-polymerisation, resulting in attenuated insulin-induced glucose transport and GLUT4 translocation. β-catenin was found to interact with M-cadherin in an insulin-dependent β-cateninS552-phosphorylation dependent manner, and loss of M-cadherin in L6-G4-myc cells attenuated insulin-induced actin-polymerisation and glucose transport. Conclusions: Our data suggest that β-catenin is a novel mediator of glucose transport in skeletal muscle and may contribute to insulin-induced actin-cytoskeleton remodelling to support GLUT4 translocation.

McKenna, A. J. (2020). Rapid evolution of starvation resistance in Drosophila: physiological and molecular mechanisms, digitalscholarship.unlv.edu.

> Page 1. UNLV Theses, Dissertations, Professional Papers, and Capstones 5-1-2020 Rapid Evolution of Starvation Resistance in Drosophila: Physiological and Molecular Mechanisms Austin Joseph McKenna Follow this and …

Meese, S., et al. (2020). "Methane emission, metabolism, and performance of Holstein dairy cows with low, medium, and high lymphocyte proliferation during transition." Journal of Dairy Science **103**(5): 4367-4377.

> This study aimed to identify interactions between state of lactation (dry or early lactating) and immune responder group (low, medium, or high) for energy metabolism traits as well as metabolic and immunological traits in dairy cows. In early lactation, when the energy priority of cows shifts toward the mammary gland, the energy available to be partitioned toward the immune system may differ among individuals. The equilibrium between energy supply from feed, digestion, and body reserve mobilization and energy expenditure with milk, immune system, methane, and heat production is delicate in this stage. Seventeen Holstein cows entering their second to fifth lactation were kept under comparable feeding, housing, and management conditions and were studied from 14 ± 6 d before calving to 11 ± 3 d after calving. Feed intake, milk yield, body condition, blood metabolites, and cortisol as well as gaseous exchange in respiration chambers were measured. The latter was used to quantify methane emission and to calculate resting metabolic rate and heat production. Subsets of blood leukocytes and peripheral blood mononuclear cells (PBMC) were monitored. Activation and proliferation of the PBMC in response to the mitogen phytohemagglutinin ante- and postpartum were assessed using the oxygen consumption rate (24-h cell culture assay) and the 3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyl tetrazolium bromide (MTT) assay (72-h cell culture assay). Cows were classified based on the in vitro proliferative response of the PBMC measured postpartum in low (n = 6), medium (n = 5), and high (n = 6) responders. We found no interaction of state of lactation with responder group for feed intake, milk yield, efficiency, metabolic traits, and immune cell activation ante- and postpartum. However, after calving, low-responder cows produced less methane per unit of body weight and per unit of energy-corrected milk compared with the other cows. This might be indicative of a low rumen fermentation intensity. Low responders might therefore suffer from a lower availability of digestible energy in early lactation and not be able to sustain the shift from immune cell activation to proliferation. If so, the selection of environmentally friendly low-methane emitters could promote phenotypes with a compromised immune response in the critical early lactation.

Mooli, R. G. R., et al. (2020). "Sustained mitochondrial biogenesis is essential to maintain caloric restriction-induced beige adipocytes." Metabolism: Clinical and Experimental **107**.

> Background: Caloric restriction (CR) delays the onset of metabolic and age-related disorders. Recent studies have demonstrated that formation of beige adipocytes induced by CR is strongly associated with extracellular remodeling in adipose tissue, decrease in adipose tissue inflammation, and improved

systemic metabolic homeostasis. However, beige adipocytes rapidly transition to white upon CR withdrawal through unclear mechanisms. Materials and methods: Six-week old C57BL6 mice were fed with 40% CR chow diet for 6 weeks. Subsequently, one group of mice was switched back to ad libitum chow diet, which was continued for additional 2 weeks. Adipose tissues were assessed histologically and biochemically for beige adipocytes. Results: Beige adipocytes induced by CR rapidly transition to white adipocytes when CR is withdrawn independent of parkin-mediated mitophagy. We demonstrate that the involution of mitochondria during CR withdrawal is strongly linked with a decrease in mitochondrial biogenesis. We further demonstrate that beige-to-white fat transition upon β3-AR agonist-withdrawal could be attenuated by CR, partly via maintenance of mitochondrial biogenesis. Conclusion: In the model of CR, our study highlights the dominant role of mitochondrial biogenesis in the maintenance of beige adipocytes. We propose that loss of beige adipocytes upon β3-AR agonist withdrawal could be attenuated by CR.

Morris, E. M., et al. (2020). "Difference in Housing Temperature-Induced Energy Expenditure Elicits Sex-Specific Diet-Induced Metabolic Adaptations in Mice." Obesity **28**(10): 1922-1931.

Objective: The aim of this study was to test whether increased energy expenditure (EE), independent of physical activity, reduces acute diet-induced weight gain through tighter coupling of energy intake to energy demand and enhanced metabolic adaptations. Methods: Indirect calorimetry and quantitative magnetic resonance imaging were used to assess energy metabolism and body composition during 7-day high-fat/high-sucrose (HFHS) feeding in male and female mice housed at divergent temperatures (20°C vs. 30°C). Results: As previously observed, 30°C housing resulted in lower total EE and energy intake compared with 20°C mice regardless of sex. Interestingly, housing temperature did not impact HFHS-induced weight gain in females, whereas 30°C male mice gained more weight than 20°C males. Energy intake coupling to EE during HFHS feeding was greater in 20°C versus 30°C housing, with females greater at both temperatures. Fat mass gain was greater in 30°C mice compared with 20°C mice, whereas females gained less fat mass than males. Strikingly, female 20°C mice gained considerably more fat-free mass than 30°C mice. Reduced fat mass gain was associated with greater metabolic flexibility to HFHS, whereas fat-free mass gain was associated with diet-induced adaptive thermogenesis. Conclusions: These data reveal that EE and sex interact to impact energy homeostasis and metabolic adaptation to acute HFHS feeding, altering weight gain and body composition change. (Figure presented.).

Morris, E. M., et al. (2020). "Reduced Liver-Specific PGC1a Increases Susceptibility for Short-Term Diet-induced Weight Gain in Male Mice." Nutrients

The central integration of peripheral neural signals is one mechanism by which systemic energy homeostasis is regulated. Previously, increased acute food intake following the chemical reduction of hepatic fatty acid oxidation and ATP levels was prevented by common hepatic branch vagotomy (HBV). However, possible offsite actions of the chemical compounds confound the precise role of liver energy metabolism. Herein, we used a hepatocyte PGC1a heterozygous (LPGC1a) mouse model, with associated reductions in mitochondrial fatty acid oxidation and respiratory capacity, to assess the role of liver energy metabolism in systemic energy homeostasis. LPGC1a male, but not female, mice had a 70% greater high-fat/high-sucrose (HFHS) diet-induced weight gain compared to wildtype (WT) mice ($p < 0.05$). The greater

weight gain was associated with altered feeding behavior and lower activity energy expenditure during the HFHS diet in LPGC1a males. WT and LPGC1a mice underwent sham surgery or HBV to assess whether vagal signaling was involved in the HFHS-induced weight gain of male LPGC1a mice. HBV increased HFHS-induced weight gain (85%, $p < 0.05$) in male WT mice, but not LPGC1a mice. These data demonstrate a sex-specific role of reduced liver energy metabolism in acute diet-induced weight gain, and the need for a more nuanced assessment of the role of vagal signaling in short-term diet-induced weight gain.

Nas, A., et al. (2020). "Impact of energy turnover on fat balance in healthy young men during energy balance, energetic restriction and overfeeding." British Journal of Nutrition **123**(1): 30-40.

Body weight control is thought to be improved when physical activity and energy intake are both high (high energy turnover (ET)). The aim of the present study was to investigate the short-term impact of ET on fat balance during zero energy balance (EB), energetic restriction (ER) and overfeeding (OF). In a randomised crossover study, nine healthy men (BMI: 23·0 (SD 2·1) kg/m2, 26·6 (SD 3·5) years) passed 3 × 3 d in a metabolic chamber: three levels of ET (low, medium and high; physical activity level = 1·3-1·4, 1·5-1·6 and 1·7-1·8) were performed at zero EB, ER and OF (100, 75 and 125 % of individual energy requirement). Different levels of ET were obtained by walking (4 km/h) on a treadmill (0, 165 and 330 min). Twenty-four-hour macronutrient oxidation and relative macronutrient balance (oxidation relative to intake) was calculated, and NEFA, 24-h insulin and catecholamine secretion were analysed as determinants of fat oxidation. During EB and OF, 24-h fat oxidation increased with higher ET. This resulted in a higher relative fat balance at medium ET (EB: +17 %, OF: +14 %) and high ET (EB: +23 %, OF: +17 %) compared with low ET (all $P < 0·05$). In contrast, ER led to a stimulation of 24-h fat oxidation irrespective of ET (no differences in relative fat balance between ET levels, $P > 0·05$). In conclusion, under highly controlled conditions, a higher ET improved relative fat balance in young healthy men during OF and EB compared with a sedentary state.

Nissinen, T. (2020). "Molecular and physiological effects of muscle wasting and its treatment by blocking myostatin and activins." JYU dissertations.

Page 1. Tuuli Nissinen JYU DISSERTATIONS 341 Molecular and Physiological Effects of Muscle Wasting and Its Treatment by Blocking Myostatin and Activins Page 2. JYU DISSERTATIONS 341 Tuuli Nissinen Molecular and Physiological Effects …

Pearson, G. L., et al. (2020). "Circadian desynchronization alters metabolic and immune responses following lipopolysaccharide inoculation in male mice." Brain, Behavior, and Immunity **88**: 220-229.

Metabolism and inflammation are linked at many levels. Sickness behaviors are elicited by the immune system's response to antigenic stimuli, and include changes in feeding and metabolism. The immune system is also regulated by the circadian (daily) clock, which generates endogenous rhythms, and synchronizes these rhythms to the light-dark cycle. Modern society has resulted in chronic misalignment or desynchronization of the circadian clock and the external environment. We have demonstrated that

circadian desynchronization (CD) in mice alters metabolic function, and also affects both peripheral and central immune responses following a low-dose lipopolysaccharide (LPS) challenge. However, it is unclear how this altered immune response impacts sickness behaviors and metabolism following challenge. To test this, we housed male mice in circadian desynchronized (10-hours light:10-hours dark) or control (12-hours light:12-hours dark) conditions for 5–6 weeks. We then challenged mice with LPS (i.p., 0.4 mg/kg) or PBS and measured changes in body mass, feeding, drinking and locomotion using a comprehensive phenotyping system. Plasma, liver, and brain were collected 36 h post-inoculation (hpi) and inflammatory messengers were measured via multiplex cytokine/chemokine array and qPCR. We find that recovery of locomotion and body mass is prolonged in CD mice following LPS challenge. Additionally, at 36 hpi the expression of several proinflammatory cytokines differ depending on pre-inoculation lighting conditions. Our findings add to the growing literature which documents how desynchronization of circadian rhythms can lead to disrupted immune responses and changes in metabolic function.

Poteko, J., et al. (2020). "Methane emissions and milk fatty acid profiles in dairy cows fed linseed, measured at the group level in a naturally ventilated housing and individually in respiration chambers." Animals **10**(6): 1-18.

The present study evaluated the effects of linseed supplementation on CH4 emission and milk fatty acid composition in dairy cows measured at the group level in an experimental dairy loose housing using a tracer gas technique and individually in tied stalls and respiration chambers. Cows (2 × 20) were maintained in two separate sections under loose-housing conditions and received a diet supplemented with extruded linseed (L) lipids (29 g·kg−1 dry matter) or a control (C) diet containing corn flour. Subsequently, 2 × 6 cows per dietary group were investigated in a tied-housing system and respiration chambers. Substantially higher proportions of favorable milk fatty acids were recovered in L cows when compared with C cows at the group level, making the analysis of bulk milk a suitable control instrument for retailers. Linseed supplementation resulted in a slightly lower diurnal course of CH4 emission intensity than the control at the group and individual levels. However, we found no more than a trend for a CH4 mitigating effect, unlike in other studies supplementing similar linseed lipid levels. Feed supplements in concentrations that lead to a significant reduction in CH4 emissions must show whether the reduction potential determined at the group and individual levels is comparable.

Qing, H., et al. (2020). "Origin and Function of Stress-Induced IL-6 in Murine Models." Cell **182**(2): 372-387.e314.

Acute psychological stress has long been known to decrease host fitness to inflammation in a wide variety of diseases, but how this occurs is incompletely understood. Using mouse models, we show that interleukin-6 (IL-6) is the dominant cytokine inducible upon acute stress alone. Stress-inducible IL-6 is produced from brown adipocytes in a beta-3-adrenergic-receptor-dependent fashion. During stress, endocrine IL-6 is the required instructive signal for mediating hyperglycemia through hepatic gluconeogenesis, which is necessary for anticipating and fueling “fight or flight” responses. This adaptation comes at the cost of enhancing mortality to a subsequent inflammatory challenge. These findings provide a mechanistic understanding of the ontogeny and adaptive purpose of IL-6 as a bona fide stress hormone coordinating systemic immunometabolic reprogramming. This brain-brown fat-liver axis

might provide new insights into brown adipose tissue as a stress-responsive endocrine organ and mechanistic insight into targeting this axis in the treatment of inflammatory and neuropsychiatric diseases.

Rathinasabapathy, A., et al. (2020). "Expression of a Human Caveolin-1 Mutation in Mice Drives Inflammatory and Metabolic Defect-Associated Pulmonary Arterial Hypertension." Frontiers in Medicine **7**.

Background: In 2012, mutations in Cav1 were found to be the driving mutation in several cases of heritable pulmonary arterial hypertension (PAH). These mutations replaced the last 21 amino acids of Cav1 with a novel 22-amino-acid sequence. Because previously only Cav1 knockouts had been studied in the context of PAH, examining the in vivo effects of this novel mutation holds promise for new understanding of the role of Cav1 in disease etiology. Methods: The new 22 amino acids created by the human mutation were knocked into the native mouse Cav1 locus. The mice underwent hemodynamic, energy balance, and inflammatory measurements, both at baseline and after being stressed with either a metabolic or an inflammatory challenge [low-dose lipopolysaccharide (LPS)]. To metabolically challenge the mice, they were injected with streptozotocin (STZ) and fed a high-fat diet for 12 weeks. Results: Very little mutant protein was found in vivo (roughly 2% of wild-type by mass spectrometry), probably because of degradation after failure to traffic from the endoplasmic reticulum. The homozygous mutants developed a mild, low-penetrance PAH similar to that described previously in knockouts, and neither baseline nor metabolic nor inflammatory stress resulted in pressures above normal in heterozygous animals. The homozygous mutants had increased lean mass and worsened oral glucose tolerance, as previously described in knockouts. Novel findings include the preservation of Cav2 and accessory proteins in the liver and the kidney, while they are lost with homozygous Cav1 mutation in the lungs. We also found that the homozygous mutants had a significantly lower tolerance to voluntary spontaneous exercise than the wild-type mice, with the heterozygous mice at an intermediate level. The mutants also had higher circulating monocytes, with both heterozygous and homozygous animals having higher pulmonary MCP1 and MCP5 proteins. The heterozygous animals also lost weight at an LPS challenge level at which the wild-type mice continued to gain weight. Conclusions: The Cav1 mutation identified in human patients in 2012 is molecularly similar to a knockout of Cav1. It results in not only metabolic deficiencies and mild pulmonary hypertension, as expected, but also an inflammatory phenotype and reduced spontaneous exercise.

Regmi, P., et al. (2020). "Early or delayed time-restricted feeding prevents metabolic impact of obesity in mice." Journal of Endocrinology **248**(1): 75-86.

Time-restricted feeding (TRF) initiated early during the dark phase prevents the metabolic consequences of a high-fat diet in rodent models. However, the metabolic consequences of delaying the initiation of TRF, akin to breakfast skipping in humans, is unclear. We assigned 8-week-old male C57BL/6J mice (n = 192) to chow or high-fat diet ad libitum (AL) for 4 weeks, before randomization to continue AL or 10 h of TRF, initiated at lights off (TRFe) or 4-h after lights off (TRFd) for a further 8 weeks. Oral glucose tolerance tests (1 g/kg), metabolic monitoring and body composition by echoMRI were performed, and tissues were collected at six time points. TRF reduced weight and fat mass vs AL, with a greater reduction in TRFe vs TRFd. TRF improved glucose tolerance and protected mice from high-fat diet-induced hepatosteatosis vs AL, with no difference between TRFe and TRFd. TRF increased the amplitude of Bmal1 , Cry1 , Per2 , Nampt , and Nocturnin mRNA levels in liver. A phase delay in Bmal1 , Cry1 , Per2 , Reverbα , Nampt , NAD, Sirt1 ,

and Nocturnin was observed in TRFd. Thus, delaying TRF limited the weight benefit and induced a phase delay in the hepatic clock, but improved metabolic health. Allowing more flexibility in when TRF is initiated may increase the translational potential of this dietary approach in humans.

Rodriguez Paris, V., et al. (2020). "Defining the impact of dietary macronutrient balance on PCOS traits." Nature Communications **11**(1).

Lifestyle, mainly dietary, interventions are first-line treatment for women with polycystic ovary syndrome (PCOS), but the optimal diet remains undefined. We combined a hyperandrogenized PCOS mouse model with a systematic macronutrient approach, to elucidate the impact of dietary macronutrients on the development of PCOS. We identify that an optimum dietary macronutrient balance of a low protein, medium carbohydrate and fat diet can ameliorate key PCOS reproductive traits. However, PCOS mice display a hindered ability for their metabolic system to respond to diet variations, and varying macronutrient balance did not have a beneficial effect on the development of metabolic PCOS traits. We reveal that PCOS traits in a hyperandrogenic PCOS mouse model are ameliorated selectively by diet, with reproductive traits displaying greater sensitivity than metabolic traits to dietary macronutrient balance. Hence, providing evidence to support the development of evidence-based dietary interventions as a promising strategy for the treatment of PCOS, especially reproductive traits.

S, L., et al. (2020). "A reduction in voluntary physical activity during pregnancy in mice is mediated by prolactin." bioRxiv.

… wheels. Our phenotyping cages 75 (Promethion, Sable Systems International) had a traditional upright wheel, while a saucer/low 76 profile wheel was used in the home cages. The upright wheel potentially takes more effort to 77 …

Sandeman, L. Y., et al. (2020). "Disabling MNK protein kinases promotes oxidative metabolism and protects against diet-induced obesity." Molecular Metabolism **42**.

Objectives: Diet-driven obesity is increasingly widespread. Its consequences pose major challenges to human health and health care systems. There are MAP kinase-interacting kinases (MNKs) in mice, MNK1 and MNK2. Studies have demonstrated that mice lacking either MNK1 or MNK2 were partially protected against high-fat diet (HFD)-induced weight gain and insulin resistance. The aims of this study were to evaluate the phenotype of mice lacking both MNKs when given an HFD, to assess whether pharmacological inhibition of MNK function also protects against diet-induced obesity (DIO) and its consequences and to probe the mechanisms underlying such protection. Methods: Male wild-type (WT) C57Bl6 mice or mice lacking both MNK1 and MNK2 (double knockout, DKO) were fed an HFD or control diet (CD) for up to 16 weeks. In a separate study, WT mice were also given an HFD for 6 weeks, after which half were treated with the recently-developed MNK inhibitor ETC-206 daily for 10 more weeks while continuing an HFD. Metabolites and other parameters were measured, and the expression of selected mRNAs and proteins was assessed. Results: MNK-DKO mice were almost completely protected from HFD-induced obesity. Higher energy expenditure (EE) in MNK-DKO mice was observed, which probably reflects

the changes in a number of genes or proteins linked to lipolysis, mitochondrial function/biogenesis, oxidative metabolism, and/or ATP consumption. The MNK inhibitor ETC-206 also prevented HFD-induced weight gain, confirming that the activity of the MNKs facilitates weight gain due to excessive caloric consumption. Conclusions: Disabling MNKs in mice, either genetically or pharmacologically, strongly prevents weight gain on a calorie-rich diet. This finding likely results from increased energy utilisation, involving greater ATP consumption, mitochondrial oxidative metabolism, and other processes.

Sharma, G., et al. (2020). "Preclinical efficacy of the GPER-selective agonist G-1 in mouse models of obesity and diabetes." Science Translational Medicine **12**(528).

Human obesity has become a global health epidemic, with few safe and effective pharmacological therapies currently available. The systemic loss of ovarian estradiol (E2) in women after menopause greatly increases the risk of obesity and metabolic dysfunction, revealing the critical role of E2 in this setting. The salutary effects of E2 are traditionally attributed to the classical estrogen receptors ERα and ERβ, with the contribution of the G protein-coupled estrogen receptor (GPER) still largely unknown. Here, we used ovariectomy- and diet-induced obesity (DIO) mouse models to evaluate the preclinical activity of GPER-selective small-molecule agonist G-1 (also called Tespria) against obesity and metabolic dysfunction. G-1 treatment of ovariectomized female mice (a model of postmenopausal obesity) reduced body weight and improved glucose homeostasis without changes in food intake, fuel source usage, or locomotor activity. G-1-treated female mice also exhibited increased energy expenditure, lower body fat content, and reduced fasting cholesterol, glucose, insulin, and inflammatory markers but did not display feminizing effects on the uterus (imbibition) or beneficial effects on bone health. G-1 treatment of DIO male mice did not elicit weight loss but prevented further weight gain and improved glucose tolerance, indicating that G-1 improved glucose homeostasis independently of its antiobesity effects. However, in ovariectomized DIO female mice, G-1 continued to elicit weight loss, reflecting possible sex differences in the mechanisms of G-1 action. In conclusion, this work demonstrates that GPER-selective agonism is a viable therapeutic approach against obesity, diabetes, and associated metabolic abnormalities in multiple preclinical male and female models.

Shay, D. A., et al. (2020). "Changes in nucleus accumbens gene expression accompany sex-specific suppression of spontaneous physical activity in aromatase knockout mice." Hormones and Behavior **121**.

Aromatase catalyzes conversion of testosterone to estradiol and is expressed in a variety of tissues, including the brain. Suppression of aromatase adversely affects metabolism and physical activity behavior, but mechanisms remain uncertain. The hypothesis tested herein was that whole body aromatase deletion would cause gene expression changes in the nucleus accumbens (NAc), a brain regulating motivated behaviors such as physical activity, which is suppressed with loss of estradiol. Metabolic and behavioral assessments were performed in male and female wild-type (WT) and aromatase knockout (ArKO) mice. NAc-specific differentially expressed genes (DEGs) were identified with RNAseq, and associations between the measured phenotypic traits were determined. Female ArKO mice had greater percent body fat, reduced spontaneous physical activity (SPA), consumed less energy, and had lower relative resting energy expenditure (REE) than WT females. Such differences were not observed in ArKO males. However, in both sexes, a top DEG was Pts, a gene encoding an enzyme necessary for catecholamine (e.g., dopamine)

biosynthesis. In comparing male and female WT mice, top DEGs were related to sexual development/fertility, immune regulation, obesity, dopamine signaling, and circadian regulation. SPA correlated strongly with Per3, a gene regulating circadian function, thermoregulation, and metabolism (r = −0.64, P = .002), which also correlated with adiposity (r = 0.54, P = .01). In conclusion, aromatase ablation leads to gene expression changes in NAc, which may in turn result in reduced SPA and related metabolic abnormalities. These findings may have significance to post-menopausal women and those treated with an aromatase inhibitor.

Shay, D. A., et al. (2020). Changes in nucleus accumbens gene expression accompany sex-specific suppression of spontaneous physical activity in aromatase knockout mice, mospace.umsystem.edu.

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Shi, H., et al. (2020). "Dihydrotestosterone (Dht) enhances wound healing of major burn injury by accelerating resolution of inflammation in mice." International Journal of Molecular Sciences **21**(17): 1-15.

Androgens have been known to inhibit cutaneous wound healing in men and male mice. However, in children with major burn injuries, a synthetic androgen was reported clinically to improve wound healing. The aim of this study is to investigate the role of dihydrotestosterone (DHT) as a new therapeutic approach in treating major burn injury. In the present study, mice received systemic androgen treatment post major burn injury. Wound healing rate and body weight were monitored over 21 days. The serum level of inflammatory cytokines/chemokines were measured using multiplex immunoassays. In addition, splenocyte enumeration was performed by flow cytometry. Healing phases of inflammation, re-epithelialization, cell proliferation and collagen deposition were also examined. In results, DHT treated mice lost less weight and displayed accelerated wound healing but has no impact on hypermetabolism.

Mice, after burn injury, displayed acute systemic inflammatory responses over 21 days. DHT treatment shortened the systemic inflammatory response with reduced splenic weight and monocyte numbers on day 14 and 21. DHT treatment also reduced wound infiltrating macrophage numbers. In conclusion, DHT treatment facilitates local wound healing by accelerating the resolution of inflammation, but not through alterations of post-burn hypermetabolic response.

Skurski, J., et al. (2020). "Loss of iRhom2 accelerates fat gain and insulin resistance in diet-induced obesity despite reduced adipose tissue inflammation." Metabolism: Clinical and Experimental **106**.

Background: Low-grade inflammation and metabolic dysregulation are common comorbidities of obesity, both of which are associated with alterations in iRhom2-regulated pro-inflammatory cytokine and epidermal growth factor receptor (EGFR) ligand signaling. Objective: Our objective was to determine the role of iRhom2 in the regulation of low-grade inflammation and metabolic dysregulation in a murine model of diet-induced obesity. Methods: Wild type (WT) and iRhom2-deficient mice were fed normal chow (NC) or a high-fat diet (HFD) starting at 5 weeks of age for up to 33 weeks. Body composition, glucose and insulin tolerance, feeding behavior, and indirect calorimetry were measured at defined time points. Adipose tissue cytokine expression and inflammatory lesions known as crown-like structures (CLS) were analyzed at the end-point of the study. Results: iRhom2-deficient mice show accelerated fat gain on a HFD, accompanied by insulin resistance. Indirect calorimetry did not demonstrate changes in energy expenditure or food intake, but locomotor activity was significantly reduced in HFD iRhom2-deficient mice. Interestingly, CLS, macrophage infiltration, and tumor necrosis factor (TNF) production were decreased in adipose tissue from HFD iRhom2-deficient mice, but circulating cytokines were unchanged. In inguinal and perigonadal fat, the EGFR ligand amphiregulin was markedly induced in HFD controls but completely prevented in iRhom2-deficient mice, suggesting a potentially dominant role of EGFR-dependent mechanisms over TNF in the modulation of insulin sensitivity. Conclusions: This study elucidates a novel role for iRhom2 as an immuno-metabolic regulator that affects adipose tissue inflammation independent of insulin resistance.

Spitler, K., et al. (2020). "ANGPTL4 from adipose, but not liver, is responsible for regulating plasma triglyceride partitioning." bioRxiv.

Elevated plasma triglyceride levels are associated with metabolic disease. Angiopoietin-like protein 4 (ANGPTL4) regulates plasma triglyceride levels by inhibiting lipoprotein lipase (LPL). ANGPTL4- deficient mice have decreased plasma triglyceride levels and increased adipose triglyceride uptake. ANGPTL4 is largely expressed by adipose and liver; therefore, we generated adipose- and liver- specific ANGPTL4 knockout mice. Mice lacking adipose ANGPTL4 recapitulated the triglyceride phenotypes of whole-body ANGPTL4 deficiency, whereas mice lacking liver ANGPTL4 had no triglyceride phenotypes. When fed a high fat diet (HFD) mice deficient in adipose ANGPTL4 gained more weight, had enhanced adipose LPL activity, and initially had improved glucose and insulin sensitivity. However, this improvement was largely lost after 6 months on HFD. Conversely, liver- deficient ANGPTL4 mice initially displayed no differences in glucose homeostasis, but began to manifest improved glucose tolerance after 6 months on HFD. We conclude that adipose derived ANGPTL4 is responsible for triglyceride regulation, while both adipose- and liver-derived ANGPTL4 may play a role in glucose homeostasis.

Stone, A. (2020). The Effects of Skeletal Muscle Specific Cpt1b Knock Out on Genetically Obese Ay Mice, digitalcommons.lsu.edu.

> … 9 2.10. Sable Systems Promethion ..... 10 2.11. Statistical Analysis … 2.10. Sable Systems Promethion Mice were put in the Sable Systems International promethion for 1 week. Food and …

Sweet, D. R., et al. (2020). "Myeloid Krüppel-like factor 2 is a critical regulator of metabolic inflammation." Nature Communications **11**(1).

> Substantial evidence implicates crosstalk between metabolic tissues and the immune system in the inception and progression of obesity. However, molecular regulators that orchestrate metaflammation both centrally and peripherally remains incompletely understood. Here, we identify myeloid Krüppel-like factor 2 (KLF2) as an essential regulator of obesity and its sequelae. In mice and humans, consumption of a fatty diet downregulates myeloid KLF2 levels. Under basal conditions, myeloid-specific KLF2 knockout mice (K2KO) exhibit increased feeding and weight gain. High-fat diet (HFD) feeding further exacerbates the K2KO metabolic disease phenotype. Mechanistically, loss of myeloid KLF2 increases metaflammation in peripheral and central tissues. A combination of pair-feeding, bone marrow-transplant, and microglial ablation implicate central and peripheral contributions to K2KO-induced metabolic dysfunction observed. Finally, overexpression of myeloid KLF2 protects mice from HFD-induced obesity and insulin resistance. Together, these data establish myeloid KLF2 as a nodal regulator of central and peripheral metabolic inflammation in homeostasis and disease.

Wang, D., et al. (2020). "LSD1 mediates microbial metabolite butyrate-induced thermogenesis in brown and white adipose tissue." Metabolism: Clinical and Experimental **102**.

> Objective: The gut microbiota regulates thermogenesis to benefit metabolic homeostasis at least partially via its metabolite butyrate, and the underlying mechanisms of this regulation are still unclear. In this study, we aim to investigate the role of lysine specific demethylase (LSD1), a histone demethylase and important regulator of thermogenesis, in mediating gut microbial metabolite butyrate regulation of thermogenesis. Methods: The antibiotic cocktail (ABX) was administrated to deplete gut microbiota. Adipose-specific LSD1 knockout mice (LSD1 aKO) were generated by crossing LSD1-lox/lox with adiponectin-cre mice and sodium butyrate and dietary fiber inulin was administrated through oral-gavage. Primary stromal vascular cells were isolated from adipose tissues and differentiated to adipocytes for studying butyrate effects on adipocyte thermogenesis. Results: The antibiotic cocktail (ABX)-mediated depletion of the gut microbiota in mice downregulated the expression of LSD1 in both brown adipose tissue (BAT) and subcutaneous white adipose tissue (scWAT) in addition to uncoupling protein 1 (UCP1) and body temperature. Gavage of the microbial metabolite butyrate in ABX-treated mice reversed the thermogenic functional impairment and LSD1 expression. The adipose-specific ablation of LSD1 in mice attenuated the butyrate-mediated induction of thermogenesis and energy expenditure. Notably, our

results showed that butyrate directly increased the expression of LSD1 and UCP1 as well as butyrate transporter monocarboxylate transporter 1 (MCT1) and catabolic enzyme acyl-CoA medium-chain synthetase 3 (ACSM3) in ex vivo cultured adipocytes. The inhibition of MCT1 blocked the effects of butyrate in adipocytes. Furthermore, the butyrate-mediated prevention of diet-induced obesity (DIO) through increased thermogenesis was attenuated in LSD1 aKO mice. Moreover, after gavaging HFD-fed mice with the dietary fiber inulin, a substrate of microbial fermentation that rapidly produces butyrate, thermogenesis in both BAT and scWAT was increased, and DIO was decreased; however, these beneficial metabolic effects were blocked in LSD1 aKO mice. Conclusions: Together, our results indicate that the microbial metabolite butyrate regulates thermogenesis in BAT and scWAT through the activation of LSD1.

Wang, Y., et al. (2020). "METTL3 is essential for postnatal development of brown adipose tissue and energy expenditure in mice." Nature Communications **11**(1).

Brown adipose tissue (BAT) undergoes rapid postnatal development and then protects against cold and obesity into adulthood. However, the molecular mechanism that determines postnatal development and maturation of BAT is largely unknown. Here we show that METTL3 (a key RNA methyltransferase) expression increases significantly in interscapular brown adipose tissue (iBAT) after birth and plays an essential role in the postnatal development and maturation of iBAT. BAT-specific deletion of Mettl3 severely impairs maturation of BAT in vivo by decreasing m6A modification and expression of Prdm16, Pparg, and Ucp1 transcripts, which leads to a marked reduction in BAT-mediated adaptive thermogenesis and promotes high-fat diet (HFD)-induced obesity and systemic insulin resistance. These data demonstrate that METTL3 is an essential regulator that controls iBAT postnatal development and energy homeostasis.

Wang, Y., et al. (2020). "IF1 connects obesity and insulin resistance through mitochondrial reprogramming in association with ANT2." bioRxiv.

IF1 (ATPIF1) is a nuclear DNA-encoded protein with an activity in the inhibition of catalytic activity of F1Fo-ATP synthase (ATPase), an enzyme for ATP synthesis in mitochondria. A role of IF1 remains unknown in the metabolic disorder in obesity. In this study, IF1 was examined in the diet-induced obese (DIO) mice and a decrease in IF1 protein was observed in several tissues including the skeletal muscle, liver and intestine in the absence of mRNA alteration. Significance of the reduction was investigated in the IF1-KO mice, in which insulin sensitivity was improved in the absence of body weight alteration on Chow diet. On a high fat diet (HFD), the IF1-KO mice gain more body weight as a result of enhanced fat tissue growth. The energy expenditure and locomotion activity were decreased in the KO mice without an alteration in food intake. The increase in insulin sensitivity remained in the obese KO mice. The colon tissue exhibited a resistance to the HFD-induced atrophy with less cell apoptosis and more secretion of GLP-1. Mitochondria exhibited an enhanced ATP production and maximal oxygen consumption without an alteration in the respiratory chain proteins. However, the ATP level was reduced in the fasting condition in the muscle as well as the liver. Mitophagy was enhanced with elevated accumulation of PINK1 and Parkin proteins in the mitochondria. The protein abundance of ADP/ATP translocase 2 (ANT2) was decreased in the inner membrane of mitochondria to account for the reduced apoptosis and enhanced mitophagy. The data suggest that the IF1 reduction in obesity leads to reprogramming of mitochondrial

metabolism in a compensatory response to maintain the insulin sensitivity through down-regulation of ANT2 protein.

Williams, A. S., et al. (2020). "Disruption of Acetyl-Lysine Turnover in Muscle Mitochondria Promotes Insulin Resistance and Redox Stress without Overt Respiratory Dysfunction." Cell Metabolism **31**(1): 131-147.e111.

This study sought to examine the functional significance of mitochondrial protein acetylation using a double knockout (DKO) mouse model harboring muscle-specific deficits in acetyl-CoA buffering and lysine deacetylation, due to genetic ablation of carnitine acetyltransferase and Sirtuin 3, respectively. DKO mice are highly susceptible to extreme hyperacetylation of the mitochondrial proteome and develop a more severe form of diet-induced insulin resistance than either single KO mouse line. However, the functional phenotype of hyperacetylated DKO mitochondria is largely normal. Of the >120 measures of respiratory function assayed, the most consistently observed traits of a markedly heightened acetyl-lysine landscape are enhanced oxygen flux in the context of fatty acid fuel and elevated rates of electron leak. In sum, the findings challenge the notion that lysine acetylation causes broad-ranging damage to mitochondrial quality and performance and raise the possibility that acetyl-lysine turnover, rather than acetyl-lysine stoichiometry, modulates redox balance and carbon flux. Williams et al. show that double knockout (DKO) mice harboring muscle-specific deficits in acetyl-CoA buffering and lysine deacetylation are susceptible to extreme mitochondrial hyperacetylation and insulin resistance. However, DKO mitochondria have normal respiratory function and increased fat oxidation. The findings suggest that acetyl-lysine turnover, not stoichiometry, regulates mitochondrial fuel use.

Woodie, L. N., et al. (2020). "Western diet-induced obesity disrupts the diurnal rhythmicity of hippocampal core clock gene expression in a mouse model." Brain, Behavior, and Immunity **88**: 815-825.

Western diet (WD) feeding disrupts core clock gene expression in peripheral tissues and contributes to WD-induced metabolic disease. The hippocampus, the mammalian center for memory, is also sensitive to WD feeding, but whether the WD disrupts its core clock is unknown. To this end, male mice were maintained on a WD for 16 weeks and diurnal metabolism, gene expression and memory were assessed. WD-induced obesity disrupted the diurnal rhythms of whole-body metabolism, markers of inflammation and hepatic gene expression, but did not disrupt diurnal expression of hypothalamic Bmal1, Npas2 and Per2. However, all measured core clock genes were disrupted in the hippocampus after WD feeding and the expression pattern of genes implicated in Alzheimer's disease and synaptic function were altered. Finally, WD feeding disrupted hippocampal memory in a task- and time-dependent fashion. Our results implicate WD-induced alterations in the rhythmicity of hippocampal gene expression in the etiology of diet-induced memory deficits.

Woodie, L. N., et al. (2020). "The physio-metabolic effects of time-restricting liquid sugar intake to six-hour windows during the mouse active phase: The effects of active phase liquid sugar consumption." Physiology and Behavior **223**.

Obesity is a major public health concern and overconsumption of unhealthy fats and sugary beverages are contributing factors. Time-restricted feeding can reduce obesity-associated pathophysiological parameters by limiting the time of food consumption; however, the effects of time-restricted sugary water consumption are unknown. To examine whether liquid calorie restriction impacts metabolic health, we measured metabolic parameters in mice provided liquid sugar at various intervals during the active phase. The control (Con) group received tap water, the adlibitum fructose-glucose (ALFG) group received ad libitumsugar water and the early fructose-glucose (EFG) and late fructose-glucose (LFG) groups received liquid sugar during the first and last six hours of the active period, respectively. Each group was given free access to chow. Zeitgeber time (ZT) notation was used to set all experimental time points to lights on as ZT 0. The ALFG group exhibited elevated body and adipose tissue weights compared to the other groups and increased hepatic steatosis compared to the Con group. The ALFG group consumed more calories than the other groups during ZT 6-11, indicating that this window may be critical in the promotion of weight gain from liquid sugar consumption. The EFG group exhibited higher levels of energy expenditure than the Con and LFG groups during the first half of the active period (ZT 12-17); however, there was no difference among the groups during the second half of the active period (ZT18-23). In contrast, the EFG group exhibited lower respiratory exchange ratio than other groups during the inactive period as well as the second half of the active period, indicating that the EFG group had greater metabolic flexibility and utilized lipids when carbohydrates from liquid sugar were not available. Additionally, the EFG group was more insulin tolerant than the ALFG and Con groups. Our results support the hypothesis that time-restricted liquid calorie restriction aids in reducing the detrimental metabolic effects of sugary drink consumption.

Yuan, Y., et al. (2020). "Exercise-induced α-ketoglutaric acid stimulates muscle hypertrophy and fat loss through OXGR1-dependent adrenal activation." The EMBO Journal **39**(7).

 Beneficial effects of resistance exercise on metabolic health and particularly muscle hypertrophy and fat loss are well established, but the underlying chemical and physiological mechanisms are not fully understood. Here, we identified a myometabolite-mediated metabolic pathway that is essential for the beneficial metabolic effects of resistance exercise in mice. We showed that substantial accumulation of the tricarboxylic acid cycle intermediate α-ketoglutaric acid (AKG) is a metabolic signature of resistance exercise performance. Interestingly, human plasma AKG level is also negatively correlated with BMI. Pharmacological elevation of circulating AKG induces muscle hypertrophy, brown adipose tissue (BAT) thermogenesis, and white adipose tissue (WAT) lipolysis in vivo. We further found that AKG stimulates the adrenal release of adrenaline through 2-oxoglutarate receptor 1 (OXGR1) expressed in adrenal glands. Finally, by using both loss-of-function and gain-of-function mouse models, we showed that OXGR1 is essential for AKG-mediated exercise-induced beneficial metabolic effects. These findings reveal an unappreciated mechanism for the salutary effects of resistance exercise, using AKG as a systemically derived molecule for adrenal stimulation of muscle hypertrophy and fat loss.

Zhang, F., et al. (2020). "Dietary Supplementation of Lauric Acid Alleviates the Irregular Estrous Cycle and the Impaired Metabolism and Thermogenesis in Female Mice Fed with High-Fat Diet (HFD)." Journal of Agricultural and Food Chemistry.

Lauric acid (LA) has been implicated in the prevention/treatment of obesity. However, the role of LA in modulating an obesity-related female reproductive disorder remains largely unknown. Here, female mice were fed a control diet, high-fat diet (HFD), or HFD supplemented with 1% LA. The results demonstrated that the HFD-induced estrous cycle irregularity and the reduction of serum follicle-stimulating hormone (FSH) were alleviated by LA supplementation. In possible mechanisms, LA supplementation led to significant increase in serum lipid metabolites such as sphingomyelin and lysophosphatidylcholine containing LA (C12:0) and the improvement of glucose metabolism in mice fed HFD. Moreover, impaired body energy metabolism and weakened brown adipose tissue (BAT) thermogenesis of HFD-fed mice were improved by LA supplementation. Together, these findings showed that LA supplementation alleviated HFD-induced estrous cycle irregularity, possibly associated with altered serum lipid metabolites, improved glucose metabolism, body energy metabolism, and BAT thermogenesis. These findings suggested the potential application of LA in alleviating obesity and its related reproductive disorders.

Zhang, K. X., et al. (2020). "Violet-light suppression of thermogenesis by opsin 5 hypothalamic neurons." Nature **585**(7825): 420-425.

The opsin family of G-protein-coupled receptors are used as light detectors in animals. Opsin 5 (also known as neuropsin or OPN5) is a highly conserved opsin that is sensitive to visible violet light1,2. In mice, OPN5 is a known photoreceptor in the retina3 and skin4 but is also expressed in the hypothalamic preoptic area (POA)5. Here we describe a light-sensing pathway in which POA neurons that express Opn5 regulate thermogenesis in brown adipose tissue (BAT). We show that Opn5 is expressed in glutamatergic warm-sensing POA neurons that receive synaptic input from several thermoregulatory nuclei. We further show that Opn5 POA neurons project to BAT and decrease its activity under chemogenetic stimulation. Opn5-null mice show overactive BAT, increased body temperature, and exaggerated thermogenesis when cold-challenged. Moreover, violet photostimulation during cold exposure acutely suppresses BAT temperature in wild-type mice but not in Opn5-null mice. Direct measurements of intracellular cAMP ex vivo show that Opn5 POA neurons increase cAMP when stimulated with violet light. This analysis thus identifies a violet light-sensitive deep brain photoreceptor that normally suppresses BAT thermogenesis.

Zhang, L., et al. (2020). "Leptin signalling on arcuate NPY neurones controls adiposity independent of energy balance or diet composition." Journal of Neuroendocrinology **32**(9).

Central action of the adipocyte hormone leptin via the neuropeptide Y (NPY) system is considered critical for energy homeostatic control. However, the precise mechanisms for this control are still not clear. To specifically investigate how leptin signalling on the NPY neurone contributes to the control of energy homeostasis, we generated an inducible adult-onset NPY neurone-specific leptin receptor (Lepr) knockout model and performed a comprehensive metabolic phenotyping study. Here, we show that the NPY neurone subpopulation that is directly responsive to leptin is not required for the inhibition of fasting-

induced hyperphagia by leptin, although it is essential for the regulation of adiposity independent of changes in energy balance or diet composition. Furthermore, under obesogenic conditions such as a high-fat diet, a lack of Lepr signalling on NPY neurones results in significant increases in food intake and concomitant reductions in energy expenditure, leading to accelerated accumulation of fat mass. Collectively, these findings support the notion that Lepr-expressing NPY neurones act as the key relay point where peripheral adipose storage information is sensed, and corresponding responses are initiated to protect adipose reserves.

Zhang, X., et al. (2020). "Sustained activation of autophagy suppresses adipocyte maturation via a lipolysis-dependent mechanism." Autophagy **16**(9): 1668-1682.

Dysregulation of macroautophagy/autophagy is implicated in obesity and insulin resistance. However, it remains poorly defined how autophagy regulates adipocyte development. Using adipose-specific rptor/raptor knockout (KO), atg7 KO and atg7 rptor double-KO mice, we show that inhibiting MTORC1 by RPTOR deficiency led to autophagic sequestration of lipid droplets, formation of LD-containing lysosomes, and elevation of basal and isoproterenol-induced lipolysis in vivo and in primary adipocytes. Despite normal differentiation at an early phase, progressive degradation and shrinkage of cellular LDs and downregulation of adipogenic markers PPARG and PLIN1 occurred in terminal differentiation of rptor KO adipocytes, which was rescued by inhibiting lipolysis or lysosome. In contrast, inactivating autophagy by depletion of ATG7 protected adipocytes against RPTOR deficiency-induced formation of LD-containing lysosomes, LD degradation, and downregulation of adipogenic markers in vitro. Ultimately, atg7 rptor double-KO mice displayed decreased lipolysis, restored adipose tissue development, and upregulated thermogenic gene expression in brown and inguinal adipose tissue compared to RPTOR-deficient mice in vivo. Collectively, our study demonstrates that autophagy plays an important role in regulating adipocyte maturation via a lipophagy and lipolysis-dependent mechanism. Abbreviations: ATG7: autophagy related 7; BAT: brown adipose tissue; CEBPB/C/EBPβ: CCAAT enhancer binding protein beta; DGAT1: diacylglycerol O-acyltransferase 1; eWAT: epididymal white adipose tissue; iWAT: inguinal white adipose tissue; KO: knockout; LD: lipid droplet; MAP1LC3/LC3: microtubule-associated protein 1 light chain 3; MTOR: mechanistic target of rapamycin kinase; MTORC1: mechanistic target of rapamycin kinase complex 1; PLIN1: perepilin 1; PNPLA2/ATGL: patatin-like phospholipase domain containing 2; PPARG/PPARγ: peroxisome proliferator activated receptor gamma; RPTOR: regulatory associated protein of MTOR complex1; TG: triglyceride; ULK1: unc-51 like kinase 1; UCP1: uncoupling protein 1; WAT: white adipose tissue.

Zidon, T. M., et al. (2020). "Effects of ERβ and ERα on OVX-induced changes in adiposity and insulin resistance." Journal of Endocrinology **245**(1): 165-178.

Loss of ovarian hormones leads to increased adiposity and insulin resistance (IR), increasing the risk for cardiovascular and metabolic diseases. The purpose of this study was to investigate whether the molecular mechanism behind the adverse systemic and adipose tissue-specific metabolic effects of ovariectomy requires loss of signaling through estrogen receptor alpha (ERα) or estrogen receptor β (ERβ). We examined ovariectomized (OVX) and ovary-intactwild-type (WT), ERα-null (αKO), and ERβ-null (βKO) female mice (age ~49 weeks; n = 7-12/group). All mice were fed a phytoestrogen-free diet (<15 mg/kg)

and either remained ovary-intact (INT) or were OVX and followed for 12 weeks. Body composition, energy expenditure, glucose tolerance, and adipose tissue gene and protein expression were analyzed. INT αKO were ~25% fatter with reduced energy expenditure compared to age-matched INT WT controls and βKO mice (all $P < 0.001$). Following OVX, αKO mice did not increase adiposity or experience a further increase in IR, unlike WT and βKO, suggesting that loss of signaling through ERα mediates OVX-induced metabolic dysfunction. In fact, OVX in αKO mice (i.e., signaling through ERβ in the absence of ERα) resulted in reduced adiposity, adipocyte size, and IR ($P < 0.05$ for all). βKO mice responded adversely to OVX in terms of increased adiposity and development of IR. Together, these findings challenge the paradigm that ERα mediates metabolic protection over ERβ in all settings. These findings lead us to suggest that, following ovarian hormone loss, ERβ may mediate protective metabolic benefits.

Adamovich, Y., et al. (2019). "Oxygen and Carbon Dioxide Rhythms Are Circadian Clock Controlled and Differentially Directed by Behavioral Signals." Cell Metabolism **29**(5): 1092-1103.e1093.

Daily rhythms in animal physiology are driven by endogenous circadian clocks in part through rest-activity and feeding-fasting cycles. Here, we examined principles that govern daily respiration. We monitored oxygen consumption and carbon dioxide release, as well as tissue oxygenation in freely moving animals to specifically dissect the role of circadian clocks and feeding time on daily respiration. We found that daily rhythms in oxygen and carbon dioxide are clock controlled and that time-restricted feeding restores their rhythmicity in clock-deficient mice. Remarkably, day-time feeding dissociated oxygen rhythms from carbon dioxide oscillations, whereby oxygen followed activity, and carbon dioxide was shifted and aligned with food intake. In addition, changes in carbon dioxide levels altered clock gene expression and phase shifted the clock. Collectively, our findings indicate that oxygen and carbon dioxide rhythms are clock controlled and feeding regulated and support a potential role for carbon dioxide in phase resetting peripheral clocks upon feeding. Adamovich et al. show that the daily regulation of oxygen consumption and carbon dioxide release is regulated by the circadian clock. Time-restricted feeding restores oxygen and carbon dioxide rhythms in clock mutants. Their findings also support a potential role for carbon dioxide in phase resetting of peripheral clocks upon feeding

Balise, V. D., et al. (2019). "Preconceptional, gestational, and lactational exposure to an unconventional oil and gas chemical mixture alters energy expenditure in adult female mice." Frontiers in Endocrinology **10**(MAY).

Previous studies conducted in our laboratory have found altered adult health outcomes in animals with prenatal exposure to environmentally relevant levels of unconventional oil and gas (UOG) chemicals with endocrine-disrupting activity. This study aimed to examine potential metabolic health outcomes following a preconception, prenatal and postnatal exposure to a mixture of 23 UOG chemicals. Prior to mating and from gestation day 1 to postnatal day 21, C57BL/6J mice were developmentally exposed to a laboratory-created mixture of 23 UOG chemicals in maternal drinking water. Body composition, spontaneous activity, energy expenditure, and glucose tolerance were evaluated in 7-month-old female offspring. Neither body weight nor body composition differed in 7-month female mice. However, females exposed to 1.5 and 150 µg/kg/day UOG mix had lower total and resting energy expenditure within the dark cycle. In the light cycle, the 1,500 µg//kg/day group had lower total energy expenditure and the 1.5 µg/kg/day group had

lower resting energy expenditure. Females exposed to the 150 µg/kg/day group had lower spontaneous activity in the dark cycle, and females exposed to the 1,500 µg/kg/day group had lower activity in the light cycle. This study reports for the first time that developmental exposure to a mixture of 23 UOG chemicals alters energy expenditure and spontaneous activity in adult female mice.

Balise, V. D., et al. (2019). "Developmental Exposure to a Mixture of Unconventional Oil and Gas Chemicals Increased Risk-Taking Behavior, Activity and Energy Expenditure in Aged Female Mice After a Metabolic Challenge." Frontiers in Endocrinology **10**.

Chemicals used in unconventional oil and gas (UOG) operations can act as endocrine disrupting chemicals and metabolic disruptors. Our lab has reported altered energy expenditure and activity in C57BL/6J mice that were preconceptionally, gestationally, and lactationally exposed via maternal drinking water to a laboratory-created mixture of 23 UOG chemicals from gestational day 1 to postnatal day 21 in 7-month-old female mice with no change in body composition. We hypothesized that allowing the mice to age and exposing them to a high fat, high sugar diet might reveal underlying changes in energy balance. To investigate whether aging and metabolic challenge would exacerbate this phenotype, these mice were aged to 12 months and given a high fat, high sugar diet (HFHSD) challenge. The short 3-day HFHSD challenge increased body weight and fasting blood glucose in all mice. Developmental exposure to the 23 UOG mixture was associated with increased activity and non-resting energy expenditure in the light cycle, increased exploratory behavior in the elevated plus maze test, and decreased sleep in 12 month female mice. Each of these effects was seen in the light cycle when mice are normally less active. Further studies are needed to better understand the behavioral changes observed after developmental exposure to UOG chemicals.

Balland, E., et al. (2019). "Leptin Signaling in the Arcuate Nucleus Reduces Insulin's Capacity to Suppress Hepatic Glucose Production in Obese Mice." Cell Reports **26**(2): 346-355.e343.

Balland et al. identify a molecular link between obesity and type 2 diabetes by demonstrating that, in obesity, leptin signaling in the CNS impairs the regulation of hepatic glucose production, leading to hyperglycemia.

Bax, E. N., et al. (2019). "Opposing effects of S-equol supplementation on metabolic and behavioral parameters in mice fed a high-fat diet." Nutrition Research **64**: 39-48.

Phytoestrogens, such as daidzein and genistein, may be used to treat various hormone-dependent disorders. Daidzein can be metabolized by intestinal microbes to S-equol. However, not all individuals possess bacteria producing this metabolite, resulting in categorization of equol vs nonequol producers. Past human and rodent studies have suggested that supplementation of this compound might yield beneficial metabolic and behavioral effects. We hypothesized that administration of S-equol to diet-induced obese male and female mice would mitigate potential diet-induced metabolic and comorbid neurobehavioral disorders. To test this possibility, we placed 5-week-old C57 mice on a high-fat diet (HFD)

to mimic the diet currently consumed by many Western adults. Animals were randomly assigned to S-equol supplementation (10 mg/kg body weight) or vehicle control group. After 4 weeks on HFD with or without S-equol supplementation, metabolic and behavioral phenotyping was performed. Although the initial hypothesis proposed that S-equol treatment would improve metabolic and neurobehavioral outcomes, this supplementation instead exacerbated aspects of HFD-induced metabolic disease, as indicated by suppressed physical activity in treated individuals, reduced energy expenditure in treated males, and serum chemistry changes (hyperglycemia in treated individuals; hyperinsulinemia and hypoleptinemia in treated males). Conversely, S-equol individuals exhibited less anxiety-like and depressive-like behaviors, as evidenced by increased exploratory time in the elevated plus maze by treated males and increased time spent mobile in the tail suspension test for treated individuals. In summary, S-equol may be beneficial in mitigating depression and anxiety disorders in individuals, but for indeterminate reasons, supplementation may worsen facets of metabolic disorders in obese individuals.

Beppu, L. Y., et al. (2019). "Blimp-1 in adipose resident Tregs controls adipocyte beiging and obesity." bioRxiv.

Crosstalk between the immune system and adipocytes is critical for maintaining tissue homeostasis and regulating chronic systemic inflammation during diet-induced obesity (DIO). How visceral adipose tissue resident regulatory T cells (aTregs) signal to adipocytes in the visceral adipose tissue (VAT) is not understood. Here we show that Treg-specific ablation of the transcriptional regulator Blimp-1 resulted in increased insulin sensitivity, decreased body weight and increased Ucp-1 in adipocytes in high fat diet (HFD)-fed mice. Mechanistically, we demonstrate that Blimp-1 drives IL-10 production in Tregs, thus suppressing beiging and energy expenditure in adipocytes. Moreover, IL-10 mRNA expression positively correlated with increasing body weight in humans. These findings reveal a surprising relationship between aTregs and adipocytes in promoting insulin resistance during excessive caloric intake, placing Blimp-1-regulated IL-10 expression by aTregs at a critical juncture in the development of obesity and its associated comorbidities in mice and humans.

Boucsein, A., et al. (2019). "Hypothalamic leptin sensitivity and health benefits of time-restricted feeding are dependent on the time of day in male mice." FASEB Journal **33**(11): 12175-12187.

Synchronization between biologic clocks and metabolism is crucial for most species. Here, we examined the ability of leptin, important in the control of energy metabolism, to induce leptin signaling at the molecular as well as the behavioral level throughout the 24-h day in mice fed either a control or a high-fat diet (HFD). Furthermore, we investigated the effects of time-restricted feeding (TRF; a limitation of HFD access to 6 h each day) on energy metabolism during different periods throughout the 24-h day. In control mice, molecular leptin sensitivity was highest at zeitgeber time (ZT)0 (lights on), declining during the light phase, and increasing during the dark phase. Surprisingly, leptin resistance in HFD-fed mice was only present from the middle of the dark to the middle of the light period. Specifically, when TRF occurred from ZT21 to ZT3 (when leptin resistance in HFD-fed mice was most profound), it resulted in a disruption of the daily rhythms of locomotor activity and energy expenditure and in increased plasma insulin levels compared with other TRF periods. These data provide evidence that leptin sensitivity is controlled by the circadian rhythm and that TRF periods may be most efficient when aligned with the leptin-sensitive

period.—Boucsein, A., Rizwan, M. Z., Tups, A. Hypothalamic leptin sensitivity and health benefits of time-restricted feeding are dependent on the time of day in male mice. FASEB J. 33, 12175–12187 (2019). www.fasebj.org.

Burke, S. J., et al. (2019). "One week of continuous corticosterone exposure impairs hepatic metabolic flexibility, promotes islet β-cell proliferation, and reduces physical activity in male C57BL/6 J mice." Journal of Steroid Biochemistry and Molecular Biology **195**.

Clinical glucocorticoid use, and diseases that produce elevated circulating glucocorticoids, promote drastic changes in body composition and reduction in whole body insulin sensitivity. Because steroid-induced diabetes is the most common form of drug-induced hyperglycemia, we investigated mechanisms underlying the recognized phenotypes associated with glucocorticoid excess. Male C57BL/6 J mice were exposed to either 100ug/mL corticosterone (cort) or vehicle in their drinking water. Body composition measurements revealed an increase in fat mass with drastically reduced lean mass during the first week (i.e., seven days) of cort exposure. Relative to the vehicle control group, mice receiving cort had a significant reduction in insulin sensitivity (measured by insulin tolerance test) five days after drug intervention. The increase in insulin resistance significantly correlated with an increase in the number of Ki-67 positive β-cells. Moreover, the ability to switch between fuel sources in liver tissue homogenate substrate oxidation assays revealed reduced metabolic flexibility. Furthermore, metabolomics analyses revealed a decrease in liver glycolytic metabolites, suggesting reduced glucose utilization, a finding consistent with onset of systemic insulin resistance. Physical activity was reduced, while respiratory quotient was increased, in mice receiving corticosterone. The majority of metabolic changes were reversed upon cessation of the drug regimen. Collectively, we conclude that changes in body composition and tissue level substrate metabolism are key components influencing the reductions in whole body insulin sensitivity observed during glucocorticoid administration.

Büsing, F., et al. (2019). "Correction: Impact of energy turnover on the regulation of glucose homeostasis in healthy subjects (Nutrition & Diabetes, (2019), 9, 1, (22), 10.1038/s41387-019-0089-6)." Nutrition and Diabetes **9**(1).

Objective
Sedentary lifestyle increases the risk of type 2 diabetes. The aim of this study was to investigate the impact of different levels of energy turnover (ET; low, medium, and high level of physical activity and the corresponding energy intake) on glucose metabolism at zero energy balance, caloric restriction, and overfeeding.

Methods
Sixteen healthy individuals (13 men, 3 women, 25.1 ± 3.9 years, BMI 24.0 ± 3.2 kg/m2) participated in a randomized crossover intervention under metabolic ward conditions. Subjects passed 3 × 3 intervention days. Three levels of physical activity (PAL: low 1.3, medium 1.6, and high 1.8 achieved by walking at 4 km/h for 0, 3 × 55, or 3 × 110 min) were compared under three levels of energy balance (zero energy balance (EB): 100% of energy requirement (Ereq); caloric restriction (CR): 75% Ereq, and overfeeding (OF):

125% Ereq). Continuous interstitial glucose monitoring, C-peptide excretion, and HOMA–IR, as well as postprandial glucose and insulin were measured.

Results
Daylong glycemia and insulin secretion did not increase with higher ET at all conditions of energy balance (EB, CR, and OF), despite a correspondingly higher CHO intake (Δ low vs. high ET: +86 to 135 g of CHO/d). At CR, daylong glycemia ($p = 0.02$) and insulin secretion ($p = 0.04$) were even reduced with high compared with low ET. HOMA–IR was impaired with OF and improved with CR, whereas ET had no effect on fasting insulin sensitivity. A higher ET led to lower postprandial glucose and insulin levels under conditions of CR and OF.

Conclusion
Low-intensity physical activity can significantly improve postprandial glycemic response of healthy individuals, independent of energy balance.

Cakir, I., et al. (2019). "Leptin receptor signaling in sim1-expressing neurons regulates body temperature and adaptive thermogenesis." Endocrinology **160**(4): 863-879.

Leptin signals to regulate food intake and energy expenditure under conditions of normative energy homeostasis. The central expression and function of leptin receptor B (LepRb) have been extensively studied during the past two decades; however, the mechanisms by which LepRb signaling dysregulation contributes to the pathophysiology of obesity remains unclear. The paraventricular nucleus of the hypothalamus (PVN) plays a crucial role in regulating energy balance as well as the neuroendocrine axes. The role of LepRb expression in the PVN in regard to the regulation of physiological function of leptin has been controversial. The single-minded homolog 1 gene (Sim1) is densely expressed in the PVN and in parts of the amygdala, making Sim1-Cre mice a useful model for examining molecular mechanisms regulating PVN function. In this study, we characterized the physiological role of LepRb in Sim1-expressing neurons using LepRb-floxed × Sim1-Cre mice. Sim1-specific LepRb-deficient mice were surprisingly hypophagic on regular chow but gained more weight upon exposure to a high-fat diet than did their control littermates. We show that Sim1-specific deletion of a single LepRb gene copy caused decreased surface and core body temperatures as well as decreased energy expenditure in ambient room temperatures in both female and male mice. Furthermore, cold-induced adaptive (nonshivering) thermogenesis is disrupted in homozygous knockout mice. A defective thermoregulatory response was associated with defective cold-induced upregulation of uncoupling protein 1 in brown adipose tissue and reduced serum T4. Our study provides novel functional evidence supporting LepRb signaling in Sim1 neurons in the regulation of body weight, core body temperature, and cold-induced adaptive thermogenesis.

Cedernaes, J., et al. (2019). "Transcriptional Basis for Rhythmic Control of Hunger and Metabolism within the AgRP Neuron." Cell Metabolism **29**(5): 1078-1091.e1075.

The alignment of fasting and feeding with the sleep/wake cycle is coordinated by hypothalamic neurons, though the underlying molecular programs remain incompletely understood. Here, we demonstrate that the clock transcription pathway maximizes eating during wakefulness and glucose production during sleep through autonomous circadian regulation of NPY/AgRP neurons. Tandem profiling of whole-cell and

ribosome-bound mRNAs in morning and evening under dynamic fasting and fed conditions identified temporal control of activity-dependent gene repertoires in AgRP neurons central to synaptogenesis, bioenergetics, and neurotransmitter and peptidergic signaling. Synaptic and circadian pathways were specific to whole-cell RNA analyses, while bioenergetic pathways were selectively enriched in the ribosome-bound transcriptome. Finally, we demonstrate that the AgRP clock mediates the transcriptional response to leptin. Our results reveal that time-of-day restriction in transcriptional control of energy-sensing neurons underlies the alignment of hunger and food acquisition with the sleep/wake state. The central molecular clock aligns feeding with the sleep/wake state. Cedernaes et al. employ RNA sequencing in AgRP neurons across different nutrient states, revealing time-of-day-dependent enrichment of circadian and bioenergetic gene networks. They discover that the behavioral and transcriptional response to leptin varies from morning to evening, as the AgRP clock coordinates the leptin response and glucose metabolism with arousal.

Clookey, S. L., et al. (2019). "Beta 3 adrenergic receptor activation rescues metabolic dysfunction in female estrogen receptor alpha-null mice." Frontiers in Physiology **10**(FEB).

Metabolic disease risk escalates following menopause. The mechanism is not fully known, but likely involves reduced signaling through estrogen receptor alpha (ERα), which is highly expressed in brown and white adipose tissue (BAT and WAT). Objective: Test the hypothesis that uncoupling protein (UCP1) activation mitigates metabolic dysfunction caused by loss of signaling through ERα. Methods: At 8 weeks of age, female ERα knock out (KO) and wild-type mice were housed at 28∘C and fed a Western-style high-fat, high sucrose diet (HFD) or a normal low-fat chow diet (NC) for 10 weeks. During the final 2 weeks, they received daily injections of CL 316,256 (CL), a selective β3 adrenergic agonist, or vehicle control (CTRL), creating eight groups: WT-CTRL, WT-CL, KO-CTRL, and KO-CL on HFD or NC; n = 4–10/group. Results: ERαKO demonstrated exacerbated HFD-induced adiposity gain ($P < 0.001$) and insulin resistance ($P = 0.006$). CL treatment improved insulin sensitivity ($P < 0.05$) and normalized ERαKO-induced adiposity increase ($P < 0.05$). In both genotypes, CL increased resting energy expenditure ($P < 0.05$) and induced WAT beiging indicated by increased UCP1 protein in both perigonadal (PGAT) and subcutaneous (SQAT) depots. These effects were attenuated under HFD conditions ($P < 0.05$). In KO, CL reduced HFD energy consumption compared to CTRL ($P < 0.05$). Remarkably, CL increased WAT ERβ protein levels of both WT and KO ($P < 0.001$), revealing CL-mediated changes in estrogen signaling may have protective metabolic effects. Conclusion: CL completely restored metabolic dysfunction in ERαKO mice. Thus, UCP1 may be a therapeutic target for treating metabolic dysfunction following loss of estrogen receptor signaling.

Coborn, J. E. (2019). Role of sex and orexin in sleep disruption induced weight gain, Curr Obes Rep.

Poor sleep time and quality associate with obesity in adults and women have a predisposition towards greater weight gain with sleep restriction. However, the mechanisms contributing to sex-specific sensitivity to weight gain following sleep loss remains unknown. Exposure to environmental noise is the only method of sleep disruption (SD) known to date that reduces sleep time and quality, stimulates weight gain and feeding and reduces distance traveled and energy expenditure (EE) (total and individual components) in male rats. The objective of this study was 1) validate the method of noise-induced sleep disruption in female rats, 2) determine mechanisms underlying the response to noise in male and female rats and 3) determine whether treatment with a sleep aid could block the effects of noise exposure

independent of sex. Overall, findings from the study described in this dissertation demonstrate the following: 1) chronic exposure to noise increases weight gain and feeding and reduces total EE due to reductions in EE during spontaneous physical activity (SPA) and sleep without altering the length of the estrous cycle in female rats; 2) stress is not the primary mechanism underlying noise-induced weight gain in either male or female rats; and 3) Suvorexant, a Federal Drug Administration (FDA) approved dual orexin receptor antagonist for insomnia, significantly ameliorates noise-induced increases in time awake independent of sex but further exacerbates noise-induced increases in sleep fragmentation for males only. Collectively, these data have implications for future studies aimed to determine sex-specific sensitivity to weight gain following sleep loss. Furthermore, these data suggest that while Suvorexant may ameliorate reductions in sleep caused by noise equally in the sexes, the drug may have differential effects on the weight gain due to SD since Suvorexant further worsened sleep quality in males but not females. (PsycINFO Database Record (c) 2018 APA, all rights reserved)

Coborn, J. E., et al. (2019). "Noise-induced sleep disruption increases weight gain and decreases energy metabolism in female rats." International Journal of Obesity **43**(9): 1759-1768.

Background/objectives: Inadequate sleep increases obesity and environmental noise contributes to poor sleep. However, women may be more vulnerable to noise and hence more susceptible to sleep disruption-induced weight gain than men. In male rats, exposure to environmental (i.e. ambient) noise disrupts sleep and increases feeding and weight gain. However, the effects of environmental noise on sleep and weight gain in female rats are unknown. Thus, this study was designed to determine whether noise exposure would disturb sleep, increase feeding and weight gain and alter the length of the estrous cycle in female rats. Subjects/methods: Female rats (12 weeks old) were exposed to noise for 17d (8 h/d during the light period) to determine the effects of noise on weight gain and food intake. In a separate set of females, estrous cycle phase and length, EEG, EMG, spontaneous physical activity and energy expenditure were recorded continuously for 27d during baseline (control, 9d), noise exposure (8 h/d, 9d) and recovery (9d) from sleep disruption. Results: Noise exposure significantly increased weight gain and food intake compared to females that slept undisturbed. Noise also significantly increased wakefulness, reduced sleep and resulted in rebound sleep during the recovery period. Total energy expenditure was significantly lower during both noise exposure and recovery due to lower energy expenditure during spontaneous physical activity and sleep. Notably, noise did not alter the estrous cycle length. Conclusions: As previously observed in male rats, noise exposure disrupted sleep and increased weight gain in females but did not alter the length of the estrous cycle. This is the first demonstration of weight gain in female rats during sleep disruption. We conclude that the sleep disruption caused by exposure to environmental noise is a significant tool for determining how sleep loss contributes to obesity in females.

Dodd, G. T., et al. (2019). "Intranasal Targeting of Hypothalamic PTP1B and TCPTP Reinstates Leptin and Insulin Sensitivity and Promotes Weight Loss in Obesity." Cell Reports **28**(11): 2905-2922.e2905.

The importance of hypothalamic leptin and insulin resistance in the development and maintenance of obesity remains unclear. The tyrosine phosphatases protein tyrosine phosphatase 1B (PTP1B) and T cell protein tyrosine phosphatase (TCPTP) attenuate leptin and insulin signaling and are elevated in the hypothalami of obese mice. We report that elevated PTP1B and TCPTP antagonize hypothalamic leptin and insulin signaling and contribute to the maintenance of obesity. Deletion of PTP1B and TCPTP in the hypothalami of obese mice enhances CNS leptin and insulin sensitivity, represses feeding, and increases browning, to decrease adiposity and improve glucose metabolism. The daily intranasal administration of a PTP1B inhibitor, plus the glucocorticoid antagonist RU486 that decreases TCPTP expression, represses feeding, increases browning, promotes weight loss, and improves glucose metabolism in obese mice. Our findings causally link heightened hypothalamic PTP1B and TCPTP with leptin and insulin resistance and the maintenance of obesity and define a viable pharmacological approach by which to promote weight loss in obesity.

Ferguson, A. L., et al. (2019). "Exposure to solar ultraviolet radiation limits diet-induced weight gain, increases liver triglycerides and prevents the early signs of cardiovascular disease in mice." Nutrition, Metabolism and Cardiovascular Diseases **29**(6): 633-638.

Background and aims: Sunlight exposure is associated with a number of health benefits including protecting us from autoimmunity, cardiovascular disease, obesity and diabetes. Animal studies have confirmed that ultraviolet (UV)-B radiation, independently of vitamin D, can limit diet-induced obesity, metabolic syndrome and atherosclerosis. The aim of this study is to investigate whether exposure to the UV radiation contained in sunlight impacts on these disease parameters. Methods and results: We have trialled an intervention with solar UV in obese and atherosclerosis-prone mice. We have discovered that solar-simulated UV can significantly limit diet-induced obesity and reduce atheroma development in mice fed a diet high in sugar and fat. The optimal regime for this benefit was exposure once a week to solar UV equivalent to approximately 30 min of summer sun. Exposure to this optimal dose of solar UV also led to a significant increase in liver triglycerides which may protect the liver from damage. Conclusion: Our results show that the UV contained in sunlight has the potential to prevent and treat chronic disease at sites distant from irradiated skin. A major health challenge going forward will be to harness the power of the sun safely, without risking an increase in skin cancers.

Freels, T. G., et al. (2019). "Vaporized cannabis extracts have reinforcing properties and support conditioned drug-seeking behavior in rats." bioRxiv.

Recent trends in cannabis legalization have increased the necessity to better understand the effects of cannabis use. Animal models involving traditional cannabinoid self-administration approaches have been notoriously difficult to establish and differences in the drug employed and its route of administration have limited the translational value of preclinical studies. To address this challenge in the field, we have developed a novel method of cannabis self-administration using response-contingent delivery of

vaporized Δ9-tetrahydrocannabinol-rich (CANTHC) or cannabidiol-rich (CANCBD) complete cannabis extracts. Male Sprague Dawley rats were trained to nosepoke for discrete puffs of CANTHC, CANCBD, or vehicle (VEH) in daily one-hour sessions. Cannabis vapor reinforcement resulted in strong discrimination between active and inactive operanda. CANTHC maintained higher response rates under fixed ratio schedules and higher break points under progressive ratio schedules compared to CANCBD or VEH, and the number of vapor deliveries positively correlated with plasma THC concentrations. Moreover, metabolic phenotyping studies revealed alterations in locomotor activity, energy expenditure, and daily food intake that are consistent with effects in human cannabis users. Furthermore, both cannabis regimens produced ecologically relevant brain concentrations of THC and CBD and CANTHC administration decreased hippocampal CB1 receptor binding. Removal of CANTHC reinforcement (but not CANCBD) resulted in a robust extinction burst and an increase in cue-induced cannabis-seeking behavior relative to VEH. These data indicate that volitional exposure to THC-rich cannabis vapor has bona fide reinforcing properties and collectively support the utility of the vapor self-administration model for the preclinical assessment of volitional cannabis intake and cannabis-seeking behaviors.

Geller, S., et al. (2019). "Tanycytes Regulate Lipid Homeostasis by Sensing Free Fatty Acids and Signaling to Key Hypothalamic Neuronal Populations via FGF21 Secretion." Cell Metabolism **30**(4): 833-844.e837.

In obesity, the increased levels of circulating lipids induce metabolic dysfunction. Thus, it is essential to determine the mechanisms behind fat storage. Here, Geller et al. demonstrate that brain-specific glial cells, the tanycytes, sense circulating lipid levels to regulate body fat storage via the production of Fgf21.

Girard, R., et al. (2019). "HNF4α is a novel regulator of intestinal glucose-dependent insulinotropic polypeptide." Scientific Reports **9**(1).

Mutations in the HNF4A gene cause MODY1 and are associated with an increased risk of Type 2 diabetes mellitus. On the other hand, incretins are hormones that potentiate reductions in blood glucose levels. Given the established role of incretin-based therapy to treat diabetes and metabolic disorders, we investigated a possible regulatory link between intestinal epithelial HNF4α and glucose-dependent insulinotropic polypeptide (GIP), an incretin that is specifically produced by gut enteroendocrine cells. Conditional deletion of HNF4α in the whole intestinal epithelium was achieved by crossing Villin-Cre and Hnf4α loxP/loxP C57BL/6 mouse models. GIP expression was measured by qPCR, immunofluorescence and ELISA. Gene transcription was assessed by luciferase and electrophoretic mobility shift assays. Metabolic parameters were analyzed by indirect calorimetry and dual-energy X-ray absorptiometry. HNF4α specific deletion in the intestine led to a reduction in GIP. HNF4α was able to positively control Gip transcriptional activity in collaboration with GATA-4 transcription factor. Glucose homeostasis and glucose-stimulated insulin secretion remained unchanged in HNF4α deficient mice. Changes in GIP production in these mice did not impact nutrition or energy metabolism under normal physiology but led to a reduction of bone area and mineral content, a well described physiological consequence of GIP deficiency. Our findings point to a novel regulatory role between intestinal HNF4α and GIP with possible functional impact on bone density.

Hägele, F. A., et al. (2019). "Appetite Control Is Improved by Acute Increases in Energy Turnover at Different Levels of Energy Balance." Journal of Clinical Endocrinology and Metabolism **104**(10): 4481-4491.

Background: Weight control is hypothesized to be improved when physical activity and energy intake are both high [high energy turnover (ET)]. Objective: The impact of three levels of ET on short-term appetite control is therefore investigated at fixed levels of energy balance. Design: In a randomized crossover trial, 16 healthy adults (25.1 ± 3.9 y of age; body mass index, 24.0 ± 3.2 kg/m2) spent three daylong protocols for four times in a metabolic chamber. Four conditions of energy balance (ad libitum energy intake, zero energy balance, -25% caloric restriction, and +25% overfeeding) were each performed at three levels of ET (PAL 1.3 low, 1.6 medium, and 1.8 high ET; by walking on a treadmill). Levels of appetite hormones ghrelin, GLP-1, and insulin (total area under the curve) were measured during 14 hours. Subjective appetite ratings were assessed by visual analog scales. Results: Compared with high ET, low ET led to decreased GLP-1 (at all energy balance conditions: $P < 0.001$) and increased ghrelin concentrations (caloric restriction and overfeeding: $P < 0.001$), which was consistent with higher feelings of hunger (zero energy balance: $P < 0.001$) and desire to eat (all energy balance conditions: $P < 0.05$) and a positive energy balance during ad libitum intake (+17.5%; $P < 0.001$). Conclusion: Appetite is regulated more effectively at a high level of ET, whereas overeating and consequently weight gain are likely to occur at low levels of ET. In contrast to the prevailing concept of body weight control, the positive impact of physical activity is independent from burning up more calories and is explained by improved appetite sensations.

Haynes, V. R., et al. (2019). "Dysferlin deficiency alters lipid metabolism and remodels the skeletal muscle lipidome in mice." Journal of Lipid Research **60**(8): 1350-1364.

Defects in the gene coding for dysferlin, a membrane-associated protein, affect many tissues, including skeletal muscles, with a resultant myopathy called dysferlinopathy. Dysferlinopathy manifests postgrowth with a progressive loss of skeletal muscle function, early intramyocellular lipid accumulation, and a striking later replacement of selective muscles by adipocytes. To better understand the changes underpinning this disease, we assessed whole-body energy homeostasis, skeletal muscle fatty acid metabolism, lipolysis in adipose tissue, and the skeletal muscle lipidome using young adult dysferlin-deficient male BLAJ mice and age-matched C57Bl/6J WT mice. BLAJ mice had increased lean mass and reduced fat mass associated with increased physical activity and increased adipose tissue lipolysis. Skeletal muscle fatty acid metabolism was remodeled in BLAJ mice, characterized by a partitioning of fatty acids toward storage rather than oxidation. Lipidomic analysis identified marked changes in almost all lipid classes examined in the skeletal muscle of BLAJ mice, including sphingolipids, phospholipids, cholesterol, and most glycerolipids but, surprisingly, not triacylglycerol. These observations indicate that an early manifestation of dysferlin deficiency is the reprogramming of skeletal muscle and adipose tissue lipid metabolism, which is likely to contribute to the progressive adverse histopathology in dysferlinopathies.

Huang, W. C., et al. (2019). "Investigation of the effects of microbiota on exercise physiological adaption, performance, and energy utilization using a gnotobiotic animal model." Frontiers in Microbiology **10**(AUG).

The wide diversity in gut microbiota that is found among individuals is affected by factors including environment, genetics, dietary habits, and lifestyle after birth. The gastrointestinal tract, the largest and most complicated in vivo ecosystem, is a natural habitat for microbe colonization. Gut microbiota acts as “metabolic organ” that interacts with the human host symbiotically and performs an important role in maintaining health. In addition to the above factors, microbiota distributions/proportions are affected by exercise and other forms of physical activity. However, diet, lifestyle, and nutritional supplementation may impede the actual analytic relationship in practice. Therefore, the purpose of this study is to understand the effects of several microbiota on physical fitness, exercise performance, energy metabolism, and biochemistries using the concept of gnotobiote based on a germ-free model. The microbes Eubacterium rectale, Lactobacillus plantarum TWK10, and Clostridium coccoides were separately inoculated into gnotobiotic animal models. Fecal analysis was regularly done for the entire duration of the experiment. The exercise capacities were measured repeatedly with and without aerobic exercise training using an exhaustive swimming test. Various fatigue-associated biochemical variables, including lactate, ammonia, glucose, lactic dehydrogenase (LDH), and creatine kinase (CK) were also measured to assess physiological adaption. In addition, metabolic phenotype was applied to record basal metabolic rate, diet, behavior, and activities. Body composition, glycogen content, and histopathology were further evaluated to assess the gnotobiotic effects. E. rectale engendered capacities, physiological adaption, and physical activities that were significantly better than other two microbes, possible due to energy regulation and bioavailability. In addition, L. plantarum TWK10 and C. coccoides were found to significantly increase the basal metabolic rate and to alter the body compositions, although no exercise capacity benefit was found in the gnotobiotic models. The E. rectale and L. plantarum gnotobiotic animals all showed normal histological observations with the exception of the C. coccoides gnotobiote, which showed the pathological observation of hepatic necrosis. The gnotobiotic model directly demonstrates the interactions between microbes and hosts, which are especially relevant and applicable to the field of sports science. This study supports the development of beneficial microbiota for application to exercise and fitness, which is an emerging area of health promotion.

Hurr, C., et al. (2019). "Liver sympathetic denervation reverses obesity-induced hepatic steatosis." Journal of Physiology **597**(17): 4565-4580.

Key points: Non-alcoholic fatty liver disease, characterized in part by elevated liver triglycerides (i.e. hepatic steatosis), is a growing health problem. In this study, we found that hepatic steatosis is associated with robust hepatic sympathetic overactivity. Removal of hepatic sympathetic nerves reduced obesity-induced hepatic steatosis. Liver sympathetic innervation modulated hepatic lipid acquisition pathways during obesity. Abstract: Non-alcoholic fatty liver disease (NAFLD) affects 1 in 3 Americans and is a significant risk factor for type II diabetes mellitus, insulin resistance and hepatic carcinoma. Characterized in part by excessive hepatic triglyceride accumulation (i.e. hepatic steatosis), the incidence of NAFLD is increasing – in line with the growing obesity epidemic. The role of the autonomic nervous system in NAFLD remains unclear. Here, we show that chronic hepatic sympathetic overactivity mediates hepatic steatosis. Direct multiunit recordings of hepatic sympathetic nerve activity were obtained in high fat diet and normal

chow fed male C57BL/6J mice. To reduce hepatic sympathetic nerve activity we utilized two approaches including pharmacological ablation of the sympathetic nerves and phenol-based hepatic sympathetic nerve denervation. Diet-induced NAFLD was associated with a nearly doubled firing rate of the hepatic sympathetic nerves, which was largely due to an increase in efferent nerve traffic. Furthermore, established high fat diet-induced hepatic steatosis was effectively reduced with pharmacological or phenol-based removal of the hepatic sympathetic nerves, independent of changes in body weight, caloric intake or adiposity. Ablation of liver sympathetic nerves was also associated with improvements in liver triglyceride accumulation pathways including free fatty acid uptake and de novo lipogenesis. These findings highlight an unrecognized pathogenic link between liver sympathetic outflow and hepatic steatosis and suggest that manipulation of the liver sympathetic nerves may represent a novel therapeutic strategy for NAFLD.

Ip, C. K., et al. (2019). "Amygdala NPY Circuits Promote the Development of Accelerated Obesity under Chronic Stress Conditions." Cell Metabolism **30**(1): 111-128.e116.

Neuropeptide Y (NPY) exerts a powerful orexigenic effect in the hypothalamus. However, extra-hypothalamic nuclei also produce NPY, but its influence on energy homeostasis is unclear. Here we uncover a previously unknown feeding stimulatory pathway that is activated under conditions of stress in combination with calorie-dense food; NPY neurons in the central amygdala are responsible for an exacerbated response to a combined stress and high-fat-diet intervention. Central amygdala NPY neuron-specific Npy overexpression mimics the obese phenotype seen in a combined stress and high-fat-diet model, which is prevented by the selective ablation of Npy. Using food intake and energy expenditure as readouts, we demonstrate that selective activation of central amygdala NPY neurons results in increased food intake and decreased energy expenditure. Mechanistically, it is the diminished insulin signaling capacity on central amygdala NPY neurons under combined stress and high-fat-diet conditions that leads to the exaggerated development of obesity.

Johnson, H. M., et al. (2019). "Glucose mediates insulin sensitivity via a hepatoportal mechanism in high-fat-fed rats." Journal of Endocrinology **241**(3): 189-199.

Poor nutrition plays a fundamental role in the development of insulin resistance, an underlying characteristic of type 2 diabetes. We have previously shown that high-fat diet-induced insulin resistance in rats can be ameliorated by a single glucose meal, but the mechanisms for this observation remain unresolved. To determine if this phenomenon is mediated by gut or hepatoportal factors, male Wistar rats were fed a high-fat diet for 3 weeks before receiving one of five interventions: high-fat meal, glucose gavage, high-glucose meal, systemic glucose infusion or portal glucose infusion. Insulin sensitivity was assessed the following day in conscious animals by a hyperinsulinaemic-euglycaemic clamp. An oral glucose load consistently improved insulin sensitivity in high-fat-fed rats, establishing the reproducibility of this model. A systemic infusion of a glucose load did not affect insulin sensitivity, indicating that the physiological response to oral glucose was not due solely to increased glucose turnover or withdrawal of dietary lipid. A portal infusion of glucose produced the largest improvement in insulin sensitivity, implicating a role for the hepatoportal region rather than the gastrointestinal tract in mediating the effect of glucose to improve lipid-induced insulin resistance. These results further deepen our understanding of

the mechanism of glucose-mediated regulation of insulin sensitivity and provide new insight into the role of nutrition in whole body metabolism.

Jurrissen, T. J., et al. (2019). "Overproduction of endothelin-1 impairs glucose tolerance but does not promote visceral adipose tissue inflammation or limit metabolic adaptations to exercise." American Journal of Physiology. Endocrinology and Metabolism **317**(3): E548-E558.

Endothelin-1 (ET-1) is a potent vasoconstrictor and proinflammatory peptide that is upregulated in obesity. Herein, we tested the hypothesis that ET-1 signaling promotes visceral adipose tissue (AT) inflammation and disrupts glucose homeostasis. We also tested if reduced ET-1 is a required mechanism by which exercise ameliorates AT inflammation and improves glycemic control in obesity. We found that 1) diet-induced obesity, AT inflammation, and glycemic dysregulation were not accompanied by significantly increased levels of ET-1 in AT or circulation in wild-type mice and that endothelial overexpression of ET-1 and consequently increased ET-1 levels did not cause AT inflammation yet impaired glucose tolerance; 2) reduced AT inflammation and improved glucose tolerance with voluntary wheel running was not associated with decreased levels of ET-1 in AT or circulation in obese mice nor did endothelial overexpression of ET-1 impede such exercise-induced metabolic adaptations; 3) chronic pharmacological blockade of ET-1 receptors did not suppress AT inflammation in obese mice but improved glucose tolerance; and 4) in a cohort of human subjects with a wide range of body mass indexes, ET-1 levels in AT, or circulation were not correlated with markers of inflammation in AT. In aggregate, we conclude that ET-1 signaling is not implicated in the development of visceral AT inflammation but promotes glucose intolerance, thus representing an important therapeutic target for glycemic dysregulation in conditions characterized by hyperendothelinemia. Furthermore, we show that the salutary effects of exercise on AT and systemic metabolic function are not contingent on the suppression of ET-1 signaling.

Kaiyala, K. J. (2019). "Do Calorimetric Results in Mice Depend on the System Being Used?" Obesity **27**(5): 689-689.

Indirect calorimetry is routinely used to assess energy expenditure, metabolism, and energy balance in rodents, and currently multiple commercial systems are in use in the scientific community. Considering this diversity, do calorimetric results depend on the system being used? In this issue of Obesity, Soto et al. (1) address this important yet complex question in work comparing outcomes measured in the Promethion and OxyMax systems. Although the systems performed quite similarly overall, enough differences were observed to warrant comments, as these systems differ greatly in a discipline where even minor details are important (2,3) and because statistical issues cloud some interpretations.

Disclosure: Dr. Kaiyala has collaborated on several occasions with Dr. John Lighton, President and Chief Scientific Officer of Sable Systems International, manufacturer of the Promethion calorimeter system

Kaiyala, K. J., et al. (2019). "Validation of an equation for energy expenditure that does not require the respiratory quotient." PLoS One **14**(2).

Background :Energy expenditure (EE) calculated from respirometric indirect calorimetry is most accurate when based on oxygen consumption (VO 2), carbon dioxide production (VCO 2) and estimated protein metabolism (PM). EE has a substantial dependence of ~7% on the respiratory quotient (RQ, VCO 2 /VO 2) and a lesser dependence on PM, yet many studies have instead estimated EE from VO 2 only while PM has often been ignored, thus reducing accuracy. In 1949 Weir proposed a method to accurately calculate EE without using RQ, which also adjusts for estimated PM based on dietary composition. This RQ - method utilizes the calorimeter airflow rate (FR), the change in fractional O 2 concentration (ΔFO 2) and the dietary protein fraction. The RQ - method has not previously been empirically validated against the standard RQ + method using both VO 2 and RQ. Our aim was to do that. Methods: VO 2 and VCO 2 were measured repeatedly in 8 mice fed a high protein diet (HPD) during exposure to different temperatures (n = 168 measurements of 24h gas exchange). The HPD-adjusted RQ + equation was: EE [kcal/time] = VO 2 [L/time]×(3.853+1.081RQ) while the corresponding RQ - equation was: EE = 4.934×FR×ΔFO 2 . Agreement was analyzed using the ratios of the RQ - to RQ + methods along with regression and Bland-Altman agreement analyses. We also evaluated the standard equation using the dietary food quotient (FQ) of 0.91 as a proxy for RQ (FQ + method). Results: Ratio analysis revealed that the mean error of the RQ - method was only 0.11 ± 0.042% while the maximum error was only 0.21%. Error using the FQ + method was 4 -and 10-fold greater, respectively. Bland-Altman analysis demonstrated that the RQ - method very slightly overestimates EE as RQ decreases. Theoretically, this error can be eliminated completely by imposing an incurrent fractional oxygen concentration at a value only slightly greater than the atmospheric level. Conclusions: The Weir 'RQ-free' method for calculating EE is a highly valid alternative to the 'gold standard' method that requires RQ. The RQ - approach permits reduced cost and complexity in studies focused on EE and provides a way to rescue EE measurement in studies compromised by faulty CO 2 measurements. Practitioners of respirometry should consider adjusting EE calculations for estimated protein metabolism based on dietary composition.

Kentish, S. J., et al. (2019). "Disruption of the light cycle ablates diurnal rhythms in gastric vagal afferent mechanosensitivity." Neurogastroenterology and Motility **31**(12).

Background: Gastric vagal afferents (GVAs) respond to mechanical stimulation, initiating satiety. These afferents exhibit diurnal fluctuations in mechanosensitivity, facilitating food intake during the dark phase in rodents. In humans, desynchrony of diurnal rhythms (eg, shift work) is associated with a higher risk of obesity. To test the hypothesis that shift work disrupts satiety signaling, the effect of a rotating light cycles on diurnal rhythms in GVA mechanosensitivity in lean and high-fat diet (HDF)-induced obese mice was determined. Methods: Male C57BL/6 mice were fed standard laboratory diet (SLD) or HFD for 12 weeks. After 4 weeks, mice were randomly allocated to a normal light (NL; 12 hour light: 12 hour dark; lights on at zeitgeber time [ZT] 0) or rotating light (RL; 3-day NL cycle, 4-day reversed light cycle [lights on: ZT12] repeated) cycle for 8 weeks. At week 12, eight mice from each group were housed in metabolic cages. After 12 weeks, ex vivo GVA recordings were taken at 3 hour intervals starting at ZT0. Key Results: SLD-RL and HFD-RL gained more weight compared to SLD-NL and HFD-NL mice, respectively. Gonadal fat pad mass was higher in SLD-RL compared to SLD-NL mice. In SLD-NL mice, tension and mucosal receptor mechanosensitivity exhibited diurnal rhythms with a peak at ZT9. These rhythms were lost in SLD-RL, HFD-NL, and HFD-RL mice and associated with dampened diurnal rhythms in food intake. Conclusions & Inferences: GVA diurnal rhythms are susceptible to disturbances in the light cycle and/or the obese state. This may underpin the observed changes in feeding behavior.

Li, H., et al. (2019). "Chronic stress induces hypersensitivity of murine gastric vagal afferents." Neurogastroenterology and Motility **31**(12).

Background: Stress exposure is known to trigger and exacerbate functional dyspepsia (FD) symptoms. Increased gastric sensitivity to food-related stimuli is widely observed in FD patients and is associated with stress and psychological disorders. The mechanisms underlying the hypersensitivity are not clear. Gastric vagal afferents (GVAs) play an important role in sensing meal-related mechanical stimulation to modulate gastrointestinal function and food intake. This study aimed to determine whether GVAs display hypersensitivity after chronic stress, and whether its interaction with leptin was altered by stress. Methods: Eight-week-old male C57BL/6 mice were exposed to unpredictable chronic mild stress or no stress (control) for 8 weeks. The metabolic rate, gastric emptying rate, and anxiety- and depression-like behaviors were determined. GVA mechanosensitivity, and its modulation by leptin, was determined using an in vitro single fiber recording technique. QRT-PCR was used to establish the levels of leptin and leptin receptor mRNA in the stomach and nodose ganglion, respectively. Key Results: The stressed mice had lower body weight and food intake, and increased anxiety-like behavior compared to the control mice. The mechanosensitivity of mucosal and tension-sensitive GVAs was higher in the stressed mice. Leptin potentiated mucosal GVA mechanosensitivity in control but not stressed mice. The expression of leptin mRNA in the gastric mucosa was lower in the stressed mice. Conclusions and Inferences: In conclusion, chronic stress enhances GVA mechanosensitivity, which may contribute to the gastric hypersensitivity in FD. In addition, the modulatory effect of leptin on GVA signaling is lost after chronic stress exposure.

Maridas, D. E., et al. (2019). "Progenitor recruitment and adipogenic lipolysis contribute to the anabolic actions of parathyroid hormone on the skeleton." FASEB Journal **33**(2): 2885-2898.

Intermittent administration of parathyroid hormone (PTH) stimulates bone formation in vivo and also suppresses the volume ofbonemarrowadipose tissue (BMAT). Incontrast, a calorie-restricted(CR) diet causes bone loss and inducesBMATin both mice andhumans.We used theCRmodel to testwhetherPTHwould reduceBMAT inmiceby both altering cell fate and inducing lipolysis ofmarrowadipocytes. Eight-week-oldmicewere placedon a control (Ctrl) diet or CR diet. At 12 wk, CR and Ctrl mice were injected daily with PTH (CR/PTH or Ctrl/PTH) or vehicle for 4wk.Two other cohortswereCRand simultaneously injected (CR+ PTHorCR+Veh) for 4wk.CRmice hadlowbonemass and increasedBMATin theproximal tibias.PTHsignificantly increased bonemass inall cohorts despite calorie restrictions. Adipocyte density and size were markedly increased with restriction of calories. PTH reduced adipocyte numbers in CR + PTH mice, whereas adipocyte size was reduced in CR/PTH-treated mice. In contrast, osteoblast number was increased 3-8-fold with PTH treatment. In vitro, bone marrow stromal cells differentiated into adipocytes and, treated with PTH, exhibited increased production of glycerol and fatty acids. Moreover, in cocultures of bonemarrowadipocyte and osteoblast progenitors, PTHstimulated the transfer of fatty acids to osteoblasts. In summary, PTHadministration toCRmice increased bonemass by shifting lineage allocation toward osteogenesis and inducing lipolysis of mature marrow adipocytes. The effects of PTH on bone marrow adiposity could enhance its anabolic actions by providing both more cells and more fuel for osteoblasts during bone formation.

Martin, A. M., et al. (2019). "The gut microbiome regulates host glucose homeostasis via peripheral serotonin." Proceedings of the National Academy of Sciences of the United States of America **116**(40): 19802-19804.

> The gut microbiome is an established regulator of aspects of host metabolism, such as glucose handling. Despite the known impacts of the gut microbiota on host glucose homeostasis, the underlying mechanisms are unknown. The gut microbiome is also a potent mediator of gut-derived serotonin synthesis, and this peripheral source of serotonin is itself a regulator of glucose homeostasis. Here, we determined whether the gut microbiome influences glucose homeostasis through effects on gut-derived serotonin. Using both pharmacological inhibition and genetic deletion of gut-derived serotonin synthesis, we find that the improvements in host glucose handling caused by antibiotic-induced changes in microbiota composition are dependent on the synthesis of peripheral serotonin.

Matthew Morris, E., et al. (2019). "Divergent energy expenditure impacts mouse metabolic adaptation to acute high-fat/high-sucrose diet producing sexually dimorphic weight gain patterns." bioRxiv.

> Objective Long-term weight gain can result from cumulative small weight increases due to short-term excess caloric intake during weekends and holidays. Increased physical activity may mediate weight gain through increases in energy expenditure (EE) and reductions in energy balance. Current methods for modulating mouse EE (e.g. - exercise, chemical uncouplers, etc.) have confounding effects. However, it is known that mouse EE linearly increases as housing temperature decreases below the thermoneutral zone. Methods To determine how robust differences in baseline EE impact 7-day changes in weight and body composition on low-fat and high-fat, high-sucrose (HFHS) diets, we performed indirect calorimetry measurements in male and female mice housed at divergent temperatures (20°C vs. 30°C). Results As expected, mice housed at 30°C have ~40% lower total EE and energy intake compared to 20°C mice regardless of diet or sex. Energy balance was increased with HFHS in all groups, with ~30% greater increases observed in 30°C versus 20°C mice. HFHS increased weight gain regardless of temperature or sex. Interestingly, no HFHS-induced weight gain differences were observed between females at different temperatures. In contrast, 30°C male mice on HFHS gained ~50% more weight than 20°C males, and ~80% more weight compared to 30°C females. HFHS increased fat mass across all groups but 2-fold higher gains occurred in 30°C mice compared to 20°C mice. Females gained ~35% less fat mass than males at both temperatures. Conclusions Together, these data reveal an interaction between divergent ambient temperature-induced EE and sex that impacted diet-induced patterns of short-term weight gain and body composition.

Miyano, C. A., et al. (2019). "Severe thermoregulatory deficiencies in mice with a deletion in the titin gene TTN." Journal of Experimental Biology **222**(9).

> Muscular dystrophy with myositis (mdm) mice carry a deletion in the N2A region of the gene for the muscle protein titin (TTN), shiver at low frequency, fail to maintain body temperatures (Tb) at ambient temperatures (Ta) <34°C, and have reduced body mass and active muscle stiffness in vivo compared with wild-type (WT) siblings. Impaired shivering thermogenesis (ST) could be due to the mutated titin protein causing more compliant muscles. We hypothesized that non-shivering thermogenesis (NST) is impaired. To characterize the response to cold exposure, we measured Tb and metabolic rate (MR) of WT and mdm mice at four nominal temperatures: 20, 24, 29 and 34°C. Subsequently, we stimulated NST with noradrenaline. Manipulation of Ta revealed an interaction between genotype and MR: mdm mice had higher MRs at 29°C and lower MRs at 24°C compared with WT mice. NST capacity was lower in mdm mice than in WT mice. Using MR data from a previous study, we compared MR of mdm mice with MR of Perognathus longimembris, a mouse species of similar body mass. Our results indicated low MR and reduced NST of mdm mice. These were more pronounced than differences between mdm and WT mice owing to body mass effects on MR and capacity for NST. Correcting MR using Q10 showed that mdm mice had lower MRs than size-matched P. longimembris, indicating that mutated N2A titin causes severe thermoregulatory defects at all levels. Direct effects of the titin mutation lead to lower shivering frequency. Indirect effects likely lead to a lower capacity for NST and increased thermal conductance through decreased body size.

Miyano, C. A., et al. (2019). Severe thermoregulatory deficiencies in mice with a deletion in the titin gene TTN, openknowledge.nau.edu.

> Muscular dystrophy with myositis (mdm) mice carry a deletion in the N2A region of the gene for the muscle protein titin (TTN), shiver at low frequency, fail to maintain body temperatures (Tb) at ambient temperatures (Ta) <34°C, and have reduced body mass and active muscle stiffness in vivo compared with wild-type (WT) siblings. Impaired shivering thermogenesis (ST) could be due to the mutated titin protein causing more compliant muscles. We hypothesized that non-shivering thermogenesis (NST) is impaired. To characterize the response to cold exposure, we measured Tb and metabolic rate (MR) of WT and mdm mice at four nominal temperatures: 20, 24, 29 and 34°C. Subsequently, we stimulated NST with noradrenaline. Manipulation of Ta revealed an interaction between genotype and MR: mdm mice had higher MRs at 29°C and lower MRs at 24°C compared with WT mice. NST capacity was lower in mdm mice than in WT mice. Using MR data from a previous study, we compared MR of mdm mice with MR of Perognathus longimembris, a mouse species of similar body mass. Our results indicated low MR and reduced NST of mdm mice. These were more pronounced than differences between mdm and WT mice owing to body mass effects on MR and capacity for NST. Correcting MR using Q10 showed that mdm mice had lower MRs than size-matched P. longimembris, indicating that mutated N2A titin causes severe thermoregulatory defects at all levels. Direct effects of the titin mutation lead to lower shivering frequency. Indirect effects likely lead to a lower capacity for NST and increased thermal conductance through decreased body size.

Murphy, K. T., et al. (2019). "MAs receptor activation slows tumor growth and attenuates muscle wasting in cancer." Cancer Research **79**(4): 706-719.

Cancer cachexia is a multifactorial syndrome characterized by a progressive loss of skeletal muscle mass associated with significant functional impairment. Cachexia robs patients of their strength and capacity to perform daily tasks and live independently. Effective treatments are needed urgently. Here, we investigated the therapeutic potential of activating the "alternative" axis of the renin-angiotensin system, involving ACE2, angiotensin-(1-7), and the mitochondrial assembly receptor (MasR), for treating cancer cachexia. Plasmid overexpression of the MasR or pharmacologic angiotensin-(1-7)/ MasR activation did not affect healthy muscle fiber size in vitro or in vivo but attenuated atrophy induced by coculture with cancer cells in vitro. In mice with cancer cachexia, the MasR agonist AVE 0991 slowed tumor development, reduced weight loss, improved locomotor activity, and attenuated muscle wasting, with the majority of these effects dependent on the orexigenic and not antitumor properties of AVE 0991. Proteomic profiling and IHC revealed that mechanisms underlying AVE 0991 effects on skeletal muscle involved miR-23a–regulated preservation of the fast, glycolytic fibers. MasR activation is a novel regulator of muscle phenotype, and AVE 0991 has orexigenic, anticachectic, and antitumorigenic effects, identifying it as a promising adjunct therapy for cancer and other serious muscle wasting conditions. Significance: These findings demonstrate that MasR activation has multiple benefits of being orexigenic, anticachectic, and antitumorigenic, revealing it as a potential adjunct therapy for cancer.

Park, B. S., et al. (2019). "Beta-Aminoisobutyric Acid Inhibits Hypothalamic Inflammation by Reversing Microglia Activation." Cells **8**(12).

Beta-aminoisobutyric acid (BAIBA), a natural thymine catabolite, is involved in the beneficial effects of exercise on metabolic disorders. In particular, it has been reported to reverse the inflammatory processes observed in the peripheral organs of animal models of obesity. Therefore, this study aimed to investigate whether BAIBA improves hypothalamic inflammation, which is also tightly coupled with the development of obesity. We observed that treatment with BAIBA effectively reversed palmitic acid-induced hypothalamic inflammation and microglial activation in vivo. Consistent with these findings, we confirmed that BAIBA reversed body weight gain and increased adiposity observed in mice fed with a high-fat diet. Collectively, the current findings evidence the beneficial impacts of BAIBA on the imbalance of energy metabolism linked to hypothalamic inflammation.

Penniman, C. M., et al. (2019). "Loss of FoxOs in muscle reveals sex-based differences in insulin sensitivity but mitigates diet-induced obesity." Molecular Metabolism **30**: 203-220.

Objective: Gender influences obesity-related complications, including diabetes. Females are more protected from insulin resistance after diet-induced obesity, which may be related to fat accumulation and muscle insulin sensitivity. FoxOs regulate muscle atrophy and are targets of insulin action, but their role in muscle insulin sensitivity and mitochondrial metabolism is unknown. Methods: We measured muscle insulin signaling, mitochondrial energetics, and metabolic responses to a high-fat diet (HFD) in male and female muscle-specific FoxO1/3/4 triple knock-out (TKO) mice. Results: In male TKO muscle,

insulin-stimulated AKT activation was decreased. AKT2 protein and mRNA levels were reduced and insulin receptor protein and IRS-2 mRNA decreased. These changes contributed to decreased insulin-stimulated glucose uptake in glycolytic muscle in males. In contrast, female TKOs maintain normal insulin-mediated AKT phosphorylation, normal AKT2 levels, and normal glucose uptake in glycolytic muscle. When challenged with a HFD, fat gain was attenuated in both male and female TKO mice, and associated with decreased glucose levels, improved glucose homeostasis, and reduced muscle triglyceride accumulation. Furthermore, female TKO mice showed increased energy expenditure, relative to controls, due to increased lean mass and maintenance of mitochondrial function in muscle. Conclusions: FoxO deletion in muscle uncovers sexually dimorphic regulation of AKT2, which impairs insulin signaling in male mice, but not females. However, loss of FoxOs in muscle from both males and females also leads to muscle hypertrophy and increases in metabolic rate. These factors mitigate fat gain and attenuate metabolic abnormalities in response to a HFD.

Rajasekaran, M., et al. (2019). "MCP-1 deficiency enhances browning of adipose tissue via increased M2 polarization." Journal of Endocrinology **242**(2): 91-101.

Obesity is strongly associated with chronic inflammation for whi ch adipose tissue macrophages play a critical role. The objective of this study is to identify monocyte chemoattractant protein-1 (MCP-1, CCL2) as a key player governing M1-M2 macrophage polarization and energy balance. We evaluated body weight, fat mass, adipocyte size and energy expenditure as well as core body temperature of Ccl2 knockout mice compared with wild-type mice. Adipose tissues, differentiated adipocyte a nd bone marrow-derived macrophages were assessed by qPCR, Western blot analysis and histochemistry. MCP-1 deficiency augmented energy expenditure by promoting browning in white adipose tissue and brown adipose tissue activity via increasing the expressions of Ucp1, Prdm16, Tnfrsf9, Ppargc1a, Nrf1 and Th and mitochondrial DNA copy number. MCP-1 abrogation promoted M2 polarization which is characterized by increased expression of Arg1, Chil3, Il10 and Klf4 whereas it decreased M1 polarization by decreased p65 nuclear translocation and attenuated expression of Itgax, Tnf and Nos2, leading to increased browning of adipocytes. Enhanced M2 polarization and attenuated M1 polarization in the absence of MCP-1 are independent. Collectively, our results suggest that the action of MCP-1 in macrophages modulates energy expenditure by impairing browning in adipose tissue.

Regué, L., et al. (2019). "IMP2 Increases Mouse Skeletal Muscle Mass and Voluntary Activity by Enhancing Autocrine Insulin-Like Growth Factor 2 Production and Optimizing Muscle Metabolism." Molecular and Cellular Biology **39**(7).

Insulin-like growth factor 2 (IGF2) mRNA binding protein 2 (IMP2) was selectively deleted from adult mouse muscle; two phenotypes were observed: decreased accrual of skeletal muscle mass after weaning and reduced wheel-running activity but normal forced treadmill performance. Reduced wheel running occurs when mice are fed a high-fat diet but is normalized when mice consume standard chow. The two phenotypes are due to altered output from different IMP2 client mRNAs. The reduced fiber size of IMP2-deficient muscle is attributable, in part, to diminished autocrine Igf2 production; basal tyrosine phosphorylation of the insulin and IGF1 receptors is diminished, and Akt1 activation is selectively reduced. Gsk3α is disinhibited, and S536-phosphorylated ε subunit of eukaryotic initiation factor 2B [eIF2Bε(S536)]

is hyperphosphorylated. Protein synthesis is reduced despite unaltered mTOR complex 1 activity. The diet-dependent reduction in voluntary exercise is likely due to altered muscle metabolism, as contractile function is normal. IMP2-deficient muscle exhibits reduced fatty acid oxidation, due to a reduced abundance of mRNA of peroxisome proliferator-activated receptor α (PPARα), an IMP2 client, and PPARα protein. IMP2-deficient muscle fibers treated with a mitochondrial uncoupler to increase electron flux, as occurs with exercise, exhibit reduced oxygen consumption from fatty acids, with higher oxygen consumption from glucose. The greater dependence on muscle glucose metabolism during increased oxygen demand may promote central fatigue and thereby diminish voluntary activity.

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Ruegsegger, G. N. (2019). Examination of nucleus accumbens mechanisms underlying the motivation for physical activity, mospace.umsystem.edu.

Physical inactivity, a primary contributor to numerous diseases including obesity, type 2 diabetes, depression, and dementia, has reached pandemic levels worldwide. Alarmingly, the percentage of individuals engaging in physical activity is low and decreasing. Accelerometry data shows that > 90% of adults fail to meet the U.S. Physical Activity Guidelines despite the excess of knowledge suggesting exercise improves health. Therefore, beginning to understand the molecular mechanisms which influence physical activity levels is imperative for the development of therapies to reduce sedentary behavior. The work presented in this dissertation made use of three independent experimental paradigms in rats to test the hypothesis that differences in the mesolimbic dopamine system associate with/cause changes in voluntary physical activity. In the first study, rats selectively bred for high (HVR) or low (LVR) voluntary wheel running distance were used to assess inherent differences in opioidergic signaling between HVR

and LVR, as well as the influence of dopamine on opioid-induced changes in voluntary wheel running. Mu-opioid receptor expression and function was increased in the nucleus accumbens (NAc) of HVR compared to LVR. Likewise, naltrexone injection decreased dopamine-related mRNA expression in mesolimbic brain regions and reduced wheel running in HVR, but not LVR. Finally, lesion of dopaminergic neurons in the NAc prevented the decrease in running following naltrexone administration in HVR, suggesting opioidergic signaling requires downstream dopaminergic activity to influence voluntary running. In the second study, the transgenerational effect of maternal Western diet (WD) on offspring voluntary wheel running was assessed. Wheel running was increased in female WD offspring from 4-7 weeks of age, but decreased running from 16- 19 weeks of age, compared to offspring from chow fed dams. These age-specific changes in wheel running are associated with the up- and down-regulation of dopamine receptor 1 in the NAc at 6 and 18 weeks of age, respectively, in WD female offspring, which in turn was negatively associated with leptin receptor mRNA in the ventral tegmental area. In the third study, age-related influences on wheel running were assessed in 8 and 14 week-old rats. In addition to a ∼60% reduction in running, RNA-sequencing revealed downregulations in networks related to cAMP-mediated signaling and synaptic plasticity in the NAc from 8 to 14 weeks-old. The down-regulations of these networks was mirrored by reductions in dendritic spine density in the NAc from 8 to 14 weeks-old. Additionally, intra-NAc injection of the Cdk5 inhibitor roscovitine, a known modulator of dendritic density and dopamine signaling, dose-dependently decreased wheel running. Despite the varying experimental models used in this dissertation, these findings collectively suggest that alterations in dopaminergic signaling in the NAc associate with, and influence, voluntary physical activity. (PsycINFO Database Record (c) 2019 APA, all rights reserved)

Sanford, D., et al. (2019). "An Intraperitoneal Treatment with Calcitonin Gene-Related Peptide (CGRP) Regulates Appetite, Energy Intake/Expenditure, and Metabolism." Journal of Molecular Neuroscience **67**(1): 28-37.

Calcitonin gene-related peptide (CGRP) is a 37-amino acid neuropeptide expressed both centrally and peripherally. CGRP has been shown to be involved in arteriolar dilation, cardiovascular regulation, pain transmission, migraine, and gastrointestinal physiology. Our current research is aimed at analyzing CGRP's impact on appetite/satiety, body metabolism, and energy homeostasis. Our study investigated the effects of a single-dose intraperitoneal (IP) treatment with CGRP on food and water consumption, energy expenditure, physical activity, respirometry, and a panel of plasma metabolic hormones in C57Bl/6 wild-type (WT) mice. After a CGRP IP injection at a dose of 2 nmol (10 µM CGRP in 200 µl of saline), a significant reduction in food intake and metabolic parameters as RQ, VCO 2 , and VO 2 was observed. CGRP-injected mice had also significantly lower total energy expenditure (TEE) with no changes in activity levels compared to vehicle-injected controls. CGRP treatment in mice induced significantly lower plasma levels of glucagon and leptin but higher levels of amylin. Our data show that a single dose of CGRP peptide significantly decreased food consumption and altered calorimetric parameters and plasma metabolic hormone levels, thus confirming that CGRP plays a pivotal role in the regulation of appetite and metabolism. Future studies are necessary to analyze CGRP's long-term impact on body metabolism and its potential effects on appetite, obesity, and metabolic disorders.

Shahmirzadi, A. A., et al. (2019). "Alpha-ketoglutarate, an endogenous metabolite, extends lifespan and compresses morbidity in aging mice." bioRxiv.

> The decline in early life mortality since the 1950s has resulted in dramatic demographic shift towards aged population. Aging manifests as a decline in health, multiple organ dysfunction and increased vulnerability to diseases, which degrades quality of life. A verity of genetic and pharmacological interventions, mostly from non-vertebrate models, have been identified that can enhance lifespan. Whether these interventions extend healthspan, the disease free and functional period of life, has only sometimes been tested and is often a matter of debate. Human aging indices have been developed to assess elements of functional decline with aging (e.g. sarcopenia, cognitive function). However, corresponding comprehensive indices in mice are seldom applied to aging studies. To probe the relationship between healthspan and lifespan extension in mammals, we performed a series of longitudinal, clinically-relevant healthspan measurements. Metabolism and aging are tightly connected and specific perturbations of nutrient-sensing pathways can enhance longevity in laboratory animals. Here we show that alpha-ketoglutarate (delivered in the form of a Calcium salt, CaAKG), a key metabolite in tricarboxylic (TCA) cycle that is reported to extend lifespan in worms, can significantly extend lifespan and healthspan in mice. AKG is involved in various fundamental processes including collagen synthesis and epigenetic changes. Due to its broad roles in multiple biological processes, AKG has been a subject of interest for researchers in various fields. AKG also influences several age-related processes, including stem cell proliferation and osteoporosis. To determine its role in mammalian aging, we administered CaAKG in 18 months old mice and determined its effect on the onset of frailty and survival, discovering that the metabolite promotes longer, healthier life associated with a decrease in levels of inflammatory factors. Interestingly the reduction in frailty was more dramatic than the increase in lifespan, leading us to propose that CaAKG compresses morbidity.

Sharma, A., et al. (2019). "Impaired skeletal muscle mitochondrial pyruvate uptake rewires glucose metabolism to drive whole-body leanness." eLife **8**.

> Metabolic cycles are a fundamental element of cellular and organismal function. Among the most critical in higher organisms is the Cori Cycle, the systemic cycling between lactate and glucose. Here, skeletal muscle-specific Mitochondrial Pyruvate Carrier (MPC) deletion in mice diverted pyruvate into circulating lactate. This switch disinhibited muscle fatty acid oxidation and drove Cori Cycling that contributed to increased energy expenditure. Loss of muscle MPC activity led to strikingly decreased adiposity with complete muscle mass and strength retention. Notably, despite decreasing muscle glucose oxidation, muscle MPC disruption increased muscle glucose uptake and whole-body insulin sensitivity. Furthermore, chronic and acute muscle MPC deletion accelerated fat mass loss on a normal diet after high fat diet-induced obesity. Our results illuminate the role of the skeletal muscle MPC as a whole-body carbon flux control point. They highlight the potential utility of modulating muscle pyruvate utilization to ameliorate obesity and type 2 diabetes.

Shi, M., et al. (2019). "The effects of supplementation with blueberry, cyanidin-3-O-β-glucoside, yoghurt and its peptides on obesity and related comorbidities in a diet-induced obese mouse model." Journal of Functional Foods **56**: 92-101.

It is widely acknowledged that type 2 diabetes mellitus (T2DM) is associated with obesity, insulin resistance and hypertension. Cyanidin-3-O-β-glucoside (C3G), an anthocyanin in blueberry, and peptides with angiotensin converting enzyme (ACE) inhibitory activity derived from yoghurt are potentially beneficial for numerous health conditions including improving insulin resistance and glucose intolerance. In this study, the synergistic/additive effects of combined supplementations with blueberry and yoghurt, and C3G and peptides were determined. Blueberry and yoghurt alone, and the combination of C3G and peptides significantly reduced both systolic and diastolic blood pressure in diet-induced obese mice. Yoghurt supplementation significantly reduced body weight, percentage body fat and improved intraperitoneal glucose tolerance. Furthermore, peptides and the combination of peptides and C3G resulted in a significant reduction of percentage body fat and improved intraperitoneal glucose tolerance. As widely available, safe and nutritious foods, blueberry and yoghurt showed therapeutic potential in the treatment of obesity, diabetes and hypertension.

Sinden, D. S., et al. (2019). "Knockout of the X-linked Fgf13 in the hypothalamic paraventricular nucleus impairs sympathetic output to brown fat and causes obesity." FASEB Journal **33**(10): 11579-11594.

Fibroblast growth factor (FGF)13, a nonsecreted, X-linked, FGF homologous factor, is differentially expressed in adipocytes in response to diet, yet Fgf13's role in metabolism has not been explored. Heterozygous Fgf13 knockouts fed normal chow and housed at 22°C showed hyperactivity accompanying reduced core temperature and obesity when housed at 30°C. Those heterozygous knockouts showed defects in thermogenesis even at 30°C and an inability to protect core temperature. Surprisingly, we detected trivial FGF13 in adipose of wild-type mice fed normal chow and no obesity in adipose-specific heterozygous knockouts housed at 30°C, and we detected an intact brown fat response through exogenous β3 agonist stimulation, suggesting a defect in sympathetic drive to brown adipose tissue. In contrast, hypothalamic-specific ablation of Fgf13 recapitulated weight gain at 30°C. Norepinephrine turnover in brown fat was reduced at both housing temperatures. Thus, our data suggest that impaired CNS regulation of sympathetic activation of brown fat underlies obesity and thermogenesis in Fgf13 heterozygous knockouts fed normal chow.—Sinden, D. S., Holman, C. D., Bare, C. J., Sun, X., Gade, A. R., Cohen, D. E., Pitt, G. S. Knockout of the X-linked Fgf13 in the hypothalamic paraventricular nucleus impairs sympathetic output to brown fat and causes obesity. FASEB J. 33, 11579–11594 (2019). www.fasebj.org.

Skinner, N. J., et al. (2019). "Chronic Light Cycle Disruption Alters Central Insulin and Leptin Signaling as well as Metabolic Markers in Male Mice." Endocrinology **160**(10): 2257-2270.

Recent evidence suggests that the circadian timing system plays a role in energy and glucose homeostasis, and disruptions to this system are a risk factor for the development of metabolic disorders. We exposed animals to a constantly shifting lighting environment comprised of a 6-hour advance, occurring every 6 days, to chronically disrupt their circadian timing system. This treatment caused a gradual increase in body

weight of 12 ± 2% after 12 phase shifts, compared with a 6 ± 1% increase in mice under control lighting conditions. Additionally, after the fifth phase shift, light cycle-disrupted (CD) animals showed a reversal in their diurnal pattern of energy homeostasis and locomotor activity, followed by a subsequent loss of this rhythm. To investigate potential molecular mechanisms mediating these metabolic alterations, we assessed central leptin and insulin sensitivity. We discovered that CD mice had a decrease in central leptin signaling, as indicated by a reduction in the number of phosphorylated signal transducer and activator of transcription 3 immunoreactive cells in the arcuate nucleus of the hypothalamus. Furthermore, CD animals exhibited a marked increase in fasting blood glucose (269.4 ± 21.1 mg/dL) compared with controls (108.8 ± 21.3 mg/dL). This dramatic increase in fasting glucose levels was not associated with an increase in insulin levels, suggesting impairments in pancreatic insulin release. Peripheral hyperglycemia was accompanied by central alterations in insulin signaling at the level of phospho Akt and insulin receptor substrate 1, suggesting that light cycle disruption alters central insulin signaling. These results provide mechanistic insights into the association between light cycle disruption and metabolic disease.

Solon-Biet, S. M., et al. (2019). "Branched-chain amino acids impact health and lifespan indirectly via amino acid balance and appetite control." Nature Metabolism **1**(5): 532-545.

Elevated branched-chain amino acids (BCAAs) are associated with obesity and insulin resistance. How long-term dietary BCAAs impact late-life health and lifespan is unknown. Here, we show that when dietary BCAAs are varied against a fixed, isocaloric macronutrient background, long-term exposure to high BCAA diets leads to hyperphagia, obesity and reduced lifespan. These effects are not due to elevated BCAA per se or hepatic mammalian target of rapamycin activation, but instead are due to a shift in the relative quantity of dietary BCAAs and other amino acids, notably tryptophan and threonine. Increasing the ratio of BCAAs to these amino acids results in hyperphagia and is associated with central serotonin depletion. Preventing hyperphagia by calorie restriction or pair-feeding averts the health costs of a high-BCAA diet. Our data highlight a role for amino acid quality in energy balance and show that health costs of chronic high BCAA intakes need not be due to intrinsic toxicity but instead are a consequence of hyperphagia driven by amino acid imbalance.

Soto, J. E., et al. (2019). "Comparison of the Effects of High-Fat Diet on Energy Flux in Mice Using Two Multiplexed Metabolic Phenotyping Systems." Obesity **27**(5): 793-802.

Objective: Multiplexed metabolic phenotyping systems are available from multiple commercial vendors, and each system includes unique design features. Although expert opinion supports strengths and weaknesses of each design, empirical data from carefully controlled studies to test the biological impact of design differences are lacking. Methods: Wild-type C57BL/6J mice of both sexes underwent phenotyping in OxyMax (Columbus Instruments International) and Promethion (Sable Systems International) systems located within the same room of a newly constructed animal research facility in a crossover design study. Phenotypes were examined under chow (2920×)-fed conditions and again after 4 weeks of 60% high-fat diet (D12492) feeding. Results: Food intake, physical activity, and respiratory gas exchange data significantly diverged between systems, depending upon sex of animals and diet supplied. Estimates of energy expenditure based on gas exchange in both systems accounted for a fraction of consumed calories that was greater in males than females. Conclusions: Design differences quantitatively

impact the assessment of metabolic end points and thus the qualitative interpretation of various interventions. Importantly, current multiplexed systems remain blind to multiple additional end points, including digestive efficiency and selected forms of energy flux (nitrogenous, anaerobic, etc.), that account for a physiologically and/or pathophysiologically significant fraction of total energy flux.

Talton, O. O., et al. (2019). "Lean maternal hyperglycemia alters offspring lipid metabolism and susceptibility to diet-induced obesity in mice." Biology of Reproduction **100**(5): 1356-1369.

We previously developed a model of gestational diabetes mellitus (GDM) in which dams exhibit glucose intolerance, insulin resistance, and reduced insulin response to glucose challenge only during pregnancy, without accompanying obesity. Here, we aimed to determine how lean gestational glucose intolerance affects offspring risk of metabolic dysfunction. One cohort of offspring was sacrificed at 19 weeks, and one at 31 weeks, with half of the second cohort placed on a high-fat, high-sucrose diet (HFHS) at 23 weeks. Exposure to maternal glucose intolerance increased weights of HFHS-fed offspring. Chow-fed offspring of GDM dams exhibited higher body fat percentages at 4, 12, and 20 weeks of age. At 28 weeks, offspring of GDM dams fed the HFHS but not the chow diet (CD) also had higher body fat percentages than offspring of controls (CON). Exposure to GDM increased the respiratory quotient (Vol CO2/Vol O2) in offspring. Maternal GDM increased adipose mRNA levels of peroxisome proliferator-activated receptor gamma (Pparg) and adiponectin (Adipoq) in 31-week-old CD-fed male offspring, and increased mRNA levels of insulin receptor (Insr) and lipoprotein lipase (Lpl) in 31-week-old male offspring on both diets. In liver at 31 weeks, mRNA levels of peroxisome proliferator-activated receptor alpha (Ppara) were elevated in CD-fed male offspring of GDM dams, and male offspring of GDM dams exhibited higher mRNA levels of Insr on both diets. Neither fasting insulin nor glucose tolerance was affected by exposure to GDM. Our findings show that GDM comprising glucose intolerance only during pregnancy programs increased adiposity in offspring, and suggests increased insulin sensitivity of subcutaneous adipose tissue.

Thakali, K. M., et al. (2019). "Metabolic Consequences of Exposure to Maternal High Fat Diet in Offspring." The FASEB Journal **33**, 591-593.

The increased risk for obesity in offspring born to obese mothers may be driven by changes in energy expenditure. To examine the effects of maternal high fat diet (HFD)-induced obesity on offspring energy expenditure, female C57BL6/J mice were fed a control (17% fat, TD95092) or HFD (45% fat, TD8811) ad libitum for 12 weeks prior to and during pregnancy and during lactation. Female mice were bred with lean male mice at 17 wk of age. At weaning, male offspring from control and HFD-dams were randomized to control (C) or HFD (H), provided ad libitum, generating 4 groups of offspring: CC, CH, HC, and HH, where the first letter corresponds to maternal diet and the second to offspring post-weaning diet. At 20 weeks of age, energy intake and expenditure (EE) were assessed in male offspring over 48 h using the Promethion Continuous Indirect Calorimetry system (Sable Systems). HFD consumption in offspring was associated with reduced respiratory exchange ratio (RER, CC vs CH: 0.89±0.02 vs 0.82±0.02; $p<0.01$, n=13–16). There was no effect of maternal HFD on offspring RER (CC vs HC, $p= 0.66$; CH vs HH, $p=0.54$). To examine the effect of maternal HFD on offspring energy expenditure, ANCOVA using offspring total body weight (measured at the beginning of the indirect calorimety experiment) as a covariate for the 48 h recording period (both light and dark cycles) was performed. When comparing the effect of maternal HFD in offspring fed either a control diet (CC vs HC) or HFD (HC vs HH) postweaning, the covariate (offspring total body weight) significantly predicted EE for both comparisons (CC vs HC, $p<0.05$; HC vs HH, $p = 0.05$), while there was no significant effect of maternal diet on EE. Offspring food intake (in both light and dark cycles) was not altered by maternal HFD (CC vs HC, $p=0.22$; CH vs HH, $p=0.21$). In control-fed offspring, maternal HFD had no effect on body weight at 20 wk of age (CC vs CH, 26.84±0.79g vs 28.91±1.03g, $p=0.12$), while in HFD-fed offspring, males exposed to maternal HFD were significantly heavier than males from control-fed dams (CH vs HH, 33.81±1.46g vs 38.42±1.59g, $p=0.04$). The observed changes in RER indicate that offspring post-weaning HFD, but not maternal HFD, is associated with a shift in substrate utilization, specifically with increased fat oxidation in response to post-weaning HFD. In the context of maternal HFD, the EE data suggest that offspring total body weight, and not maternal obesity may be driving offspring changes in energy expenditure.

Trefts, E., et al. (2019). "Energy metabolism couples hepatocyte integrin-linked kinase to liver glucoregulation and postabsorptive responses of mice in an age-dependent manner." American Journal of Physiology - Endocrinology and Metabolism **316**(6): E1118-E1135.

Integrin-linked kinase (ILK) is a critical intracellular signaling node for integrin receptors. Its role in liver development is complex, as ILK deletion at E10.5 (before hepatocyte differentiation) results in biochemical and morphological differences that resolve as mice age. Nevertheless, mice with ILK depleted specifically in hepatocytes are protected from the hepatic insulin resistance during obesity. Despite the potential importance of hepatocyte ILK to metabolic health, it is unknown how ILK controls hepatic metabolism or glucoregulation. The present study tested the role of ILK in hepatic metabolism and glucoregulation by deleting it specifically in hepatocytes, using a cre-lox system that begins expression at E15.5 (after initiation of hepatocyte differentiation). These mice develop the most severe morphological and glucoregulatory abnormalities at 6 wk, but these gradually resolve with age. After identifying when the deletion of ILK caused a severe metabolic phenotype, in depth studies were performed at this time point to define the metabolic programs that coordinate control of glucoregulation that are regulated by

ILK. We show that 6-wk-old ILK-deficient mice have higher glucose tolerance and decreased net glycogen synthesis. Additionally, ILK was shown to be necessary for transcription of mito-chondrial-related genes, oxidative metabolism, and maintenance of cellular energy status. Thus, ILK is required for maintaining hepatic transcriptional and metabolic programs that sustain oxidative metabolism, which are required for hepatic maintenance of glucose homeostasis.

Wang, Z., et al. (2019). "Role of SOCS3 in POMC neurons in metabolic and cardiovascular regulation." American Journal of Physiology - Regulatory Integrative and Comparative Physiology **316**(4): R338-R351.

Suppressor of cytokine signaling 3 (SOCS3) is a negative regulator of leptin signaling. We previously showed that the chronic effects of leptin on blood pressure (BP) and glucose regulation are mediated by stimulation of proopiomelanocortin (POMC) neurons. In this study we examined the importance of endogenous SOCS3 in POMC neurons in control of metabolic and cardiovascular function and potential sex differences. Male and female SOCS3flox/flox/POMC-Cre mice in which SOCS3 was selectively deleted in POMC neurons and control SOCS3flox/flox mice were studied during a control diet (CD) or a high-fat diet (HFD) and during chronic leptin infusion. Body weight was lower in male and female SOCS3flox/flox/POMC-Cre than control mice fed the CD, despite similar food intake. Male SOCS3flox/flox/POMC-Cre mice exhibited increased energy expenditure. BP and heart rate were similar in male and female SOCS3flox/flox/POMC-Cre and control mice fed the CD. HFD-fed male and female SOCS3flox/flox/POMC-Cre mice showed attenuated weight gain. HFD-induced elevations in baseline BP and BP responses to an air-jet stress test were greater in female SOCS3flox/flox/ POMC-Cre than control mice. Chronic leptin infusion produced similar responses for food intake, body weight, oxygen consumption, blood glucose, BP, and heart rate in all groups. Thus SOCS3 deficiency in POMC neurons influences body weight regulation in the setting of CD and HFD and differentially affects BP and energy balance in a sex-specific manner but does not amplify the dietary, glycemic, or cardiovascular effects of leptin.

Yap, Y. W., et al. (2019). "Restriction of essential amino acids dictates the systemic response to dietary protein dilution." bioRxiv.

Dietary protein dilution (DPD) promotes metabolic remodelling and health but the precise nutritional components driving this response remain elusive. Here we demonstrate that dietary amino acids (AA) are sufficient and necessary to drive the response to DPD. In particular, the restriction of dietary essential AA (EAA) supply, but not non-EAA, drives the systemic metabolic response to total AA deprivation. Furthermore, systemic deprivation of Thr and Trp, independent of total AA supply, are both adequate and necessary to confer the systemic metabolic response to both diet, and genetic AA-transport loss, driven AA restriction. Thr is also potentially limiting in low-protein diet fed humans, and dietary Thr restriction (DTR) retarded the development of obesity-associated metabolic dysfunction in mice. Liver-derived fibroblast growth factor 21 was required for the metabolic remodelling with DTR. Strikingly, hepatocyte-selective establishment of Thr biosynthetic capacity reversed the systemic response to DTR. Taken together, our studies demonstrate that the restriction of EAA are sufficient and necessary to confer the systemic metabolic effects of DPD.

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Yuan, Y., et al. (2019). "α-ketoglutaric acid stimulates muscle hypertrophy and fat loss through OXGR1-dependent adrenal activation." bioRxiv.

> Beneficial effects of resistance exercise on metabolic health and particularly muscle hypertrophy and fat loss are well established, but the underlying chemical and physiological mechanisms are not fully understood. Here we identified a myometabolite-mediated metabolic pathway that is essential for the beneficial metabolic effects of resistance exercise in vivo. We showed that substantial accumulation of the tricarboxylic acid cycle intermediate α-ketoglutaric acid (AKG) is a metabolic signature of resistance exercise performance. Interestingly, human plasma AKG level is also negatively correlated with BMI. Pharmacological elevation of circulating AKG induces muscle hypertrophy, brown adipose tissue (BAT) thermogenesis, and white adipose tissue (WAT) lipolysis in vivo. We further found that AKG stimulates the adrenal release of adrenaline through 2-oxoglutarate receptor 1 (OXGR1) expressed in adrenal glands. Finally, by using both loss-of-function and gain-of-function mouse models, we showed that OXGR1 is essential for AKG-mediated exercise-induced beneficial metabolic effects. These findings reveal an unappreciated mechanism for the salutary effects of resistance exercise, using AKG as a systemically-derived molecule for adrenal stimulation of muscle hypertrophy and fat loss.

Zaidi, M. (2019). "Compositions and methods for reducing adiposity by inhibiting fsh/fshr." US Patent App. 16/317,352(WO 2018/022505 A1).

> The perimenopausal transition in women is associated with increases in total body fat and decrements in energy expenditure and physical activity, all of which impact quality of life. Late perimenopause in particular is characterized by a sharp increase in visceral adiposity, which coincides with the emergence of disrupted energy balance and reduced physical activity.

Perimenopause is also characterized by relatively stable estrogen and rising levels of follicle stimulating hormone (FSH), which become elevated as the ability to procreate ceases. FSH is a glycoprotein hormone synthesized and secreted by the pituitary. It causes the synthesis and secretion of estrogen by interacting with its receptor, the FSH receptor, on the follicular cell of the ovary. Estrogen levels, in turn, control FSH release from the pituitary through a well-known feedback mechanism. Thus, when estrogen rises, FSH falls. FSH has, however, never been implicated directly in causing an increase in adiposity. Accordingly, there exists a need to identify additional factors that contribute to increase in visceral adiposity and an urgent need for treatments to reduce such.

Zecharia, D., et al. (2019). "Postnatal administration of leptin antagonist mitigates susceptibility to obesity under high-fat diet in male αMUPA mice." American Journal of Physiology. Endocrinology and Metabolism **317**(5): E783-E793.

Perturbations in postnatal leptin signaling have been associated with altered susceptibility to diet-induced obesity (DIO) under high-fat-diet (HFD), albeit with contradicting evidence. Previous studies have shown that alpha murine urokinase-type plasminogen activator (αMUPA) mice have a higher and longer postnatal leptin surge compared with their wild types (WTs) as well as lower body weight and food intake under regular diet (RD). Here we explored αMUPA's propensity for DIO and the effect of attenuating postnatal leptin signaling with leptin antagonist (LA) on energy homeostasis under both RD and HFD. Four-day-old αMUPA pups were treated on alternate days until postnatal day 18 with either vehicle or LA (10 or 20 mg·day-1·kg-1) and weaned into RD or HFD. Compared with RD-fed αMUPA males, HFD-fed αMUPA males showed higher energy intake, even when corrected for body weight difference, and became hyperinsulinemic and obese. Additionally, HFD-fed αMUPA males gained body weight at a higher rate than their WTs mainly because of strain differences in energy expenditure. LA administration did not affect strain differences under RD but attenuated αMUPA's hyperinsulinemia and DIO under HFD, most likely by mediating energy expenditure. Together with our previous findings, these results suggest that αMUPA's leptin surge underlies its higher susceptibility to obesity under HFD, highlighting the role of leptin-related developmental processes in inducing obesity in a postweaning obesogenic environment, at least in αMUPA males. This study therefore supports the use of αMUPA mice for elucidating developmental mechanisms of obesity and the efficacy of early-life manipulations via leptin surge axis in attenuating DIO.

Zhang, F., et al. (2019). "Calcium Supplementation Alleviates High-Fat Diet-Induced Estrous Cycle Irregularity and Subfertility Associated with Concomitantly Enhanced Thermogenesis of Brown Adipose Tissue and Browning of White Adipose Tissue." Journal of Agricultural and Food Chemistry **67**(25): 7073-7081.

Obesity has been demonstrated as a disruptor of female fertility. Our previous study showed the antiobesity effects of calcium on HFD-fed male mice. However, the role of calcium in alleviating reproductive dysfunction of HFD-fed female mice remains unclear. Here, we found that HFD led to estrus cycle irregularity (longer cycle duration and shorter estrus period) and subfertility (longer conception time, lower fertility index, and less implantations) in mice. However, the HFD-induced reproductive

abnormality was alleviated by calcium supplementation. Additionally, calcium supplementation enhanced activation/thermogenesis of BAT and browning of WAT in HFD-fed mice. Consequently, the abnormality of energy metabolism and glucose homeostasis induced by HFD were improved by calcium supplementation, with elevated metabolic rates and core temperature. In conclusion, these data showed that calcium supplementation alleviated HFD-induced estrous cycle irregularity and subfertility associated with concomitantly enhanced BAT thermogenesis and WAT browning, suggesting the potential application of calcium in improving obesity-related reproductive disorders.

Zhang, W., et al. (2019). "GDF5 Promotes White Adipose Tissue Thermogenesis via p38 MAPK Signaling Pathway." DNA and Cell Biology **38**(11): 1303-1312.

Growth differentiation factor 5 (GDF5) was reported to regulate brown adipogenesis; however, its effects on insulin sensitivity, full metabolic syndrome spectrum, and the thermogenesis in subcutaneous white adipose tissue (sWAT) have not been elucidated yet. We thus generated fatty acid-binding protein 4 (Fabp4)-GDF5 transgenic (TG) mice and showed that GDF5 TG mice developed a relative lean phenotype on a high-fat diet (HFD) and showed increased insulin sensitivity. Over expression of GDF5 in adipose tissues greatly promoted the thermogenic process in sWAT after cold or β3-agonist treatment. In TG mice, sWAT showed an important thermogenic effect as the thermogenic gene expression was markedly increased, which was consistent with the typical features of beige adipocytes. Moreover, knockdown of the protein GDF5 impaired browning program in sWAT after thermogenic stimuli. Enhanced mitogen-activated protein kinase (MAPK)/activating transcription factor 2 (ATF2) signaling was also identified in sWAT of HFD-fed GDF5 mice, and thermogenesis in mature adipocytes induced by GDF5 protein could be partly blocked by a p38 MAPK inhibitor. Taken together, our data suggest that GDF5 could improve insulin sensitivity and prevent metabolic syndrome, the adaptive thermogenesis in sWAT could mediate the obesity resistance effects of GDF5 in mice and partially resulted in the activation of the p38 MAPK signaling pathway.

Alleleyn, A. M. E., et al. (2018). "The effect of an encapsulated nutrient mixture on food intake and satiety: A double-blind randomized cross-over proof of concept study." Nutrients **10**(11).

Activation of the intestinal brake by infusing nutrients into the distal small intestine with catheters inhibits food intake and enhances satiety. Encapsulation of macronutrients, which protects against digestion in the proximal gastrointestinal tract, can be a non-invasive alternative to activate this brake. In this study, we investigate the effect of oral ingestion of an encapsulated casein and sucrose mixture (active) targeting the distal small intestine versus a control product designed to be released in the stomach on food intake, satiety, and plasma glucose concentrations. Fifty-nine volunteers received the active and control product on two separate test days. Food intake was determined during an ad libitum meal 90 min after ingestion of the test product. Visual analogue scale scores for satiety and blood samples for glucose analysis were collected at regular intervals. Ingestion of the active product decreased food intake compared to the control product (655 kcal compared with 699 kcal, respectively, $p < 0.05$). The area under the curve (AUC) for hunger was decreased ($p < 0.05$) and AUC for satiety was increased ($p < 0.01$) after ingestion of the active product compared to the control product. Ingestion of an encapsulated protein-carbohydrate mixture resulted in inhibition of food intake compared to a non-encapsulated control product.

Alvarez, B., et al. (2018). "Effects on Hedonic Feeding, Energy Expenditure and Balance of the Non-opioid Peptide DYN-A2-17." Neuroscience **371**: 337-345.

The dynorphin (DYN) peptide family includes opioid and non-opioid peptides, yet the physiological role of the non-opioid DYN peptides remains poorly understood. Recent evidence shows that administering the non-opioid peptide DYN-A2-17 into the paraventricular hypothalamic nucleus (PVN) simultaneously increased short-term intake of standard rodent chow and spontaneous physical activity (SPA). The present studies aimed to expand upon the mechanisms and role of DYN-A2-17 on food intake and energy expenditure. Injection of DYN-A2-17 in PVN increased SPA, energy expenditure and wheel running in the absence of food. Repeated DYN-A2-17 injection in PVN increased short-term chow intake, but this effect habituated over time and failed to alter cumulative food intake, body weight or adiposity. Pre-treatment with a CRF receptor antagonist into PVN blocked the effects of DYN-A2-17 on food intake while injection of DYN-A2-17 in PVN increased plasma ACTH. Finally, as DYN peptides are co-released with orexin peptides, we compared the effects of DYN-A2-17 to orexin-A and the opioid peptide DYN-A1-13 on food choice and intake in PVN when palatable snacks and chow were available. DYN-A1-13 selectively increased intake of palatable snacks. DYN-A2-17 and orexin-A decreased palatable snack intake while orexin-A also increased chow intake. These findings demonstrate that the non-opioid peptide DYN-A2-17 acutely regulates physical activity, energy expenditure and food intake without long-term effects on energy balance. These data also propose different roles of opioid, non-opioid DYN and orexin peptides on food choice and intake when palatable and non-palatable food options are available.

Araujo, E. J. d. A., et al. (2018). "Su1208 - Is There Peri-Neuronal Net in the Enteric Nervous System?" Gastroenterology **154**(6): S-503.

All transcribed genes for PNN molecules are present in the GIT of mice, specifically HAS2, CD44, VCAN, TNX, TNC, HAPLN1 and HAPLN4. However, these molecules do not build PNN as described in the CNS, since we found no WFA labelling. These results suggest that the composition and/or organization of PNN in the ENS is different to the CNS.

Brown, J. D., et al. (2018). "Oleoylethanolamide modulates glucagon-like peptide-1 receptor agonist signaling and enhances exendin-4-mediated weight loss in obese mice." American Journal of Physiology. Regulatory, Integrative and Comparative Physiology **315**(4): R595-R608.

Long-acting glucagon-like peptide-1 (GLP-1) receptor (GLP-1R) agonists (GLP-1RA), such as exendin-4 (Ex4), promote weight loss. On the basis of a newly discovered interaction between GLP-1 and oleoylethanolamide (OEA), we tested whether OEA enhances GLP-1RA-mediated anorectic signaling and weight loss. We analyzed the effect of GLP-1+OEA and Ex4+OEA on canonical GLP-1R signaling and other proteins/pathways that contribute to the hypophagic action of GLP-1RA (AMPK, Akt, mTOR, and glycolysis). We demonstrate that OEA enhances canonical GLP-1R signaling when combined with GLP-1 but not with Ex4. GLP-1 and Ex4 promote phosphorylation of mTOR pathway components, but OEA does not enhance this effect. OEA synergistically enhanced GLP-1- and Ex4-stimulated glycolysis but did not augment the hypophagic action of GLP-1 or Ex4 in lean or diet-induced obese (DIO) mice. However, the

combination of Ex4+OEA promoted greater weight loss in DIO mice than Ex4 or OEA alone during a 7-day treatment. This was due in part to transient hypophagia and increased energy expenditure, phenotypes also observed in Ex4-treated DIO mice. Thus, OEA augments specific GLP-1RA-stimulated signaling but appears to work in parallel with Ex4 to promote weight loss in DIO mice. Elucidating cooperative mechanisms underlying Ex4+OEA-mediated weight loss could, therefore, be leveraged toward more effective obesity therapies.

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Campisi, J., et al. (2018). "Transgenic mouse for determining the role of senescent cells in cancer." US Patent

This invention provides a transgenic mouse with a p16INK4a promoter sequence that controls expression of a protein such that it is expressed preferentially in senescent cells. The protein either directly induces apoptosis, or converts a prodrug to a cytotoxic compound. In addition, the mouse is injected with syngeneic tumor cells, or has second transgene that causes tumors to form. Removing senescent cells from the mouse may result in the formation of fewer tumors.

Carvalho, A. L., et al. (2018). "High fat diet attenuates hyperglycemia, body composition changes, and bone loss in male streptozotocin-induced type 1 diabetic mice." Journal of Cellular Physiology **233**(2): 1585-1600.

There is a growing and alarming prevalence of obesity and the metabolic syndrome in type I diabetic patients (T1DM), particularly in adolescence. In general, low bone mass, higher fracture risk, and

increased marrow adipose tissue (MAT) are features of diabetic osteopathy in insulin-deficient subjects. On the other hand, type 2 diabetes (T2DM) is associated with normal or high bone mass, a greater risk of peripheral fractures, and no change in MAT. Therefore, we sought to determine the effect of weight gain on bone turnover in insulin-deficient mice. We evaluated the impact of a 6-week high-fat (HFD) rich in medium chain fatty acids or low-fat diet (LFD) on bone mass and MAT in a streptozotocin (STZ)-induced model using male C57BL/6J mice at 8 weeks of age. Dietary intervention was initiated after diabetes confirmation. At the endpoint, lower non-fasting glucose levels were observed in diabetic mice fed with high fat diet compared to diabetic mice fed the low fat diet (STZ-LFD). Compared to euglycemic controls, the STZ-LFD had marked polydipsia and polyphagia, as well as reduced lean mass, fat mass, and bone parameters. Interestingly, STZ-HFD mice had higher bone mass, namely less cortical bone loss and more trabecular bone than STZ-LFD. Thus, we found that a HFD, rich in medium chain fatty acids, protects against bone loss in a T1DM mouse model. Whether this may also translate to T1DM patients who are overweight or obese in respect to maintenance of bone mass remains to be determined through longitudinal studies.

Chen, X., et al. (2018). "The diabetes gene and wnt pathway effector TCF7L2 regulates adipocyte development and function." Diabetes **67**(4): 554-568.

The gene encoding for transcription factor 7-like 2 (TCF7L2) is the strongest type 2 diabetes mellitus (T2DM) candidate gene discovered to date. The TCF7L2 protein is a key transcriptional effector of the Wnt/b-catenin signaling pathway, which is an important developmental pathway that negatively regulates adipogenesis. However, the precise role that TCF7L2 plays in the development and function of adipocytes remains largely unknown. Using a combination of in vitro approaches, we first show that TCF7L2 protein is increased during adipogenesis in 3T3-L1 cells and primary adipocyte stem cells and that TCF7L2 expression is required for the regulation of Wnt signaling during adipogenesis. Inactivation of TCF7L2 protein by removing the high-mobility group (HMG)-box DNA binding domain in mature adipocytes in vivo leads to whole-body glucose intolerance and hepatic insulin resistance. This phenotype is associated with increased subcutaneous adipose tissue mass, adipocyte hypertrophy, and inflammation. Finally, we demonstrate that TCF7L2 mRNA expression is downregulated in humans with impaired glucose tolerance and adipocyte insulin resistance, highlighting the translational potential of these findings. In summary, our data indicate that TCF7L2 has key roles in adipose tissue development and function that may reveal, at least in part, how TCF7L2 contributes to the pathophysiology of T2DM.

Christie, S., et al. (2018). "A rotating light cycle promotes weight gain and hepatic lipid storage in mice." American Journal of Physiology - Gastrointestinal and Liver Physiology **315**(6): G932-G942.

Processes involved in regulation of energy balance and intermediary metabolism are aligned to the light-dark cycle. Shift-work and high-fat diet (HFD)-induced obesity disrupt circadian rhythmicity and are associated with increased risk of nonalcoholic fatty liver disease. This study aimed to determine the effect of simulating shift work on hepatic lipid accumulation in lean and HFD mice. C57BL/6 mice fed a standard laboratory diet (SLD) or HFD for 4 wk were further allocated to a normal light (NL) cycle (lights on: 0600–1800) or rotating light (RL) cycle [3 days NL and 4 days reversed (lights on: 1800–0600) repeated] for 8 wk. Tissue was collected every 3 h beginning at 0600. HFD mice gained more weight than SLD mice, and RL

mice gained more weight than NL mice. SLD-NL and HFD-NL mice, but not RL mice, were more active, had higher respiratory quotients, and consumed/expended more energy during the dark phase compared with the light phase. Blood glucose and plasma cholesterol and triglyceride concentrations were elevated in HFD and SLD-RL compared with SLD-NL mice. Hepatic glycogen was elevated in HFD compared with SLD mice. Hepatic triglycerides were elevated in SLD-RL and HFD mice compared with SLD-NL. Circadian rhythmicity of hepatic acetyl-CoA carboxylase (ACACA) mRNA was phase shifted in SLD-RL and HFD-NL and lost in HFD-RL mice. Hepatic ACACA protein was reduced in SLD-RL and HFD mice compared with SLD-NL mice. Hepatic adipose triglyceride lipase was elevated in HFD-NL compared with SLD-NL but lower in RL mice compared with NL mice irrespective of diet. In conclusion, an RL cycle model of shift work promotes weight gain and hepatic lipid storage even in lean conditions. NEW & NOTEWORTHY In this publication we describe the effects of a rotating light cycle model of shift work in lean and high-fat diet-induced obese mice on body mass, diurnal patterns of energy intake and expenditure, and hepatic lipid storage. The data indicate that modeling shift work, via a rotating light cycle, promotes weight gain and hepatic lipid accumulation even in mice on a standard laboratory diet.

Clayton, Z. (2018). The Role of Pik3r1 in the Regulation of Adipose Tissue Insulin Sensitivity, scholarsbank.uoregon.edu.

… Food Intake and Total Energy Expenditure. Using a metabolic monitoring system (Promethion, Sable Systems, Las Vegas, NV), we measured cage behavior (food intake, water intake and activity), oxygen, carbon dioxide and water vapor during the 12:12-h …

Clayton, Z. S. and C. E. McCurdy (2018). "Short-term thermoneutral housing alters glucose metabolism and markers of adipose tissue browning in response to a high-fat diet in lean mice." American Journal of Physiology - Regulatory Integrative and Comparative Physiology **315**(4): R627-R637.

Systemic insulin resistance and glucose intolerance occur with as little as 3 days of a high-fat diet (HFD) in mice and humans; the mechanisms that initiate acute insulin resistance are unknown. Most laboratories house mice at 22°C, which is below their thermoneutral temperature (~30°C). Cold stress has been shown to increase white adipose tissue (WAT) browning, alter lipid trafficking, and impair immune function, whereas energy intake and expenditure decrease with increasing ambient temperature; importantly, dysregulation of these parameters has been strongly linked to obesity-induced insulin resistance. Therefore, we compared acute changes in glucose metabolism and the metabolic phenotype in lean mice in response to a control diet or HFD housed at standard vivarium (22°C) and thermoneutral (30°C) temperatures. Glucose intolerance occurred following 1 or 5 days of HFD and was independent of housing temperature or adiposity; however, the reduction in tissue-specific glucose clearance with HFD diverged by temperature with reduced brown adipose tissue (BAT) glucose uptake at 22°C but reduced soleus glucose uptake at 30°C. Fasting glucose, food intake, and energy expenditure were significantly lower at 30°C, independent of diet. Additionally, markers of browning in both BAT and inguinal subcutaneous WAT, but not perigonadal epididymal WAT, decreased at 30°C. Together, we find housing temperature has a significant impact on the cellular pathways that regulate glucose tolerance in response to an acute HFD exposure. Thus, even short-term changes in housing temperature should be highly considered in interpretation of metabolic studies in mice.

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Clookey, S. (2018). Estrogen signaling and UCP1: exploring mechanisms to improve metabolic health, mospace.umsystem.edu.

> Page 1. ESTROGEN SIGNALING AND UCP1: EXPLORING MECHANISMS TO IMPROVE METABOLIC HEALTH A Thesis presented to the Faculty of the Graduate School at the University of Missouri-Columbia In Partial Fulfillment of the Requirement for the Degree …

Clookey, S. L., et al. (2018). "Increased susceptibility to OVX-associated metabolic dysfunction in UCP1-null mice." Journal of Endocrinology **239**(2): 107-120.

> Premenopausal females are protected against adipose tissue inflammation and insulin resistance, until loss of ovarian hormone production (e.g., menopause). There is some evidence that females have greater brown adipose tissue (BAT) thermogenic capacity. Because BAT mass correlates inversely with insulin resistance, we hypothesized that increased uncoupling protein 1 (UCP1) expression contributes to the superior metabolic health of females. Given that UCP1 transiently increases in BAT following ovariectomy (OVX), we hypothesized that UCP1 may ebufferf OVX-mediated metabolic dysfunction. Accordingly, female UCP1-knockout (KO) and WT mice received OVX or sham (SHM) surgeries at 12 weeks of age creating four groups (n = 10/group), which were followed for 14 weeks and compared for body weight

and adiposity, food intake, energy expenditure and spontaneous physical activity (metabolic chambers), insulin resistance (HOMA-IR, ADIPO-IR and glucose tolerance testing) and adipose tissue phenotype (histology, gene and protein expression). Two-way ANOVA was used to assess the main effects of genotype (G), OVX treatment (O) and genotype by treatment (GxO) interactions, which were considered significant when $P \leq 0.05$. UCP1KO mice experienced a more adverse metabolic response to OVX than WT. Whereas OVX-induced weight gain was not synergistically greater for KO compared to WT (GxO, NS), OVX-induced insulin resistance was significantly exacerbated in KO compared to WT (GxO for HOMA-IR, $P < 0.05$). These results suggest UCP1 is protective against metabolic dysfunction associated with loss of ovarian hormones and support the need for more research into therapeutics to selectively target UCP1 for prevention and treatment of metabolic dysfunction following ovarian hormone loss.

Cooper, M. A., et al. (2018). "Intrinsic Activity of C57BL/6 Substrains Associates with High-Fat Diet-Induced Mechanical Sensitivity in Mice." Journal of Pain **19**(11): 1285-1295.

Pain is significantly impacted by the increasing epidemic of obesity and the metabolic syndrome. Our understanding of how these features impact pain is only beginning to be developed. Herein, we have investigated how small genetic differences among C57BL/6 mice from 2 different commercial vendors lead to important differences in the development of high-fat diet-induced mechanical sensitivity. Two substrains of C57BL/6 mice from Jackson Laboratories (Bar Harbor, ME; C57BL/6J and C57BL/6NIH), as well as C57BL/6 from Charles Rivers Laboratories (Wilmington, MA; C57BL/6CR) were placed on high-fat diets and analyzed for changes in metabolic features influenced by high-fat diet and obesity, as well as measures of pain-related behaviors. All 3 substrains responded to the high-fat diet; however, C57BL/6CR mice had the highest weights, fat mass, and impaired glucose tolerance of the 3 substrains. In addition, the C57BL/6CR mice were the only strain to develop significant mechanical sensitivity over the course of 8 weeks. Importantly, the C57BL/6J mice were protected from mechanical sensitivity, which may be based on increased physical activity compared with the other 2 substrains. These findings suggest that activity may play a powerful role in protecting metabolic changes associated with a high-fat diet and that these may also be protective in pain-associated changes as a result of a high-fat diet. These findings also emphasize the importance of selection and transparency in choosing C57BL/6 substrains in pain-related research. Perspective: Obesity and the metabolic syndrome play an important role in pain. This study identifies key differences in the response to a high-fat diet among substrains of C57BL/6 mice and differences in intrinsic physical activity that may influence pain sensitivity. The results emphasize physical activity as a powerful modulator of obesity-related pain sensitivity.

Cumnock, K., et al. (2018). "Host Energy Source Is Important for Disease Tolerance to Malaria." Current Biology **28**(10): 1635-1642.e1633.

Pathologic infections are accompanied by a collection of short-term behavioral perturbations collectively termed sickness behaviors [1, 2]. These include changes in body temperature, reduced eating and drinking, and lethargy and mimic behaviors of animals in torpor and hibernation [1, 3–6]. Sickness behaviors are important, pathogen-specific components of the host response to infection [1, 3, 7–9]. In particular, host anorexia has been shown to be beneficial or detrimental depending on the infection [7, 8]. While these studies have illuminated the effects of anorexia on infection, they consider this behavior

in isolation from other behaviors and from its effects on host metabolism and energy. Here, we explored the temporal dynamics of multiple sickness behaviors and their effect on host energy and metabolism throughout infection. We used the Plasmodium chabaudi AJ murine model of malaria as it causes severe pathology from which most animals recover. We found that infected animals did become anorexic, skewing their metabolism toward fatty acid oxidation and ketosis. Metabolism of fats requires oxygen for the production of ATP. In this model, animals also suffer severe anemia, limiting their ability to carry oxygen concurrent with their switch toward fatty acid metabolism. We reasoned that the combination of anorexia and anemia would increase pressure on glycolysis as a critical energy pathway because it does not require oxygen. Treating infected mice when anorexic with the glycolytic inhibitor 2-deoxyglucose (2DG) reduced survival; treating animals with glucose improved survival. Peak parasite loads were unchanged, demonstrating changes in disease tolerance. Parasite clearance was reduced with 2DG treatment, suggesting altered resistance. Cumnock et al. find that mice suffering from malaria face metabolic challenges created by their symptoms. The mice become anorexic and try to burn fat, requiring oxygen. The mice also become anemic, limiting oxygen transport. This suggests that glycolysis is a critical energy pathway. Manipulation of glycolysis modulates survival of the infected mice.

Deem, J. D., et al. (2018). "Leptin regulation of core body temperature involves mechanisms independent of the thyroid axis." American Journal of Physiology - Endocrinology and Metabolism **315**(4): E552-E564.

The ability to maintain core temperature within a narrow range despite rapid and dramatic changes in environmental temperature is essential for the survival of free-living mammals, and growing evidence implicates an important role for the hormone leptin. Given that thyroid hormone plays a major role in thermogenesis and that circulating thyroid hormone levels are reduced in leptin-deficient states (an effect partially restored by leptin replace-ment), we sought to determine the extent to which leptin's role in thermogenesis is mediated by raising thyroid hormone levels. To this end, we 1) quantified the effect of physiological leptin replacement on circulating levels of thyroid hormone in leptin-deficient ob/ob mice, and 2) determined if the effect of leptin to prevent the fall in core temperature in these animals during cold exposure is mimicked by administration of a physiological replacement dose of triiodothyronine (T3). We report that, as with leptin, normalization of circulating T3 levels is sufficient both to increase energy expenditure, respiratory quotient, and ambulatory activity and to reduce torpor in ob/ob mice. Yet, unlike leptin, infusing T3 at a dose that normalizes plasma T3 levels fails to prevent the fall of core temperature during mild cold exposure. Because thermal conductance (e.g., heat loss to the envi-ronment) was reduced by administration of leptin but not T3, leptin regulation of heat dissipation is implicated as playing a uniquely important role in thermoregulation. Together, these findings identify a key role in thermoregulation for leptin-mediated suppression of thermal conduction via a mechanism that is independent of the thyroid axis.

Desai, A., et al. (2018). "Regulation of fatty acid trafficking in liver by thioesterase superfamily member 1." Journal of Lipid Research **59**(2): 368-379.

Thioesterase superfamily member 1 (Them1) is an acyl-CoA thioesterase that is highly expressed in brown adipose tissue, where it functions to suppress energy expenditure. Lower Them1 expression levels in the

liver are upregulated in response to high-fat feeding. Them1/ mice are resistant to diet-induced obesity, hepatic steatosis, and glucose intolerance, but the contribution of Them1 in liver is unclear. To examine its liver-specific functions, we created conditional transgenic mice, which, when bred to Them1/ mice and activated, expressed Them1 exclusively in the liver. Mice with liver-specific Them1 expression exhibited no changes in energy expenditure. Rates of fatty acid oxidation were increased, whereas hepatic VLDL triglyceride secretion rates were decreased by hepatic Them1 expression. When fed a high-fat diet, Them1 expression in liver promoted excess steatosis in the setting of reduced rates of fatty acid oxidation and preserved glycerolipid synthesis. Liver-specific Them1 expression did not influence glucose tolerance or insulin sensitivity, but did promote hepatic gluconeogenesis in high-fat-fed animals. This was attributable to the generation of excess fatty acids, which activated PPAR and promoted expression of gluconeogenic genes. These findings reveal a regulatory role for Them1 in hepatocellular fatty acid trafficking.

DiTacchio, K. A., et al. (2018). "Hepatic JARID1a ablation disrupts the transcription adaptation to feeding and alters systemic metabolism." bioRxiv.

The liver is a key regulator of systemic energy homeostasis whose proper function is dependent on the circadian clock. Here, we show that livers deficient in the oscillator component JARID1a exhibit a dysregulation of genes involved in energy metabolism. Importantly, we find that mice that lack hepatic JARID1a have decreased lean body mass, decreased respiratory exchange ratios, faster production of ketones and increased glucose production in response to fasting. Finally, we find that JARID1a loss compromises the response of the hepatic transcriptome to nutrient availability. In all, ablation of hepatic JARID1a disrupts the coordination of hepatic metabolic programs with whole-body consequences.

Eggink, H. M., et al. (2018). "Chronic infusion of taurolithocholate into the brain increases fat oxidation in mice." Journal of Endocrinology **236**(2): 85-97.

Bile acids can function in the postprandial state as circulating signaling molecules in the regulation of glucose and lipid metabolism via the transmembrane receptor TGR5 and nuclear receptor FXR. Both receptors are present in the central nervous system, but their function in the brain is unclear. Therefore, we investigated the effects of intracerebroventricular (i.c.v.) administration of taurolithocholate (tLCA), a strong TGR5 agonist, and GW4064, a synthetic FXR agonist, on energy metabolism. We determined the effects of chronic i.c.v. infusion of tLCA, GW4064, or vehicle on energy expenditure, body weight and composition as well as tissue specific fatty acid uptake in mice equipped with osmotic minipumps. We found that i.c.v. administration of tLCA (final concentration in cerebrospinal fluid: 1 µM) increased fat oxidation (tLCA group: 0.083 ± 0.006 vs control group: 0.036 ± 0.023 kcal/h, F = 5.46, P = 0.04) and decreased fat mass (after 9 days of tLCA infusion: 1.35 ± 0.13 vs controls: 1.96 ± 0.23 g, P = 0.03). These changes were associated with enhanced uptake of triglyceride-derived fatty acids by brown adipose tissue and with browning of subcutaneous white adipose tissue. I.c.v. administration of GW4064 (final concentration in cerebrospinal fluid: 10 µM) did not affect energy metabolism, body composition nor bile acid levels, negating a role of FXR in the central nervous system in metabolic control. In conclusion, bile acids such as tLCA may exert metabolic effects on fat metabolism via the brain.

Ehrlicher, S. E., et al. (2018). "Skeletal muscle autophagy remains responsive to hyperinsulinemia and hyperglycemia at higher plasma insulin concentrations in insulin-resistant mice." Physiological Reports **6**(14).

Skeletal muscle autophagy is suppressed by insulin, but it is not clear if such suppression is altered with insulin resistance. We investigated if the inhibitory action of insulin on autophagy remains intact despite insulin resistance to glucose metabolism. C57BL/6J mice consumed either a low-fat (10% fat) diet as control or high-fat (60% fat) diet for 12 weeks to induce insulin resistance. Following a 5-hour fast, mice underwent either hyperinsulinemic-euglycemic, hyperinsulinemic-hyperglycemic, or saline infusion to test the effect of insulin on autophagy markers in the quadriceps muscle (n = 8–10 per diet and clamp condition). Mice were anesthetized by sodium pentobarbital for tissue collection after 2 h of infusion. Despite the high-fat group having lower insulin-stimulated glucose uptake, both low-fat and high-fat groups had similar autophagosome abundance during hyperinsulinemic conditions. The lipidation of microtubule-associated proteins 1A/1B light chain 3B (LC3II/LC3I) was decreased in hyperinsulinemia versus saline control ($P < 0.01$) in low-fat (−54%) and high-fat groups (−47%), demonstrating similar suppression of autophagy between diet groups. Mitochondrial-associated LC3II was greater in the high-fat compared to the low-fat group ($P = 0.045$) across clamp conditions, suggesting a greater localization of autophagosomes with mitochondria. L6 myotubes were treated with insulin and rapamycin to determine the role of mechanistic target of rapamycin complex-1 (mTORC1) in insulin-mediated suppression of autophagy. Inhibition of mTORC1 blunted the decline of LC3II/LC3I with insulin by 40%, suggesting mTORC1 partially mediates the insulin action to suppress autophagy. Collectively, autophagy remained responsive to the suppressive effects of insulin in otherwise insulin-resistant and obese mice.

Evans, M. C., et al. (2018). "Leptin and insulin do not exert redundant control of metabolic or emotive function via dopamine neurons." Hormones and Behavior **106**: 93-104.

Leptin and insulin's hunger-suppressing and activity-promoting actions on hypothalamic neurons are well characterized, yet the mechanisms by which they modulate the midbrain dopamine system to influence energy balance remain less clear. A subset of midbrain dopamine neurons express receptors for leptin (Lepr) and insulin (Insr). Leptin-dopamine signaling reduces running reward and homecage activity. However, dopamine-specific deletion of Lepr does not affect body weight or food intake in mice. We hypothesized insulin-dopamine signaling might compensate for disrupted leptin-dopamine signaling. To investigate the degree to which insulin and leptin exert overlapping (i.e. redundant) versus discrete control over dopamine neurons, we generated transgenic male and female mice exhibiting dopamine-specific deletion of either Lepr (Lepr KO), Insr (Insr KO) or both Lepr and Insr (Dbl KO) and assessed their feeding behavior, voluntary activity, and energy expenditure compared to control mice. No differences in body weight, daily food intake, energy expenditure or hyperphagic feeding of palatable chow were observed between Lepr, Insr or Dbl KO mice and control mice. However, consistent with previous findings, Lepr KO (but not Insr or Dbl KO) male mice exhibited significantly increased running wheel activity compared to controls. These data demonstrate that insulin and leptin do not exert redundant control of dopamine neuron-mediated modulation of energy balance. Furthermore, our results indicate neither leptin nor insulin plays a critical role in the modulation of dopamine neurons regarding hedonic feeding behavior or anxiety-related behavior.

Farzi, A., et al. (2018). "Arcuate nucleus and lateral hypothalamic cart neurons in the mouse brain exert opposing effects on energy expenditure." eLife **7**.

Cocaine- and amphetamine-regulated transcript (CART) is widely expressed in the hypothalamus and an important regulator of energy homeostasis; however, the specific contributions of different CART neuronal populations to this process are not known. Here, we show that depolarization of mouse arcuate nucleus (Arc) CART neurons via DREADD technology decreases energy expenditure and physical activity, while it exerts the opposite effects in CART neurons in the lateral hypothalamus (LHA). Importantly, when stimulating these neuronal populations in the absence of CART, the effects were attenuated. In contrast, while activation of CART neurons in the LHA stimulated feeding in the presence of CART, endogenous CART inhibited food intake in response to Arc CART neuron activation. Taken together, these results demonstrate anorexigenic but anabolic effects of CART upon Arc neuron activation, and orexigenic but catabolic effects upon LHA-neuron activation, highlighting the complex and nuclei-specific functions of CART in controlling feeding and energy homeostasis.

Fletcher, J. A., et al. (2018). "Fibroblast growth factor 21 increases hepatic oxidative capacity but not physical activity or energy expenditure in hepatic peroxisome proliferator-activated receptor γ coactivator-1α-deficient mice." Experimental Physiology **103**(3): 408-418.

New Findings: What is the central question of this study? Does a reduction in hepatic peroxisome proliferator-activated receptor γ coactivator-1α (PGC-1α), which has been observed in an insulin-resistant obese state, impair the ability of fibroblast growth factor 21 (FGF21) to modulate metabolism? What is the main finding and its importance? A deficit in hepatic PGC-1α does not compromise the ability of FGF21 to increase hepatic fatty acid oxidation; however, the effects of FGF21 to regulate whole-body metabolism (i.e. total and resting energy expenditure), as well as ambulatory activity, were altered when hepatic PGC-1α was reduced. Abstract: Fibroblast growth factor 21 (FGF21) treatment drives metabolic improvements, including increased metabolic flux and reduced hepatic steatosis, but the mechanisms responsible for these effects remain to be elucidated fully. We tested whether a targeted reduction in hepatic peroxisome proliferator-activated receptor γ coactivator-1α (PGC-1α), which has been shown to occur with obesity, had a negative impact on the metabolic effects of FGF21. We infused FGF21 (1 mg kg−1 day−1) or saline in chow-fed wild-type (WT) and liver-specific PGC-1α heterozygous (LPGC-1α) mice for 4 weeks. Administration of FGF21 lowered serum insulin and cholesterol ($P \leq 0.05$) and tended to lower free fatty acids ($P = 0.057$). The LPGC-1α mice exhibited reduced complete hepatic fatty acid oxidation (FAO; LPGC-1α, 1788 ± 165 nmol g−1 h−1 compared with WT, 2572 ± 437 nmol g−1 h−1; $P < 0.001$), which was normalized by FGF21 treatment (2788 ± 519 nmol g−1 h−1; $P < 0.001$). FGF21 also increased hepatic incomplete FAO by 12% in both groups and extramitochondrial FAO by 89 and 56% in WT and LPGC-1α mice, respectfully ($P = 0.001$), and lowered hepatic triacylglycerol by 30–40% ($P < 0.001$). Chronic treatment with FGF21 lowered body weight and fat mass ($P < 0.05$), while increasing food consumption ($P < 0.05$), total energy expenditure [7.3 ± 0.60 versus 6.6 ± 0.39 kcal (12 h)−1 in WT mice; $P = 0.009$] and resting energy expenditure [5.4 ± 0.89 versus 4.6 ± 0.21 kcal (12 h)−1 in WT mice; $P = 0.005$]. Interestingly, FGF21 only increased ambulatory activity in the WT mice ($P = 0.03$), without a concomitant increase in non-resting energy expenditure. In conclusion, although reduced hepatic PGC-1α expression was not necessary for FGF21 to increase FAO, it does appear to mediate FGF21-induced changes in total and resting energy expenditure and ambulatory activity in lean mice.

Gabalski, A., et al. (2018). "Su1209 - Adrenomedullin (ADM) Regulates Feeding Behavior, Energy Balance and Metabolic Hormone Profile." Gastroenterology **154**(6): S-503.

Introduction: Adrenomedullin (ADM) is a 52 amino acid peptide abundantly expressed throughout the gastrointestinal tract, in which it has been shown to inhibit gastric secretion and emptying as well as to modulate intestinal mucosa absorption, circulation, healing and motility. Our aims are to investigate the peripheral effects of ADM treatment on food and water intake, feeding behavior, respirometry, energy expenditure and metabolic hormones. Method(s): C57/Bl6 wild type (16 males and 15 females), age and sex matched mice (10-12 weeks old) were studied. Mice were fed a standard diet, temperature; humidity and light/dark cycle were kept consistent. Mice were divided in two groups; single housed in Promethion metabolic cages (Sable Systems, Las Vegas, NV) and after 5 days of acclimation, parameters recording in parallel was initiated. Non-restricted and ad libitum access to food hopper and water were allowed throughout the study. Murine ambulatory activity and physical positions were detected with XYZ beam arrays using a beam spacing of 0.25 cm. Respiratory gases were measured with an integrated fuel cell oxygen analyzer, spectrophotometric CO2 analyzer, and capacitive water vapor partial pressure analyzer. One group was injected intraperitoneally with a 10muM dose of ADM while the other group was injected with saline as control. VO2, VCO2, Respiratory Quotient (RQ), as well as food and water consumption, total energy expenditure (TEE) and physical activity were measured continuously for a 48-hour period after each injection. Result(s): An ADM ip treatment with (10 muM in 200 mul of saline) in WT mice significantly increased VH2O content within the metabolic cage during the post injection12-hour light cycle interval as compared to saline injected controls (245.5+/- 45.78 vs. 69.98+/-4.63; p=0.001). The respirometry data revealed a significantly higher VCO2 in the ADM injected mice (411.2+/-24.69 vs 368.6+/- 12.67; p=0.01) during the 12 hour dark cycle post injection. No significant differences were measured in food and water intake between the two groups. Plasma metabolic hormone measurements in mice 1 hour post injection showed that ADM treated animals had lower levels of Ghrelin (670 vs.1301), Glucagon (64.1 vs. 222.9) and Resistin (6988 vs.9622.2) in mice independant of sex. Conclusion(s): ADM injected in WT mice significantly increases VH2O content. This is the first study to demonstrate that ADM regulates energy balance, body metabolism and metabolic hormones.

Ghamari-Langroudi, M., et al. (2018). "Regulation of energy rheostasis by the melanocortin-3 receptor." Science Advances **4**(8).

Like most homeostatic systems, adiposity in mammals is defended between upper and lower boundary conditions. While leptin and melanocortin-4 receptor (MC4R) signaling are required for defending energy set point, mechanisms controlling upper and lower homeostatic boundaries are less well understood. In contrast to the MC4R, deletion of the MC3R does not produce measurable hyperphagia or hypometabolism under normal conditions. However, we demonstrate that MC3R is required bidirectionally for controlling responses to external homeostatic challenges, such as caloric restriction or calorie-rich diet. MC3R is also required for regulated excursion from set point, or rheostasis, during pregnancy. Further, we demonstrate a molecular mechanism: MC3R provides regulatory inputs to melanocortin signaling, acting presynaptically on agouti-related protein neurons to regulate -

aminobutyric acid release onto anorexigenic MC4R neurons, exerting boundary control on the activity of MC4R neurons. Thus, the MC3R is a critical regulator of boundary controls on melanocortin signaling, providing rheostatic control on energy storage.

Ghielmetti, V., et al. (2018). "Food intake and energy expenditure in growing cats with and without a predisposition to overweight." Journal of Animal Physiology and Animal Nutrition **102**(5): 1401-1410.

Overweight and obesity are multifactorial diseases caused by an imbalance in energy metabolism. An underlying genetic predisposition is often a factor in these conditions. In the cat breeding family of the Institute of Animal Nutrition at the Vetsuisse Faculty, University of Zurich, a segregating overweight phenotype with a genetic contribution was observed. From this breeding family, 26 kittens were followed from birth up to 8 months of age. During this time, food intake was measured using an automatic feeding station, and energy expenditure was investigated using indirect calorimetry at the ages of 4 and 6 months. Dual-energy X-ray absorptiometry (DEXA) was performed and blood glucose, leptin and insulin were measured at the ages of 4, 6 and 8 months. The kittens were also weighed daily for the first 2 weeks of life, every second day until weaning and once per week until 8 months of age. The body condition score (BCS) was evaluated monthly between 2 and 8 months of age. The main finding of this study is that a predisposition to overweight is connected to a higher food intake early in life, with no significant alterations in energy expenditure. The leptin blood levels were related to body fat percentage, and insulin sensitivity did not seem to be affected.

Grandl, F., et al. (2018). "Evidence for increasing digestive and metabolic efficiency of energy utilization with age of dairy cattle as determined in two feeding regimes." Animal **12**(3): 515-527.

The changes taking place with age in energy turnover of dairy cattle are largely unknown. It is unclear whether the efficiency of energy utilization in digestion (characterized by faecal and methane energy losses) and in metabolism (characterized by urine and heat energy losses) is altered with age. In the present study, energy balance data were obtained from 30 lactating Brown Swiss dairy cows aged between 2 and 10 years, and 12 heifers from 0.5 to 2 years of age. In order to evaluate a possible dependence of age effects on diet type, half of the cattle each originated from two herds kept at the same farm, which were fed either on a forage-only diet or on the same forage diet but complemented with 5 kg/day of concentrate since their first calving. During 2 days, the gaseous exchange of the animals was quantified in open-circuit respiration chambers, followed by an 8-day period of feed, faeces, urine and milk collection. Daily amounts and energy contents were used to calculate complete energy balances. Age and feeding regime effects were analysed by parametric regression analysis where BW, milk yield and hay proportion in forage as consumed were considered as covariates. Relative to intake of gross energy, the availability of metabolizable energy (ME) increased with age. This was not the result of an increasing energy digestibility, but of proportionately lower energy losses with methane (following a curvilinear relationship with the greatest losses in middle-aged cows) and urine (continuously declining). The efficiency of utilization of ME for milk production (k l) increased with age. Potential reasons include an increase in the propionate-to-acetate ratio in the rumen because of a shift away from fibre degradation and methane formation as well as lower urine energy losses. The greater k l allowed older cows to accrete more energy reserves in the body. As expected, offering concentrate enhanced digestibility,

metabolizability and metabolic utilization of energy. Age and feeding regime did not interact significantly. In conclusion, older cows seem to have digestive and metabolic strategies to use dietary energy to a certain degree more efficiently than younger cows.

Huck, I. (2018). Role of HNF4a in Liver Regeneration, Liver Cancer Pathogenesis and Global Metabolism, kuscholarworks.ku.edu.

Page 1. Role of HNF4α in Liver Regeneration, Liver Cancer Pathogenesis and Global Metabolism By Ian Huck ©2018 Ian Huck Submitted to the graduate degree program in Pharmacology, Toxicology and Therapeutics and …

Huck, I., et al. (2018). "Hepatocyte-specific hepatocyte nuclear factor 4 alpha (HNF4α) deletion decreases resting energy expenditure by disrupting lipid and carbohydrate homeostasis." bioRxiv.

Hepatocyte Nuclear Factor 4 alpha (HNF4α) is required for hepatocyte differentiation and regulates expression of genes involved in lipid and carbohydrate metabolism including those that control VLDL secretion and gluconeogenesis. Whereas previous studies have focused on specific genes regulated by HNF4α in metabolism, its overall role in whole body energy utilization has not been studied. In this study, we used indirect calorimetry to determine the effect of hepatocyte-specific HNF4α deletion (HNF4α-KO) in mice on whole body energy expenditure (EE) and substrate utilization in fed, fasted, and high fat diet (HFD) conditions. HNF4α-KO had reduced resting EE during fed conditions and higher rates of carbohydrate oxidation with fasting. HNF4αKO mice exhibited decreased body mass caused by fat mass depletion despite no change in energy intake and evidence of positive energy balance. HNF4α-KO mice were able to upregulate lipid oxidation during HFD suggesting that their metabolic flexibility was intact. However, only hepatocyte specific HNF4α-KO mice exhibited significant reduction in basal metabolic rate and spontaneous activity during HFD. Consistent with previous studies, hepatic gene expression in HNF4α-KO supports decreased gluconeogenesis and decreased VLDL export and hepatic β-oxidation in HNF4α-KO livers across all feeding conditions. Together, our data suggest deletion of hepatic HNF4α increases dependence on dietary carbohydrates and endogenous lipids for energy during fed and fasted conditions by inhibiting hepatic gluconeogenesis, hepatic lipid export, and intestinal lipid absorption resulting in decreased whole body energy expenditure. These data clarify the role of hepatic HNF4α on systemic metabolism and energy homeostasis.

Kentish, S. J., et al. (2018). "Time-restricted feeding prevents ablation of diurnal rhythms in gastric vagal afferent mechanosensitivity observed in high-fat diet-induced obese mice." Journal of Neuroscience **38**(22): 5088-5095.

Mechanosensitive gastric vagal afferents (GVAs) are involved in the regulation of food intake. GVAs exhibit diurnal rhythmicity in their response to food-related stimuli, allowing time of day-specific satiety signaling. This diurnal rhythmicity is ablated in high-fat-diet (HFD)-induced obesity. Time-restricted feeding (TRF) has a strong influence on peripheral clocks. This study aimed to determine whether diurnal patterns in GVA mechanosensitivity are entrained by TRF. Eight-week-old male C57BL/6 mice (N = 256) were fed a

standard laboratory diet (SLD) or HFD for 12 weeks. After 4 weeks of diet acclimatization, the mice were fed either ad libitum or only during the light phase [Zeitgeber time (ZT) 0–12] or dark phase (ZT12–24) for 8 weeks. A subgroup of mice from all conditions (n = 8/condition) were placed in metabolic cages. After 12 weeks, ex vivo GVA recordings were taken at 3 h intervals starting at ZT0. HFD mice gained more weight than SLD mice. TRF did not affect weight gain in the SLD mice, but decreased weight gain in the HFD mice regardless of the TRF period. In SLD mice, diurnal rhythms in food intake were inversely associated with diurnal rhythmicity of GVA mechanosensitivity. These diurnal rhythms were entrained by the timing of food intake. In HFD mice, diurnal rhythms in food intake and diurnal rhythmicity of GVA mechanosensitivity were dampened. Loss of diurnal rhythmicity in HFD mice was abrogated by TRF. In conclusion, diurnal rhythmicity in GVA responses to food-related stimuli can be entrained by food intake. TRF prevents the loss of diurnal rhythmicity that occurs in HFD-induced obesity.

Kwan, J. R., et al. (2018). "Obese Mice Are Protected From Increased Energy Intake in Response to Voluntary Wheel Running." The FASEB Journal **32**: 822-853.

Diseases of metabolism pose an increasing threat to public health that has led to greater demand for effective treatments. Obesity is a highly prevalent metabolic disease that afflicts over 100 million Americans and increases the risk of developing diabetes and cardiovascular disease. Lifestyle modifications that incorporate exercise are a standard prescription for obesity, but its effectiveness is limited due to variability in compliance and physiological adaptations (e.g. compensatory increases in energy intake). Previous studies have shown that voluntary exercise fails to induce weight loss in high fat fed mice, but little is known regarding the onset of exercise. Here, we tested the hypothesis that chronic overnutrition from high fat feeding will mitigate the compensatory increase in food intake that commonly accompanies increased energy expenditure from voluntary exercise. Starting at 12 weeks of age, male C57BL/6J mice were weight matched, singly housed and fed a chow (n=8) or high fat diet (HFD; 60% calories from fat; n=6) for 4 weeks. At 18 weeks of age, mice were housed in a Promethion Metabolic Analyzer to measure energy expenditure, food intake and physical activity. Experiments were performed at 21°C with running wheels locked for four days then unlocked for the subsequent nine days. Body composition was assessed both prior to and immediately following indirect calorimetry experiments. Ethoscan (Sable Systems) analysis was performed to examine voluntary wheel running (VWR) distance, duration and speed and measured the time and distance for off-wheel activities (i.e. roaming and feeding). HF-fed mice were heavier (WT: 26.9 ± 1.1 vs. HF: 36.8 ± 4.2 g; $p < 0.05$) and had greater fat mass (WT: 3.5 ± 0.6 vs. HF: 11.1 ± 3.1 g; $p < 0.05$) than chow-fed mice prior to energy balance studies. VWR caused a decrease in whole body (WT: −0.3 ± 0.8 vs. HF: −4.0 ± 1.3 g; $p > 0.05$) and fat mass (WT: 0.3 ± 0.6 vs. HF: −3.0 ± 0.9 g; $p < 0.05$) in HFD-fed, but not in chow-fed mice. VWR distance, duration, and speed were not affected by diet. VWR increased energy expenditure (EE) with both diets equally, but energy intake (EI) only increased in chow-fed mice. VWR decreased overall off-wheel activity (OWA) regardless of diet. These data demonstrate that 9 days of VWR cause weight loss in HFD-fed mice but not mice fed a chow-diet. Because VWR did not vary between diets, the efficacy of VWR in HFD-fed mice could instead be attributed to resistance to specific behavioral compensations (i.e. increased food intake) that occur with the introduction of VWR in lean mice.

Ladyman, S. R., et al. (2018). "Energy homeostasis and running wheel activity during pregnancy in the mouse." Physiology and Behavior **194**: 83-94.

Pregnancy and lactation are metabolically challenging states, where the mother must supply all the energy requirements for the developing fetus and growing pups respectively. The aim of the current study was to characterize many aspects of energy homeostasis before and during pregnancy in the mouse, and to examine the role of voluntary activity on changes in energy expenditure during pregnancy. In a secondary aim, we evaluate measures of energy homeostasis during pregnancy in mice that successfully reared their litter or in mice that went on to abandon their litter, to determine if an impairment in pregnancy-induced adaptation of energy homeostasis might underlie the abandonment of pups soon after birth. During pregnancy, food intake was increased, characterized by increased meal size and duration but not number of meals per day. The duration of time spent inactive, predicted to indicate sleep behaviour, was increased both early and late in pregnancy compared to pre-pregnancy levels. Increased x + y beam breaks, as a measure of activity increased during pregnancy and this reflected an increase in ambulatory behaviour in mid pregnancy and an increase in non-ambulatory movement in late pregnancy. Energy expenditure, as measured by indirect calorimetry, increased across pregnancy, likely due to the growth and development of fetal tissue. There was also a dramatic reduction in voluntary wheel running as soon as the mice became pregnant. Compared with successful pregnancies and lactations, pregnancies where pups were abandoned soon after birth were associated with reduced body weight gain and an increase in running wheel activity at the end of pregnancy, but no difference in food intake or energy expenditure. Overall, during pregnancy there are multiple adaptations to change energy homeostasis, resulting in partitioning of provisions of energy to the developing fetus and storing energy for future metabolic demands.

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during pregnancy there are multiple adaptations to change energy homeostasis, resulting in partitioning of provisions of energy to the developing fetus and storing energy for future metabolic demands.

Lantier, L., et al. (2018). "SIRT2 knockout exacerbates insulin resistance in high fat-fed mice." PLoS One **13**(12).

The NAD + -dependent deacetylase SIRT2 is unique amongst sirtuins as it is effective in the cytosol, as well as the mitochondria. Defining the role of cytosolic acetylation state in specific tissues is difficult since even physiological effects at the whole body level are unknown. We hypothesized that genetic SIRT2 knockout (KO) would lead to impaired insulin action, and that this impairment would be worsened in HF fed mice. Insulin sensitivity was tested using the hyperinsulinemic-euglycemic clamp in SIRT2 KO mice and WT littermates. SIRT2 KO mice exhibited reduced skeletal muscle insulin-induced glucose uptake compared to lean WT mice, and this impairment was exacerbated in HF SIRT2 KO mice. Liver insulin sensitivity was unaffected in lean SIRT2 KO mice. However, the insulin resistance that accompanies HF-feeding was worsened in SIRT2 KO mice. It was notable that the effects of SIRT2 KO were largely disassociated from cytosolic acetylation state, but were closely linked to acetylation state in the mitochondria. SIRT2 KO led to an increase in body weight that was due to increased food intake in HF fed mice. In summary, SIRT2 deletion in vivo reduces muscle insulin sensitivity and contributes to liver insulin resistance by a mechanism that is unrelated to cytosolic acetylation state. Mitochondrial acetylation state and changes in feeding behavior that result in increased body weight correspond to the deleterious effects of SIRT2 KO on insulin action.

Lark, D. S., et al. (2018). "Reduced nonexercise activity attenuates negative energy balance in mice engaged in voluntary Exercise." Diabetes **67**(5): 831-840.

Exercise alone is often ineffective for treating obesity despite the associated increase in metabolic requirements. Decreased nonexercise physical activity has been implicated in this resistance to weight loss, but the mechanisms responsible are unclear. We quantified the metabolic cost of nonexercise activity, or "off-wheel" activity (OWA), and voluntary wheel running (VWR) and examined whether changes in OWA during VWR altered energy balance in chow-fed C57BL/6J mice (n = 12). Energy expenditure (EE), energy intake, and behavior (VWR and OWA) were continuously monitored for 4 days with locked running wheels followed by 9 days with unlocked running wheels. Unlocking the running wheels increased EE as a function of VWR distance. The metabolic cost of exercise (kcal/m traveled) decreased with increasing VWRspeed. Unlockingthe wheelledtoanegativeenergy balance but also decreased OWA, which was predicted to mitigate the expected changeinenergy balanceby∼45%. A novel behavioral circuit involved repeated bouts of VWR, and roaming was discovered and represented novel predictors of VWR behavior. The integrated analysis described here reveals that the weight loss effects of voluntary exercise can be countered by a reduction in nonexercise activity.

Lee, N. J., et al. (2018). "Osteoglycin, a novel coordinator of bone and glucose homeostasis." Molecular Metabolism **13**: 30-44.

Objective: The skeleton, which is strongly controlled by endocrine factors, has recently been shown to also play an active endocrine role itself, specifically influencing energy metabolism. However, much less is known about this role. Therefore, we sought to identify novel endocrine factors involved in the regulation of both bone mass and whole-body glucose homeostasis. Methods: We used transcriptomic and proteomic analysis of Y1 receptor deficient osteoblasts combined with the generation of a novel osteoglycin deficient mouse model and performed comprehensive in vivo phenotype profiling, combined with osteoglycin administration in wildtype mice and human studies. Results: Here we identify a novel role for osteoglycin, a secreted proteoglycan, in coordinating bone accretion with changes in energy balance. Using an osteoglycin knockout mouse model, we show that at a whole body level, osteoglycin acts to suppress bone formation and modulate whole body energy supplies by altering glucose uptake through changes in insulin secretion and sensitivity, as well as by altering food intake through central signaling. Examining humans following gastric surgery as a model of negative energy balance, we show that osteoglycin is associated with BMI and lean mass as well as changes in weight, BMI, and glucose levels. Conclusions: Thus, we identify osteoglycin as a novel factor involved in the regulation of energy homeostasis and identify a role for it in facilitating the matching of bone acquisition to alterations in energy status.

Lee, N. J., et al. (2018). "Central RANK signalling in NPY neurons alters bone mass in male mice." Neuropeptides **68**: 75-83.

RANKL signalling known to be important for the control of bone mass, has recently also been implicated in the brain to control thermoregulation, however, it is not known which neuronal pathways are involved and whether other aspects of energy homeostasis are also affected. Here we show that selective deletion of RANK from NPY neurons down-regulated NPY mRNA expression in the hypothalamus. While comprehensive phenotyping of germline-induced NPY neuron specific RANK deficient mice revealed no significant changes in physical or metabolic parameters, adult onset deletion of RANK from NPY neurons led to a significant increase in fat mass and a decrease in whole body bone mineral content and bone mineral density. Intriguingly, when these conditional knockout mice were placed on a high fat diet, body weight and fat mass did not differ to control mice. However, they were able to significantly increase their bone mass to match their increased body weight, an ability that was lacking in control mice. Taken together, results from this study demonstrate that RANK signalling in NPY neurons is involved in modulating NPY levels and through that matching bone mass to body weight.

Li, M. D., et al. (2018). "Adipocyte OGT governs diet-induced hyperphagia and obesity." Nature Communications **9**(1).

Palatable foods (fat and sweet) induce hyperphagia, and facilitate the development of obesity. Whether and how overnutrition increases appetite through the adipose-to-brain axis is unclear. O-linked beta-D-N-acetylglucosamine (O-GlcNAc) transferase (OGT) couples nutrient cues to O-GlcNAcylation of intracellular proteins at serine/threonine residues. Chronic dysregulation of O-GlcNAc signaling contributes to metabolic diseases. Here we show that adipocyte OGT is essential for high fat diet-induced hyperphagia, but is dispensable for baseline food intake. Adipocyte OGT stimulates hyperphagia by transcriptional activation of de novo lipid desaturation and accumulation of N-arachidonyl ethanolamine

(AEA), an endogenous appetite-inducing cannabinoid (CB). Pharmacological manipulation of peripheral CB1 signaling regulates hyperphagia in an adipocyte OGT-dependent manner. These findings define adipocyte OGT as a fat sensor that regulates peripheral lipid signals, and uncover an unexpected adipose-to-brain axis to induce hyperphagia and obesity.

Luo, X., et al. (2018). "Expression of STING Is Increased in Liver Tissues From Patients With NAFLD and Promotes Macrophage-Mediated Hepatic Inflammation and Fibrosis in Mice." Gastroenterology **155**(6): 1971-1984.e1974.

Background & Aims: Transmembrane protein 173 (TMEM173 or STING) signaling by macrophage activates the type I interferon-mediated innate immune response. The innate immune response contributes to hepatic steatosis and non-alcoholic fatty liver disease (NAFLD). We investigated whether STING regulates diet-induced in hepatic steatosis, inflammation, and liver fibrosis in mice. Methods: Mice with disruption of Tmem173 (STINGgt) on a C57BL/6J background, mice without disruption of this gene (controls), and mice with disruption of Tmem173 only in myeloid cells were fed a standard chow diet, a high-fat diet (HFD; 60% fat calories), or a methionine- and choline-deficient diet (MCD). Liver tissues were collected and analyzed by histology and immunohistochemistry. Bone marrow cells were isolated from mice, differentiated into macrophages, and incubated with 5,6-dimethylxanthenone-4-acetic acid (DMXAA; an activator of STING) or cyclic guanosine monophosphate–adenosine monophosphate (cGAMP). Macrophages or their media were applied to mouse hepatocytes or human hepatic stellate cells (LX2) cells, which were analyzed for cytokine expression, protein phosphorylation, and fat deposition (by oil red O staining after incubation with palmitate). We obtained liver tissues from patients with and without NAFLD and analyzed these by immunohistochemistry. Results: Non-parenchymal cells of liver tissues from patients with NAFLD had higher levels of STING than cells of liver tissues from patients without NAFLD. STINGgt mice and mice with disruption only in myeloid cells developed less severe hepatic steatosis, inflammation, and/or fibrosis after the HFD or MCD than control mice. Levels of phosphorylated c-Jun N-terminal kinase and p65 and mRNAs encoding tumor necrosis factor and interleukins 1B and 6 (markers of inflammation) were significantly lower in liver tissues from STINGgt mice vs control mice after the HFD or MCD. Transplantation of bone marrow cells from control mice to STINGgt mice restored the severity of steatosis and inflammation after the HFD. Macrophages from control, but not STINGgt, mice increased markers of inflammation in response to lipopolysaccharide and cGAMP. Hepatocytes and stellate cells cocultured with STINGgt macrophages in the presence of DMXAA or incubated with the medium collected from these macrophages had decreased fat deposition and markers of inflammation compared with hepatocytes or stellate cells incubated with control macrophages. Conclusions: Levels of STING were increased in liver tissues from patients with NAFLD and mice with HFD-induced steatosis. In mice, loss of STING from macrophages decreased the severity of liver fibrosis and the inflammatory response. STING might be a therapeutic target for NAFLD.

Mantey, S. A., et al. (2018). "Su1207 - The Bombesin Receptor Subtype3 (BRS-3) Agonist, MK-5046, Functions as an Allosteric Agonist in Rat, Mouse, Human but Varies in Basis for Allosterism." Gastroenterology **154**(6): S-503.

Mice were divided in two groups; single housed in Promethion metabolic cages (Sable Systems, Las Vegas, NV) and after 5 days of acclimation, parameters recording in parallel was initiated.

Martinez-Huenchullan, S. F., et al. (2018). "Differential metabolic effects of constant moderate versus high intensity interval training in high-fat fed mice: possible role of muscle adiponectin." Physiological Reports **6**(4).

Exercise regimens may have differing effects in the presence of obesity. In addition to being fat derived, adiponectin has recently been described as a myokine that regulates insulin sensitivity, which may link to exercise-related metabolic benefits in obesity. Whether skeletal muscle adiponectin varies in different exercise modalities is unclear. This study investigated the comparative effects of 10 weeks of endurance constant-moderate intensity exercise (END) with high intensity interval training (HIIT), on metabolic outcomes, including muscle adiponectin in a mouse model of diet-induced obesity. Ten-week-old male C57BL/6 mice were fed a high-fat diet (HFD) (45% FAT) or standard CHOW diet ab libitum and underwent one of three training regimes: (1) no exercise, (2) END, or (3) HIIT (8 bouts of 2.5 min with eight periods of rest of 2.5 min) for 10 weeks (3 × 40 min sessions/week). Chow-fed mice acted as controls. Compared with HFD alone, both training programs similarly protected against body weight gain (HFD = 45 ± 2; END = 37 ± 2; HIIT = 36 ± 2 g), preserved lean/fat tissue mass ratio (HFD = 0.64 ± 0.09; END = 0.34 ± 0.13; HIIT = 0.33 ± 0.13), and improved blood glucose excursion during an insulin tolerance test (HFD = 411 ± 54; END = 350 ± 57; HIIT = 320 ± 66 arbitrary units [AU]). Alterations in fasting glycemia, insulinemia, and AST/ALT ratios were prevented only by END. END, but not HIIT increased skeletal muscle adiponectin mRNA (14-fold; $P < 0.05$) and increased protein content of high molecular weight (HMW) adiponectin (3.3-fold), whereas HIIT induced a milder increase (2.4-fold). Compared with HFD, neither END nor HIIT altered circulating low (LMW) or high (HMW) molecular weight adiponectin forms. Furthermore, only END prevented the HFD downregulation of PGC1α ($P < 0.05$) mRNA levels downstream of muscle adiponectin. These data show that different training programs affect muscle adiponectin to differing degrees. Together these results suggest that END is a more effective regimen to prevent HFD-induced metabolic disturbances in mice.

Pei, Y., et al. (2018). "Regulation of adipose tissue inflammation by adenosine 2A receptor in obese mice." Journal of Endocrinology **239**(3): 365-376.

Adenosine 2A receptor (A2AR) exerts anti-inflammatory effects. However, the role of A2AR in obesity-associated adipose tissue inflammation remains to be elucidated. The present study examined the expression of A2AR in adipose tissue of mice with diet-induced obesity and determined the effect of A2AR disruption on the status of obesity-associated adipose tissue inflammation. WT C57BL/6J mice and A2AR-disrupted mice were fed a high-fat diet (HFD) for 12 weeks to induce obesity and adipose tissue inflammation. In vitro, bone marrow-derived macrophages from A2AR-disrupted mice and WT control mice were treated with palmitate and examined for macrophage proinflammatory activation. Compared with that of low-fat diet (LFD)-fed WT mice, A2AR expression in adipose tissue of HFD-fed WT mice was increased significantly and was present predominantly in adipose tissue macrophages. The increase in adipose tissue A2AR expression in HFD-fed mice was accompanied with increased phosphorylation states of c-Jun N-terminal kinase 1 p46 and nuclear factor kappa B p65 and mRNA levels of interleukin (Il)-1beta,

Il6 and tumor necrosis factor alpha. In A2AR-disrupted mice, HFD feeding induced significant increases in adipose tissue inflammation, indicated by enhanced proinflammatory signaling and increased proinflammatory cytokine expression, and adipose tissue insulin resistance, indicated by a decrease in insulin-stimulated Akt phosphorylation relative to those in WT mice. Lastly, A2AR disruption enhanced palmitate-induced macrophage proinflammatory activation. Taken together, these results suggest that A2AR plays a protective role in obesity-associated adipose tissue inflammation, which is attributable to, in large part, A2AR suppression of macrophage proinflammatory activation.

Ravussin, Y., et al. (2018). "Evidence for a Non-leptin System that Defends against Weight Gain in Overfeeding." Cell Metabolism **28**(2): 289-299.e285.

Weight is defended so that increases or decreases in body mass elicit responses that favor restoration of one's previous weight. While much is known about the signals that respond to weight loss and the central role that leptin plays, the lack of experimental systems studying the overfed state has meant little is known about pathways defending against weight gain. We developed a system to study this physiology and found that overfed mice defend against increased weight gain with graded anorexia but, unlike weight loss, this response is independent of circulating leptin concentration. In overfed mice that are unresponsive to orexigenic stimuli, adipose tissue is transcriptionally and immunologically distinct from fat of ad libitum-fed obese animals. These findings provide evidence that overfeeding-induced obesity alters adipose tissue and central responses in ways that are distinct from ad libitum obesity and activates a non-leptin system to defend against weight gain. Following overfeeding, mice decrease food intake and return to their previous weight. The signal or set of signals that underlie this response is not known. Ravussin et al. have developed a model to investigate overfeeding in mice and provide evidence for a leptin-independent system that defends against body weight gain.

Reber, J., et al. (2018). "Non-invasive Measurement of Brown Fat Metabolism Based on Optoacoustic Imaging of Hemoglobin Gradients." Cell Metabolism **27**(3): 689-701.e684.

Metabolism is a fundamental process of life. However, non-invasive measurement of local tissue metabolism is limited today by a deficiency in adequate tools for in vivo observations. We designed a multi-modular platform that explored the relation between local tissue oxygen consumption, determined by label-free optoacoustic measurements of hemoglobin, and concurrent indirect calorimetry obtained during metabolic activation of brown adipose tissue (BAT). By studying mice and humans, we show how video-rate handheld multi-spectral optoacoustic tomography (MSOT) in the 700–970 nm spectral range enables non-invasive imaging of BAT activation, consistent with positron emission tomography findings. Moreover, we observe BAT composition differences between healthy and diabetic tissues. The study consolidates hemoglobin as a principal label-free biomarker for longitudinal non-invasive imaging of BAT morphology and bioenergetics in situ. We also resolve water and fat components in volunteers, and contrast MSOT readouts with magnetic resonance imaging data. Reber et al. employed label-free multi-spectral optoacoustic tomography to non-invasively image BAT and WAT in mice and humans and resolve BAT activation based on hemoglobin gradients. The 700–970 nm spectral range further enabled identification of BAT composition using lipid and water signatures.

Reichenbach, A., et al. (2018). "Carnitine acetyltransferase (Crat) in hunger-sensing AgRP neurons permits adaptation to calorie restriction." FASEB Journal **32**(12): 6923-6933.

> Hunger-sensing agouti-related peptide (AgRP) neurons ensure survival by adapting metabolism and behavior to low caloric environments. This adaption is accomplished by consolidating food intake, suppressing energy expenditure, and maximizing fat storage (nutrient partitioning) for energy preservation. The intracellular mechanisms responsible are unknown. Here we report that AgRP carnitine acetyltransferase (Crat) knockout (KO) mice exhibited increased fatty acid utilization and greater fat loss after 9 d of calorie restriction (CR). No differences were seen in mice with ad libitum food intake. Eleven days ad libitum feeding after CR resulted in greater food intake, rebound weight gain, and adiposity in AgRP Crat KO mice compared with wild-type controls, as KO mice act to restore pre-CR fat mass. Collectively, this study highlights the importance of Crat in AgRP neurons to regulate nutrient partitioning and fat mass during chronically reduced caloric intake. The increased food intake, body weight gain, and adiposity in KO mice after CR also highlights the detrimental and persistent metabolic consequence of impaired substrate utilization associated with CR. This finding may have significant implications for postdieting weight management in patients with metabolic diseases.

Rubin, J., et al. (2018). "Su1210 - Low Rates of H. Pylori Eradication Testing and Cure Rates in Usual Care." Gastroenterology **154**(6): S-503-S-504.

> . Mice were divided in two groups; single housed in Promethion metabolic cages (Sable Systems, Las Vegas, NV) and after 5 days of acclimation, parameters recording in parallel was initiated …

Rubinow, K., et al. (2018). "Androgen receptor deficiency in monocytes/macrophages does not alter adiposity or glucose homeostasis in male mice." Asian Journal of Andrology **20**(3): 276-283.

> Androgen deprivation in men leads to increased adiposity, but the mechanisms underlying androgen regulation of fat mass have not been fully defined. Androgen receptor (AR) is expressed in monocytes/macrophages, which are resident in key metabolic tissues and influence energy metabolism in surrounding cells. Male mice bearing a cell-specific knockout of the AR in monocytes/macrophages (M-ARKO) were generated to determine whether selective loss of androgen signaling in these cells would lead to altered body composition. Wild-type (WT) and M-ARKO mice (12-22 weeks of age, n = 12 per group) were maintained on a regular chow diet for 8 weeks and then switched to a high-fat diet for 8 additional weeks. At baseline and on both the regular chow and high-fat diets, no differences in lean mass or fat mass were observed between groups. Consistent with the absence of differential body weight or adiposity, no differences in food intake (3.0 ± 0.5 g per day for WT mice vs 2.8 ± 0.4 g per day for M-ARKO mice) or total energy expenditure (0.6 ± 0.1 Kcal h-1 for WT mice vs 0.5 ± 0.1 Kcal h-1 for M-ARKO mice) were evident between groups during high-fat feeding. Liver weight was greater in M-ARKO than that in WT mice (1.5 ± 0.1 g vs 1.3 ± 0.0 g, respectively, P = 0.02). Finally, M-ARKO mice did not exhibit impairments in glucose tolerance or insulin sensitivity relative to WT mice at any study time point. In aggregate, these findings suggest that AR signaling specifically in monocytes/macrophages does not contribute to the regulation of systemic energy balance, adiposity, or insulin sensitivity in male mice.

Sloan, D. K., et al. (2018). "Estrogen effects on oxytocinergic pathways that regulate food intake." Hormones and Behavior **105**: 128-137.

Multiple stimulatory and inhibitory neural circuits control eating, and these circuits are influenced by an array of hormonal, neuropeptide, and neurotransmitter signals. For example, estrogen and oxytocin (OT) both are known to decrease food intake, but the mechanisms by which these signal molecules influence eating are not fully understood. These studies investigated the interaction between estrogen and OT in the control of food intake. RT-qPCR studies revealed that 17β-estradiol benzoate (EB)-treated rats showed a two-fold increase in OT mRNA in the paraventricular nucleus of the hypothalamus (PVN) compared to Oil-treated controls. Increased OT mRNA expression may increase OT protein levels, and immunohistochemistry studies showed that EB-treated rats had more intense OT labeling in the nucleus of the solitary tract (NTS), a region known to integrate signals for food intake. Food intake measurements showed that EB treatment reduced food intake, as expected. EB-treated rats lost weight over the course of the experiment, as expected, and EB-treated rats that received the highest dose of OT lost more weight than EB-treated rats that did not receive OT. Finally, OT antagonist administered to EB-treated rats reversed the effect of EB on food intake, suggesting that estrogen effects to decrease food intake may involve the oxytocinergic pathway.

Small, L., et al. (2018). "Thermoneutral housing does not influence fat mass or glucose homeostasis in C57BL/6 mice." Journal of Endocrinology **239**(3): 313-324.

One major factor affecting physiology often overlooked when comparing data from animal models and humans is the effect of ambient temperature. The majority of rodent housing is maintained at ~22°C, the thermoneutral temperature for lightly clothed humans. However, mice have a much higher thermoneutral temperature of ~30°C, consequently data collected at 22°C in mice could be influenced by animals being exposed to a chronic cold stress. The aim of this study was to investigate the effect of housing temperature on glucose homeostasis and energy metabolism of mice fed normal chow or a high-fat, obesogenic diet (HFD). Male C57BL/6J(Arc) mice were housed at standard temperature (22°C) or at thermoneutrality (29°C) and fed either chow or a 60% HFD for 13 weeks. The HFD increased fat mass and produced glucose intolerance as expected but this was not exacerbated in mice housed at thermoneutrality. Changing the ambient temperature, however, did alter energy expenditure, food intake, lipid content and glucose metabolism in skeletal muscle, liver and brown adipose tissue. Collectively, these findings demonstrate that mice regulate energy balance at different housing temperatures to maintain whole-body glucose tolerance and adiposity irrespective of the diet. Despite this, metabolic differences in individual tissues were apparent. In conclusion, dietary intervention in mice has a greater impact on adiposity and glucose metabolism than housing temperature although temperature is still a significant factor in regulating metabolic parameters in individual tissues.

Staffas, A., et al. (2018). "Nutritional Support from the Intestinal Microbiota Improves Hematopoietic Reconstitution after Bone Marrow Transplantation in Mice." Cell Host and Microbe **23**(4): 447-457.e444.

Bone marrow transplantation (BMT) offers curative potential for patients with high-risk hematologic malignancies, but the post-transplantation period is characterized by profound immunodeficiency. Recent studies indicate that the intestinal microbiota not only regulates mucosal immunity, but can also contribute to systemic immunity and hematopoiesis. Using antibiotic-mediated microbiota depletion in a syngeneic BMT mouse model, here we describe a role for the intestinal flora in hematopoietic recovery after BMT. Depletion of the intestinal microbiota resulted in impaired recovery of lymphocyte and neutrophil counts, while recovery of the hematopoietic stem and progenitor compartments and the erythroid lineage were largely unaffected. Depletion of the intestinal microbiota also reduced dietary energy uptake and visceral fat stores. Caloric supplementation through sucrose in the drinking water improved post-BMT hematopoietic recovery in mice with a depleted intestinal flora. Taken together, we show that the intestinal microbiota contribute to post-BMT hematopoietic reconstitution in mice through improved dietary energy uptake. Intestinal bacteria can exert effects on systemic hematopoiesis. Staffas et al. show that the intestinal flora contributes to hematopoietic recovery after bone marrow transplantation (BMT) through improved dietary energy uptake. The findings suggest possible clinical intervention strategies for improved BMT outcomes.

Stroh, M. A., et al. (2018). "NCB5OR Deficiency in the Cerebellum and Midbrain Leads to Dehydration and Alterations in Thirst Response, Fasted Feeding Behavior, and Voluntary Exercise in Mice." Cerebellum **17**(2): 152-164.

Cytosolic NADH-cytochrome-b5-oxidoreductase (NCB5OR) is ubiquitously expressed in animal tissues. We have previously reported that global ablation of NCB5OR in mice results in early-onset lean diabetes with decreased serum leptin levels and increased metabolic and feeding activities. The conditional deletion of NCB5OR in the mouse cerebellum and midbrain (conditional knock out, CKO mice) results in local iron dyshomeostasis and altered locomotor activity. It has been established that lesion to or removal of the cerebellum leads to changes in nutrient organization, visceral response, feeding behavior, and body weight. This study assessed whether loss of NCB5OR in the cerebellum and midbrain altered feeding or metabolic activity and had an effect on serum T3, cortisol, prolactin, and leptin levels. Metabolic cage data revealed that 16 week old male CKO mice had elevated respiratory quotients and decreased respiratory water expulsion, decreased voluntary exercise, and altered feeding and drinking behavior compared to wild-type littermate controls. Most notably, male CKO mice displayed higher consumption of food during refeeding after a 48-h fast. Echo MRI revealed normal body composition but decreased total water content and hydration ratios in CKO mice. Increased serum osmolality measurements confirmed the dehydration status of male CKO mice. Serum leptin levels were significantly elevated in male CKO mice while prolactin, T3, and cortisol levels remain unchanged relative to wild-type controls, consistent with elevated transcript levels for leptin receptors (short form) in the male CKO mouse cerebellum. Taken together, these findings suggest altered feeding response post starvation as a result of NCB5OR deficiency in the cerebellum.

Treat, M. D., et al. (2018). "Extreme physiological plasticity in a hibernating basoendothermic mammal, Tenrec ecaudatus." Journal of Experimental Biology **221**(20).

Physiological plasticity allows organisms to respond to diverse conditions. However, can being too plastic actually be detrimental? Malagasy common tenrecs, Tenrec ecaudatus, have many plesiomorphic traits and may represent a basal placental mammal. We established a laboratory population of T. ecaudatus and found extreme plasticity in thermoregulation and metabolism, a novel hibernation form, variable annual timing, and remarkable growth and reproductive biology. For instance, tenrec body temperature (Tb) may approximate ambient temperature to as low as 12°C even when tenrecs are fully active. Conversely, tenrecs can hibernate with Tb of 28°C. During the active season, oxygen consumption may vary 25-fold with little or no change in Tb. During the austral winter, tenrecs are consistently torpid but the depth of torpor may vary. A righting assay revealed that Tb contributes to but does not dictate activity status. Homeostatic processes are not always linked, e.g. a hibernating tenrec experienced a ∼34%decrease in heart rate while maintaining constant body temperature and oxygen consumption rates. Tenrec growth rates vary but young may grow ∼40-fold in the 5 weeks until weaning and may possess indeterminate growth as adults. Despite all of this profound plasticity, tenrecs are surprisingly intolerant of extremes in ambient temperature (<8 or >34°C). We contend that while plasticity may confer numerous energetic advantages in consistently moderate environments, environmental extremes may have limited the success and distribution of plastic basal mammals.

Wahl, D., et al. (2018). "Comparing the Effects of Low-Protein and High-Carbohydrate Diets and Caloric Restriction on Brain Aging in Mice." Cell Reports **25**(8): 2234-2243.e2236.

Calorie restriction (CR) increases lifespan and improves brain health in mice. Ad libitum low-protein, high-carbohydrate (LPHC) diets also extend lifespan, but it is not known whether they are beneficial for brain health. We compared hippocampus biology and memory in mice subjected to 20% CR or provided ad libitum access to one of three LPHC diets or to a control diet. Patterns of RNA expression in the hippocampus of 15-month-old mice were similar between mice fed CR and LPHC diets when we looked at genes associated with longevity, cytokines, and dendrite morphogenesis. Nutrient-sensing proteins, including SIRT1, mTOR, and PGC1α, were also influenced by diet; however, the effects varied by sex. CR and LPHC diets were associated with increased dendritic spines in dentate gyrus neurons. Mice fed CR and LPHC diets had modest improvements in the Barnes maze and novel object recognition. LPHC diets recapitulate some of the benefits of CR on brain aging. Calorie restriction (CR) and ad libitum low-protein, high-carbohydrate (LPHC) diets improve cardiometabolic health in mice. Wahl et al. show that, like healthspan, CR and LPHC diets positively affect hippocampus biology in mice by influencing hippocampus gene expression, nutrient-sensing pathways, dendritic morphology, and cognition.

Wang, B., et al. (2018). "Effects of eucalyptus oil and anise oil supplementation on rumen fermentation characteristics, methane emission, and digestibility in sheep." Journal of Animal Science **96**(8): 3460-3470.

The objective of this study was to evaluate antimethanogenic activity of eucalyptus oil (EUC) and anise oil (ANI) in vitro and in vivo using sheep as a model. In vitro study was conducted using batch culture technique, each of EUC and ANI were added at 0, 50, 100, 200, or 400 mg/L of fermentation media with substrate containing 60% corn-based concentrate and 40% hay (DM basis). Total gas production (GP) linearly ($P < 0.01$) decreased with increasing ANI, whereas the GP was not affected with EUC addition. Supplementation of ANI and EUC linearly ($P < 0.01$) decreased total methane production and methane proportion in total gas. Total VFA and ammonia-nitrogen (NH3- N) concentration linearly ($P < 0.01$) decreased with increasing ANI supplementation. For the in vivo study, a replicated 3 × 3 Latin square design was carried out using six ruminal cannulated Du Han hybrid sheep (BW, 64.5 ± 8.56 kg) with 22 d of periods. Three treatments were control diet (consisted of 60% corn-based concentrate and 40% Chinese wildrye hay), EUC (control diet supplemented with 0.5 g EUC/d per head), and ANI (control diet supplemented with 0.5 g ANI/d per head). Each period consisted of 14 d for adaption and 8 d for sampling and data collection. Supplementation of EUC and ANI had no effects on feed intake and apparent nutrient digestibility. Ruminal NH3-N concentration was greater with EUC ($P < 0.01$) and ANI ($P = 0.03$) than control. Urinal allantoin output was less ($P < 0.05$) in sheep fed EUC and ANI than control animals. Methane emission was less ($P = 0.03$) in sheep fed ANI than sheep fed EUC, and a tendency of decrease for an eduction in this parameter was found for sheep fed with ANI ($P = 0.08$) compared to control. The in vitro results indicated a reduction of methane production with both EUC and ANI but in a dose-dependant manner. Supplementation of ANI tended to reduce ruminal methane production without adversely affecting rumen fermentation characteristics, nutrient intake, and digestibility, suggesting potential inhibition of ruminal methane emission in sheep supplemented with ANI.

Wang, S., et al. (2018). "Supplementation of Pelleted Hazel (Corylus avellana) Leaves Decreases Methane and Urinary Nitrogen Emissions by Sheep at Unchanged Forage Intake." Scientific Reports **8**(1).

This study is the first to quantify the effects of hazel (Corylus avellana) leaves on methane and urinary nitrogen emissions, digestibility, nitrogen and the energy balance of ruminants. Four experimental pellets were produced with 0, 30% and 60% hazel leaves, the latter also with 4% polyethylene glycol. Hazel leaves gradually replaced lucerne. The diet was composed of the pellets and grass hay (80%: 20%). Six adult sheep were allocated to all four treatments in a 6 × 4 crossover design. Including hazel leaves did not affect the feed intake, but it decreased the apparent digestibility of organic matter and fibre, especially at the high level. Methane emission was reduced by up to 25 to 33% per day, per unit of intake and per unit of organic matter digested. Urinary nitrogen excretion decreased by 33 to 72% with increasing levels of hazel leaves. The treatment with polyethylene glycol demonstrated that tannins in hazel leaves caused significant shares of the effects. In conclusion, the current results indicated a significant potential of hazel leaves as forage for ruminants to mitigate methane and urinary nitrogen emissions. Even high dietary hazel leaf proportions were palatable. The lower digestibility needs to be compensated with easily digestible diet ingredients.

Wilmanns, J. C., et al. (2018). "Metformin Intervention Prevents Cardiac Dysfunction in a murine model of Adult Congenital Heart Disease." bioRxiv.

> Congenital heart disease (CHD) is the most frequent birth defect worldwide and the number of adult patients with CHD, now referred to as ACHD, is increasing. However the mechanisms whereby ACHD predisposes patients to heart dysfunction are still unclear. ACHD is strongly associated with metabolic syndrome, but how ACHD interacts with poor modern lifestyle choices and other comorbidities, such as hypertension, obesity and diabetes, is mostly unknown. Using a genetic mouse model of ACHD we showed that ACHD mice placed under metabolic stress (high fat diet) displayed decreased heart function. Comprehensive physiological, biochemical and molecular analysis showed that ACHD hearts exhibited early changes in energy metabolism that preceded cardiac dysfunction. Restoration of metabolic balance by metformin prevented the development of heart dysfunction in ACHD mice. This study reveals that early metabolic impairment reinforces heart dysfunction in ACHD predisposed individuals and diet or pharmacological interventions can be used to modulate heart function and attenuate heart failure and may be an important avenue for intervention in ACHD.

Wilmanns, J. C., et al. (2018). Metformin Intervention Prevents Cardiac Dysfunction in a murine model of Adult Congenital Heart Disease, core.ac.uk.

> Congenital heart disease (CHD) is the most frequent birth defect worldwide and the number of adult patients with CHD, now referred to as ACHD, is increasing. However the mechanisms whereby ACHD predisposes patients to heart dysfunction are still unclear. ACHD is strongly associated with metabolic syndrome, but how ACHD interacts with poor modern lifestyle choices and other comorbidities, such as hypertension, obesity and diabetes, is mostly unknown. Using a genetic mouse model of ACHD we showed that ACHD mice placed under metabolic stress (high fat diet) displayed decreased heart function. Comprehensive physiological, biochemical and molecular analysis showed that ACHD hearts exhibited early changes in energy metabolism that preceded cardiac dysfunction. Restoration of metabolic balance by metformin prevented the development of heart dysfunction in ACHD mice. This study reveals that early metabolic impairment reinforces heart dysfunction in ACHD predisposed individuals and diet or pharmacological interventions can be used to modulate heart function and attenuate heart failure and may be an important avenue for intervention in ACHD.

Woodie, L. N., et al. (2018). "Restricted feeding for 9 h in the active period partially abrogates the detrimental metabolic effects of a Western diet with liquid sugar consumption in mice." Metabolism: Clinical and Experimental **82**: 1-13.

> Background: Obesity is a major public health concern that can result from diets high in fat and sugar, including sugar sweetened beverages. A proposed treatment for dietary-induced obesity is time-restricted feeding (TRF), which restricts consumption of food to specific times of the 24-hour cycle. Although TRF shows great promise to prevent obesity and the development of chronic disease, the effects of TRF to reverse metabolic changes and the development of NAFLD in animal models of a Western diet with sugary water consumption is not known. Objective: The objective of the current study was to evaluate the role

of TRF in the treatment of obesity and NAFLD through examination of changes in metabolic and histopathologic parameters. Methods: To better understand the role of TRF in the treatment of obesity and NAFLD, we investigated the metabolic phenotype and NAFLD parameters in a mouse model of NAFLD in which obesity and liver steatosis are induced by a Western Diet (WD): a high-fat diet of lard, milkfat and Crisco with sugary drinking water. Mice were subjected to a short-term (4-weeks) and long-term (10-weeks) TRF in which food was restricted to 9 h at night. Results: Prior to TRF treatment, the WD mice had increased body mass, and exhibited less activity, and higher average daytime energy expenditure (EE) than chow fed mice. Approximately 4- and 10-weeks following TFR treatment, WD-TRF had moderate but not statistically significant weight loss compared to WD-ad libitum (WD-AL) mice. There was a modest but significant reduction in the inguinal adipose tissue weight in both WD-TRF groups compared to the WD-AL groups; however, there was no difference in epididymal and retroperitoneal adipose tissue mass or adipocyte size distribution. In contrast, the diet-induced increase in normalized liver tissue weight, hepatic triglyceride, and NAFLD score was partially abrogated in the 4-week WD-TRF mice, while systemic insulin resistance was partially abrogated and glucose intolerance was completely abrogated in the 10-week WD-TRF mice. Importantly, WD-induced metabolic dysfunction (substrate utilization, energy expenditure, and activity) was partially abrogated by 4- and 10-week TRF. Conclusions: Our results support the hypothesis that TRF aids in reducing the detrimental metabolic effects of consuming a WD with sugary drinking water but does not ameliorate obesity.

Zhang, X. and C. Paul (2018). "PO-264 Target Mitochondria Metabolism and Energy Expenditure in Muscle Atrophy, Sarcopenia and Cachexia." Exercise Biochemistry Review **1**(5).

Objective Muscle atrophy is a common and clinically important outcome of many diseases and hospitalization bed rest. Mechanical unloading of skeletal muscles may result in a rapid loss of muscle mass and mitochondria dysfunction. And the recovery from disuse muscle atrophy is usually complete in young healthy adults but is delayed and often incomplete in older patients. However, the mechanisms underlying poor recovery of aged muscle following disuse remain to be delineated. Recent evidence suggests that mitochondrial energetics play an important role in regulation of muscle mass. To this end, we employed multiple approaches to address the role of mitochondrial function and metabolism in muscle atrophy and sarcopenia. And we are also engaged to develop novel mitochondria-targeted antioxidant peptides to mitigate this mitochondria-related functional impairment by ROS and improve the recovery from muscle atrophy and cachexia. Methods Tail Suspension Hind Limb Unloading for adult mice (6-month-old) and aged mice (22-24month old). Cardiometabolic Phenotyping : Measurement of fat and lean mass (g) is accomplished using a LF90II TD-NMR (Bruker, Madison, WI). Locomotor activity measurements are obtained using a Promethion Mouse Multiplexed Metabolic System(Sable Systems International, Las Vegas, NV).Mitochondrial Respiration :Respirometry assays were conducted using an Oxygraph-2k (Oroboros Instruments, Innsbruck, Austria), Mitochondria H2O2 emission was measured with Amplex Red reagent which reacts with H2O2 to produce the stable fluorescent compound resorufin. Resorufin fluorescence was monitored using a Fluorescence. A continuous, spectrophotometric assay was utilized to measure mitochondrial calcium retention capacity (CRC) within soleus fiber bundles. RNA Sequencing and Informatics, KEGG Pathway and Upstream Regulator Analysis, Real-Time Quantitative-PCR. Multidimensional mass spectrometry-based shotgun lipidomics was employed to measure and characterize the lipid patterns inmouse soleus muscle. Metabolomics Acylcarnitines A panel of acylcarnitines was quantitated by LC/ MS/MS (Agilent 1290 HPLC/6490 triple quadrupole mass spectrometer). Amino Acids. Quantitation of amino acids was achieved using LC/MS/MS (Agilent 1290

HPLC/6490 triple quadrupole mass spectrometer). Results Old mice have impaired early recovery of soleus mass following unloading induced muscle atrophy. And mitochondrial function and metabolism does not Improve during early recovery in aged Mice. Divergent metabolomic response was found between adult and aged Mice during unloading and recovery. However, transcriptomic response to unloading and reloading is similar in adult and aged Mice. Conclusions Here, we report that aged mice with low muscle mass and low glucose clearance rate also display poor early recovery of muscle mass after disuse muscle atrophy. We used unbiased and targeted approaches to identify changes in energy metabolism gene expression, metabolite pools and mitochondrial phenotype and show for the first time that persistent mitochondrial dysfunction, dysregulated fatty acid β-oxidation and elevated H2O2 emission occur concomitantly with poor early recovery of muscle mass following a period of disuse in old mice. Importantly, this is linked to more severe whole-body insulin resistance, as determined by insulin tolerance test. Our findings also showed cellular metabolic changes during muscle atrophy and sarcopenia may induce higher production of oxygen radicals that play a significant role in the progression of age-related sarcopenia. These findings suggest that muscle fuel metabolism and mitochondrial energetics could be a focus for mining therapeutic targets to improve recovery of muscle mass following periods of disuse. We are engaged to develop novel mitochondria-targeted antioxidant peptides to mitigate this mitochondria-related functional impairment by ROS and improve the recovery from muscle atrophy and cachexia. Co-administration of other targeted compounds was also promising to improve recovery from muscle atrophy and cachexia.

Zhang, X., et al. (2018). "Impaired Mitochondrial Energetics Characterize Poor Early Recovery of Muscle Mass Following Hind Limb Unloading in Old Mice." Journals of Gerontology - Series A Biological Sciences and Medical Sciences **73**(10): 1313-1322.

The progression of age-related sarcopenia can be accelerated by impaired recovery of muscle mass following periods of disuse due to illness or immobilization. However, the mechanisms underlying poor recovery of aged muscle following disuse remain to be delineated. Recent evidence suggests that mitochondrial energetics play an important role in regulation of muscle mass. Here, we report that 22- to 24-month-old mice with low muscle mass and low glucose clearance rate also display poor early recovery of muscle mass following 10 days of hind limb unloading. We used unbiased and targeted approaches to identify changes in energy metabolism gene expression, metabolite pools and mitochondrial phenotype, and show for the first time that persistent mitochondrial dysfunction, dysregulated fatty acid β-oxidation, and elevated H 2 O 2 emission occur concomitantly with poor early recovery of muscle mass following a period of disuse in old mice. Importantly, this is linked to more severe whole-body insulin resistance, as determined by insulin tolerance test. The findings suggest that muscle fuel metabolism and mitochondrial energetics could be a focus for mining therapeutic targets to improve recovery of muscle mass following periods of disuse in older animals.

Anunciado-Koza, R. P., et al. (2017). "Diet-induced adipose tissue expansion is mitigated in mice with a targeted inactivation of mesoderm specific transcript (Mest)." PLoS One **12**(6).

Interindividual variation of white adipose tissue (WAT) expression of mesoderm specific transcript (Mest), a paternally-expressed imprinted gene belonging to the α/β-hydrolase fold protein family, becomes

apparent among genetically inbred mice fed high fat diet (HFD) and is positively associated with adipose tissue expansion (ATE). To elucidate a role for MEST in ATE, mice were developed with global and adipose tissue inactivation of Mest. Mice with homozygous (MestgKO) and paternal allelic (MestpKO) inactivation of Mest were born at expected Mendelian frequencies, showed no behavioral or physical abnormalities, and did not perturb expression of the Mest locus-derived microRNA miR-335. MestpKO mice fed HFD showed reduced ATE and adipocyte hypertrophy, improved glucose tolerance, and reduced WAT expression of genes associated with hypoxia and inflammation compared to littermate controls. Remarkably, caloric intake and energy expenditure were unchanged between genotypes. Mice with adipose tissue inactivation of Mest were phenotypically similar to MestpKO, supporting a role for WAT MEST in ATE. Global profiling of WAT gene expression of HFD-fed control and MestpKO mice detected few differences between genotypes; nevertheless, genes with reduced expression in MestpKO mice were associated with immune processes and consistent with improved glucose homeostasis. Ear-derived mesenchymal stem cells (EMSC) from MestgKO mice showed no differences in adipogenic differentiation compared to control cells unless challenged by shRNA knockdown of Gpat4, an enzyme that mediates lipid accumulation in adipocytes. Reduced adipogenic capacity of EMSC from MestgKO after Gpat4 knockdown suggests that MEST facilitates lipid accumulation in adipocytes. Our data suggests that reduced diet-induced ATE in MEST-deficient mice diminishes hypoxia and inflammation in WAT leading to improved glucose tolerance and insulin sensitivity. Since inactivation of Mest in mice has minimal additional effects aside from reduction of ATE, an intervention that mitigates MEST function in adipocytes is a plausible strategy to obviate obesity and type-2-diabetes.

Arguin, G., et al. (2017). "The loss of P2X7 receptor expression leads to increase intestinal glucose transit and hepatic steatosis." Scientific Reports **7**(1).

In intestinal epithelial cells (IEC), it was reported that the activation of the P2X7 receptor leads to the internalization of the glucose transporter GLUT2, which is accompanied by a reduction of IEC capacity to transport glucose. In this study, we used P2rx7 -/- mice to decipher P2X7 functions in intestinal glucose transport and to evaluate the impacts on metabolism. Immunohistochemistry analyses revealed the presence of GLUT2 at the apical domain of P2rx7-/- jejunum enterocytes. Positron emission tomography and biodistribution studies demonstrated that glucose was more efficiently delivered to the circulation of knockout animals. These findings correlated with increase blood glucose, insulin, triglycerides and cholesterol levels. In fact, P2rx7-/- mice had increased serum triglyceride and cholesterol levels and displayed glucose intolerance and resistance to insulin. Finally, P2rx7-/- mice developed a hepatic steatosis characterized by a reduction of Acaca, Acacb, Fasn and Acox1 mRNA expression, as well as for ACC and FAS protein expression. Our study suggests that P2X7 could play a central role in metabolic diseases.

Belenchia, A. M., et al. (2017). "In utero vitamin D deficiency predisposes offspring to long-term adverse adipose tissue effects." Journal of Endocrinology **234**(3): 301-313.

The fetal period represents an important window of susceptibility for later obesity and metabolic disease. Maternal vitamin D deficiency (VDD) during pregnancy is a global concern that may have long-lasting

consequences on offspring metabolic health. We sought to determine whether a VDD in utero environment affects fetal adipose tissue development and offspring metabolic disease predisposition in adulthood. Furthermore, we sought to explore the extent to which the VDD intrauterine environment interacts with genetic background or postnatal environment to influence metabolic health. Eight-week-old P0 female C57BL/6J mice were fed either a VDD diet or sufficient diet (VDS) from four weeks before pregnancy (periconception) then bred to male Avy/a mice. Females were maintained on the diets throughout gestation. At weaning, Avy/a and a/a male F1 offspring were randomized to low-fat (LFD) or high-fat diet (HFD) until 19 weeks of age, at which point serum and adipose tissue were harvested for analyses. Mice born to VDD dams weighed less at weaning than offspring born to VDS dams but experienced rapid weight gain in the four weeks post weaning, and acquired a greater ratio of perigonadal (PGAT) to subcutaneous (SQAT) than control offspring. Additionally, these mice were more susceptible to HFD-induced adipocyte hypertrophy. Offspring of VDD dams also had greater expression of Pparg transcript. These novel findings demonstrate that in utero VDD, an easily correctable but highly prevalent health concern, predisposes offspring to long-term adipose tissue consequences and possible adverse metabolic health complications.

Bunney, P. E., et al. (2017). "Orexin activation counteracts decreases in nonexercise activity thermogenesis (NEAT) caused by high-fat diet." Physiology and Behavior **176**: 139-148.

Overweight and obesity result from an imbalance between caloric intake and energy expenditure, including expenditure from spontaneous physical activity (SPA). Changes in SPA and resulting changes in non-exercise activity thermogenesis (NEAT) likely interact with diet to influence risk for obesity. However, previous research on the relationship between diet, physical activity, and energy expenditure has been mixed. The neuropeptide orexin is a driver of SPA, and orexin neuron activity can be manipulated using DREADDs (Designer Receptors Exclusively Activated by Designer Drugs). We hypothesized that HFD decreases SPA and NEAT, and that DREADD-mediated activation of orexin neuron signaling would abolish this decrease and produce an increase in NEAT instead. To test these ideas, we characterized behaviors to determine the extent to which access to a high-fat diet (HFD) influences the proportion and probability of engaging in food intake and activity. We then measured NEAT following access to HFD and following a DREADD intervention targeting orexin neurons. Two cohorts of orexin-cre male mice were injected with an excitatory DREADD virus into the caudal hypothalamus, where orexin neurons are concentrated. Mice were then housed in continuous metabolic phenotyping cages (Sable Promethion). Food intake, indirect calorimetry, and SPA were automatically measured every second. For cohort 1 (n = 8), animals were given access to chow, then switched to HFD. For cohort 2 (n = 4/group), half of the animals were given access to HFD, the other access to chow. Then, among animals on HFD, orexin neurons were activated following injections of clozapine n-oxide (CNO). Mice on HFD spent significantly less time eating ($p < 0.01$) and more time inactive compared to mice on chow ($p < 0.01$). Following a meal, mice on HFD were significantly more likely to engage in periods of inactivity compared to those on chow ($p < 0.05$). NEAT was decreased in animals on HFD, and was increased to the NEAT level of control animals following activation of orexin neurons with DREADDs. Food intake (kilocalories) was not significantly different between mice on chow and HFD, yet mice on chow expended more energy per unit of SPA, relative to that in mice consuming HFD. These results suggest that HFD consumption reduces SPA and NEAT, and increases inactivity following a meal. Together, the data suggest a change in the efficiency of energy expenditure based upon diet, such that SPA during HFD burns fewer calories compared to SPA on a standard chow diet.

Campos, C. A., et al. (2017). "Cancer-induced anorexia and malaise are mediated by CGRP neurons in the parabrachial nucleus." Nature Neuroscience **20**(7): 934-942.

Anorexia is a common manifestation of chronic diseases, including cancer. Here we investigate the contribution to cancer anorexia made by calcitonin gene-related peptide (CGRP) neurons in the parabrachial nucleus (PBN) that transmit anorexic signals. We show that CGRPPBN neurons are activated in mice implanted with Lewis lung carcinoma cells. Inactivation of CGRPPBN neurons before tumor implantation prevents anorexia and loss of lean mass, and their inhibition after symptom onset reverses anorexia. CGRPPBN neurons are also activated in Apcmin/+ mice, which develop intestinal cancer and lose weight despite the absence of reduced food intake. Inactivation of CGRPPBN neurons in Apcmin/+ mice permits hyperphagia that counteracts weight loss, revealing a role for these neurons in a 'nonanorexic' cancer model. We also demonstrate that inactivation of CGRPPBN neurons prevents lethargy, anxiety and malaise associated with cancer. These findings establish CGRPPBN neurons as key mediators of cancer-induced appetite suppression and associated behavioral changes.

Coborn, J. E., et al. (2017). "Role of orexin-A in the ventrolateral preoptic area on components of total energy expenditure." International Journal of Obesity **41**(8): 1256-1262.

Background:Identifying whether components of total energy expenditure (EE) are affected by orexin receptor (OXR1 and OXR2) stimulation or antagonism with dual orexin receptor antagonists (DORAs) has relevance for obesity treatment. Orexin receptor stimulation reduces weight gain by increasing total EE and EE during spontaneous physical activity (SPA).Objective:The purpose of this study was to determine if a DORA (TCS-1102) in the ventrolateral preoptic area (VLPO) reduced orexin-A-induced arousal, SPA, total EE and EE during sleep, rest, wake and SPA and whether the DORA alone reduced total EE and its components. We hypothesized that: (1) a DORA would reduce orexin-A induced increases in arousal, SPA, components of total EE, reductions in sleep and the EE during sleep and (2) the DORA alone would reduce baseline (non-stimulated) SPA and total EE.Subjects/Methods:Sleep, wakefulness, SPA and EE were determined after microinjection of the DORA (TCS-1102) and orexin-A in the VLPO of male Sprague-Dawley rats with a unilateral cannula targeted towards the VLPO. Individual components of total EE were determined based on time-stamped data.Results:The DORA reduced orexin-A-induced increases in arousal, SPA, total EE and EE during SPA, wake, rest and sleep 1 h post injection ($P<0.05$). Orexin-A significantly reduced sleep and significantly increased EE during sleep 1 h post injection ($P<0.05$). Furthermore, the DORA alone significantly reduced total EE, EE during sleep (NREM and REM) and resting EE 2 h post injection ($P<0.05$).Conclusions:These data suggest that orexin-A reduces weight gain by stimulating total EE through increases in EE during SPA, rest and sleep. Residual effects of the DORA alone include decreases in total EE and EE during sleep and rest, which may promote weight gain.

Cooper, M. A., et al. (2017). "Modulation of diet-induced mechanical allodynia by metabolic parameters and inflammation." Journal of the Peripheral Nervous System **22**(1): 39-46.

Dietary-associated diseases have increased tremendously in our current population, yet key molecular changes associated with high-fat diets that cause clinical pre-diabetes, obesity, hyperglycemia, and

peripheral neuropathy remain unclear. This study examines molecular and metabolic aspects altered by voluntary exercise and a high-fat diet in the mouse dorsal root ganglion. Mice were examined for changes in mRNA and proteins encoding anti-inflammatory mediators, metabolic-associated molecules, and pain-associated ion channels. Proteins involved in the synaptosomal complex and pain-associated TRP ion channels decrease in the dorsal root ganglion of high-fat exercise animals relative to their sedentary controls. Exercise reversed high-fat diet induced mechanical allodynia without affecting weight gain, elevated blood glucose, and utilization of fat as a fuel source. Independent of weight or fat mass changes, high-fat exercised mice display reduced inflammation-associated mRNAs. The benefits of exercise on abnormal peripheral nerve function appear to occur independent of systemic metabolic changes, suggesting that the utilization of fats and inflammation in the peripheral nervous system may be key for diet-induced peripheral nerve dysfunction and the response to exercise.

Cross, T. W. L., et al. (2017). "Soy improves cardiometabolic health and cecal microbiota in female low-fit rats." Scientific Reports **7**(1).

Phytoestrogen-rich soy is known to ameliorate menopause-associated obesity and metabolic dysfunction for reasons that are unclear. The gut microbiota have been linked with the development of obesity and metabolic dysfunction. We aimed to determine the impact of soy on cardiometabolic health, adipose tissue inflammation, and the cecal microbiota in ovariectomized (OVX) rats bred for low-running capacity (LCR), a model that has been previously shown to mimic human menopause compared to sham-operated (SHM) intact control LCR rats. In this study, soy consumption, without affecting energy intake or physical activity, significantly improved insulin sensitivity and body composition of OVX rats bred for low-running capacity. Furthermore, soy significantly improved blood lipid profile, adipose tissue inflammation, and aortic stiffness of LCR rats. Compared to a soy-free control diet, soy significantly shifted the cecal microbial community of LCR rats, resulting in a lower Firmicutes:Bacteroidetes ratio. Correlations among metabolic parameters and cecal bacterial taxa identified in this study suggest that taxa Prevotella, Dorea, and Phascolarctobacterium may be taxa of interest. Our results suggest that dietary soy ameliorates adiposity, insulin sensitivity, adipose tissue inflammation, and arterial stiffness and exerts a beneficial shift in gut microbial communities in a rat model that mimics human menopause.

Demaria, M., et al. (2017). "Cellular senescence promotes adverse effects of chemotherapy and cancer relapse." Cancer Discovery **7**(2): 165-176.

Cellular senescence suppresses cancer by irreversibly arresting cell proliferation. Senescent cells acquire a proinflammatory senescence-associated secretory phenotype. Many genotoxic chemotherapies target proliferating cells nonspecifically, often with adverse reactions. In accord with prior work, we show that several chemotherapeutic drugs induce senescence of primary murine and human cells. Using a transgenic mouse that permits tracking and eliminating senescent cells, we show that therapy-induced senescent (TIS) cells persist and contribute to local and systemic inflammation. Eliminating TIS cells reduced several short-and long-term effects of the drugs, including bone marrow suppression, cardiac dysfunction, cancer recurrence, and physical activity and strength. Consistent with our findings in mice, the risk of chemotherapy-induced fatigue was significantly greater in humans with increased expression of a senescence marker in T cells prior to chemotherapy. These findings suggest that senescent cells can

cause certain chemotherapy side effects, providing a new target to reduce the toxicity of anticancer treatments. SIGNIFICANCE: Many genotoxic chemotherapies have debilitating side effects and also induce cellular senescence in normal tissues. The senescent cells remain chronically present where they can promote local and systemic inflammation that causes or exacerbates many side effects of the chemotherapy.

Den Hartigh, L. J., et al. (2017). "Metabolically distinct weight loss by 10,12 CLA and caloric restriction highlight the importance of subcutaneous white adipose tissue for glucose homeostasis in mice." PLoS One **12**(2).

Background Widely used as a weight loss supplement, trans-10,cis-12 conjugated linoleic acid (10,12 CLA) promotes fat loss in obese mice and humans, but has also been associated with insulin resistance. Objective We therefore sought to directly compare weight loss by 10,12 CLA versus caloric restriction (CR, 15±25%), an acceptable healthy method of weight loss, to determine how 10,12 CLAmediated weight loss fails to improve glucose metabolism. Methods Obese mice with characteristics of human metabolic syndrome were either supplemented with 10,12 CLA or subjected to CR to promote weight loss. Metabolic endpoints such as energy expenditure, glucose and insulin tolerance testing, and trunk fat distribution were measured. Results By design, 10,12 CLA and CR caused equivalent weight loss, with greater fat loss by 10,12 CLA accompanied by increased energy expenditure, reduced respiratory quotient, increased fat oxidation, accumulation of alternatively activated macrophages, and browning of subcutaneous white adipose tissue (WAT). Moreover, 10,12 CLA-supplemented mice better defended their body temperature against a cold challenge. However, 10,12 CLA concurrently induced the detrimental loss of subcutaneous WAT without reducing visceral WAT, promoted reduced plasma and WAT adipokine levels, worsened hepatic steatosis, and failed to improve glucose metabolism. Obese mice undergoing CR were protected from subcutaneous-specific fat loss, had improved hepatic steatosis, and subsequently showed the expected improvements in WAT adipokines, glucose metabolism and WAT inflammation. Conclusions These results suggest that 10,12 CLA mediates the preferential loss of subcutaneous fat that likely contributes to hepatic steatosis and maintained insulin resistance, despite significant weight loss and WAT browning in mice. Collectively, we have shown that weight loss due to 10,12 CLA supplementation or CR results in dramatically different metabolic phenotypes, with the latter promoting a healthier form of weight loss.

DePorter, D. P., et al. (2017). "Partial Sleep Deprivation Reduces the Efficacy of Orexin-A to Stimulate Physical Activity and Energy Expenditure." Obesity **25**(10): 1716-1722.

Objective: Sufficient sleep is required for weight maintenance. Sleep deprivation due to noise exposure stimulates weight gain by increasing hyperphagia and reducing energy expenditure (EE). Yet the mechanistic basis underlying the weight gain response is unclear. Orexin-A promotes arousal and negative energy balance, and orexin terminals project to the ventrolateral preoptic area (VLPO), which is involved in sleep-to-wake transitions. To determine whether sleep deprivation reduces orexin function in VLPO and to test the hypothesis that sleep deprivation would attenuate the orexin-A-stimulated increase in arousal, physical activity (PA), and EE. Methods: Electroencephalogram, electromyogram, distance traveled, and EE were determined in male Sprague-Dawley rats following orexin-A injections into VLPO

both before and after acute (12-h) and chronic (8 h/d, 9 d) sleep deprivation by noise exposure. Results: Orexin-A in the VLPO significantly increased arousal, PA, total EE, and PA-related EE and reduced sleep and respiratory quotient before sleep deprivation. In contrast to after acute sleep deprivation in which orexin-A failed to stimulate EE during PA only, orexin-A failed to significantly increase arousal, PA, fat oxidation, total EE, and PA-related EE after chronic sleep deprivation. Conclusions: Sleep deprivation may reduce sensitivity to endogenous stimuli that enhance EE due to PA and thus stimulate weight gain.

Dodd, G. T., et al. (2017). "A Hypothalamic Phosphatase Switch Coordinates Energy Expenditure with Feeding." Cell Metabolism **26**(2): 375-393.e377.

Beige adipocytes can interconvert between white and brown-like states and switch between energy storage versus expenditure. Here we report that beige adipocyte plasticity is important for feeding-associated changes in energy expenditure and is coordinated by the hypothalamus and the phosphatase TCPTP. A fasting-induced and glucocorticoid-mediated induction of TCPTP, inhibited insulin signaling in AgRP/NPY neurons, repressed the browning of white fat and decreased energy expenditure. Conversely feeding reduced hypothalamic TCPTP, to increase AgRP/NPY neuronal insulin signaling, white adipose tissue browning and energy expenditure. The feeding-induced repression of hypothalamic TCPTP was defective in obesity. Mice lacking TCPTP in AgRP/NPY neurons were resistant to diet-induced obesity and had increased beige fat activity and energy expenditure. The deletion of hypothalamic TCPTP in obesity restored feeding-induced browning and increased energy expenditure to promote weight loss. Our studies define a hypothalamic switch that coordinates energy expenditure with feeding for the maintenance of energy balance.

Dorfman, M. D., et al. (2017). "Sex differences in microglial CX3CR1 signalling determine obesity susceptibility in mice." Nature Communications **8**.

Female mice are less susceptible to the negative metabolic consequences of high-fat diet feeding than male mice, for reasons that are incompletely understood. Here we identify sex-specific differences in hypothalamic microglial activation via the CX3CL1-CX3CR1 pathway that mediate the resistance of female mice to diet-induced obesity. Female mice fed a high-fat diet maintain CX3CL1-CX3CR1 levels while male mice show reductions in both ligand and receptor expression. Female Cx3cr1 knockout mice develop 'male-like' hypothalamic microglial accumulation and activation, accompanied by a marked increase in their susceptibility to diet-induced obesity. Conversely, increasing brain CX3CL1 levels in male mice through central pharmacological administration or virally mediated hypothalamic overexpression converts them to a 'female-like' metabolic phenotype with reduced microglial activation and body-weight gain. These data implicate sex differences in microglial activation in the modulation of energy homeostasis and identify CX3CR1 signalling as a potential therapeutic target for the treatment of obesity.

Douglass, J. D., et al. (2017). "Astrocyte IKKβ/NF-κB signaling is required for diet-induced obesity and hypothalamic inflammation." Molecular Metabolism **6**(4): 366-373.

Objective Obesity and high fat diet (HFD) consumption in rodents is associated with hypothalamic inflammation and reactive gliosis. While neuronal inflammation promotes HFD-induced metabolic dysfunction, the role of astrocyte activation in susceptibility to hypothalamic inflammation and diet-induced obesity (DIO) remains uncertain. Methods Metabolic phenotyping, immunohistochemical analyses, and biochemical analyses were performed on HFD-fed mice with a tamoxifen-inducible astrocyte-specific knockout of IKKβ (GfapCreERIkbkbfl/fl, IKKβ-AKO), an essential cofactor of NF-κB-mediated inflammation. Results IKKβ-AKO mice with tamoxifen-induced IKKβ deletion prior to HFD exposure showed equivalent HFD-induced weight gain and glucose intolerance as Ikbkbfl/fl littermate controls. In GfapCreERTdTomato marker mice treated using the same protocol, minimal Cre-mediated recombination was observed in the mediobasal hypothalamus (MBH). By contrast, mice pretreated with 6 weeks of HFD exposure prior to tamoxifen administration showed substantially increased recombination throughout the MBH. Remarkably, this treatment approach protected IKKβ-AKO mice from further weight gain through an immediate reduction of food intake and increase of energy expenditure. Astrocyte IKKβ deletion after HFD exposure—but not before—also reduced glucose intolerance and insulin resistance, likely as a consequence of lower adiposity. Finally, both hypothalamic inflammation and astrocytosis were reduced in HFD-fed IKKβ-AKO mice. Conclusions These data support a requirement for astrocytic inflammatory signaling in HFD-induced hyperphagia and DIO susceptibility that may provide a novel target for obesity therapeutics.

Evans, M. C. and G. M. Anderson (2017). "Dopamine neuron-restricted leptin receptor signaling reduces some aspects of food reward but exacerbates the obesity of leptin receptor-deficient male mice." Endocrinology **158**(12): 4246-4256.

The contribution of leptin-induced modulation of dopamine neurons to feeding behavior and energy homeostasis remains unclear. Midbrain dopamine neurons regulate the reward value of food, and direct leptin administration to the midbrain reduces food intake. However, selective deletion of leptin receptors (Leprs) from dopamine neurons has no effect on body weight, food intake, or hedonic responses, suggesting that leptin acts indirectly or demonstrating that sufficient compensation occurs to mask any direct leptin-dopamine effects. To further explore the role of direct Lepr-dopamine neuron signaling in the control of feeding behavior and energy homeostasis, we generated mice in which Leprs were expressed exclusively in dopamine transporter (DAT)-expressing neurons (LeprDAT). We then assessed weekly body weight, daily food intake, hyperphagic feeding, and leptin-induced suppression of feeding in the LeprDAT mice compared with their Lepr-deficient (LeprNULL) and wild-type control (LeprCON) littermates. We also used metabolic cages to characterize running wheel activity, home-cage activity, and total energy expenditure. As expected, LeprNULL mice exhibited increased body weight and food intake compared with LeprCON mice. LeprDAT male mice exhibited acute leptin-induced suppression of food intake and reduced hedonic feeding but also exhibited significantly increased postweaning body weight gain compared with the LeprNULL mice. This was associated with significantly reduced home-cage activity counts, although no differences in food intake were observed between the LeprDAT and LeprNULL mice. These data demonstrate that restoring Lepr signaling exclusively in dopamine neurons reduces some aspects of food reward and activity but does not ameliorate the obesity phenotype of Lepr-deficient mice.

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Fujimoto, D., et al. (2017). "Usefulness of Linked Color Imaging System in Endoscopic Diagnosis of Sessile Serrated Adenoma/Polyp: A Novel Image Enhanced Technique." Gastroenterology **152**(5): S81-S82.

Aims: It has been reported that 10 to 15% of colorectal polyps are missed in colonoscopic examinations. Particularly, colorectal polyps in the right-side colon are reportedly missed in colonoscopy. Sessile serrated adenoma/polyp (SSA/P) predominantly occurs in the rightside colon, and is a putative precursor lesion of the cancer with microsatellite instability. It is more difficult to detect SSA/P with conventional colonoscopy than adenomatous polyp because it is flat and isochronous. Although new image-enhanced endoscopic (IEE) technology methods such as blue laser imaging (BLI), BLI-bright, and linked color imaging (LCI) have been developed, their utility for the detection of colorectal polyps including SSA/P has not yet been determined. This study aimed to evaluate the utility of BLI, BLI-bright, and LCI for SSA/P detection in still images and in a prospective randomized controlled trial (RCT). Methods: A group of 6 of expert and non-expert endoscopists read 200 endoscopic still images containing SSA/P lesions using white light (WLI), BLI, BLI-bright and LCI. Detection rate of SSA/P in each imaging mode was evaluated. For detectability, images were scored on a 4-point scale, and individual scores were added in each mode. Color differences between SSA/Ps and background mucosa were calculated according to the color space method. A prospective RCT of tandem colonoscopy with WLI and LCI was also performed. Patients with SSA/P and those with a history of SSA/P that had been endoscopically removed were enrolled and randomly allocated to WLI-LCI or LCI-WLI groups. Additional endoscopic detection rates for SSA/P were compared between the 2 groups. Results: LCI showed the highest SSA/P detection rate and detectability score among

the 4 modes for both expert and non-expert endoscopists. The detection rates and detectability score with LCI were significantly higher than those with WLI both for expert and non-expert endoscopists. The color difference of SSA/P in LCI (17.6±7.3) was the highest, and was significantly higher than in WLI (11.2±6.0). In the RCT, a total of 44 patients (22 each in the WLI-LCI and LCI-WLI groups) underwent colonoscopy. The additional detection rate of SSA/P in the second inspection in the WLI-LCI group (21.6%, 95%CI 8.4-34.9%) was significantly higher than in the LCI-WLI group (3.2%, 95%CI-3.4-9.4%, p=0.03). The prevalence of additional SSA/P lesions in the second inspection was significantly higher in the WLI-LCI group (7/22) versus the LCI-WLI group (1/22, p=0.03). The smaller, non-mucus and isochroous SSA/Ps in the transverse colon were detected more frequently in the second inspection with LCI. Conclusions: LCI mode was the most sensitive for SSA/P detection in colonoscopy among WLI, BLI, BLI-bright and LCI in both expert and non-expert endoscopists. RCT demonstrated superiority of LCI to WLI for SSA/P detection in colonoscopy.

Grobe, J. L. (2017). "Comprehensive assessments of energy balance in mice." Methods in Molecular Biology **1614**: 123-146.

Increasing evidence supports a major role for the renin-angiotensin system (RAS) in energy balance physiology. The RAS exists as a circulating system but also as a local paracrine/autocrine signaling mechanism in target tissues including the gastrointestinal tract, the brain, the kidney, and distinct adipose beds. Through activation of various receptors in these target tissues, the RAS contributes to the control of food intake behavior, digestive efficiency, spontaneous physical activity, and aerobic and anaerobic resting metabolism. Although the assortment of methodologies available to assess the various aspects of energy balance can be daunting for an investigator new to this area, a relatively straightforward array of entry-level and advanced methodologies can be employed to comprehensively and quantitatively dissect the effects of experimental manipulations on energy homeostasis. Such methodologies and a simple initial workflow for the use of these methods are described in this chapter, including the use of metabolic caging systems, bomb calorimetry, body composition analyzers, respirometry systems, and direct calorimetry systems. Finally, a brief discussion of the statistical analyses of metabolic data is included.

Haywood, N. J., et al. (2017). "Insulin-like growth factor binding protein 1 could improve glucose regulation and insulin sensitivity through its RGD domain." Diabetes **66**(2): 287-299.

Low circulating levels of insulin-like growth factor binding protein 1 (IGFBP-1) are associated with insulin resistance and predict the development of type 2 diabetes. IGFBP-1 can affect cellular functions independently of IGF binding through an Arg-Gly-Asp (RGD) integrin-binding motif. Whether causal mechanisms underlie the favorable association of high IGFBP-1 levels with insulin sensitivity and whether these could be exploited therapeutically remain unexplored. We used recombinant IGFBP-1 and a synthetic RGD-containing hexapeptide in complementary in vitro signaling assays and in vivo metabolic profiling in obese mice to investigate the effects of IGFBP-1 and its RGD domain on insulin sensitivity, insulin secretion, and whole-body glucose regulation. The RGD integrin-binding domain of IGFBP-1, through integrin engagement, focal adhesion kinase, and integrin-linked kinase, enhanced insulin sensitivity and insulin secretion in C2C12 myotubes and INS-1 832/13 pancreatic β-cells. Both acute administration and chronic infusion of an RGD synthetic peptide to obese C57BL/6 mice improved glucose

clearance and insulin sensitivity. These favorable effects on metabolic homeostasis suggest that the RGD integrin-binding domain of IGFBP-1 may be a promising candidate for therapeutic development in the field of insulin resistance.

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Johnson, S. A., et al. (2017). "Effects of a maternal high-fat diet on offspring behavioral and metabolic parameters in a rodent model." Journal of Developmental Origins of Health and Disease **8**(1): 75-88.

Maternal diet-induced obesity can cause detrimental developmental origins of health and disease in offspring. Perinatal exposure to a high-fat diet (HFD) can lead to later behavioral and metabolic disturbances, but it is not clear which behaviors and metabolic parameters are most vulnerable. To address this critical gap, biparental and monogamous oldfield mice (Peromyscus polionotus), which may better replicate most human societies, were used in the current study. About 2 weeks before breeding, adult females were placed on a control or HFD and maintained on the diets throughout gestation and lactation. F1 offspring were placed at weaning (30 days of age) on the control diet and spatial learning and memory, anxiety, exploratory, voluntary physical activity, and metabolic parameters were tested when they reached adulthood (90 days of age). Surprisingly, maternal HFD caused decreased latency in initial and reverse Barnes maze trials in male, but not female, offspring. Both male and female HFD-fed offspring showed increased anxiogenic behaviors, but decreased exploratory and voluntary physical activity. Moreover, HFD offspring demonstrated lower resting energy expenditure (EE) compared with controls. Accordingly, HFD offspring weighed more at adulthood than those from control fed dams, likely the result of reduced physical activity and EE. Current findings indicate a maternal HFD may increase obesity susceptibility in offspring due to prenatal programming resulting in reduced physical activity and EE later in life. Further work is needed to determine the underpinning neural and metabolic mechanisms by which a maternal HFD adversely affects neurobehavioral and metabolic pathways in offspring.

Kothari, V., et al. (2017). "High fat diet induces brain insulin resistance and cognitive impairment in mice." Biochimica et Biophysica Acta - Molecular Basis of Disease **1863**(2): 499-508.

High fat diet-induced obesity is associated with insulin resistance (IR) and other chronic, diet related illnesses, including dementia. Alzheimer disease is the most common form of dementia, and is characterized by the presence of amyloid plaques and neurofibrillary tangles in brain. This study was designed to determine whether diet-induced changes in peripheral insulin sensitivity could contribute to alterations in brain insulin signaling and cognitive functions. Six week old, male C57BL/6NHsd mice were randomly assigned a high fat diet (40% energy from fat) with 42 g/L liquid sugar (HFS) added to the drinking water or a normal chow diet (12% energy from fat) for 14 weeks. Metabolic phenotypes were characterized for energy expenditure, physical activity, and food intake, and glucose and insulin tolerance tests. In addition, we examined the changes in protein expression related to brain insulin signaling and cognitive function. Mice fed HFS exhibited a statistically significant increase in obesity, and lower glucose and insulin tolerance as compared to animals fed the normal chow diet. In brain, HFS elicited IR as evidenced by a significant decrease in tyrosine phosphorylation of insulin receptor and an increase serine phosphorylation of IRS-1. These changes were accompanied by inflammatory (NFκB, JNK) and stress responses (p38 MAPK, CHOP) in whole brain lysate. In addition, HFS mouse brain exhibited biochemical changes related to increased amyloid beta deposition and neurofibrillary tangle formation, and decreased synaptic plasticity. These results suggested changes in insulin sensitivity might contribute to cognitive impairment associated with the HFS diet in mice.

Leggett, C. B., et al. (2017). "Obesogenic Drugs and their Effects on Post-Laparoscopic Sleeve Gastrectomy Outcomes." Gastroenterology **152**(5): S82-S83.

Background: Laparoscopic sleeve gastrectomy (LSG) is considered an effective, low-risk surgical treatment option for obesity. Nevertheless, there is great variance in weight loss after the procedure. Many medications used to treat routine medical problems are obesogenic (e.g. amitriptyline, gabapentin, propranolol, etc.). Our aim is to determine whether obesogenic drugs negatively affect weight loss outcomes post-LSG. Methods: This is a retrospective study of 323 patients within UCSD Healthcare System that underwent LSG between March 2007-March 2016. We identified a list of 43 drugs classified as obesogenic by The Endocrine Society (Apovian et al., 2015). Patients were divided into two groups based on their prescription drug regimens. Patients prescribed obesogenic medications for periods of at least 3 months within one-year post-LSG were added to our “meds group” whereas patients without any obesogenic medications on their prescription drug regimens were added to our “control group.” We recorded patient weight before surgery and at 3, 6, 9, and 12 months post-LSG and calculated percent excess weight loss (%EWL) for these time points. We collected information on comorbidities and demographics as well as prescription drug regimens at each time point. T-tests were used to compare the %EWL of each group at the time points of interest and results were confirmed with a multivariable regression to identify potential confounders. Results: A total of 177 patients (55%) were on prescribed obesogenic medications during the one-year post-LSG timeframe. By 12 months post-LSG we had weight data on 84 patients from our meds group and 61 patients from our control group. Patients prescribed obesogenic medications at any time within one year post-LSG had less weight loss compared to patients who were never prescribed obesogenic medications post-LSG (%EWL 57.3 ± 2.38 n = 84, 65.6 ± 3.34 n = 61, respectively, p = 0.015; Figure 1). This difference in %EWL between the two groups could not be

attributed to differences in age, gender, or the following co-morbidities: depression, anxiety, bipolar disorder, seizures/epilepsy, insulin resistance, diabetes, dyslipidemia, or hypertension. Secondary analysis was done to assess weight loss outcomes of specific subsets of patients within our meds group. Of note, patients who were on obesogenic medications during the entire year post-LSG had even less weight loss (%EWL 55.5 ± 2.59 n = 72, p = 0.01) when compared to patients on no obesogenic medications. Patients who were on obesogenic antipsychotics were most resistant to weight loss post-LSG (%EWL 42.1 n=7, p=0.02). Patients who were on obesogenic anticonvulsants (n=24) had an average%EWL of 51.5% compared to patients without obesogenic medications (p=0.03). Conclusions: The use of obesogenic medications may negatively impact weight loss outcomes post-LSG.

Lighton, J. R. B. (2017). "Limitations and requirements for measuring metabolic rates: A mini review." European Journal of Clinical Nutrition **71**(3): 301-305.

Metabolic measurement of humans and model animals is an important aspect of biomedicine. Particularly, in the case of model animals, the limitations of currently widely used metabolic measurement methods are not widely understood. In this mini-review, I explain the theoretical underpinnings of flow-through respirometry as a linear time-invariant system, and the (usually serious) distortions of metabolic data caused by the interaction of chamber volume and flow rate. These can be ameliorated by increasing the flow rate through the chamber, though this is at the expense of the magnitude of the O 2 depletion and CO 2 enhancement signals from which metabolic rates are calculated. If achieved, however, the improvement in temporal response that follows higher flow rates can be marked, and allows confident and accurate measurement of resting and active energy expenditure. Applications of this approach in multiplexing gas signals from multiple cages, and in human room calorimetry, are also discussed.

Liu, P., et al. (2017). "Blocking FSH induces thermogenic adipose tissue and reduces body fat." Nature **546**(7656): 107-112.

Menopause is associated with bone loss and enhanced visceral adiposity. A polyclonal antibody that targets the β-subunit of the pituitary hormone follicle-stimulating hormone (Fsh) increases bone mass in mice. Here, we report that this antibody sharply reduces adipose tissue in wild-type mice, phenocopying genetic haploinsufficiency for the Fsh receptor gene Fshr. The antibody also causes profound beiging, increases cellular mitochondrial density, activates brown adipose tissue and enhances thermogenesis. These actions result from the specific binding of the antibody to the β-subunit of Fsh to block its action. Our studies uncover opportunities for simultaneously treating obesity and osteoporosis.

Maridas, D. E., et al. (2017). "IGFBP4 Is required for adipogenesis and influences the distribution of adipose depots." Endocrinology **158**(10): 3488-3500.

> Insulinlike growth factor (IGF) I induces adipogenesis in vitro. IGF-binding protein 4 (IGFBP4) is highly expressed in adipocytes and osteoblasts and is inhibitory of IGFs in vitro.We previously reported that Igfbp4 null mice (Igfbp42/2) had decreased fat proportions at 8 and 16 weeks of age. However, the mechanism leading to the reduced adiposity remains unknown. The purpose of this study was to elucidate how IGFBP4 mediates adipose tissue development in vivo. Our results showed that inguinal and gonadal white adipose tissue (gWAT) from Igfbp42/2 mice had decreased weights and Pparg expression. Cultures of primary bone marrow stromal cells (BMSCs) and ear mesenchymal stem cells (eMSCs) from mutant mice showed reduced adipogenesis. Both BMSCs and eMSC had a strong induction of Igfbp4 expression during adipogenesis. Furthermore, the increase in phosphorylated Akt (p-Akt), a downstream target of IGF-I signaling, in wild-Type cells, was blunted in mutant eMSCs. On a high-fat diet (HFD) there were sexual differences in adipocyte expansion of Igfbp42/2 mice. Mutant males gained weight by expanding their white fat depots. However, Igfbp42/2 female mice were protected against diet-induced obesity. Ovariectomized Igfbp42/2 female mice gained weight in a manner similar to that seen in ovariectomized controls. Thus, Igfbp4 is required for inguinal fat expansion in female mice but not in male mice. However, gWAT expansion, which is prevented by estrogen during HFD, does not require Igfbp4.

Mina, A. I., et al. (2017). "CalR: A Web-based Analysis Tool for Indirect Calorimetry Experiments." bioRxiv.

> We report a web-based tool for analysis of indirect calorimetry experiments which measure physiological energy balance. CalR easily imports raw data files, generates plots, and determines the most appropriate statistical tests for interpretation. Analysis with the general linear model (which includes ANOVA and ANCOVA) allows for flexibility to interpret experiments of obesity and thermogenesis. Users may also produce standardized output files of an experiment which can be shared and subsequently re-evaluated using CalR. This framework will provide the transparency necessary to enhance consistency and reproducibility in experiments of energy expenditure. CalR analysis software will greatly increase the speed and efficiency with which metabolic experiments can be organized, analyzed according to accepted norms, and reproduced—and will likely become a standard tool for the field. CalR is accessible at https://CalR.bwh.harvard.edu. Graphical Abstract

Nas, A., et al. (2017). "Impact of breakfast skipping compared with dinner skipping on regulation of energy balance and metabolic risk." American Journal of Clinical Nutrition **105**(6): 1351-1361.

> Background: Meal skipping has become an increasing trend of the modern lifestyle that may lead to obesity and type 2 diabetes. Objective: We investigated whether the timing of meal skipping impacts these risks by affecting circadian regulation of energy balance, glucose metabolism, and postprandial inflammatory responses. Design: In a randomized controlled crossover trial, 17 participants [body mass index (in kg/m2): 23.7 ± 4.6] underwent 3 isocaloric 24-h interventions (55%, 30%, and 15% carbohydrate, fat, and protein, respectively): a breakfast skipping day (BSD) and a dinner skipping day (DSD) separated

by a conventional 3-meal-structure day (control). Energy and macronutrient balance was measured in a respiration chamber. Postprandial glucose, insulin, and inflammatory responses in leukocytes as well as 24-h glycemia and insulin secretion were analyzed. Results: When compared with the 3-meal control, 24-h energy expenditure was higher on both skipping days (BSD: +41 kcal/d; DSD: +91 kcal/d; both P<0.01), whereas fat oxidation increased on the BSD only (+16 g/d; P<0.001). Spontaneous physical activity, 24-h glycemia, and 24-h insulin secretion did not differ between intervention days. The postprandial homeostasis model assessment index (+54%) and glucose concentrations after lunch (+46%) were, however, higher on the BSD than on the DSD (both P<0.05). Concomitantly, a longer fasting period with breakfast skipping also increased the inflammatory potential of peripheral blood cells after lunch. Conclusions: Compared with 3 meals/d, meal skipping increased energy expenditure. In contrast, higher postprandial insulin concentrations and increased fat oxidation with breakfast skipping suggest the development of metabolic inflexibility in response to prolonged fasting that may in the long term lead to low-grade inflammation and impaired glucose homeostasis. This trial was registered at clinicaltrials.gov as NCT02635139. Am J Clin Nutr 2017;105:1351-61.

Park, Y. M., et al. (2017). "Voluntary Running Attenuates Metabolic Dysfunction in Ovariectomized Low-Fit Rats." Medicine and Science in Sports and Exercise **49**(2): 254-264.

Introduction Ovariectomy and high-fat diet (HFD) worsen obesity and metabolic dysfunction associated with low aerobic fitness. Exercise training mitigates metabolic abnormalities induced by low aerobic fitness, but whether the protective effect is maintained after ovariectomy and HFD is unknown. Purpose This study determined whether, after ovariectomy and HFD, exercise training improves metabolic function in rats bred for low intrinsic aerobic capacity. Methods Female rats selectively bred for low (LCR) and high (HCR) intrinsic aerobic capacity (n = 30) were ovariectomized, fed HFD, and randomized to either a sedentary (SED) or voluntary wheel running (EX) group. Resting energy expenditure, glucose tolerance, and spontaneous physical activity were determined midway through the experiment, whereas body weight, wheel running volume, and food intake were assessed throughout the study. Body composition, circulating metabolic markers, and skeletal muscle gene and protein expression were measured at sacrifice. Results EX reduced body weight and adiposity in LCR rats (-10% and -50%, respectively; P < 0.05) and, unexpectedly, increased these variables in HCR rats (+7% and +37%, respectively; P < 0.05) compared with their respective SED controls, likely because of dietary overcompensation. Wheel running volume was approximately fivefold greater in HCR than LCR rats, yet EX enhanced insulin sensitivity equally in LCR and HCR rats (P < 0.05). This EX-mediated improvement in metabolic function was associated with thee gene upregulation of skeletal muscle interleukin-6 and interleukin-10. EX also increased resting energy expenditure, skeletal muscle mitochondrial content (oxidative phosphorylation complexes and citrate synthase activity), and adenosine monophosphate-activated protein kinase activation similarly in both lines (all P <0.05). Conclusion Despite a fivefold difference in running volume between rat lines, EX similarly improved systemic insulin sensitivity, resting energy expenditure, and skeletal muscle mitochondrial content and adenosine monophosphate-activated protein kinase activation in ovariectomized LCR and HCR rats fed HFD compared with their respective SED controls.

Parrish, J. B. and J. A. Teske (2017). "Acute partial sleep deprivation due to environmental noise increases weight gain by reducing energy expenditure in rodents." Obesity **25**(1): 141-146.

Objective: Chronic partial sleep deprivation (SD) by environmental noise exposure increases weight gain and feeding in rodents, which contrasts weight loss after acute SD by physical methods. This study tested whether acute environmental noise exposure reduced sleep and its effect on weight gain, food intake, physical activity, and energy expenditure (EE). It was hypothesized that acute exposure would (1) increase weight gain and feeding and (2) reduce sleep, physical activity, and EE (total and individual components); and (3) behavioral changes would persist throughout recovery from SD. Methods: Three-month old male Sprague-Dawley rats slept ad libitum, were noise exposed (12-h light cycle), and allowed to recover (36 h). Weight gain, food intake, sleep/wake, physical activity, and EE were measured. Results: Acute environmental noise exposure had no effect on feeding, increased weight gain ($P < 0.01$), and reduced sleep ($P < 0.02$), physical activity ($P < 0.03$), total EE ($P < 0.05$), and several components ($P < 0.05$). Reductions in EE and physical activity persisted during recovery. Conclusions: Reductions in EE during sleep, rest, and physical activity reduce total EE and contribute to weight gain during acute SD and recovery from SD. These data emphasize the importance of increasing physical activity after SD to prevent obesity.

Phillips, D. J. (2017). Exploring the Effects of Circadian Disruption on Metabolic Function and Sleep, search.proquest.com.

The circadian system plays an important role in regulating nearly every process in the body, keeping them in sync with each other, as well as the outside environment. However, artificial lighting and an “always on” society have led to the breakdown between the circadian system and the solar day. One of the most perceptible outputs of the circadian clock, particularly when it is not working properly, is the timing of the sleep-wake cycle. However, circadian rhythms are found in nearly every physiological system. Thus, it is no surprise that circadian and sleep disruption is associated with negative health outcomes, including metabolic dysregulation and obesity. Therefore, understanding how circadian disruption (CD) alters regulation of the sleep-wake cycle and metabolic function is imperative. We induced environmental CD by housing adult male mice in an altered light-dark (LD) cycle with a period of 20h (10hr light, 10hr dark). We used a number of recording techniques to probe metabolic, circadian, and sleep function in normal lighting conditions (LD12:12, 12hr light, 12hr dark) compared to environmental CD (LD10:10). Our model of CD induces a number of changes in both metabolic function and sleep. We find a marked increase in adiposity and decreased glucose tolerance following CD. Diurnal variation in feeding and respiratory quotient are diminished during CD. In addition, there are significant changes in sleep timing and quality. Our work also probed how disrupting the circadian clock alters the ability of an organism to respond to additional stressors. To do this, we applied an immune challenge to circadian disrupted mice, and found that the immune response to lipopolysaccharide challenge was greatly perturbed in CD mice. Conversely, we explored how genetic predisposition to being “stress sensitive” may change the response to CD. To accomplish this, we used a humanized mouse model carrying a val66met polymorphism (Val = normal, and Met = mutant) in the brain derived neurotrophic factor (BDNF) gene, which causes a stress-sensitive phenotype. Val mice show a loss in niche appropriate sleep timing and flattening of delta power in CD (as expected), whereas Met mice maintain both. Contrary to our initial hypothesis of increased vulnerability to CD in mice that are “stress sensitive”, these data suggest the Met mutation may protect against the

effects of CD. Together, the following chapters will explore how circadian disruption affects sleep, metabolic, and immune function (Chapters 2 and 3), and further, the role of BDNF in modulating the effects of circadian disruption (Chapter 4).

Rifai, O. A., et al. (2017). "Proprotein convertase furin regulates osteocalcin and bone endocrine function." Journal of Clinical Investigation **127**(11): 4104-4117.

Osteocalcin (OCN) is an osteoblast-derived hormone that increases energy expenditure, insulin sensitivity, insulin secretion, and glucose tolerance. The cDNA sequence of OCN predicts that, like many other peptide hormones, OCN is first synthesized as a prohormone (pro-OCN). The importance of pro-OCN maturation in regulating OCN and the identity of the endopeptidase responsible for pro-OCN cleavage in osteoblasts are still unknown. Here, we show that the proprotein convertase furin is responsible for pro-OCN maturation in vitro and in vivo. Using pharmacological and genetic experiments, we also determined that furin-mediated pro-OCN cleavage occurred independently of its γ-carboxylation, a posttranslational modification that is known to hamper OCN endocrine action. However, because pro-OCN is not efficiently decarboxylated and activated during bone resorption, inactivation of furin in osteoblasts in mice resulted in decreased circulating levels of undercarboxylated OCN, impaired glucose tolerance, and reduced energy expenditure. Furthermore, we show that Furin deletion in osteoblasts reduced appetite, a function not modulated by OCN, thus suggesting that osteoblasts may secrete additional hormones that regulate different aspects of energy metabolism. Accordingly, the metabolic defects of the mice lacking furin in osteoblasts became more apparent under pair-feeding conditions. These findings identify furin as an important regulator of bone endocrine function.

Rising, R., et al. (2017). "Validation of whole room indirect calorimeters: Refinement of current methodologies." Physiological Reports **5**(22).

Whole room indirect calorimeter (WRIC) validation techniques consist of propane combustion (PC) or infusion of mixed carbon dioxide (CO2) and nitrogen (N2) by a precision blender (PB). To determine the best method, PC of 6, 10, 22-h and PB infusions of 6, 10, and 14-h, were conducted. The 14-h infusion consisted of two metabolic settings. Energy expenditure (EE; kJ), ventilation (V; liters/min) of oxygen (VO2), VCO2, and respiratory quotient (VCO2/VO2) obtained from the WRIC were extrapolated to the respective test durations and compared to similarly calculated values. Moreover, accurate equations (AE) were derived to correct infusions for additional N2. As a final evaluation of a PC validated WRIC, weight maintenance (WM), energy balance (EB), respiratory quotient (RQ), and food quotients (FQ) were determined in 22 subjects who had repeat 24-h EE measurements. Statistical analyses ($P < 0.05$) were conducted (SPSS, version 23). Significant differences in RQ existed between PC and stoichiometry after 6-h. Errors for the rest of the PC tests ranged from –1.5 ± 2.4 (VCO2) to 2.8 ± 4.6% (EE). When compared with the WRIC, all uncorrected metabolic parameters for six and 10-h PB infusions were significantly different with errors from –12.8 ± 1.6 (VO2) to 6.0 ± 2.8% (RQ). The AE reduced the magnitude of errors to –12.4 ± 1.5 (RQ) to 2.2 ± 3.0% (RQ). The PB infusion with two settings showed similar performance. No differences in WM, EB, RQ, or FQ existed in the subjects. In conclusion, 10-h PC tests are sufficient for validating WRICs.

Ruegsegger, G. N., et al. (2017). "Maternal Western diet age-specifically alters female offspring voluntary physical activity and dopamine- and leptin-related gene expression." FASEB Journal **31**(12): 5371-5383.

Prenatal overnutrition affects development into adulthood and influences risk of obesity. We assessed the transgenerational effect of maternal Western diet (WD) consumption on offspring physical activity. Voluntary wheel running was increased in juvenile (4–7 wk of age), but decreased in adult (16–19 wk of age), F1 female WD offspring. In contrast, no wheel-running differences in F1 male offspring were observed. Increased wheel running in juvenile female WD offspring was associated with up-regulated dopamine receptor (DRD)-1 and -2 in the nucleus accumbens (NAc) and with down-regulated Lepr in the ventral tegmental area (VTA). Conversely, decreased wheel running by adult female WD offspring was associated with down-regulated DRD1 in the NAc and with up-regulated Lepr in the VTA. Body fat, leptin, and insulin were increased in male, but not in female, F1 WD offspring. Recombinant virus (rAAV) leptin antagonism in the VTA decreased wheel running in standard diet but not in WD F1 female offspring. Analysis of F2 offspring found no differences in wheel running or adiposity in male or female offspring, suggesting that changes in the F1 generation were related to in utero somatic reprogramming. Our findings indicate prenatal WD exposure leads to age-specific changes in voluntary physical activity in female offspring that are differentially influenced by VTA leptin antagonism.

Sanford, D., et al. (2017). "CGRP Significantly Regulates Appetite, Energy Intake and Metabolism Peripherally." Gastroenterology **152**(5): S82-S82.

Introduction: Obesity is a worldwide epidemic and in the U.S. affects 31% of adults and causes over 280,000 deaths yearly. Currently, there are no effective treatments for appetite disorders and obesity. Gastrointestinal neuropeptides have been shown to modulate appetite and food intake. Calcitonin Gene-Related Peptide (CGRP) and its receptor, calcitonin receptor-like receptor (CLR) are localized in the gastrointestinal tract and centrally where CGRP has anorexigenic effects when injected intraventricularly. Our hypothesis is that CGRP is a peripheral regulator of appetite, feeding behavior and metabolism. The aim of this study is to understand the mechanisms by which peripherally administered CGRP regulates appetite and metabolism. Methods: C57/Bl6 age matched wild type (WT) mice (10-12 weeks old), 31 M and 15 F were studied. Mice were fed a standard diet, divided in two groups, single housed in Promethion metabolic cages (Sable Systems) and after acclimation, parameters were recorded. Mice had non-restricted, ad libitum access to food and water. Ambulatory activity and physical positions were detected with XYZ beam arrays. Respiratory gases were measured with an integrated fuel cell analyzer. The first group was injected intraperitoneally with CGRP (10µM) while the second group with saline as control. VO2, VCO2, Respiratory Quotient (RQ), as well as food and water consumption, total energy expenditure (TEE) and physical activity were measured continuously for a 48-hour period after injection. Results: A CGRP IP treatment with (10 µM in 200 µl of saline) in WT mice significantly reduced cumulative food intake during the first 4 hours of the dark phase of feeding as compared to saline control 0.6999 vs. 0.9578 g ($P < 0.05$). Cumulatively, during the first 720 minutes after CGRP treatment the mice had a decrease in RQ, 307.1±12.10 vs. 323.3±15.11 ($P < 0.001$), VCO2, 432.0±68.16 vs. 529.8±118.4 ($P < 0.01$) and VO2, 490.5±70.64 vs. 577.0±138.4 ($P < 0.05$). A 30 minutes interval analysis revealed in CGRP injected mice a significant decrease in RQ, VCO2 and VO2 at different intervals between 30 and 390 minutes. Lastly, CGRP injected mice had a significant decrease in TEE at 60' ($P < 0.05$) and 150' ($P < 0.05$) minutes as well as an

increased fine activity at 30 mins (P < 0.01). Conclusions: CGRP, injected peripherally in WT mice, significantly suppressed appetite and food intake for 4 hours and decreased VO2, VCO2 and RQ. These data show that CGRP plays a key anorexigenic role peripherally. This is the first report demonstrating a role for peripherally administered CGRP in the regulation of appetite, feeding behavior and metabolism. Characterization of the pathways by which CGRP regulate appetite and metabolism are important to understand and suggest that CGRP could be used to develop new pharmacological protocols to treat human appetite and obesity disorders.

Sorensen, J. C., et al. (2017). "BGP-15 protects against oxaliplatin-induced skeletal myopathy and mitochondrial reactive oxygen species production in mice." Frontiers in Pharmacology **8**(APR).

Chemotherapy is a leading intervention against cancer. Albeit highly effective, chemotherapy has a multitude of deleterious side-effects including skeletal muscle wasting and fatigue, which considerably reduces patient quality of life and survivability. As such, a defense against chemotherapy-induced skeletal muscle dysfunction is required. Here we investigate the effects of oxaliplatin (OXA) treatment in mice on the skeletal muscle and mitochondria, and the capacity for the Poly ADP-ribose polymerase (PARP) inhibitor, BGP-15, to ameliorate any pathological side-effects induced by OXA. To do so, we investigated the effects of 2 weeks of OXA (3 mg/kg) treatment with and without BGP-15 (15 mg/kg). OXA induced a 15% (p < 0.05) reduction in lean tissue mass without significant changes in food consumption or energy expenditure. OXA treatment also altered the muscle architecture, increasing collagen deposition, neutral lipid and Ca2+ accumulation; all of which were ameliorated with BGP-15 adjunct therapy. Here, we are the first to show that OXA penetrates the mitochondria, and, as a possible consequence of this, increases mtROS production. These data correspond with reduced diameter of isolated FDB fibers and shift in the fiber size distribution frequency of TA to the left. There was a tendency for reduction in intramuscular protein content, albeit apparently not via Murf1 (atrophy)- or p62 (autophagy)- dependent pathways. BGP-15 adjunct therapy protected against increased ROS production and improved mitochondrial viability 4-fold and preserved fiber diameter and number. Our study highlights BGP-15 as a potential adjunct therapy to address chemotherapy-induced skeletal muscle and mitochondrial pathology.

Sorg, D., et al. (2017). "The agreement between two next-generation laser methane detectors and respiration chamber facilities in recording methane concentrations in the spent air produced by dairy cows." Computers and Electronics in Agriculture **143**: 262-272.

In this study, the handheld laser methane detector (LMD) was discussed as a tool for estimating the methane emissions of individual dairy cows by measuring the profiles of the exhaled air. Data obtained with the most recent generation of the device were compared with those of indirect open-circuit respiration chambers, which are commonly used to quantify methane emissions from ruminants. Data from two LaserMethane Mini-Green LMD units (Tokyo Gas Engineering Solutions) exhibited high agreement with those from four respiration chambers, two at the AgroVet-Strickhof, Eschikon, Lindau (Switzerland) and two at the Leibniz Institute for Farm Animal Biology (FBN) Dummerstorf (Germany). The results were determined using Pearson and concordance correlations and the Bland–Altman method. An inverse regression analysis was used to predict the amount of methane in the chambers from the LMD data. The two LMD units also agreed well with each other in the same respiration chamber and under

farm conditions. Both the LMDs and chambers were suitable for detecting differences in mean methane concentrations in the spent air produced by dairy cows during different cow activities in the chamber ($p < 0.05$). Therefore, the most recent LMD model can reliably quantify the dynamics of methane concentrations in the air produced by dairy cows, although the devices were originally designed to detect gas leaks with high methane concentrations in industrial applications. Further studies are needed to investigate the utility of the current LMD technology in measuring the methane profiles directly at the animal's nostrils to quantify methane emissions from dairy cows and other ruminants.

Su, J., et al. (2017). "PKA-RIIB deficiency induces brown fatlike adipocytes in inguinal WAT and promotes energy expenditure in male FVB/NJ mice." Endocrinology **158**(3): 578-591.

Obesity has become the most common metabolic disorder worldwide. Promoting brown adipose tissue (BAT) and beige adipose tissue formation, and therefore, a functional increase in energy expenditure, may counteract obesity. Mice lacking type IIb regulatory subunit of adenosine 30,5' cyclic monophosphate (cAMP)-dependent protein kinase A (PKA-RIIB) display reduced adiposity and resistance to diet-induced obesity. PKA-RIIB, encoded by the Prkar2b gene, is most abundant in BAT and white adipose tissue (WAT) and in the brain. In this study, we show that mice lacking PKARIIB have increased energy expenditure, limited weight gain, and improved glucose metabolism. PKA-RIIB deficiency induces brownlike adipocyte in inguinal WAT (iWAT). PKA-RIIB deficiency also increases the expression of uncoupling protein 1 and other thermogenic genes in iWAT and primary preadipocytes from iWAT through a mechanism involving increased PKA activity, which is represented by increased phosphorylation of PKA substrate, cAMP response element binding protein, and P38 mitogen-activated protein kinase. Our study provides evidence for the role of PKA-RIIB deficiency in regulating thermogenesis in WAT, which may potentially have therapeutic implications for the treatment of obesity and related metabolic disorders.

Swerdlow, R., et al. (2017). O-GlcNAc Regulation of Mitochondrial Function and Energy Metabolism, kuscholarworks.ku.edu.

O-GlcNAc is a post-translational modification (PTM) of a single N-acetylglucosamine sugar attachment on serine or threonine residues of nuclear, cytoplasmic, and mitochondrial proteins. Two opposing enzymes facilitate the modification; O-GlcNAc transferase (OGT) adds the modification, while O-GlcNAcase (OGA) removes it. The addition and the removal of O-GlcNAc, termed O-GlcNAc cycling, is often a dynamic process sensitive to changes in the cellular environment. Disruptions in O-GlcNAcylation contribute to diseases such as diabetes, cancer, and neurodegeneration. Accumulative chronic dysfunctional mitochondria also lead to the development of disease; and importantly, O-GlcNAcylation regulates mitochondrial function. In order to test our first hypothesis that disruptions in O-GlcNAc cycling affect mitochondrial function by changing the mitochondrial proteome, we employed a proteomics screen using SH-SY5Y neuroblastoma cells. We found that OGT and OGA overexpression severely disrupted the mitochondrial proteome, including proteins involved in the respiratory chain and TCA cycle. Furthermore, mitochondrial morphology in the over-expressing cells had disorganized cristae and altered shape and size. Both cellular respiration and glycolysis is impaired. These data support that O-GlcNAc cycling was essential for the proper regulation of mitochondrial function. We next investigated how sustained elevations in cellular O-GlcNAc levels would alter the metabolic profile of the cell. We elevated cellular O-

GlcNAc levels by either treating SH-SY5Y cells with low levels of glucosamine (GlcN), the metabolic substrate of OGT, or the OGA inhibitor Thiamet-G (TMG). We found cellular respiration was altered and ATP levels were lower in these cells with sustained elevated O-GlcNAc. Additionally, these cells produce significantly less reactive oxygen species (ROS). Both GlcN and TMG treated cells have elongated mitochondria, while mitochondrial fusion/fission protein expressions were decreased. RNA-sequencing analysis showed that the transcriptome is reprogrammed and NRF2 anti-oxidant response is down-regulated. Importantly, sustained O-GlcNAcylation in mice brain and liver validated the metabolic phenotypes seen in cells, whereas liver OGT knockdown elevated ROS levels, impaired mitochondrial respiration, and increased NRF2 anti-oxidant response. Furthermore, we discovered from an indirect calorimetric study that sustained elevated O-GlcNAc promoted weight loss and lowered respiration, skewing mice toward using carbohydrates as their main energy source. Here, our results demonstrated that sustained elevation in O-GlcNAcylation, coupled with increased OGA expression, reprograms energy metabolism and can potentially impact the development of metabolic diseases. Altogether, these studies provide new evidence supporting the role of O-GlcNAc as a critical regulator of mitochondrial function and energy metabolism.

Talton, O. O. (2017). Models of gestational diabetes and offspring outcomes in mice, mospace.umsystem.edu.

Gestational diabetes mellitus (GDM) is the most common pregnancy disorder. GDM pregnancies result in offspring that are more likely to develop metabolic syndrome in adolescence than the background population. As offspring experience these adverse effects during their reproductive years, GDM has the potential to propagate disease for many generations. Hyperleptinemia, a key characteristic of both GDM and maternal obesity has not been studied in isolation to determine its role in programming offspring outcomes. Hyperglycemia in the absence of obesity has also not been widely modeled without surgical or chemical means. My research goal was to study the offspring outcomes of these two facets of GDM in C57B6 mice. We observed that maternal hyperleptinemia improved offspring insulin sensitivity, and protected the offspring from developing glucose intolerance. These outcomes were partly mediated by reduced fatty acid accumulation in the liver. Our findings suggest that maternal hyperleptinemia is protective of offspring glucose control. Maternal hyperglycemia in lean dams increased offspring adiposity while glucose tolerance was unchanged. This effect was mediated by a preference for glucose over lipids for substrate utilization, and multiple gene expression changes in the male adipose tissue and liver. Our results indicate that lean maternal hyperglycemia results in metabolically healthy obesity in offspring. This work demonstrates that GDM in lean women may not negatively affect glucose tolerance, and that maternal hyperleptinemia may mediate this, through improving insulin sensitivity. It supports other data that suggest that the liver and adipose tissue are key regulators of whole body metabolism.

Tan, E. P., et al. (2017). "Sustained O-GlcNAcylation reprograms mitochondrial function to regulate energy metabolism." Journal of Biological Chemistry **292**(36): 14940-14962.

Dysfunctional mitochondria and generation of reactive oxygen species (ROS) promote chronic diseases, which have spurred interest in the molecular mechanisms underlying these conditions. Previously, we have demonstrated that disruption of post-translational modification of proteins with β-linked N-acetylglucosamine (O-GlcNAcylation) via overexpression of the O-GlcNAc-regulating enzymes O-GlcNAc

transferase (OGT) or O-GlcNAcase (OGA) impairs mitochondrial function. Here, we report that sustained alterations in O-GlcNAcylation either by pharmacological or genetic manipulation also alter metabolic function. Sustained O-GlcNAcelevation inSH-SY5Yneuroblastoma cells increased OGA expression and reduced cellular respiration and ROS generation. Cells with elevated O-GlcNAc levels had elongated mitochondria and increased mitochondrial membranepotential, and RNA-sequencing analysis indicated transcriptome reprogramming and down-regulation of the NRF2-mediated antioxidant response. Sustained O-GlcNAcylation in mouse brain and liver validated the metabolic phenotypes observed in the cells, and OGT knockdown in the liver elevated ROS levels, impaired respiration, and increased the NRF2 antioxidant response. Moreover, elevated O-GlcNAc levels promoted weight loss and lowered respiration in mice and skewed the mice toward carbohydrate-dependent metabolism as determined by indirect calorimetry. In summary, sustained elevation in O-GlcNAcylation coupled with increased OGA expression reprograms energy metabolism, a finding that has potential implications for the etiology, development, and management of metabolic diseases.

Vu, J. P., et al. (2017). "Long-term intake of a high-protein diet affects body phenotype, metabolism, and plasma hormones in mice." Journal of Nutrition **147**(12): 2243-2251.

Background: High-protein diets (HPDs) recently have been used to obtain body weight and fat mass loss and expand muscle mass. Several studies have documented that HPDs reduce appetite and food intake. Objective: Our goal was to determine the long-term effects of an HPD on body weight, energy intake and expenditure, and metabolic hormones. Methods: Male C57BL/6 mice (8 wk old) were fed either an HPD (60% of energy as protein) or a control diet (CD; 20% of energy as protein) for 12 wk. Body composition and food intakes were determined, and plasma hormone concentrations were measured in mice after being fed and after overnight feed deprivation at several time points. Results: HPD mice had significantly lower body weight (in means ± SEMs; 25.73 ± 1.49 compared with 32.5 ± 1.31 g; P = 0.003) and fat mass (9.55% ± 1.24% compared with 15.78% ± 2.07%; P = 0.05) during the first 6 wk compared with CD mice, and higher lean mass throughout the study starting at week 2 (85.45% ± 2.25% compared with 75.29% ± 1.90%; P = 0.0001). Energy intake, total energy expenditure, and respiratory quotient were significantly lower in HPD compared with CD mice as shown by cumulative energy intake and eating rate. Water vapor was significantly higher in HPD mice during both dark and light phases. In HPD mice, concentrations of leptin [feed-deprived: 41.31 ± 11.60 compared with 3041 ± 683 pg/mL (P = 0.0004); postprandial: 112.5 ± 102.0 compared with 8273 ± 1415 pg/mL (P < 0.0001)] and glucagon-like peptide 1 (GLP-1) [feed-deprived: 5.664 ± 1.44 compared with 21.31 ± 1.26 pg/mL (P = < 0.0001); postprandial: 6.54 ± 2.13 compared with 50.62 ± 11.93 pg/mL (P = 0.0037)] were significantly lower, whereas postprandial glucagon concentrations were higher than in CD-fed mice. Conclusions: In male mice, the 12-wk HPD resulted in short-term body weight and fat mass loss, but throughout the study preserved body lean mass and significantly reduced energy intake and expenditure as well as leptin and GLP-1 concentrations while elevating postprandial glucagon concentrations. This study suggests that long-term use of HPDs may be an effective strategy to decrease energy intake and expenditure and to maintain body lean mass.

Wichert, B., et al. (2017). Neue Daten zum Energieumsatz von wachsenden Katzen, zora.uzh.ch.

> … Während der Messphasen wurden die Volumen der Gase O, CO und Methan mit einem Gasanalysator (Promethion, GA-4, Sable Systems Europe GmbH, Berlin, Germany) gemessen. Der Luftdurchfluss wurde auf 60 l/min (Promethion …

Wilson, C. H., et al. (2017). "Caspase-2 deficiency enhances whole-body carbohydrate utilisation and prevents high-fat diet-induced obesity." Cell Death and Disease **8**(10): e3136-e3136.

> Caspase-2 has been shown to be involved in metabolic homeostasis. Here, we show that caspase-2 deficiency alters basal energy metabolism by shifting the balance in fuel choice from fatty acid to carbohydrate usage. At 4 weeks of age, whole-body carbohydrate utilisation was increased in Casp2-/- mice and was maintained into adulthood. By 17 weeks of age, Casp2-/- mice had reduced white adipose mass, smaller white adipocytes decreased fasting blood glucose and plasma triglycerides but maintained normal insulin levels. When placed on a 12-week high-fat diet (HFD), Casp2-/- mice resisted the development of obesity, fatty liver, hyperinsulinemia and insulin resistance. In addition, HFD-fed Casp2-/- mice had reduced white adipocyte hypertrophy, apoptosis and expansion of both subcutaneous and visceral adipose depots. Increased expression of UCP1 and the maintenance of adiponectin levels in white adipose tissue of HFD-fed Casp2-/- mice indicated increased browning and adipocyte hyperplasia. We found that while the preference for whole-body carbohydrate utilisation was maintained, HFD-fed Casp2-/- mice were not impaired in their ability to switch to utilising fats as a fuel source. Our findings suggest that caspase-2 impacts basal energy metabolism by regulating adipocyte biology and fat expansion, most likely via a non-apoptotic function. Furthermore, we show that caspase-2 deficiency shifts the balance in fuel choice towards increased carbohydrate utilisation and propose that this is due to mild energy stress. As a consequence, Casp2-/- mice show an adaptive remodelling of adipose tissue that protects from HFD-induced obesity and improves glucose homeostasis while paradoxically increasing their susceptibility to oxidative stress induced damage and premature ageing.

Winn, N. C., et al. (2017). "Loss of UCP1 exacerbates western diet-induced glycemic dysregulation independent of changes in body weight in female mice." American Journal of Physiology - Regulatory Integrative and Comparative Physiology **312**(1): R74-R84.

> We tested the hypothesis that female mice null for uncoupling protein 1 (UCP1) would have increased susceptibility to Western diet-induced “whitening” of brown adipose tissue (AT) and glucose intolerance. Six-week-old C57BL/6J wild-type (WT) and UCP1 knockout (UCP1-/-) mice, housed at 25°C, were randomized to either a control diet (10% kcal from fat) or Western diet (45% kcal from fat and 1% cholesterol) for 28 wk. Loss of UCP1 had no effect on energy intake, energy expenditure, spontaneous physical activity, weight gain, or visceral white AT mass. Despite similar susceptibility to weight gain compared with WT, UCP1-/- exhibited whitening of brown AT evidenced by a striking ~500% increase in mass and appearance of large unilocular adipocytes, increased expression of genes related to inflammation, immune cell infiltration, and endoplasmic reticulum/oxidative stress ($P < 0.05$), and decreased mitochondrial subunit protein (COX I, II, III, and IV, $P < 0.05$), all of which were exacerbated by

Western diet (P < 0.05). UCP1-/- mice also developed liver steatosis and glucose intolerance, which was worsened by Western diet. Collectively, these findings demonstrate that loss of UCP1 exacerbates Western diet-induced whitening of brown AT, glucose intolerance, and induces liver steatosis. Notably, the adverse metabolic manifestations of UCP1-/- were independent of changes in body weight, visceral adiposity, and energy expenditure. These novel findings uncover a previously unrecognized metabolic protective role of UCP1 that is independent of its already established role in energy homeostasis.

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Zhang, X., et al. (2017). "Metabolite profile and mitochondrial energetics characterize poor early recovery of muscle mass following hind limb unloading in old mice." bioRxiv.

The progression of age-related sarcopenia can be accelerated by impaired recovery of muscle mass following periods of disuse due to illness or immobilization. The molecular underpinnings of poor recovery of aging muscle following disuse remain largely unknown. However, recent evidence suggests that mitochondrial energetics may play an important role. Here, we report that 22-24 month old mice with low muscle mass and insulin resistance display poor early recovery of muscle mass following 10 days of hind limb unloading. We took an unbiased approach to identify changes in energy metabolism gene expression and metabolite pools and show for the first time that persistent mitochondrial dysfunction, dysregulated fatty acid β-oxidation and elevated H2O2 emission underlie poor early recovery of muscle mass following a period of disuse in old mice. Importantly, this is linked to more severe whole-body insulin resistance. The findings suggest that muscle fuel metabolism and mitochondrial energetics should be a focus for mining therapeutic targets to improve recovery of muscle mass following periods of disuse in older animals.

Blevins, J. E., et al. (2016). "Chronic CNS oxytocin signaling preferentially induces fat loss in high-fat diet-fed rats by enhancing satiety responses and increasing lipid utilization." American Journal of Physiology - Regulatory Integrative and Comparative Physiology **310**(7): R640-R658.

Based largely on a number of short-term administration studies, growing evidence suggests that central oxytocin is important in the regulation of energy balance. The goal of the current work is to determine whether long-term third ventricular (3V) infusion of oxytocin into the central nervous system (CNS) is effective for obesity prevention and/or treatment in rat models. We found that chronic 3V oxytocin infusion between 21 and 26 days by osmotic minipumps both reduced weight gain associated with the progression of high-fat diet (HFD)-induced obesity and elicited a sustained reduction of fat mass with no decrease of lean mass in rats with established diet-induced obesity. We further demonstrated that these chronic oxytocin effects result from 1) maintenance of energy expenditure at preintervention levels despite ongoing weight loss, 2) a reduction in respiratory quotient, consistent with increased fat oxidation, and 3) an enhanced satiety response to cholecystokinin-8 and associated decrease of meal size. These weightreducing effects persisted for approximately 10 days after termination of 3V oxytocin administration and occurred independently of whether sucrose was added to the HFD. We conclude that long-term 3V administration of oxytocin to rats can both prevent and treat dietinduced obesity.

Blevins, J. E., et al. (2016). "Chronic CNS oxytocin signaling preferentially induces fat loss in high-fat diet-fed rats by enhancing satiety responses and increasing lipid utilization." American Journal of Physiology - Regulatory Integrative and Comparative Physiology **310**(7): R640-R658.

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Ceddia, R. P., et al. (2016). "The PGE2 EP3 receptor regulates diet-induced adiposity in male Mice." Endocrinology **157**(1): 220-232.

Mice carrying a targeted disruption of the prostaglandin E2 (PGE2) E-prostanoid receptor 3 (EP3) gene, Ptger3, werefed a high-fat diet (HFD), or a micronutrientmatchedcontrol diet, to investigate the effects of disrupted PGE2-EP3 signaling on diabetes in a setting of diet-induced obesity. Although no differences in

body weight were seen in mice fed the control diet, when fed a HFD, EP3-/- mice gained more weight relative to EP3+/+ mice. Overall, EP3-/- mice had increased epididymal fat mass and adipocyte size; paradoxically, a relative decrease in both epididymal fat pad mass and adipocyte size was observed in the heaviest EP3-/- mice. The EP3-/- mice had increased macrophage infiltration, TNF-κ, monocyte chemoattractant protein-1, IL-6 expression, and necrosis in their epididymal fat pads as compared with EP3+/+ animals. Adipocytes isolated from EP3+/+ or EP3-/- mice were assayed for the effect of PGE2-evoked inhibition of lipolysis. Adipocytes isolated from EP3-/- mice lacked PGE2-evoked inhibition of isoproterenol stimulated lipolysis compared with EP3+/+. EP3-/- mice fed HFD had exaggerated ectopic lipid accumulation in skeletal muscle and liver, with evidence of hepatic steatosis. Both blood glucose and plasma insulin levels were similar between genotypes on a control diet, but when fed HFD, EP3-/- mice becamehyperglycemic and hyperinsulinemicwhencompared with EP3+/+ fed HFD, demonstrating a more severe insulin resistance phenotype in EP3-/-. These results demonstrate that when fed a HFD, EP3-/- mice have abnormal lipid distribution, developing excessive ectopic lipid accumulation and associated insulin resistance.

Grandl, F., et al. (2016). "Biological implications of longevity in dairy cows: 2. Changes in methane emissions and efficiency with age." Journal of Dairy Science **99**(5): 3472-3485.

Previous studies indicated that absolute CH4 emissions and CH4 yield might increase and that milk production efficiency might decrease with age in cattle. Both would make strategies to increase longevity in dairy cattle less attractive. These aspects were experimentally determined in Brown Swiss cattle distributed continuously across a large age range. Thirty lactating dairy cows (876-3,648 d of age) received diets consisting of hay, corn silage, and grass pellets supplemented with 0 or 5 kg of concentrate per day. Twelve heifers (199-778 d of age) received hay only. Cows and heifers were members of herds subjected to the 2 different feeding regimens (with or without concentrate) for the past 10 yr. Methane emissions were measured individually for 2 d in open-circuit respiration chambers, followed by quantifying individual feed intake and milk yield over 8 d. Additional data on digestibility, rumination time, and passage time of feed of all experimental animals were available. Regression analyses were applied to evaluate effects of age and feeding regimen. Body weight, milk yield, and the hay proportion of forage dry matter intake were considered as covariates. Methane emissions per unit of intake, body weight, and milk yield were significantly related to age. Their development in the cows with age was characterized by an increase to maximum at around 2,000 d of age, followed by a decline. This response was not accompanied by corresponding age-related changes in intake, chewing activity, digesta passage time, and digestibility of organic matter, which would have explained shifts in CH4. However, fiber digestibility showed a similar change with age as methane emissions, resulting in quite stable methane emissions per unit of digestible fiber. As expected, methane emissions intensity per unit of milk produced was greater by 8% without concentrate than with concentrate, but no difference was noted in the response to age when the animals were subjected to different feeding regimens. The efficiency of milk production was only marginally influenced by age and diet, and no different response was observed for age in the 2 dietary regimens. In conclusion, life cycle analyses of milk production systems focusing on longevity should consider changing methane yields with age in addition to the variation in environmental costs for replacements of culled cows.

Henderson, S. J., et al. (2016). "Robust anti-obesity and metabolic effects of a dual GLP-1/glucagon receptor peptide agonist in rodents and non-human primates." Diabetes, Obesity and Metabolism **18**(12): 1176-1190.

Aims: To characterize the pharmacology of MEDI0382, a peptide dual agonist of glucagon-like peptide-1 (GLP-1) and glucagon receptors. Materials and methods: MEDI0382 was evaluated in vitro for its ability to stimulate cAMP accumulation in cell lines expressing transfected recombinant or endogenous GLP-1 or glucagon receptors, to potentiate glucose-stimulated insulin secretion (GSIS) in pancreatic β-cell lines and stimulate hepatic glucose output (HGO) by primary hepatocytes. The ability of MEDI0382 to reduce body weight and improve energy balance (i.e. food intake and energy expenditure), as well as control blood glucose, was evaluated in mouse models of obesity and healthy cynomolgus monkeys following single and repeated daily subcutaneous administration for up to 2 months. Results: MEDI0382 potently activated rodent, cynomolgus and human GLP-1 and glucagon receptors and exhibited a fivefold bias for activation of GLP-1 receptor versus the glucagon receptor. MEDI0382 produced superior weight loss and comparable glucose lowering to the GLP-1 peptide analogue liraglutide when administered daily at comparable doses in DIO mice. The additional fat mass reduction elicited by MEDI0382 probably results from a glucagon receptor-mediated increase in energy expenditure, whereas food intake suppression results from activation of the GLP-1 receptor. Notably, the significant weight loss elicited by MEDI0382 in DIO mice was recapitulated in cynomolgus monkeys. Conclusions: Repeated administration of MEDI0382 elicits profound weight loss in DIO mice and non-human primates, produces robust glucose control and reduces hepatic fat content and fasting insulin and glucose levels. The balance of activities at the GLP-1 and glucagon receptors is considered to be optimal for achieving weight and glucose control in overweight or obese Type 2 diabetic patients.

Kaiyala, K. J., et al. (2016). "Physiological role for leptin in the control of thermal conductance." Molecular Metabolism **5**(10): 892-902.

Objective To investigate the role played by leptin in thermoregulation, we studied the effects of physiological leptin replacement in leptin-deficient ob/ob mice on determinants of energy balance, thermogenesis and heat retention under 3 different ambient temperatures. Methods The effects of housing at 14 °C, 22 °C or 30 °C on core temperature (telemetry), energy expenditure (respirometry), thermal conductance, body composition, energy intake, and locomotor activity (beam breaks) were measured in ob/ob mice implanted subcutaneously with osmotic minipumps at a dose designed to deliver a physiological replacement dose of leptin or its vehicle-control. Results As expected, the hypothermic phenotype of ob/ob mice was partially rescued by administration of leptin at a dose that restores plasma levels into the physiological range. This effect of leptin was not due to increased energy expenditure, as cold exposure markedly and equivalently stimulated energy expenditure and induced activation of brown adipose tissue irrespective of leptin treatment. Instead, the effect of physiological leptin replacement to raise core body temperature of cold-exposed ob/ob mice was associated with reduced thermal conductance, implying a physiological role for leptin in heat conservation. Finally, both leptin- and vehicle-treated ob/ob mice failed to match energy intake to expenditure during cold exposure, resulting in weight loss. Conclusions The physiological effect of leptin to reduce thermal conductance contributes to maintenance of core body temperature under sub-thermoneutral conditions.

Kang, L., et al. (2016). "Integrin-linked kinase in muscle is necessary for the development of insulin resistance in diet-induced obese mice." Diabetes **65**(6): 1590-1600.

Diet-induced muscle insulin resistance is associated with expansion of extracellular matrix (ECM) components, such as collagens, and the expression of collagenbinding integrin, α2β1. Integrins transduce signals from ECM via their cytoplasmic domains, which bind to intracellular integrin-binding proteins. The integrinlinked kinase (ILK)-PINCH-parvin (IPP) complex interacts with the cytoplasmic domain of b-integrin subunits and is critical for integrin signaling. In this study we defined the role of ILK, a key component of the IPP complex, in diet-induced muscle insulin resistance. Wild-type (ILKlox/lox) and muscle-specific ILK-deficient (ILKlox/loxHSAcre) mice were fed chow or a high-fat (HF) diet for 16 weeks. Body weight was not different between ILKlox/lox and ILKlox/loxHSAcre mice. However, HF-fed ILKlox/lox HSAcre mice had improved muscle insulin sensitivity relative to HF-fed ILKlox/lox mice, as shown by increased rates of glucose infusion, glucose disappearance, and muscle glucose uptake during a hyperinsulinemic-euglycemic clamp. Improved muscle insulin action in the HF-fed ILKlox/loxHSAcre mice was associated with increased insulin-stimulated phosphorylation of Akt and increased muscle capillarization. These results suggest that ILK expression in muscle is a critical component of diet-induced insulin resistance, which possibly acts by impairing insulin signaling and insulin perfusion through capillaries.

Karaman, S., et al. (2016). "Transgenic overexpression of VEGF-C induces weight gain and insulin resistance in mice." Scientific Reports **6**.

Obesity comprises great risks for human health, contributing to the development of other diseases such as metabolic syndrome, type 2 diabetes and cardiovascular disease. Previously, obese patients were found to have elevated serum levels of VEGF-C, which correlated with worsening of lipid parameters. We recently identified that neutralization of VEGF-C and-D in the subcutaneous adipose tissue during the development of obesity improves metabolic parameters and insulin sensitivity in mice. To test the hypothesis that VEGF-C plays a role in the promotion of the metabolic disease, we used K14-VEGF-C mice that overexpress human VEGF-C under control of the keratin-14 promoter in the skin and monitored metabolic parameters over time. K14-VEGF-C mice had high levels of VEGF-C in the subcutaneous adipose tissue and gained more weight than wildtype littermates, became insulin resistant and had increased ectopic lipid accumulation at 20 weeks of age on regular mouse chow. The metabolic differences persisted under high-fat diet induced obesity. These results indicate that elevated VEGF-C levels contribute to metabolic deterioration and the development of insulin resistance, and that blockade of VEGF-C in obesity represents a suitable approach to alleviate the development of insulin resistance.

Kentish, S. J., et al. (2016). "High-fat diet-induced obesity ablates gastric vagal afferent circadian rhythms." Journal of Neuroscience **36**(11): 3199-3207.

Rats with high-fat diet (HFD)-induced obesity increase daytime eating, suggesting an alteration in circadian food intake mechanisms. Gastric vagal afferents (GVAs) respond to mechanical stimuli to initiate satiety. These signals are dampened in HFD mice and exhibit circadian variations inversely with food intake in lean mice. Furthermore, leptin shows circadian variation in its circulating level and is able to

modulateGVAmechanosensitivity. However, whether leptin's ability to modulateGVAsoccurs in a circadian manner is unknown. Therefore, we investigated whether changes in the circadian intake of food in HFD-induced obesity is associated with a disruption inGVA circadian rhythms. Eight-week-old male C57BL/6 mice were fed a standard laboratory diet (SLD) or a HFD for 12 weeks. A subgroup of SLD andHFDmice were housed in metabolic cages. After 12 weeks, ex vivoGVArecordings were taken at 3 h intervals starting at zeitgeber time 0 (ZT0) and stomach content was measured. After 12 weeks, HFD mice consumed more food during the light phase through larger and more frequent meals compared with SLD mice. SLD mice exhibited circadian fluctuation in stomach content, which peaked at ZT18 and reached a nadir at ZT9. At these time points, both tension and mucosal receptor mechanosensitivity were the lowest and highest, respectively. HFD mice exhibited little circadian variation in stomach content or GVA mechanosensitivity. Leptin potentiated mucosal receptor mechanosensitivity only in SLD mice and with reduced potency during the dark phase. In conclusion, loss of circadian variation in GVA signaling may underpin changes in eating behavior in HFD-induced obesity.

Knani, I., et al. (2016). "Targeting the endocannabinoid/CB1 receptor system for treating obesity in Prader–Willi syndrome." Molecular Metabolism **5**(12): 1187-1199.

Objective Extreme obesity is a core phenotypic feature of Prader–Willi syndrome (PWS). Among numerous metabolic regulators, the endocannabinoid (eCB) system is critically involved in controlling feeding, body weight, and energy metabolism, and a globally acting cannabinoid-1 receptor (CB1R) blockade reverses obesity both in animals and humans. The first-in-class CB1R antagonist rimonabant proved effective in inducing weight loss in adults with PWS. However, it is no longer available for clinical use because of its centrally mediated, neuropsychiatric, adverse effects. Methods We studied eCB 'tone' in individuals with PWS and in the Magel2-null mouse model that recapitulates the major metabolic phenotypes of PWS and determined the efficacy of a peripherally restricted CB1R antagonist, JD5037 in treating obesity in these mice. Results Individuals with PWS had elevated circulating levels of 2-arachidonoylglycerol and its endogenous precursor and breakdown ligand, arachidonic acid. Increased hypothalamic eCB 'tone', manifested by increased eCBs and upregulated CB1R, was associated with increased fat mass, reduced energy expenditure, and decreased voluntary activity in Magel2-null mice. Daily chronic treatment of obese Magel2-null mice and their littermate wild-type controls with JD5037 (3 mg/kg/d for 28 days) reduced body weight, reversed hyperphagia, and improved metabolic parameters related to their obese phenotype. Conclusions Dysregulation of the eCB/CB1R system may contribute to hyperphagia and obesity in Magel2-null mice and in individuals with PWS. Our results demonstrate that treatment with peripherally restricted CB1R antagonists may be an effective strategy for the management of severe obesity in PWS.

Labbé, S. M., et al. (2016). "MTORC1 is Required for Brown Adipose Tissue Recruitment and Metabolic Adaptation to Cold." Scientific Reports **6**.

In response to cold, brown adipose tissue (BAT) increases its metabolic rate and expands its mass to produce heat required for survival, a process known as BAT recruitment. The mechanistic target of rapamycin complex 1 (mTORC1) controls metabolism, cell growth and proliferation, but its role in regulating BAT recruitment in response to chronic cold stimulation is unknown. Here, we show that cold

activates mTORC1 in BAT, an effect that depends on the sympathetic nervous system. Adipocyte-specific mTORC1 loss in mice completely blocks cold-induced BAT expansion and severely impairs mitochondrial biogenesis. Accordingly, mTORC1 loss reduces oxygen consumption and causes a severe defect in BAT oxidative metabolism upon cold exposure. Using in vivo metabolic imaging, metabolomics and transcriptomics, we show that mTORC1 deletion impairs glucose and lipid oxidation, an effect linked to a defect in tricarboxylic acid (TCA) cycle activity. These analyses also reveal a severe defect in nucleotide synthesis in the absence of mTORC1. Overall, these findings demonstrate an essential role for mTORC1 in the regulation of BAT recruitment and metabolism in response to cold.

Lacraz, G., et al. (2016). "Deficiency of interleukin-15 confers resistance to obesity by diminishing inflammation and enhancing the thermogenic function of adipose tissues." PLoS One **11**(9).

Objective: IL-15 is an inflammatory cytokine secreted by many cell types. IL-15 is also produced during physical exercise by skeletal muscle and has been reported to reduce weight gain in mice. Contrarily, our findings on IL-15 knockout (KO) mice indicate that IL-15 promotes obesity. The aim of this study is to investigate the mechanisms underlying the pro-obesity role of IL-15 in adipose tissues. Methods: Control and IL-15 KO mice were maintained on high fat diet (HFD) or normal control diet. After 16 weeks, body weight, adipose tissue and skeletal mass, serum lipid levels and gene/protein expression in the adipose tissues were evaluated. The effect of IL-15 on thermogenesis and oxygen consumption was also studied in primary cultures of adipocytes differentiated from mouse preadipocyte and human stem cells. Results: Our results show that IL-15 deficiency prevents diet-induced weight gain and accumulation of lipids in visceral and subcutaneous white and brown adipose tissues. Gene expression analysis also revealed elevated expression of genes associated with adaptive thermogenesis in the brown and subcutaneous adipose tissues of IL-15 KO mice. Accordingly, oxygen consumption was increased in the brown adipocytes from IL-15 KO mice. In addition, IL-15 KO mice showed decreased expression of pro-inflammatory mediators in their adipose tissues. Conclusions: Absence of IL-15 results in decreased accumulation of fat in the white adipose tissues and increased lipid utilization via adaptive thermogenesis. IL-15 also promotes inflammation in adipose tissues that could sustain chronic inflammation leading to obesity-associated metabolic syndrome.

Li, A. J., et al. (2016). "Deletion of GPR40 fatty acid receptor gene in mice blocks mercaptoacetate-induced feeding." American Journal of Physiology - Regulatory Integrative and Comparative Physiology **310**(10): R968-R974.

Both increased and decreased fatty acid (FA) availability contribute to control of food intake. For example, it is well documented that intestinal FA reduces feeding by triggering enterondocrine secretion of satietogenic pep-tides, such as cholecystokinin (CCK) and glucagon-like peptide-1 (GLP-1). In contrast, mechanisms by which decreased FA availability increase feeding are not well understood. Over the past three decades substantial research related to FA availability and increased feeding has involved use of the orexigenic compound mercaptoacetate (MA). Because MA reportedly inhibits FA oxidation, it has been assumed that reduced FA oxidation accounts for the orexigenic action of MA. Recently, however, we demonstrated that MA antagonizes G proteincoupled receptor 40 (GPR40), a membrane receptor for long and medium chain FA. We also demonstrated that, by antagonizing GPR40, MA inhibits GLP-1 secretion

and attenuates vagal afferent activation by FA. Because both vagal afferent activation and GLP-1 inhibit food intake, we postulated that inhibition of GPR40 by MA might underlie the orexigenic action of MA. We tested this hypothesis using male and female GPR40 knockout (KO) and wild-type (WT) mice. Using several testing protocols, we found that MA increased feeding in WT, but not GPR40 KO mice, and that GPR40 KO mice gained more weight than WT on a high-fat diet. Metabolic monitoring after MA or saline injection in the absence of food did not reveal significant differences in respiratory quotient or energy expenditure between treatment groups or genotypes. These results support the hypothesis that MA stimulates food intake by blocking FA effects on GPR40.

Liao, C. Y., et al. (2016). "Rapamycin Reverses Metabolic Deficits in Lamin A/C-Deficient Mice." Cell Reports **17**(10): 2542-2552.

The role of the mTOR inhibitor, rapamycin, in regulation of adiposity remains controversial. Here, we evaluate mTOR signaling in lipid metabolism in adipose tissues of Lmna−/− mice, a mouse model for dilated cardiomyopathy and muscular dystrophy. Lifespan extension by rapamycin is associated with increased body weight and fat content, two phenotypes we link to suppression of elevated energy expenditure. In both white and brown adipose tissue of Lmna−/− mice, we find that rapamycin inhibits mTORC1 but not mTORC2, leading to suppression of elevated lipolysis and restoration of thermogenic protein UCP1 levels, respectively. The short lifespan and metabolic phenotypes of Lmna−/− mice can be partially rescued by maintaining mice at thermoneutrality. Together, our findings indicate that altered mTOR signaling in Lmna−/− mice leads to a lipodystrophic phenotype that can be rescued with rapamycin, highlighting the effect of loss of adipose tissue in Lmna−/− mice and the consequences of altered mTOR signaling.

Liu, T.-W. (2016). Effects of diet and loss of ovarian hormone production on adiposity, inflammation, cecal integrity, and gut microbial communities of rodent models, ideals.illinois.edu.

Menopause is an age-related loss of ovarian hormone production that has been linked with obesity-associated metabolic dysfunction, increased visceral adiposity, and inflammation, although the mechanisms remain unclear. Obesity has been strongly linked with profound shifts in the gastrointestinal (GI) microbiota, disrupted gut barrier function, and inflammation, but little is known regarding the involvement of the GI tract in obesity associated with loss of ovarian hormone production. Herein, we hypothesized that GI tract and the gut microbiota are involved in the weight gain and inflammation that occurs with the loss of ovarian hormone production and that dietary interventions may affect these responses. The objective of aim 1 was to evaluate the impact of soy on metabolic health, adipose tissue inflammation, and the cecal microbiota in ovariectomized (OVX) rats bred for low-running capacity (LCR), a model that has been previously shown to mimic human menopause. Forty 27-wk old LCR rats were either OVX or sham-operated (SHM) and fed either soy-rich (soy) or soy-free (control) diets for 28 wk. Soy consumption reduced ($p<0.05$) body weight gain, adiposity, circulating cholesterol concentrations, and improved insulin sensitivity of LCR rats. Principal coordinates analysis (PCoA) of weighted and unweighted UniFrac distances of cecal microbiota revealed a sharp separation ($p<0.05$) between soy- and control-fed groups. The soy-fed group had a lower ($p<0.001$) Firmicutes:Bacteroidetes ratio compared to control. The objective of aim 2 was to determine the energy metabolism, lipid accumulation, and inflammation in

adipose and liver tissues of OVX female C57BL/6J mice in response to a high-fat diet (HFD). Forty 10-wk-old female C57BL/6J mice were fed either a high-fat diet (HFD; 60% kcal from fat) or a low-fat diet (LFD; 10% kcal from fat). After a 2-wk acclimation period, mice underwent surgical intervention (OVX or SHM). As expected, OVX mice fed HFD had substantially greater ($p<0.05$) body weight gain, adiposity, and hepatic triglyceride concentrations than OVX mice fed LFD. Compared to intact controls, ovariectomy led to greater ($p<0.05$) adipose and hepatic tissue inflammation, macrophage infiltration, oxidative stress, hindered insulin signaling and glucose uptake, and altered lipid and energy metabolism. Moreover, HFD feeding of OVX mice led to greater ($p<0.05$) inflammation and macrophage infiltration in gonadal adipose tissue. The degree of adiposity and inflammation resulting from HFD in OVX mice vs. SHM mice was dramatically greater than that observed in OVX mice vs. SHM control mice fed LFD. The objective of aim 3 was to examine the cecal microbial communities and barrier function in OVX or SHM mice fed a HFD or LFD for 12 wk. OVX/HFD mice had greater ($p<0.05$) serum lipopolysaccharide-binding protein than OVX/LFD mice. Cecal expression of inflammatory genes was not elevated due to ovariectomy, but the expression of B cell leukemia/lymphoma 2 (BCL2) was greater ($p<0.05$) in OVX mice than SHM mice, indicating greater apoptosis associated with loss of ovarian hormone production. Cecal permeability was not different among treatment groups. However, OVX mice had lower ($p<0.05$) cecal expression of occludin, claudin3, and AMP-activated protein kinase (AMPK) than SHM mice, suggesting that the cecal integrity was compromised due to loss of ovarian hormone production. Lower cecal expression of farnesoid X receptor (FXR) and fibroblast growth factor (FGF15) was observed in the OVX mice compared to SHM, suggesting an interaction between estrogen and the FXR-FGF15 pathway that is known to affect bile acid synthesis. PCoA of weighted and unweighted UniFrac distances of cecal microbiota revealed a distinct separation ($p<0.05$) between mice fed LFD and HFD. Despite the profound physiological changes of OVX/HFD vs. SHM/HFD mice, differential clustering of microbial communities was only observed between OVX/LFD and SHM/LFD mice. HFD promoted a greater ($p<0.05$) Firmicutes:Bacteroidetes ratio and lower ($p<0.05$) species richness of the cecal microbial community. Ovariectomy led to greater ($p<0.05$) abundance of Lactobacillus and lower ($p<0.05$) relative abundance of Oscillospira, Ruminococcus, and an undefined genus in the order Clostridiales. Differential clustering of the cecal microbial community was observed only between OVX and SHM mice fed a LFD, suggesting that the impact of ovariectomy on the cecal microbiota was masked by the HFD intervention. Thus, the objective of aim 4 was to determine the bacterial beta-glucuronidase activity level and the longitudinal shifts of the gut microbiota following the loss of ovarian function and progression of obesity in mice fed a HFD. Fecal pellets were collected at baseline (wk 0, prior to ovariectomy surgery but 2 wk after diet interventions were initiated) and 4-, 8-, and 12-wk post-surgerical intervention. Fecal beta-glucuronidase activity was elevated ($p<0.05$) in mice of the SHM/HFD group compared to those in the SHM/LFD group, but it was not different than mice in the OVX groups. PCoA of weighted UniFrac distances of fecal microbiota revealed a distinct separation ($p<0.05$) between diets. However, SHM and OVX mice only clustered differently in those fed a LFD. The Firmicutes:Bacteroidetes ratio was elevated at wk 8 and wk 12 of those fed the HFD, indicating that this elevation was due to increased adiposity instead of ovariectomy per se. The relative abundance of Clostridium and an undefined genus in the family Clostridiaceae was elevated in the OVX/HFD group at wk 4, but not SHM/HFD, indicating that this change may be due to the loss of ovarian hormone production. Our results indicate that ovariectomy impacts the composition of the gut microbial community, even though the signal was much weaker than that due to dietary intervention. However, ovariectomy promotes disrupted gut barrier function and the alteration of FXR and FGF15 in the cecum, suggesting that signaling and regulation of inflammation and bile acid metabolism in the GI tract were impacted by ovariectomy. Metagenomic, transcriptomic, and metabolomic analyses will need to be conducted to better examine the involvement of the gut microbiome and host-microbial interactions in ovariectomy- and menopausal-associated obesity.

Nguyen, K. P., et al. (2016). "Feeding Experimentation Device (FED): A flexible open-source device for measuring feeding behavior." Journal of Neuroscience Methods **267**: 108-114.

Background: Measuring food intake in rodents is a conceptually simple yet labor-intensive and temporally-imprecise task. Most commonly, food is weighed manually, with an interval of hours or days between measurements. Commercial feeding monitors are excellent, but are costly and require specialized caging and equipment. New method: We have developed the Feeding Experimentation Device (FED): a low-cost, open-source, home cage-compatible feeding system. FED utilizes an Arduino microcontroller and open-source software and hardware. FED dispenses a single food pellet into a food well where it is monitored by an infrared beam. When the mouse removes the pellet, FED logs the timestamp to a secure digital (SD) card and dispenses a new pellet into the well. Post-hoc analyses of pellet retrieval timestamps reveal high-resolution details about feeding behavior. Results: FED is capable of accurately measuring food intake, identifying discrete trends during light and dark-cycle feeding. Additionally, we show the utility of FED for measuring increases in feeding resulting from optogenetic stimulation of agouti-related peptide neurons in the arcuate nucleus of the hypothalamus. Comparison to existing methods: With a cost of ~$350 per device, FED is >10× cheaper than commercially available feeding systems. FED is also self-contained, battery powered, and designed to be placed in standard colony rack cages, allowing for monitoring of true home cage feeding behavior. Moreover, FED is highly adaptable and can be synchronized with emerging techniques in neuroscience, such as optogenetics, as we demonstrate here. Conclusions: FED allows for accurate, precise monitoring of feeding behavior in a home cage setting.

Panaro, B. L. (2016). Roles of the melanocortin-4 receptor in gut-brain communication, Vanderbilt University.

Globally, obesity prevalence among the population has been rapidly increasing and has reached epidemic levels in many developed nations. In the United States, as of 2012, greater than one-third of adults and 17% of children are obese with a body mass index (BMI) of greater than 30 [1]. Along with obesity comes increased risk for a number of comorbidities including type-2 diabetes, cardiovascular disease, cancer, and stroke [2]. The obesity epidemic was responsible for an estimated $147 billion in annual medical costs in the United States alone in 2008 [3], suggesting that there are vast public health and economic consequences to the epidemic that threaten the nation. These harrowing statistics emphasize the importance of investigation into the underlying mechanisms that control body weight among individuals so that we may understand and eventually treat human obesity and reverse the current trends in order to restore a healthy population. (PsycINFO Database Record (c) 2016 APA, all rights reserved)

Park, Y. M., et al. (2016). "Ovariectomized Highly Fit Rats Are Protected against Diet-Induced Insulin Resistance." Medicine and Science in Sports and Exercise **48**(7): 1259-1269.

Introduction In the absence of exercise training, rats selectively bred for high intrinsic aerobic capacity (high-capacity running (HCR)) are protected against ovariectomy (OVX)-induced insulin resistance (IR) and obesity compared with those bred for low intrinsic aerobic capacity (low-capacity running (LCR)). Purpose This study determined whether OVX HCR rats remain protected with exposure to high-fat diet (HFD)

compared with OVX LCR rats. Methods Female HCR and LCR rats (n = 36; age, 27-33 wk) underwent OVX and were randomized to a standard chow diet (NC, 5% kcal fat) or HFD (45% kcal fat) ad libitum for 11 wk. Total energy expenditure, resting energy expenditure, spontaneous physical activity (SPA), and glucose tolerance were assessed midway, whereas fasting circulating metabolic markers, body composition, adipose tissue distribution, and skeletal muscle adenosine monophosphate-Activated protein kinase (AMPK), and mitochondrial markers were assessed at sacrifice. Results Both HCR and LCR rats experienced HFD-induced increases in total and visceral adiposity after OVX. Despite similar gains in adiposity, HCR rats were protected from HFD-induced IR and reduced total energy expenditure observed in LCR rats ($P < 0.05$). This metabolic protection was likely attributed to a compensatory increase in SPA and associated preservation of skeletal muscle AMPK activity in HCR; however, HFD significantly reduced SPA and AMPK activity in LCR ($P < 0.05$). In both lines, HFD reduced citrate synthase activity, gene expression of markers of mitochondrial biogenesis (tFAM, NRF1, and PGC-1), and protein levels of mitochondrial oxidative phosphorylation complexes I, II, IV, and V in skeletal muscle (all $P < 0.05$). Conclusion After OVX, HCR and LCR rats differentially respond to HFD such that HCR increase while LCR decrease SPA. This "physical activity compensation" likely confers protection from HFD-induced IR and reduced energy expenditure in HCR rats.

Perriotte-Olson, C., et al. (2016). "Nanoformulated copper/zinc superoxide dismutase reduces adipose inflammation in obesity." Obesity **24**(1): 148-156.

Objective An intimate association exists between oxidative stress and inflammation. Because adipose tissue (AT) inflammation is intricately linked to metabolic disorders, it was hypothesized that reducing oxidative stress would be effective in ameliorating AT inflammation in obesity. Methods Wild-type mice were fed a high-fat diet (HF) for 8 weeks followed by a 2-week treatment with nanoformulated copper/zinc superoxide dismutase (NanoSOD). The mice were divided into: 1) chow diet, 2) HF, and 3) HF + NanoSOD. Results The HF + NanoSOD-treated mice showed a significant decrease in plasma and liver triglycerides when compared with HF-fed mice. Interestingly, NanoSOD reduced the expression of macrophage and inflammatory markers in visceral AT (VAT) and stromal cells derived from VAT. Moreover, the activation of proinflammatory signaling pathways, in particular, the extracellular signal-regulated kinases, was blunted in VAT on NanoSOD treatment. However, markers of oxidative stress were not altered significantly in the HF + NanoSOD group in the experimental conditions. Pretreatment of either macrophages or adipocytes significantly reduced the inflammatory response invoked in an in vitro coculture system, further supporting the role of NanoSOD in inhibiting obesity-linked inflammation. Conclusions This data suggest that NanoSOD is effective not only in reducing AT macrophage accumulation and AT inflammation but also in promoting triglyceride metabolism in obesity.

Ringling, R. E., et al. (2016). "Loss of Nlrp3 does not protect mice from western diet-induced adipose tissue inflammation and glucose intolerance." PLoS One **11**(9).

We tested the hypothesis that loss of Nlrp3 would protect mice from Western diet-induced adipose tissue (AT) inflammation and associated glucose intolerance and cardiovascular complications. Five-week old C57BL6J wild-type (WT) and Nlrp3 knockout (Nlrp3-/-) mice were randomized to either a control diet (10% kcal from fat) or Western diet (45% kcal from fat and 1% cholesterol) for 24 weeks (n = 8/group). Contrary

to our hypothesis that obesitymediated white AT inflammation is Nlrp3-dependent, we found that Western diet-induced expression of AT inflammatory markers (i.e., Cd68, Cd11c, Emr1, Itgam, Lgals, Il18, Mcp1, TNF, Ccr2, Ccl5 mRNAs, and Mac-2 protein) were not accompanied by increased caspase-1 cleavage, a hallmark feature of NLRP3 inflammasome activation. Furthermore, Nlrp3 null mice were not protected from Western diet-induced white or brown AT inflammation. Although Western diet promoted glucose intolerance in both WT and Nlrp3-/-mice, Nlrp3-/-mice were protected from Western diet-induced aortic stiffening. Additionally, Nlrp3-/-mice exhibited smaller cardiomyocytes and reduced cardiac fibrosis, independent of diet. Collectively, these findings suggest that presence of the Nlrp3 gene is not required for Western diet-induced AT inflammation and/or glucose intolerance; yet Nlrp3 appears to play a role in potentiating arterial stiffening, cardiac hypertrophy and fibrosis.

Rising, R., et al. (2016). "A New Whole Room Indirect Calorimeter for Measurement of the Energetics of Exercise." Journal of exercise physiology online **19**(6): 156-169.

The purpose of this study was to compare the accuracy of exercise energy expenditure (EXEE) measurements from a metabolic cart (HG_MC) to that obtained with a new exercise whole room indirect calorimeter (EX_WRIC). First, the HG_MC and the EX_WRIC were subjected to 10, 30-min ethanol (99.8% purity) and propane (99.5% purity) combustion validations, respectively, for EE, ventilation rates (liters) of oxygen (VO2), carbon dioxide (VCO2), and the respiratory quotient (RQ; VCO2/VO2). Then, 15 healthy adults (13 men and 2 women) cycled at 65% age predicted heart rate max for random determination of their EXEE, VO2, VCO2 and RQ after a 12-hr fast with both the HG-MC and EX_WRIC. Comparing stoichiometry to combustion, the HG_MC underestimated EE ($P<0.05$), VO2 ($P<0.05$), VCO2 ($P<0.05$), and RQ ($P<0.05$) while no differences were found for the EX_WRIC. The EXEE and VO2 were lower ($P<0.05$) while RQ was greater ($P<0.05$) when measured with the HG_MC versus the EX_WRIC. The EX_WRIC was more accurate than the HG_MC without the related tethered connections.

Stroh, M. A. (2016). A characterization of deficits associated with loss of NCB5OR in the mouse brain, kuscholarworks.ku.edu.

The simplest approach to the study of an event is to first consider that of the simplest cause. When investigating the mechanisms governing idiopathic diseases, this generally takes the form of an ab initio genetic approach, often in search of a single genetic culprit. To date, this genetic ‘smoking gun’ has remained elusive for many affected by diabetes mellitus and a number of neurodegenerative diseases. With no single gene, or even subset of genes, found to be causative in all cases, other approaches to studying the etiology and treatment of these diseases seem reasonable. One such approach is considering trends consistently observed in diseases closely correlated with one another. In the cases of diabetes mellitus and neurodegenerative diseases, overlapping themes of mitochondrial influence or dysfunction and iron dyshomeostasis are apparent and relatively consistent. This might suggest that gene networks involved in the maintenance of mitochondrial and iron related pathways are etiologically important. Thus, this dissertation focuses on a reductase, NCB5OR, whose absence has been shown to result in diabetes mellitus, mitochondrial dysfunction, and altered iron metabolism in mice. Specifically, we focus on the effects of NCB5OR deficiency on mouse neural tissue as a means of exploring genes and pathways known to result in these overlapping trends. In order to study the effects of NCB5OR deficiency on neural tissue

and pathways we used mice globally deficient for NCB5OR (GKO) and also developed a conditional knockout mouse that inactivates NCB5OR in the cerebellum and midbrain (CKO). Using either of these models, three questions were addressed: What effect does NCB5OR deficiency in the mouse cerebellum and midbrain have on iron homeostasis and locomotor behavior? What effect does loss of NCB5OR in cerebellohypothalamic circuitry have on feeding behavior and metabolism? Does loss of NCB5OR affect major neurotransmitters in the brains of mice globally deficient for NCB5OR? Chapter 1 details background information on the MIND (mitochondria, iron, neurodegeneration, and diabetes) paradigm, which provides the context in which these studies were conducted. Although over 100 neurodegenerative diseases have been classified, the majority of the background presented will use Alzheimer's disease (AD) as the model complex, idiopathic neurodegenerative disease, providing a focused example of the MIND paradigm framework. Also discussed is the incidence of diabetes accompanied by neuropathy and neurodegeneration along with neurodegenerative disorders prone to development of diabetes. Mouse models containing multiple facets of this overlap are also described alongside current molecular trends attributed to both diseases. A detailed background pertaining to NCB5OR as well as known phenotypes and preliminary observations associated with its absence are presented. Finally, a review of the cerebellum and its contribution to motor and higher order cognitive processes is presented so as to help better understand why the cerebellum was chosen as the primary model system for the study of NCB5OR in neural tissue. Chapters 2-4 address the above three questions with studies aimed at initial characterization of the effects of NCB5OR deficiency. Briefly, results from Chapter 2 demonstrate an altered state of iron homeostasis in the mouse cerebellum devoid of NCB5OR. Additionally, analysis of locomotor behavior revealed altered locomotor activity, proprioception, and sensitivity to harmaline-induced tremor in CKO mice. Chapter 3 explores metabolic and behavioral changes in CKO mice which reveal the complex nature of NCB5OR deficiency on neural pathways that participate in feeding behavior and neural regulation of metabolism. Finally, Chapter 4 presents data that suggest that the absence of NCB5OR does not affect levels of serotonin (5-HT), dopamine (DA), γ-aminobutyric acid (GABA), or glutamate (Glut) in the cerebellum but does increase levels of DA in the frontal cortex of mice globally deficient for NCB5OR. The primary purpose of this work is to contribute to the understanding of the complex nature and etiology of idiopathic neurological disease by providing evidence emphasizing the importance of genes whose function influences iron and metabolic homeostasis. It is hoped that the data presented here helps to shed light on pathways and genetic networks whose composite function could provide insight into the development of complex neurological disease.

Wang, Q. P., et al. (2016). "Sucralose Promotes Food Intake through NPY and a Neuronal Fasting Response." Cell Metabolism **24**(1): 75-90.

Non-nutritive sweeteners like sucralose are consumed by billions of people. While animal and human studies have demonstrated a link between synthetic sweetener consumption and metabolic dysregulation, the mechanisms responsible remain unknown. Here we use a diet supplemented with sucralose to investigate the long-term effects of sweet/energy imbalance. In flies, chronic sweet/energy imbalance promoted hyperactivity, insomnia, glucose intolerance, enhanced sweet taste perception, and a sustained increase in food and calories consumed, effects that are reversed upon sucralose removal. Mechanistically, this response was mapped to the ancient insulin, catecholamine, and NPF/NPY systems and the energy sensor AMPK, which together comprise a novel neuronal starvation response pathway. Interestingly, chronic sweet/energy imbalance promoted increased food intake in mammals as well, and this also occurs through an NPY-dependent mechanism. Together, our data show that chronic

consumption of a sweet/energy imbalanced diet triggers a conserved neuronal fasting response and increases the motivation to eat.

Welly, R. J., et al. (2016). "Comparison of Diet versus Exercise on Metabolic Function and Gut Microbiota in Obese Rats." Medicine and Science in Sports and Exercise **48**(9): 1688-1698.

Cardiometabolic impairments that begin early in life are particularly critical, because they often predict metabolic dysfunction in adulthood. Obesity, high-fat diet (HFD), and inactivity are all associated with adipose tissue (AT) inflammation and insulin resistance (IR), major predictors of metabolic dysfunction. Recent evidence has also associated the gut microbiome with cardiometabolic health. Purpose The objective of this study is to compare equal energy deficits induced by exercise and caloric reduction on cardiometabolic disease risk parameters including AT inflammation, IR, and gut microbiota changes during HFD consumption. Methods Obesity-prone rats fed HFD were exercise trained (Ex, n = 10) or weight matched to Ex via caloric reduction although kept sedentary (WM, n = 10), and compared with ad libitum HFD-fed (Sed, n = 10) rats for IR, systemic energetics and spontaneous physical activity (SPA), adiposity, and fasting metabolic parameters. Visceral, infcutaneous, periaortic, and brown AT (BAT), liver, aorta, and cecal digesta were examined. Results Despite identical reductions in adiposity, Ex, but not WM, improved IR, increased SPA by approximately 26% ($P < 0.05$ compared with WM and Sed), and reduced LDL cholesterol ($P < 0.05$ compared with Sed). WM and Ex both reduced inflammatory markers in all AT depots and aorta, whereas only Ex increased indicators of mitochondrial function in BAT. Ex significantly increased the relative abundance of cecal Streptococcaceae and decreased S24-7 and one undefined genus in Rikenellaceae; WM induced similar changes but did not reach statistical significance. Conclusions Both Ex and WM reduced AT inflammation across depots, whereas Ex caused more robust changes to gut microbial communities, improved IR, increased fat oxidation, increased SPA, and increased indices of BAT mitochondrial function. Our findings add to the growing body of literature indicating that there are weight-loss-independent metabolic benefits of exercise.

Wong, M. L., et al. (2016). "Inflammasome signaling affects anxiety- and depressive-like behavior and gut microbiome composition." Molecular Psychiatry **21**(6): 797-805.

The inflammasome is hypothesized to be a key mediator of the response to physiological and psychological stressors, and its dysregulation may be implicated in major depressive disorder. Inflammasome activation causes the maturation of caspase-1 and activation of interleukin (IL)-1β and IL-18, two proinflammatory cytokines involved in neuroimmunomodulation, neuroinflammation and neurodegeneration. In this study, C57BL/6 mice with genetic deficiency or pharmacological inhibition of caspase-1 were screened for anxiety- and depressive-like behaviors, and locomotion at baseline and after chronic stress. We found that genetic deficiency of caspase-1 decreased depressive- and anxiety-like behaviors, and conversely increased locomotor activity and skills. Caspase-1 deficiency also prevented the exacerbation of depressive-like behaviors following chronic stress. Furthermore, pharmacological caspase-1 antagonism with minocycline ameliorated stress-induced depressive-like behavior in wild-type mice. Interestingly, chronic stress or pharmacological inhibition of caspase-1 per se altered the fecal microbiome in a very similar manner. When stressed mice were treated with minocycline, the observed gut microbiota changes included increase in relative abundance of Akkermansia spp. and Blautia spp.,

which are compatible with beneficial effects of attenuated inflammation and rebalance of gut microbiota, respectively, and the increment in Lachnospiracea abundance was consistent with microbiota changes of caspase-1 deficiency. Our results suggest that the protective effect of caspase-1 inhibition involves the modulation of the relationship between stress and gut microbiota composition, and establishes the basis for a gut microbiota-inflammasome-brain axis, whereby the gut microbiota via inflammasome signaling modulate pathways that will alter brain function, and affect depressive- and anxiety-like behaviors. Our data also suggest that further elucidation of the gut microbiota-inflammasome-brain axis may offer novel therapeutic targets for psychiatric disorders.

Chan, C. B., et al. (2015). "Activation of muscular TrkB by its small molecular agonist 7,8-dihydroxyflavone sex-dependently regulates energy metabolism in diet-induced obese mice." Chemistry and Biology **22**(3): 355-368.

Summary Chronic activation of brain-derived neurotrophic factor (BDNF) receptor TrkB is a potential method to prevent development of obesity, but the short half-life and nonbioavailable nature of BDNF hampers validation of the hypothesis. We report here that activation of muscular TrkB by the BDNF mimetic, 7,8-dihydroxyflavone (7,8-DHF), is sufficient to protect the development of diet-induced obesity in female mice. Using in vitro and in vivo models, we found that 7,8-DHF treatment enhanced the expression of uncoupling protein 1 (UCP1) and AMP-activated protein kinase (AMPK) activity in skeletal muscle, which resulted in increased systemic energy expenditure, reduced adiposity, and improved insulin sensitivity in female mice fed a high-fat diet. This antiobesity activity of 7,8-DHF is muscular TrkB-dependent as 7,8-DHF cannot mitigate diet-induced obesity in female muscle-specific TrkB knockout mice. Hence, our data reveal that chronic activation of muscular TrkB is useful in alleviating obesity and its complications.

DeMambro, V. E., et al. (2015). "Igfbp2 deletion in ovariectomized mice enhances energy expenditure but accelerates bone loss." Endocrinology **156**(11): 4129-4140.

Previously, we reported sexually dimorphic bone mass and body composition phenotypes in Igfbp2-/- mice (-/-), where male mice exhibited decreased bone and increased fat mass, whereas female mice displayed increased bone but no changes in fat mass. To investigate the interaction between IGF-binding protein (IGFBP)-2 and estrogen, we subjected Igfbp2 -/- and +/+ female mice to ovariectomy (OVX) or sham surgery at 8 weeks of age. At 20 weeks of age, mice underwent metabolic cage analysis and insulin tolerance tests before killing. At harvest, femurs were collected for microcomputed tomography, serum for protein levels, brown adipose tissue (BAT) and inguinal white adipose tissue (IWAT) adipose depots for histology, gene expression, and mitochondrial respiration analysis of whole tissue. In +/+ mice, serum IGFBP-2 dropped 30% with OVX. In the absence of IGFBP-2, OVX had no effect on preformed BAT; however, there was significant "browning" of the IWAT depot coinciding with less weight gain, increased insulin sensitivity, lower intraabdominal fat, and increased bone loss due to higher resorption and lower formation. Likewise, afterOVX,energy expenditure, physical activityandBATmitochondrial respirationweredecreased less in the OVX-/- compared with OVX+/+. Mitochondrial respiration of IWAT was reduced in OVX+/+ yet remained unchanged in OVX-/- mice. These changes were associated with significant increases in Fgf21 and Foxc2 expression, 2 proteins known for their insulin sensitizing and

browning ofWATeffects.Weconclude that estrogen deficiency has a profound effect on body and bone composition in the absence of IGFBP-2 and may be related to changes in fibroblast growth factor 21.

Flynn, C. R., et al. (2015). "Bile diversion to the distal small intestine has comparable metabolic benefits to bariatric surgery." Nature Communications **6**.

Roux-en-Y gastric bypass (RYGB) is highly effective in reversing obesity and associated diabetes. Recent observations in humans suggest a contributing role of increased circulating bile acids in mediating such effects. Here we use a diet-induced obesity (DIO) mouse model and compare metabolic remission when bile flow is diverted through a gallbladder anastomosis to jejunum, ileum or duodenum (sham control). We find that only bile diversion to the ileum results in physiologic changes similar to RYGB, including sustained improvements in weight, glucose tolerance and hepatic steatosis despite differential effects on hepatic gene expression. Circulating free fatty acids and triglycerides decrease while bile acids increase, particularly conjugated tauro-β-muricholic acid, an FXR antagonist. Activity of the hepatic FXR/FGF15 signalling axis is reduced and associated with altered gut microbiota. Thus bile diversion, independent of surgical rearrangement of the gastrointestinal tract, imparts significant weight loss accompanied by improved glucose and lipid homeostasis that are hallmarks of RYGB.

Johnson, S. A., et al. (2015). "Sex-dependent effects of developmental exposure to bisphenol A and ethinyl estradiol on metabolic parameters and voluntary physical activity." Journal of Developmental Origins of Health and Disease **6**(6): 539-552.

Endocrine disrupting chemicals (EDC) have received considerable attention as potential obesogens. Past studies examining obesogenic potential of one widespread EDC, bisphenol A (BPA), have generally focused on metabolic and adipose tissue effects. However, physical inactivity has been proposed to be a leading cause of obesity. A paucity of studies has considered whether EDC, including BPA, affects this behavior. To test whether early exposure to BPA and ethinyl estradiol (EE, estrogen present in birth control pills) results in metabolic and such behavioral disruptions, California mice developmentally exposed to BPA and EE were tested as adults for energy expenditure (indirect calorimetry), body composition (echoMRI) and physical activity (measured by beam breaks and voluntary wheel running). Serum glucose and metabolic hormones were measured. No differences in body weight or food consumption were detected. BPA-exposed females exhibited greater variation in weight than females in control and EE groups. During the dark and light cycles, BPA females exhibited a higher average respiratory quotient than control females, indicative of metabolizing carbohydrates rather than fats. Various assessments of voluntary physical activity in the home cage confirmed that during the dark cycle, BPA and EE-exposed females were significantly less active in this setting than control females. Similar effects were not observed in BPA or EE-exposed males. No significant differences were detected in serum glucose, insulin, adiponectin and leptin concentrations. Results suggest that females developmentally exposed to BPA exhibit decreased motivation to engage in voluntary physical activity and altered metabolism of carbohydrates v. fats, which could have important health implications.

Kaiyala, K. J., et al. (2015). "Leptin signaling is required for adaptive changes in food intake, but not energy expenditure, in response to different thermal conditions." PLoS One **10**(3).

> Survival of free-living animals depends on the ability to maintain core body temperature in the face of rapid and dramatic changes in their thermal environment. If food intake is not adjusted to meet the changing energy demands associated with changes of ambient temperature, a serious challenge to body energy stores can occur. To more fully understand the coupling of thermoregulation to energy homeostasis in normal animals and to investigate the role of the adipose hormone leptin to this process, comprehensive measures of energy homeostasis and core temperature were obtained in leptin-deficient ob/ob mice and their wild-type (WT) littermate controls when housed under cool (14° C), usual (22° C) or thermoneutral (30° C) conditions. Our findings extend previous evidence that WT mice robustly defend normothermia in response to either a lowering (14° C) or an increase (30° C) of ambient temperature without changes in body weight or body composition. In contrast, leptin-deficient, ob/ob mice fail to defend normothermia at ambient temperatures lower than thermoneutrality and exhibit marked losses of both body fat and lean mass when exposed to cooler environments (14° C). Our findings further demonstrate a strong inverse relationship between ambient temperature and energy expenditure in WT mice, a relationship that is preserved in ob/ob mice. However, thermal conductance analysis indicates defective heat retention in ob/ob mice, irrespective of temperature. While a negative relationship between ambient temperature and energy intake also exists in WT mice, this relationship is disrupted in ob/ob mice. Thus, to meet the thermoregulatory demands of different ambient temperatures, leptin signaling is required for adaptive changes in both energy intake and thermal conductance. A better understanding of the mechanisms coupling thermoregulation to energy homeostasis may lead to the development of new approaches for the treatment of obesity.

Li, M. (2015). Regulation of the Circadian Clock and Appetite by O-GIcNAc Transferase, Yale University, 2015.

> Regulation of the Circadian Clock and Appetite by O-GIcNAc Transferase. Abstract. Calorie overload and circadian rhythm disorders contribute to the pathogenesis of diabetes and obesity. Nutrient flux into the hexosamine biosynthesis …

Liu, T. W., et al. (2015). "Physical activity differentially affects the cecal microbiota of ovariectomized female rats selectively bred for high and low aerobic capacity." PLoS One **10**(8).

> The gut microbiota is considered a relevant factor in obesity and associatedmetabolic diseases, for which postmenopausal women are particularly at risk. Increasing physical activity has been recognized as an efficacious approach to prevent or treat obesity, yet the impact of physical activity on the microbiota remains under-investigated.We examined the impacts of voluntary exercise on hostmetabolism and gut microbiota in ovariectomized (OVX) high capacity (HCR) and low capacity running (LCR) rats. HCR and LCR rats (age = 27wk) were OVX and fed a high-fat diet (45%kcal fat) ad libitum and housed in cages equipped with (exercise, EX) or without (sedentary, SED) running wheels for 11wk (n = 7-8/group).We hypothesized that increased physical activity would hinder weight gain, increasemetabolic health and shift the

microbiota of LCR rats, resulting in populationsmore similar to that of HCR rats. Animals were compared for characteristic metabolic parameters including body composition, lipid profile and energy expenditure; whereas cecal digesta were collected for DNA extraction. 16S rRNA gene-based amplicon IlluminaMiSeq sequencing was performed, followed by analysis using QIIME 1.8.0 to assess cecal microbiota. Voluntary exercise decreased body and fat mass, and normalized fasting NEFA concentrations of LCR rats, despite only running one-third the distance of HCR rats. Exercise, however, increased food intake, weight gain and fat mass of HCR rats. Exercise clustered the gut microbial community of LCR rats, which separated them from the other groups. Assessments of specific taxa revealed significant ($p<0.05$) line by exercise interactions including shifts in the abundances of Firmicutes, Proteobacteria, and Cyanobacteria. Relative abundance of Christensenellaceae family was higher ($p = 0.026$) in HCR than LCR rats, and positively correlated ($p<0.05$) with food intake, body weight and running distance. These findings demonstrate that exercise differentially impacts hostmetabolism and gut microbial communities of female HCR and LCR rats without ovarian function. Copyright:

Mavanji, V., et al. (2015). "Promotion of wakefulness and energy expenditure by orexin-a in the ventrolateral preoptic area." Sleep **38**(9): 1361-1370.

Study Objectives: The ventrolateral preoptic area (VLPO) and the orexin/hypocretin neuronal system are key regulators of sleep onset, transitions between vigilance states, and energy homeostasis. Reciprocal projections exist between the VLPO and orexin/hypocretin neurons. Although the importance of the VLPO to sleep regulation is clear, it is unknown whether VLPO neurons are involved in energy balance. The purpose of these studies was to determine if the VLPO is a site of action for orexin-A, and which orexin receptor subtype(s) would mediate these effects of orexin-A. We hypothesized that orexin-A in the VLPO modulates behaviors (sleep and wakefulness, feeding, spontaneous physical activity [SPA]) to increase energy expenditure. Design and Measurements: Sleep, wakefulness, SPA, feeding, and energy expenditure were determined after orexin-A microinjection in the VLPO of male Sprague-Dawley rats with unilateral cannulae targeting the VLPO. We also tested whether pretreatment with a dual orexin receptor antagonist (DORA, TCS-1102) or an OX2R antagonist (JNJ-10397049) blocked the effects of orexin-A on the sleep/wake cycle or SPA, respectively. Results: Orexin-A injected into the VLPO significantly increased wakefulness, SPA, and energy expenditure (SPA-induced and total) and reduced NREM sleep and REM sleep with no effect on food intake. Pretreatment with DORA blocked the increase in wakefulness and the reduction in NREM sleep elicited by orexin-A, and the OX2R antagonist reduced SPA stimulated by orexin-A. Conclusions: These data show the ventrolateral preoptic area is a site of action for orexin-A, which may promote negative energy balance by modulating sleep/wakefulness and stimulating spontaneous physical activity and energy expenditure.

Motyl, K. J., et al. (2015). "Propranolol attenuates risperidone-induced trabecular bone loss in female mice." Endocrinology **156**(7): 2374-2383.

Atypical antipsychotic (AA) drugs cause significant metabolic side effects, and clinical data are emerging that demonstrate increased fracture risk and bone loss after treatment with the AA, risperidone (RIS). The pharmacology underlying the adverse effects on bone is unknown. However, RIS action in the central nervous system could be responsible because the sympathetic nervous system (SNS) is known to uncouple

bone remodeling. RIS treatment in mice significantly lowered trabecular bone volume fraction (bone volume/total volume), owing to increased osteoclast-mediated erosion and reduced osteoblast-mediated bone formation. Daytime energy expenditure was also increased and was temporally associated with the plasma concentration of RIS. Even a single dose of RIS transiently elevated expression of brown adipose tissue markers of SNS activity and thermogenesis, Pgc1a and Ucp1. Rankl, an osteoclast recruitment factor regulated by the SNS, was also increased 1 hour after a single dose of RIS. Thus, we inferred that bone loss from RIS was regulated, at least in part, by the SNS. To test this, we administered RIS or vehicle to mice that were also receiving the nonselective β-blocker propranolol. Strikingly, RIS did not cause any changes in trabecular bone volume/total volume, erosion, or formation while propranolol was present. Furthermore, β2-adrenergic receptor null (Adrb2-/-) mice were also protected from RIS-induced bone loss. This is the first report to demonstrate SNS-mediated bone loss from any AA. Because AA medications are widely prescribed, especially to young adults, clinical studies are needed to assess whether β-blockers will prevent bone loss in this vulnerable population.

Rising, R., et al. (2015). "Evaluation of a new whole room indirect calorimeter specific for measurement of resting metabolic rate." Nutrition and Metabolism **12**(1).

Background: The most common methods for obtaining human resting metabolic rate (RMR) use either a ventilated hood connected to a metabolic cart (VH-MC) or calculation by many prediction equations utilizing the person's height and weight. These methods may be inherently inaccurate. The objective of this study is to compare the accuracy for the measurement of RMR by three methods: a new whole room indirect calorimeter specific for this purpose (RMR-WRIC), VH-MC and calculation by the Mifflin equation (ME). First, the VH-MC (Vmax Encore 2900, Carefusion Inc, San Diego, CA) and RMR-WRIC (Promethion GA-6/FG-1, Sable Systems Intl, Las Vegas, NV) were subjected to 10, one-hour ethanol (99.8 % purity) and propane (99.5 % purity) combustion tests, respectively, for simulated metabolic measurements. Thereafter, 40 healthy adults (22 M/18 F, 78.0 ± 24.5 kg, BMI = 25.6 ± 4.8, age 36.6 ± 13.4 years) had one-hour RMR (kcal), ventilation (liters) rates of oxygen (VO2), carbon dioxide (VCO2) and RQ (VCO2/VO2) measured after a 12-h fast with both the VH- MC and the RMR-WRIC in a randomized fashion. The resting state was documented by heart rate. The RMR was also calculated using the ME, which was compared to both the RMR-WRIC and the VH-MC. All simulated and human metabolic data were extrapolated to 24-h and analyzed (SPSS, Ver. 22). Results: Comparing stoichiometry to actual combustion, the VH-MC underestimated simulated RMR ($p < 0.05$), VO2 ($p < 0.05$), VCO2 ($p < 0.05$) and the RQ. Similarly the RMR-WRIC underestimated simulated RMR ($p < 0.05$) and VO2 while overestimating VCO2 and the RQ. There was much greater variability in the simulated metabolic data between combustion and the VH-MC as compared to that of the RMR-WRIC. With regards to the volunteers, the RMR, RQ, VO2 and VCO2 determined by the VH-MC tended to be lower in comparison to these measurements determined by the RMR-WRIC. Finally, RMR calculated utilizing the ME was significantly ($p < 0.05$) less than the RMR-WRIC but similar to that obtained by the VH-MC. Conclusion: The RMR-WRIC was more accurate and precise than either the VH-MC or ME, which has implications for determining energy requirements for individuals participating in weight loss or nutrition rehabilitation programs.

Rising, R., et al. (2015). Evaluation of a new whole room indirect calorimeter specific for measurement of resting metabolic rate, Wiley Online Library.

Background: The most common methods for obtaining human resting metabolic rate (RMR) use either a ventilated hood connected to a metabolic cart (VH-MC) or calculation by many prediction equations utilizing the person's height and weight. These methods may be inherently inaccurate. The objective of this study is to compare the accuracy for the measurement of RMR by three methods: a new whole room indirect calorimeter specific for this purpose (RMR-WRIC), VH-MC and calculation by the Mifflin equation (ME). First, the VH-MC (Vmax Encore 2900, Carefusion Inc, San Diego, CA) and RMR-WRIC (Promethion GA-6/FG-1, Sable Systems Intl, Las Vegas, NV) were subjected to 10, one-hour ethanol (99.8 % purity) and propane (99.5 % purity) combustion tests, respectively, for simulated metabolic measurements. Thereafter, 40 healthy adults (22 M/18 F, 78.0 ± 24.5 kg, BMI = 25.6 ± 4.8, age 36.6 ± 13.4 years) had one-hour RMR (kcal), ventilation (liters) rates of oxygen (VO2), carbon dioxide (VCO2) and RQ (VCO2/VO2) measured after a 12-h fast with both the VH- MC and the RMR-WRIC in a randomized fashion. The resting state was documented by heart rate. The RMR was also calculated using the ME, which was compared to both the RMR-WRIC and the VH-MC. All simulated and human metabolic data were extrapolated to 24-h and analyzed (SPSS, Ver. 22). Results: Comparing stoichiometry to actual combustion, the VH-MC underestimated simulated RMR ($p < 0.05$), VO2 ($p < 0.05$), VCO2 ($p < 0.05$) and the RQ. Similarly the RMR-WRIC underestimated simulated RMR ($p < 0.05$) and VO2 while overestimating VCO2 and the RQ. There was much greater variability in the simulated metabolic data between combustion and the VH-MC as compared to that of the RMR-WRIC. With regards to the volunteers, the RMR, RQ, VO2 and VCO2 determined by the VH-MC tended to be lower in comparison to these measurements determined by the RMR-WRIC. Finally, RMR calculated utilizing the ME was significantly ($p < 0.05$) less than the RMR-WRIC but similar to that obtained by the VH-MC. Conclusion: The RMR-WRIC was more accurate and precise than either the VH-MC or ME, which has implications for determining energy requirements for individuals participating in weight loss or nutrition rehabilitation programs.

Stohn, J. P., et al. (2015). "Cthrc1 controls adipose tissue formation, body composition, and physical activity." Obesity **23**(8): 1633-1642.

Objective This study investigated the effects of loss of Cthrc1 on adipogenesis, body composition, metabolism, physical activity, and muscle physiology. Methods Complete metabolic and activity monitoring as well as grip strength measurements and muscle myography was performed in Cthrc1 null and wildtype mice. Results Compared to wildtypes, Cthrc1 null mice had similar body weights but significantly reduced energy expenditure, decreased lean mass, and increased fat mass, especially visceral fat. In vitro studies demonstrated that Cthrc1 inhibited adipocyte differentiation as well as PPAR and CREB reporter activity, while preadipocytes isolated from Cthrc1 null mice exhibited enhanced adipogenic differentiation. Voluntary physical activity in Cthrc1 null mice as assessed by wheel running was reduced to approximately half the distance covered by wildtypes. Reduced grip strength was observed in Cthrc1 null mice at the age of 15 weeks or older with reduced performance and mass of hyphenate muscle. In the brain, Cthrc1 expression was most prominent in neurons of thalamic and hypothalamic nuclei with evidence for secretion into the circulation in the median eminence. Conclusions Our data indicate that Cthrc1 regulates body composition through inhibition of adipogenesis. In addition, central Cthrc1 may be a mediator of muscle function and physical activity.

Zhou, H., et al. (2015). "Berardinelli-Seip congenital lipodystrophy 2 regulates adipocyte lipolysis, browning, and energy balance in adult animals." Journal of Lipid Research **56**(10): 1912-1925.

Mutations in BSCL2/SEIPIN cause Berardinelli-Seip congenital lipodystrophy type 2 (BSCL2), but the mechanisms whereby Bscl2 regulates adipose tissue function are unclear. Here, we generated adipose tissue (mature) Bscl2 knockout (Ad-mKO) mice, in which Bscl2 was specifically ablated in adipocytes of adult animals, to investigate the impact of acquired Bscl2 deletion on adipose tissue function and energy balance. Ad-mKO mice displayed reduced adiposity and were protected against high fat diet induced obesity, but not insulin resistance or hepatic steatosis. Gene expression profiling and biochemical assays revealed increased lipolysis and fatty acid oxidation in white adipose tissue (WAT) and brown adipose tissue , as well as browning of WAT, owing to induction of cAMP/protein kinase A signaling upon Bscl2 deletion. Interestingly, Bscl2 deletion reduced food intake and downregulated adipose β3-adrenergic receptor (ADRB3) expression. Impaired ADRB3 signaling partially offsets upregulated browning-induced energy expenditure and thermogenesis in Ad-mKO mice housed at ambient temperature. However, this counterregulatory response was abrogated under thermoneutral conditions, resulting in even greater body mass loss in AdmKO mice. These findings suggest that Bscl2 regulates adipocyte lipolysis and β-adrenergic signaling to produce complex effects on adipose tissues and whole-body energy balance.

Bornstein, S., et al. (2014). "FGF-21 and skeletal remodeling during and after lactation in C57BL/6J mice." Endocrinology **155**(9): 3516-3526.

Lactation is associated with significant alterations in both body composition and bone mass. Systemic and local skeletal factors such as receptor activator of nuclear factor κ-B ligand (RANKL), PTHrP, calcitonin,andestrogen areknownto regulateboneremodeling duringandafter lactation. Fibroblast growth factor 21 (FGF-21) may function as an endocrine factor to regulate body composition changes during lactation by inducing gluconeogenesis and fatty acid oxidation. In this study, we hypothesized that the metabolic changes during lactation were due in part to increased circulating FGF-21, which in turn could accentuate bone loss.Welongitudinally characterized body composition in C57BL/6J (B6) mice during (day 7 and day 21 of lactation) and after normal lactation (day 21 postlactation). At day 7 of lactation, areal bone density declined by 10% (P <.001), bone resorption increased (P<.0001), percent fat decreased by 20%, energy expenditure increased (P<.01), and markers of brown-like adipogenesis were suppressed in the inguinal depot and in preformed brown adipose tissue. At day 7 of lactation there was a 2.4-fold increase in serum FGF-21 vs baseline (P <.0001), a 8-fold increase in hepatic FGF-21 mRNA (P <.03), a 2-fold increase in undercarboxylated osteocalcin (Glu13 OCn) (P<.01), and enhanced insulin sensitivity. Recovery of total areal bone density was noted at day 21 of lactation, whereas the femoral trabecular bone volume fraction was still reduced (P <.01). Because FGF-21 levels rose rapidly at day 7 of lactation in B6 lactating mice,wenext examined lactating mice with a deletion in the Fgf21 gene. Trabecular and cortical bone masses were maintained throughout lactation in FGF-21-/-mice, and pup growth was normal. Compared with lactating control mice, lactating FGF-21-/-mice exhibited an increase in bone formation, but no change in bone resorption. In conclusion, in addition to changes in calciotropic hormones, systemic FGF-21 plays a role in skeletal remodeling and changes in body composition during lactation in B6 mice. Copyright

Cappel, D. A. (2014). Metabolic health with obesity: a novel role for cholesteryl ester transfer protein, Vanderbilt University.

Chen, C. C. W. and K. C. Welch (2014). "Hummingbirds can fuel expensive hovering flight completely with either exogenous glucose or fructose." Functional Ecology **28**(3): 589-600.

Summary: Hummingbirds have specialized on a diet consisting almost exclusively of a mixture of sucrose, glucose and fructose found in floral nectar. Previous studies have shown that hummingbirds can fuel energetically expensive hovering flight almost exclusively using recently ingested sucrose. However, the relative capacities for the direct utilization of glucose and fructose by hovering hummingbirds remain unknown. 13C-enriched solutions of glucose and fructose were fed to ruby-throated hummingbirds (Archilochus colubris) separately. Along with simultaneous measurements of gas exchange during hovering we collected exhaled breath samples using feeder-mask respirometry and analysed these to determine the isotopic signatures of exhaled carbon dioxide. We found that hovering hummingbirds transition from exclusively oxidizing endogenous fatty acids when fasted, to oxidizing newly ingested carbohydrates when given access to either glucose or fructose solutions. We then switched hummingbirds to the respective unlabelled solutions of glucose or fructose to estimate carbohydrate turnover kinetics. During the period of availability of enriched solutions, the percentage of metabolism supported by exogenous sugar increased from 0% to near 100% in some individuals. On average, hummingbirds fuelled 81% and 88% of their metabolism during hovering flight with exogenous glucose and fructose, respectively. The amount of energy ingested, fractional turnover of ingested sugars in the pool of actively metabolized substrates, amount oxidized, energy expended and proportion of hovering metabolism supported by each hexose were all similar between glucose and fructose. By foraging frequently and fuelling hovering flight directly with ingested monosaccharides hummingbirds avoid the energetic tax associated with the cost of synthesis of fats from these sugars prior to their oxidation. Remarkably, hovering hummingbirds are able to utilize fructose and glucose equally, a physiological feat which no mammals are thought to match, and one that suggests novel physiological capacities for the oxidation of fructose by active muscle tissues in hummingbirds. The data presented here indicate hummingbirds enhance net energy intake though specialization of diet, behaviour, and, uniquely, metabolic physiology.

Chen, Z., et al. (2014). "Incorporation of therapeutically modified bacteria into Gut microbiota inhibits obesity." Journal of Clinical Investigation **124**(8): 3391-3406.

Metabolic disorders, including obesity, diabetes, and cardiovascular disease, are widespread in Westernized nations. Gut microbiota composition is a contributing factor to the susceptibility of an individual to the development of these disorders; therefore, altering a person's microbiota may ameliorate disease. One potential microbiome-altering strategy is the incorporation of modified bacteria that express therapeutic factors into the gut microbiota. For example, N-acylphosphatidylethanolamines

(NAPEs) are precursors to the N-acylethanolamide (NAE) family of lipids, which are synthesized in the small intestine in response to feeding and reduce food intake and obesity. Here, we demonstrated that administration of engineered NAPE-expressing E. coli Nissle 1917 bacteria in drinking water for 8 weeks reduced the levels of obesity in mice fed a high-fat diet. Mice that received modified bacteria had dramatically lower food intake, adiposity, insulin resistance, and hepatosteatosis compared with mice receiving standard water or control bacteria. The protective effects conferred by NAPE-expressing bacteria persisted for at least 4 weeks after their removal from the drinking water. Moreover, administration of NAPE-expressing bacteria to TallyHo mice, a polygenic mouse model of obesity, inhibited weight gain. Our results demonstrate that incorporation of appropriately modified bacteria into the gut microbiota has potential as an effective strategy to inhibit the development of metabolic disorders.

Csóka, B., et al. (2014). "A2B Adenosine receptors prevent insulin resistance by inhibiting adipose tissue inflammation via maintaining alternative macrophage activation." Diabetes **63**(3): 850-866.

Obesity causes increased classical and decreased alternative macrophage activation, which in turn cause insulin resistance in target organs. Because A2B adenosine receptors (ARs) are important regulators of macrophage activation, we examined the role of A2B ARs in adipose tissue inflammation and insulin resistance. A2B AR deletion impaired glucose and lipid metabolism in mice fed chow but not a high-fat diet, which was paralleled by dysregulation of the adipokine system, and increased classical macrophage activation and inhibited alternative macrophage activation. The expression of alternative macrophage activation- specific transcriptions factors, including CCAAT/enhancer-binding protein-β, interferon regulatory factor 4, and peroxisome proliferator- activated receptor-γ, was decreased in adipose tissue of A2B AR-deficient mice. Furthermore, in in vitro studies, we found that stimulation of A2B ARs suppressed free fatty acid-induced deleterious inflammatory and metabolic activation of macrophages. Moreover, AR activation upregulated the interleukin-4-induced expression of CCAAT/enhancer-binding protein-β, interferon regulatory factor 4, and peroxisome proliferator- activated receptor-γ in macrophages. Altogether, our results indicate that therapeutic strategies targeting A2B ARs hold promise for preventing adipose tissue inflammation and insulin resistance. © 2014 by the American Diabetes Association..

Kang, L., et al. (2014). "Matrix metalloproteinase 9 opposes diet-induced muscle insulin resistance in mice." Diabetologia **57**(3): 603-613.

Aims/hypothesis: Increased extracellular matrix (ECM) collagen is a characteristic of muscle insulin resistance. Matrix metalloproteinase (MMP) 9 is a primary enzyme that degrades collagen IV (ColIV). As a component of the basement membrane, ColIV plays a key role in ECM remodelling. We tested the hypotheses that genetic deletion of MMP9 in mice increases muscle ColIV, induces insulin resistance in lean mice and worsens diet-induced muscle insulin resistance. Methods: Wild-type (Mmp9 +/+) and Mmp9-null (Mmp9 -/-) mice were chow or high-fat (HF) fed for 16 weeks. Insulin action was measured by the hyperinsulinaemic-euglycaemic clamp in conscious weight-matched surgically catheterised mice. Results: Mmp9 -/- and HF feeding independently increased muscle ColIV. ColIV in HF-fed Mmp9 -/- mice was further increased. Mmp9 -/- did not affect fasting insulin or glucose in chow- or HF-fed mice. The glucose infusion rate (GIR), endogenous glucose appearance (EndoRa) and glucose disappearance (Rd)

rates, and a muscle glucose metabolic index (Rg), were the same in chow-fed Mmp9 +/+ and Mmp9 -/- mice. In contrast, HF-fed Mmp9 -/- mice had decreased GIR, insulin-stimulated increase in Rd and muscle Rg. Insulin-stimulated suppression of EndoRa, however, remained the same in HF-fed Mmp9 -/- and Mmp9 +/+ mice. Decreased muscle Rg in HF-fed Mmp9 -/- was associated with decreased muscle capillaries. Conclusions/interpretation: Despite increased muscle ColIV, genetic deletion of MMP9 does not induce insulin resistance in lean mice. In contrast, this deletion results in a more profound state of insulin resistance, specifically in the skeletal muscle of HF-fed mice. These results highlight the importance of ECM remodelling in determining muscle insulin resistance in the presence of HF diet. © 2013 Springer-Verlag Berlin Heidelberg.

Shechter, A., et al. (2014). "Postprandial thermogenesis and substrate oxidation are unaffected by sleep restriction." International Journal of Obesity **38**(9): 1153-1158.

Background/objectives: The extent to which alterations in energy expenditure (EE) in response to sleep restriction contribute to the short sleep-obesity relationship is not clearly defined. Short sleep may induce changes in resting metabolic rate (RMR), thermic effect of food (TEF) and postprandial substrate oxidation. Subjects/methods: Ten females (age and body mass index: 22-43 years and 23.4-28 kg m-2) completed a randomized, crossover study assessing the effects of short (4 h per night) and habitual (8 h per night) sleep duration on fasting and postprandial RMR and respiratory quotient (RQ). Measurements were taken after three nights using whole-room indirect calorimetry. The TEF was assessed over a 6-h period following consumption of a high-fat liquid meal. Results: Short versus habitual sleep did not affect RMR (1.01±0.05 and 0.97±0.04 kcal min-1; P=0.23). Fasting RQ was significantly lower after short versus habitual sleep (0.84±0.01 and 0.88±0.01; P=0.028). Postprandial EE (short: 1.13±0.04 and habitual: 1.10±0.04, P=0.09) and RQ (short: 0.88±0.01 and habitual: 0.88±0.01, P=0.50) after the high-fat meal were not different between conditions. TEF was similar between conditions (0.24±0.02 kcal min-1 in both; P=0.98), as was the ~6-h incremental area under the curve (1.16±0.10 and 1.17±0.09 kcal min-1 × 356 min after short and habitual sleep, respectively; P=0.92). Conclusions: Current findings observed in non-obese healthy premenopausal women do not support the hypothesis that alterations in TEF and postprandial substrate oxidation are major contributors to the higher rate of obesity observed in short sleepers. In exploring a role of sleep duration on EE, research should focus on potential alterations in physical activity to explain the increased obesity risk in short sleepers.

Sugawara, E. and H. Nikaido (2014). Properties of AdeABC and AdeIJK efflux systems of Acinetobacter baumannii compared with those of the AcrAB-TolC system of Escherichia coli, digital.lib.washington.edu.

Acinetobacter baumannii contains RND-family efflux systems AdeABC and AdeIJK, which pump out a wide range of antimicrobial compounds, as judged from the MIC changes occurring upon deletion of the responsible genes. However, these studies may miss changes because of the high backgrounds generated by the remaining pumps and by β-lactamases, and it is unclear how the activities of these pumps compare quantitatively with those of the well-studied AcrAB-TolC system of Escherichia coli. We expressed adeABC and adeIJK of A. baumannii, as well as E. coli acrAB, in an E. coli host from which acrAB was deleted. The A. baumannii pumps were functional in E. coli, and the MIC changes that were observed largely confirmed

the substrate range already reported, with important differences. Thus, the AdeABC system pumped out all β-lactams, an activity that was often missed in deletion studies. When the expression level of the pump genes was adjusted to a similar level for a comparison with AcrAB-TolC, we found that both A. baumannii efflux systems pumped out a wide range of compounds, but AdeABC was less effective than AcrAB-TolC in the extrusion of lipophilic β-lactams, novobiocin, and ethidium bromide, although it was more effective at tetracycline efflux. AdeIJK was remarkably more effective than a similar level of AcrAB-TolC in the efflux of β-lactams, novobiocin, and ethidium bromide, although it was less so in the efflux of erythromycin. These results thus allow us to compare these efflux systems on a quantitative basis, if we can assume that the heterologous systems are fully functional in the E. coli host.

Vieira-Potter, V. J., et al. (2014). "Female rats selectively bred for high intrinsic aerobic fitness are protected from ovariectomy-associated metabolic dysfunction." American Journal of Physiology - Regulatory Integrative and Comparative Physiology **308**(6): R530-R542.

Ovariectomized rodents model human menopause in that they rapidly gain weight, reduce spontaneous physical activity (SPA), and develop metabolic dysfunction, including insulin resistance. How contrasting aerobic fitness levels impacts ovariectomy (OVX)-associated metabolic dysfunction is not known. Female rats selectively bred for high and low intrinsic aerobic fitness [high-capacity runners (HCR) and low-capacity runners (LCR), respectively] were maintained under sedentary conditions for 39 wk. Midway through the observation period, OVX or sham (SHM) operations were performed providing HCR-SHM, HCR-OVX, LCR-SHM, and LCR-OVX groups. Glucose tolerance, energy expenditure, and SPA were measured before and 4 wk after surgery, while body composition via dual-energy X-ray absorptiometry and adipose tissue distribution, brown adipose tissue (BAT), and skeletal muscle phenotype, hepatic lipid content, insulin resistance via homeostatic assessment model of insulin resistance and AdipoIR, and blood lipids were assessed at death. Remarkably, HCR were protected from OVX-associated increases in adiposity and insulin resistance, observed only in LCR. HCR rats were ~30% smaller, had ~70% greater spontaneous physical activity (SPA), consumed ~10% more relative energy, had greater skeletal muscle proliferator-activated receptor coactivator 1-alpha, and ~40% more BAT. OVX did not increase energy intake and reduced SPA to the same extent in both HCR and LCR. LCR were particularly affected by an OVX-associated reduction in resting energy expenditure and experienced a reduction in relative BAT; resting energy expenditure correlated positively with BAT across all animals ($r = 0.6$; $P < 0.001$). In conclusion, despite reduced SPA following OVX, high intrinsic aerobic fitness protects against OVX-associated increases in adiposity and insulin resistance. The mechanism may involve preservation of resting energy expenditure.

Cappel, D. A., et al. (2013). "Cholesteryl ester transfer protein protects against insulin resistance in obese female mice." Molecular Metabolism **2**(4): 457-467.

Cholesteryl ester transfer protein (CETP) shuttles lipids between lipoproteins, culminating in cholesteryl ester delivery to liver and increased secretion of cholesterol as bile. Since gut bile acids promote insulin sensitivity, we aimed to define if CETP improves insulin sensitivity with high-fat feeding. CETP and non-transgenic mice of both sexes became obese. Female but not male CETP mice had increased ileal bile acid levels versus non-transgenic littermates. CETP expression protected female mice from insulin resistance

but had a minimal effect in males. In liver, female CETP mice showed activation of bile acid-sensitive pathways including Erk1/2 phosphorylation and Fxr and Shp gene expression. In muscle, CETP females showed increased glycolysis, increased mRNA for Dio2, and increased Akt phosphorylation, known effects of bile acid signaling. These results suggest that CETP can ameliorate insulin resistance associated with obesity in female mice, an effect that correlates with increased gut bile acids and known bile-signaling pathways. © 2013 .

Motyl, K. J., et al. (2013). "Altered thermogenesis and impaired bone remodeling in Misty mice." Journal of Bone and Mineral Research **28**(9): 1885-1897.

Fat mass may be modulated by the number of brown-like adipocytes in white adipose tissue (WAT) in humans and rodents. Bone remodeling is dependent on systemic energy metabolism and, with age, bone remodeling becomes uncoupled and brown adipose tissue (BAT) function declines. To test the interaction between BAT and bone, we employed Misty (m/m) mice, which were reported be deficient in BAT. We found that Misty mice have accelerated age-related trabecular bone loss and impaired brown fat function (including reduced temperature, lower expression of Pgc1a, and less sympathetic innervation compared to wild-type (+/ +)). Despite reduced BAT function, Misty mice had normal core body temperature, suggesting heat is produced from other sources. Indeed, upon acute cold exposure (4°C for 6 hours), inguinal WAT from Misty mice compensated for BAT dysfunction by increasing expression of Acadl, Pgc1a, Dio2, and other thermogenic genes. Interestingly, acute cold exposure also decreased Runx2 and increased Rankl expression in Misty bone, but only Runx2 was decreased in wild-type. Browning of WAT is under the control of the sympathetic nervous system (SNS) and, if present at room temperature, could impact bone metabolism. To test whether SNS activity could be responsible for accelerated trabecular bone loss, we treated wild-type and Misty mice with the β-blocker, propranolol. As predicted, propranolol slowed trabecular bone volume/total volume (BV/TV) loss in the distal femur of Misty mice without affecting wild-type. Finally, the Misty mutation (a truncation of DOCK7) also has a significant cell-autonomous role. We found DOCK7 expression in whole bone and osteoblasts. Primary osteoblast differentiation from Misty calvaria was impaired, demonstrating a novel role for DOCK7 in bone remodeling. Despite the multifaceted effects of the Misty mutation, we have shown that impaired brown fat function leads to altered SNS activity and bone loss, and for the first time that cold exposure negatively affects bone remodeling. Copyright © 2013 American Society for Bone and Mineral Research.

Shechter, A., et al. (2013). "Experimental sleep curtailment causes wake-dependent increases in 24-h energy expenditure as measured by whole-room indirect calorimetry1-4." American Journal of Clinical Nutrition **98**(6): 1433-1439.

Background: Epidemiologic evidence has shown a link between short sleep and obesity. Clinical studies suggest a role of increased energy intake in this relation, whereas the contributions of energy expenditure (EE) and substrate utilization are less clearly defined. Objective: Our aim was to investigate the effects of sleep curtailment on 24-h EE and respiratory quotient (RQ) by using whole room indirect calorimetry under fixed-meal conditions. Design: Ten females aged 22-43 y with a BMI (in kg/m2) of 23.4- 27.5 completed a randomized, crossover study. Participants were studied under short- (4 h/night) and habitual- (8 h/night) sleep conditions for 3 d, with a 4-wk washout period between visits. Standardized

weight-maintenance meals were served at 0800, 1200, and 1900 with a snack at 1600. Measures included EE and RQ during the sleep episode on day 2 and continuously over 23 h on day 3. Results: Short compared with habitual sleep resulted in significantly higher (±SEM) 24-h EE (1914.0 ± 62.4 compared with 1822.1 ± 43.8 kcal; P = 0.012). EE during the scheduled sleep episode (0100-0500 and 2300-0700 in short- And habitual-sleep conditions, respectively) and across the waking episode (0800- 2300) were unaffected by sleep restriction. RQ was unaffected by sleep restriction. Conclusions: Short compared with habitual sleep is associated with an increased 24-h EE of ~92 kcal (~5%)-lower than the increased energy intake observed in prior sleep-curtailment studies. This finding supports the hypothesis that short sleep may predispose to weight gain as a result of an increase in energy intake that is beyond the modest energy costs associated with prolonged nocturnal wakefulness. This trial was registered at clinicaltrials.gov as NCT01751581. Am J Clin Nutr 2013;98:1433-9. © 2013 American Society for Nutrition.

Speakman, J. R. (2013). "Measuring energy metabolism in the mouse - theoretical, practical, and analytical considerations." Frontiers in Physiology **4 MAR**.

The mouse is one of the most important model organisms for understanding human genetic function and disease. This includes characterization of the factors that influence energy expenditure and dysregulation of energy balance leading to obesity and its sequelae. Measuring energy metabolism in the mouse presents a challenge because the animals are small, and in this respect it presents similar challenges to measuring energy demands in many other species of small mammal. This paper considers some theoretical, practical, and analytical considerations to be considered when measuring energy expenditure in mice. Theoretically total daily energy expenditure is comprised of several different components: basal or resting expenditure, physical activity, thermoregulation, and the thermic effect of food. Energy expenditure in mice is normally measured using open flow indirect calorimetry apparatus. Two types of system are available - one of which involves a single small Spartan chamber linked to a single analyzer, which is ideal for measuring the individual components of energy demand. The other type of system involves a large chamber which mimics the home cage environment and is generally configured with several chambers/analyzer. These latter systems are ideal for measuring total daily energy expenditure but at present do not allow accurate decomposition of the total expenditure into its components. The greatest analytical challenge for mouse expenditure data is how to account for body size differences between individuals. This has been a matter of some discussion for at least 120 years. The statistically most appropriate approach is to use analysis of covariance with individual aspects of body composition as independent predictors. © 2013 Speakman.

Chin, C., et al. (2012). Carbohydrate Oxidation in Fueling Hovering Flight in the Ruby- Throated Hummingbird (Archilochus colubris) by Carbohydrate Oxidation in Fueling Hovering Flight in the Ruby, tspace.library.utoronto.ca.

… Mass measurements were reported at 0.5s intervals during data collection to a laptop computer for recording via IM1 Promethion (Sable Systems International) using Expedata software (v. 1.3.20, Sable Systems International) …

Kaiyala, K. J., et al. (2012). "Acutely decreased thermoregulatory energy expenditure or decreased activity energy expenditure both acutely reduce food intake in mice." PLoS One **7**(8).

Despite the suggestion that reduced energy expenditure may be a key contributor to the obesity pandemic, few studies have tested whether acutely reduced energy expenditure is associated with a compensatory reduction in food intake. The homeostatic mechanisms that control food intake and energy expenditure remain controversial and are thought to act over days to weeks. We evaluated food intake in mice using two models of acutely decreased energy expenditure: 1) increasing ambient temperature to thermoneutrality in mice acclimated to standard laboratory temperature or 2) exercise cessation in mice accustomed to wheel running. Increasing ambient temperature (from 21°C to 28°C) rapidly decreased energy expenditure, demonstrating that thermoregulatory energy expenditure contributes to both light cycle (40±1%) and dark cycle energy expenditure (15±3%) at normal ambient temperature (21°C). Reducing thermoregulatory energy expenditure acutely decreased food intake primarily during the light cycle (65±7%), thus conflicting with the delayed compensation model, but did not alter spontaneous activity. Acute exercise cessation decreased energy expenditure only during the dark cycle (14±2% at 21°C; 21±4% at 28°C), while food intake was reduced during the dark cycle (0.9±0.1 g) in mice housed at 28°C, but during the light cycle (0.3±0.1 g) in mice housed at 21°C. Cumulatively, there was a strong correlation between the change in daily energy expenditure and the change in daily food intake (R2 = 0.51, p<0.01). We conclude that acutely decreased energy expenditure decreases food intake suggesting that energy intake is regulated by metabolic signals that respond rapidly and accurately to reduced energy expenditure. © 2012 Kaiyala et al.

Morton, G. J., et al. (2012). "Peripheral oxytocin suppresses food intake and causes weight loss in diet-induced obese rats." American Journal of Physiology - Endocrinology and Metabolism **302**(1).

Growing evidence suggests that oxytocin plays an important role in the regulation of energy balance and that central oxytocin administration induces weight loss in diet induced obese (DIO) animals. To gain a better understanding of how oxytocin mediates these effects, we examined feeding and neuronal responses to oxytocin in animals rendered obese following exposure to either a high-fat (HFD) or low-fat diet (LFD). Our findings demonstrate that peripheral administration of oxytocin dose dependently reduces food intake and body weight to a similar extent in rats maintained on either diet. Moreover, the effect of oxytocin to induce weight loss remained intact in leptin receptor-deficient Koletsky (fa k/fa k) rats relative to their lean littermates. To determine whether systemically administered oxytocin activates hindbrain areas that regulate meal size, we measured neuronal c-Fos induction in the nucleus of the solitary tract (NTS) and area postrema (AP). We observed a robust neuronal response to oxytocin in these hindbrain areas that was unexpectedly increased in rats rendered obese on a HFD relative to lean, LFD-fed controls. Finally, we report that repeated daily peripheral administration of oxytocin in DIO animals elicited a sustained reduction of food intake and body weight while preventing the reduction of energy expenditure characteristic of weight-reduced animals. These findings extend recent evidence suggesting that oxytocin circumvents leptin resistance and induces weight-loss in DIO animals through a mechanism involving activation of neurons in the NTS and AP, key hindbrain areas for processing satiety-related inputs. © 2012 the American Physiological Society.

Lighton, J. R. B. and L. G. Halsey (2011). "Flow-through respirometry applied to chamber systems: Pros and cons, hints and tips." Comparative Biochemistry and Physiology - A Molecular and Integrative Physiology **158**(3): 265-275.

Flow-through respirometry is a powerful, accurate methodology for metabolic measurement that is applicable to organisms spanning a body mass range of many orders of magnitude. Concentrating on flow-through respirometry that utilizes a chamber to contain the experimental animals, we describe the most common flow measurement and control methodologies (push, pull and stop-flow) and their associated advantages and disadvantages. Objective methods for calculating air flow rates through the chamber, based on the body mass and taxon of the experimental organism, are presented. Techniques for removing the effect of water vapor dilution, including the direct measurement of water vapor pressure and mathematical compensation for its presence, are described and evaluated, as are issues surrounding the analysis of one or both of the respiratory gases (oxygen and carbon dioxide), and issues related to the mathematical correction of wash-out phenomena (response correction). Two important biomedical applications of flow-through respirometry (metabolic phenotyping and room calorimetry) are discussed in detail, and we conclude with a list of suggestions aimed primarily at investigators starting out in applying flow-through respirometry.