SABLE SYSTEMS INTERNATIONAL

METHODS, TECHNIQUES AND APPLICATIONS

Metabolic and Behavioral Measurement

By Scientists, For Scientists

Mouse Graded Exercise Test: Determining $\dot{V}O_2$ max

APPLICATION NOTE

The gold standard for measuring cardiovascular fitness is the graded exercise test (GXT). Typical fitness parameters of GXT include $\dot{V}O_2$ max, metabolic crossover, and anaerobic threshold – of which $\dot{V}O_2$ max is the most widely accepted measure of cardiovascular fitness. A simple definition of $\dot{V}O_2$ max is the maximum rate of oxygen consumed despite an increase in exertion. Here we describe a graded exercise test to determine $\dot{V}O_2$ max using our automated mouse metabolic treadmill.

Typical Issues in Measuring Mouse $\dot{V}O_2$ max

- **Indirect calorimetry not optimized for graded exercise tests**
- **Human variability in running the tests**
- **Inconsistent test results**

Many physiological systems contribute to $\dot{V}O_2$ max/ aerobic fitness, including cardiovascular, muscular, pulmonary, and neuronal systems. The graded exercise test enables researchers to study each system and their components to gain a basic understanding of the physiological response to exercise with the aim of reducing the prevalence of metabolic diseases (such as obesity and diabetes) as well as improving quality of life as one ages.

The GXT combines an exercise to achieve maximal exertion with an indirect calorimeter for the measurement of VO_2 (rate of oxygen consumed), VCO_2 (rate of carbon dioxide produced), and the calculation of the respiratory exchange ratio (VCO_2/VO_2). Maximal exertion is achieved by the rapid stepwise increase of exercise workload.

There are established treadmill GXT protocols to determine $\dot{V}O_2$ max. These include the Bruce, Balke and Ellestad protocols. These protocols consist of variations on the stepwise increase in speed and incline and are similar between humans and mice. Simultaneous with the increasing exercise workload, the participant's VO_2 is measured by indirect calorimetry. $\dot{V}O_2$ max is the point at which VO_2 does not increase despite an increase in exertion. Additional parameters obtained may include time to exhaustion, anaerobic threshold, metabolic crossover, and recovery time.

For mice, the GXT can be performed using a mouse metabolic treadmill, which is a small treadmill enclosed in a chamber and connected to an indirect calorimeter. To increase consistency of the GXT results, the mouse is acclimated by 2-3 training sessions. Table 1 describes one acclimation protocol. Each protocol may be different depending on the specifics of the study.

Table 1: Animal acclimation to treadmill protocol. Acclimation was performed by three training sessions with 60 hours rest between each session.

Stage	Speed (meter/ min)	Inclination (degrees)	Duration (min)
1	5	0	3 to 5
2	10	0	5 to 10
3	20	0	5 to 10
4	15 to 25	0 to 20°	5 to 10

The Sable Systems metabolic treadmill is programmable and automated. This allows for the use of standardized protocols for consistent results and the versatility to be programmed to fit the research requirements. Figure 1 illustrates the graded exercise test protocol used for the determination of $\dot{V}O_2$ max.

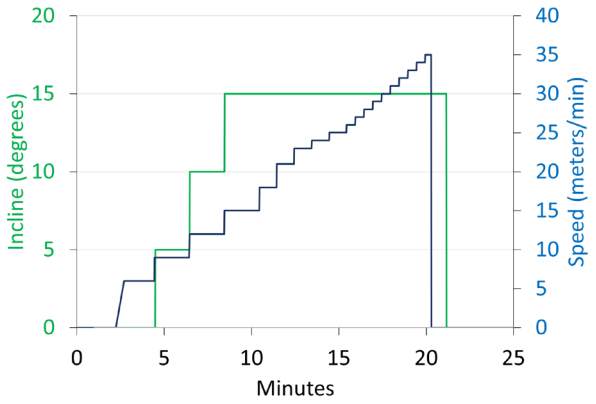

Figure 1: Illustration of the graded exercise test protocol showing the automated stepwise increase in treadmill speed in meters/minute and treadmill incline in degrees. Protocols range in duration from 12 to 25 minutes to minimize fatigue effects. The protocol also depends on the specifics of the study, e.g., the mouse model. The $\dot{V}O_2$ max protocol was adapted from Petrosino JM et.al.[1]

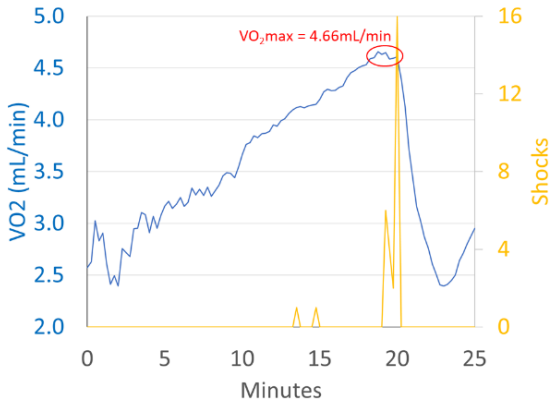

Figure 2: $\dot{V}O_2$ max and time to exhaustion determined by the measurement of VO_2. A shock grid induces running and signals (light and auditory signals) to the observer when the mouse contacts the grid. This enables the observer to assess the condition of the mouse and to stop the test when exhaustion is acheived. High-resolution respirometry measurements were acquired concurrent with the treadmill graded exercise test. Note that baseline measurements are acquired before and after the run so that all key metabolic events (such as achieving $\dot{V}O_2$ max and recovery) are captured and not lost due to baselining during the test. In line with the treadmill GXT protocol, VO_2 increased linearly. VO_2 plateaued between 18.5 and 20 minutes and $\dot{V}O_2$ max calculated as the average VO_2 during the plateau (VO_2 max = 4.66 ml/min). Time to exhaustion occurred at the 20-minute mark, as determined by the rapid shock count increase, and the treadmill run was manually ended. However, respirometry measurements continued, enabling the evaluation of recovery.

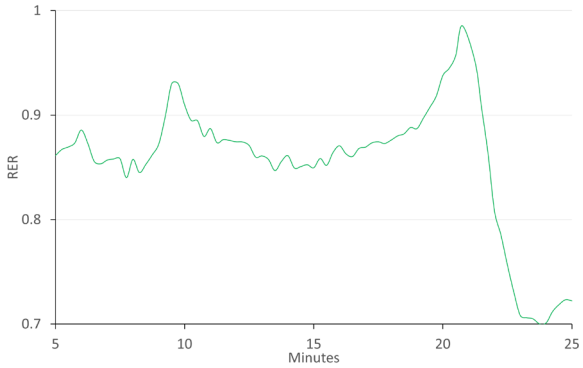

Figure 3: Relative fuel utilization is represented by the respiratory exchange ratio. The respiratory exchange ratio (RER) is the ratio of VCO_2 and VO_2 (VCO_2/VO_2). An RER of 0.7 means that lipids are being oxidized and an RER of 1.0 means that carbohydrates are being oxidized. During strenuous activity RER values between 0.7 and 1.0 represent a mix of lipid and carbohydrate oxidation. An abrupt increase in RER indicates the anaerobic threshold where aerobic energy production starts to be supplemented by anaerobic mechanisms.[2] Both anaerobic threshold and metabolic crossover (time point shift from lipid to carbohydrate oxidation) may be calculated from the RER data.

1. Petrosino JM, Heiss VJ, Maurya SK, et al. Graded Maximal Exercise Testing to Assess Mouse Cardio-Metabolic Phenotypes. *PLoS One*. 2016;11(2):e0148010. Published 2016 Feb 9.

2. Wasserman K. The anaerobic threshold measurement in exercise testing. *Clin Chest Med*. 1984 Mar;5(1):77-88. PMID: 6723245.)

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