Exercise assessment of transgenic models of human cardiovascular disease

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Bernstein, Daniel. Exercise assessment of transgenic models of human cardiovascular disease. Physiol Genomics 13: 217–226, 2003; 10.1152/physiolgenomics.00188.2002.—Exercise provides one of the most severe, yet physiological, stresses to the intact cardiovascular system and is a major determinant of the utilization of metabolic substrates. The adaptations to exercise are the result of a coordinated response of multiple organ systems, including cardiovascular, pulmonary, endocrine-metabolic, immunologic, and skeletal muscle. With the proliferation of genetically altered murine models of cardiovascular disease, the importance of developing methods of accurate physiological phenotyping is critical. There are numerous examples of transgenic models in which the baseline cardiovascular phenotype is unchanged or minimally changed from the wild type, only to become manifest during the stress of exercise testing. In this review, we cover the basics of the murine cardiovascular response to exercise and the importance of attending to strain differences, compare different exercise methodologies (constant workload treadmill, incremental workload treadmill, swimming) and hemodynamic monitoring systems, and examine the murine response to exercise conditioning. Several examples where exercise studies have contributed to the elucidation of cardiovascular phenotypes are reviewed: the β-adrenergic receptor knockouts, phospholamban knockout, dystrophin knockout (mdx), and the mutant α-myosin heavy chain (R403Q) transgenic.

β-adrenergic receptor knockout; skeletal muscle; phospholamban knockout; dystrophin knockout; mutant α-myosin heavy chain transgenic

OVER THE PAST DECADE, there has been a proliferation of genetically modified animal models of human cardiovascular disease. Despite adaptation of transgenic technology to larger mammals, the mouse remains the most commonly used species for transgenic studies and continues to be the only species in which targeted genetic deletions are possible. The addition of technologies allowing tissue-specific knockouts as well as the ability to turn gene expression on and off again at will enhances our ability to study genetic regulation of the cardiovascular system. Some phenotypes, however, may be extremely subtle. Thus thorough evaluation of genetically altered murine models requires the ability to study all components of murine cardiovascular physiology. The development of techniques allowing the evaluation of mice in an awake, unrestrained state has advanced the study of true resting cardiovascular dynamics and has also allowed the introduction of exercise testing to study cardiovascular response during stressed states. Exercise studies provide valuable information about how the cardiovascular system responds under maximally stimulated conditions, with the possibility of uncovering phenotypes not observed at rest. Given the expense and time involved in creating genetically altered murine models of human cardiovascular disease, it is not unreasonable to suggest that most if not all models should be evaluated during both resting and exercise-stressed conditions.

Adaptations to Exercise: A Brief Review

Exercise provides one of the most severe physiological stresses to the cardiovascular system, yet one which is highly clinically relevant. The organism’s response to exercise is the result of the coordinated response of multiple organ systems, including the heart, lungs, peripheral vasculature, exercising muscle, and the neuroendocrine system. However, the cardiovascular system is most directly affected by exercise and is responsible for many of the important acute changes. For an excellent review see the references by Rowell (54, 55), Janicki et al. (26), and Laughlin et al. (29).
**Oxygen consumption.** The ultimate goal of cardiovascular adaptation to exercise is the delivery of adequate oxygen and other metabolic substrates to the working muscle, as well as the removal of carbon dioxide and other waste products. The success of the cardiovascular system in achieving this goal can be assessed by measuring the balance between tissue oxygen delivery and tissue oxygen consumption. Tissue oxygen consumption \((\dot{V}_\text{O}_2)\) is most commonly estimated by measuring oxygen consumption through ventilation \((\dot{V}_\text{O}_2)\), and at equilibrium, these two values are identical. The Fick equation describes the relationship between oxygen consumption and the major indices of cardiovascular performance

\[ \dot{V}_\text{O}_2 = CO \times (C\text{ao}_2 - C\text{vo}_2) = HR \times SV \times (C\text{ao}_2 - C\text{vo}_2) \]

where \(\dot{V}_\text{O}_2\) = oxygen consumption; \(CO = \) cardiac output; \(C\text{ao}_2 = \) arterial oxygen content; \(C\text{vo}_2 = \) mixed venous oxygen content; \(HR = \) heart rate; and \(SV = \) stroke volume. With increasing exercise workloads, muscle oxygen consumption increases. The increased demand is met through increases in cardiac output and in the extraction of oxygen from the blood, measured by the arterial-venous oxygen content difference \((C\text{ao}_2 - C\text{vo}_2)\).

**Stroke volume.** One of the initial cardiovascular responses to low levels of exercise is mediated through an increase in cardiac output, the product of left ventricular stroke volume and heart rate. The increase in stroke volume with exercise is largely due to increases in ventricular filling (preload) and contractility and to a decrease in arterial resistance (afterload). Preload is increased by a combination of venoconstriction, the pumping action of contracting peripheral muscles \((55, 59, 60)\), and by an increase in ventricular compliance \((33, 45, 60)\). Ventricular contractility is increased, independent of changes in preload and afterload, through an increase in sympathetic stimulation mediated by cardiac \(\beta\)-adrenergic receptors \((48, 55)\). Afterload is decreased due to a redistribution of blood flow to skin and working muscle \((8)\), mediated by the effects of local vasodilators such as potassium, lactate, bradykinins, hypoxia, and hypercarbia \((54)\). Increases in stroke volume result in small increases in cardiac output, primarily during low levels of exercise.

Rowell, in his 1974 review \((54)\), suggests the use of this revised formula for the calculation of \(\dot{V}_\text{O}_2\)

\[ \dot{V}_\text{O}_2 = (C\text{ao}_2 - C\text{vo}_2) \times \frac{MBP}{TPR} \]

where \(MBP = \) mean blood pressure, and \(TPR = \) total peripheral resistance. Substituting \(MBP/TPR\) for cardiac output emphasizes the importance of peripheral vascular impedance in regulating cardiac performance.

**Blood pressure.** During dynamic exercise, systolic blood pressure increases significantly while diastolic blood pressure decreases slightly, resulting in only a slight increase in mean arterial blood pressure \((64)\). Thus the large increase in cardiac output during exercise must be closely matched (ventricular-vascular coupling) by a similar decrease in systemic vascular resistance in order to maintain a stable mean blood pressure. The changes in blood pressure (as well as other cardiovascular parameters) with static exercise are dramatically different; therefore, static exercise is rarely used for evaluation of cardiovascular status.

**Heart rate.** Heart rate is regulated by the balance between sympathetic and parasympathetic tone. Exercise increases sympathetic tone and attenuates parasympathetic tone, resulting in tachycardia. During the early (low intensity) phases of exercise, the major mechanism for the increase in heart rate is withdrawal of parasympathetic tone, mediated through the vagus nerve. With moderate to high-intensity exercise, a more gradual increase in heart rate occurs due to an increase in sympathetic stimulation, mediated via direct cardiac sympathetic innervation as well as circulating catecholamines \((50)\). The accurate assessment of resting heart rate, while relatively easy in humans, is more difficult in animals and most difficult in mice (see below).

**Arterial-venous oxygen content difference.** Oxygen supply to working muscle is increased during exercise by an increase in tissue oxygen extraction. This is reflected by the arterial-venous oxygen content difference \((C\text{ao}_2 - C\text{vo}_2)\). At rest, total body oxygen extraction is relatively low, ranging between 25–30%. During exercise, oxygen extraction can increase to as high as 80–85%. This is accomplished by the redistribution of blood flow to working muscle, where the \(Q\text{O}_2\) is very high compared with other organs. With the increase in blood flow to working muscle, capillary transit time is maintained by the opening of previously constricted capillary beds, thus allowing time for adequate oxygen extraction. Further enhancement of tissue oxygen delivery is mediated by a rightward shift in the hemoglobin-oxygen dissociation curve, due to local factors such as acidemia, hypoxemia, and hyperthermia.

**Measurement Of Exercise Capacity**

**Exercise capacity.** Exercise capacity is the maximal amount of exercise work achievable by an organism. It is usually quantified by parameters such as the “maximal workload” achieved during exercise or the “maximal duration” of exercise. These variables are heavily influenced by motivation, which can be a significant confounding variable in human as well as animal studies.

**Aerobic exercise capacity.** Aerobic exercise capacity is another method of assessing exercise capacity, based on quantifiable indices of physiological performance. It is usually measured using metabolic parameters such as maximal oxygen consumption \((\dot{V}_\text{O}_2 \text{ max})\) or anaerobic threshold \((\text{AT})\). Measures of aerobic exercise capacity are less influenced by motivational factors and thus offer a more reliable assessment of the true physiological response to exercise.

**Maximal oxygen consumption.** It is important to make the distinction between maximal oxygen consumption, known as \(\dot{V}_\text{O}_2 \text{ max}\), and oxygen consumption at peak exercise, known as peak \(\dot{V}_\text{O}_2\) or maximum \(\dot{V}_\text{O}_2\), as they do not measure the same parameter. Maximum
\( \dot{V}O_2 \) is simply the value of oxygen consumption at the time immediately before exercise ceases, whereas \( \dot{V}O_2 \text{max} \) represents the true metabolic limit to the consumption of oxygen. During incremental exercise testing, it can be assumed that \( \dot{V}O_2 \text{max} \) has been reached when \( \dot{V}O_2 \) fails to increase despite further increases in work intensity (30, 47, 58). There is still a lack of consensus, however, regarding the validity of \( \dot{V}O_2 \text{max} \) measurements as a representation of true oxidative capacity because of the difficulty in determining when an experimental subject can truly exercise no longer. This holds true for humans as well as experimental animals such as mice.

**Anaerobic threshold.** AT is the point at which anaerobic metabolism contributes significantly to overall energy production. AT can be estimated by calculating the respiratory exchange ratio (RER), which is simply the ratio of \( \dot{V}CO_2 \) to \( \dot{V}O_2 \). In the human, resting RER is usually \( \sim 0.8 \). When the RER rises above 1.0, it represents the point at which \( \dot{CO}_2 \) production exceeds \( \dot{O}_2 \) consumption, or the AT (63). Breath-by-breath respiratory analysis can also be used to estimate AT, by determining the point at which the minute ventilation to carbon dioxide production ratio (VE/\( \dot{V}CO_2 \)) begins to increase; however, this is not feasible in small animals. Determination of the “lactate threshold” is another method of measuring AT (49). The need for repeat samples at various levels of workload makes this less feasible, although not impossible, in mice.

**Motivational issues in murine exercise physiology.** Motivation is an important confounding variable when assessing the murine exercise response (10). Mice will normally run as part of their daily activity if provided the opportunity to do so (e.g., by placing an exercise wheel in their cage); however, there are substantial differences between exercise capacities derived from voluntary wheel running and from treadmill exercise testing where running is motivated, usually by an electric shock. Lerman et al. (32) evaluated seven inbred strains of mice using both techniques and found a poor correlation between estimations of exercise capacity achieved with each method. Although age differences between the mice may have factored into these results, we would like to emphasize the importance of controlling both motivation and strain in any exercise study.

**Exercise conditioning.** Exercise conditioning is the result of multiple physiological adaptations which work in concert to optimize performance during repeated exercise stress (26, 29). There are several ways to measure the effects of conditioning, including increased exercise capacity (distance or duration of exercise) and alterations in \( \dot{V}O_2 \text{max} \) and heart rate at maximal exercise. In humans, exercise conditioning results in a significant increase in cardiac output, primarily as a result of an increase in stroke volume (55). Although total body arteriovenous oxygen difference does not change with conditioning, there are alterations in skeletal muscle metabolism which enhance oxygen extraction locally. Experimental conditions in which repeated exercise studies are being performed on individual subjects may need to take into account the effects of conditioning, as this adaptation does occur in the mouse.

**The Murine Cardiovascular Response To Exercise**

Many of the techniques used in murine exercise physiology were initially developed for studies in the rat. There is an extensive volume of data on rat cardiovascular responses to both acute and chronic exercise and, more recently, interesting studies using selective breeding to develop animals with traits such as running endurance (18, 23). As there are significant differences between rats and humans in the exercise response, there are also significant differences between rat and mouse responses, so that the reader is cautioned not to generalize across species. If transgenic or gene knockout technologies are not required, then the rat may be the preferred model for exercise studies of cardiovascular performance because of the easier means of instrumentation.

**Heart rate.** One cannot begin to assess exercise heart rate in the mouse without understanding the inherent variability and difficulties in measuring resting heart rate in this species (10). There is wide variability in the literature over the definition of normal resting heart rate in the mouse, with published values ranging from as low as 200 to as high as 700 beats/min. This wide variability reflects primarily differences in the use of anesthesia and restraint, acclimatization, recovery time from surgery, strain, as well as activity state of the mouse during recording. (1, 2, 6, 16, 20, 25, 35, 57). Studies in awake mice without restraint are more consistent in reporting resting heart rate between 450–550 beats/min (4, 11, 28, 52, 62). Desai et al. (11) documented the marked changes in murine heart rates during different normal activities (Table 1).

The murine heart rate response to exercise is partly dependent on the mode of exercise. Using forced swimming, Kaplan et al. (27) found a maximal heart rate of 650 beats/min occurring after only 3 min and followed by a plateau. This swimming protocol is thus a form of constant workload exercise, resulting in submaximal levels of exercise intensity. Desai et al. (11), using chronically instrumented mice during graded treadmill exercise, demonstrated linear increases in heart rate with increasing workloads (Fig. 1, top) and peak heart rates as high as 840 beats/min.

The high resting rate in the mouse and the small tachycardic response to atropine suggest a low resting vagal tone (11, 62); however, during the early phase of

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**Table 1. Effect of activity on Resting HR and BP in the mouse**

<table>
<thead>
<tr>
<th>Activity State</th>
<th>HR, beats/min</th>
<th>Mean BP, mmHg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sleep</td>
<td>398 ± 3</td>
<td>109 ± 12</td>
</tr>
<tr>
<td>Quiet rest</td>
<td>463 ± 13</td>
<td>113 ± 8</td>
</tr>
<tr>
<td>Groom/feed</td>
<td>541 ± 17</td>
<td>127 ± 11</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 5 mice of the 129 strain. HR, heart rate; BP, blood pressure.
incremental treadmill exercise, there is a rapid increase in heart rate compared with subsequent exercise (Fig. 1). In humans and other large mammals, this initial rapid increase in heart rate is due to vagal withdrawal, whereas the subsequent gradual rise in heart rate is due to sympathetic stimulation (55). Similarly, in mice this initial phase of heart rate response (~1/3 of the total heart rate response to exercise) is due to vagal withdrawal, as mice lacking β-adrenergic receptors still manifest this early tachycardic response but lack the later, more gradual increase (53).

**Blood pressure.** Resting blood pressure measurements are subject to the same variability and difficulty in measurement as those for heart rate. For exercise testing, direct measurement of arterial blood pressure using a chronically indwelling fluid-filled arterial catheter is easily achieved (11). Totally implantable telemetric blood pressure systems are also available for mice (PhysioTel Transmitters; Data Sciences International, St. Paul, MN), although the size and weight of the device can influence the exercise response (see below). As for other parameters, there are strain differences in blood pressure which must be accounted for in any experimental design (11). The baroreflex is also present in mice and may influence experimental results (41).

During exercise using an incremental treadmill protocol, mice demonstrate a mean blood pressure response that is qualitatively similar to that seen in humans and larger mammals (11). There is a rise in blood pressure at the onset of exercise, followed by a plateau (Fig. 1, top). This blood pressure response is different than that in humans using cuff blood pressure measurements (where systolic blood pressure rises more dramatically); however, it is identical to the human response where mean blood pressures were measured centrally (11, 17).

**Oxygen consumption.** Mice have significantly higher weight-corrected values of resting VO₂ than larger mammals (9, 11, 65). Similar to the variation seen in murine heart rate and blood pressure measurements, routine activities such as grooming or feeding and stresses such as handling can increase VO₂ by up to 50% (9).

During exercise, VO₂ can be measured easily using a commercially available open circuit volumetric gas analysis system (Oxymax System; Columbus Instruments, Columbus, OH) coupled to a sealed chamber treadmill (Fig. 1, bottom). The standard for measuring VO₂ max is the inability to further increase VO₂ in response to increasing workload (47). Reported VO₂ max values in mice range from 132–294 ml·kg⁻¹·min⁻¹. Part of this variability is strain related; however, even within a particular strain, VO₂ max may differ depending on the season and on where the animal was bred (we have noticed higher VO₂ max in identical strain mice obtained from an outside laboratory compared with those bred in our own animal facility). When measuring VO₂ during exercise, it is important to leave adequate time between measurements to achieve a steady-state (58). VO₂ max during swimming exercise has also been measured but has not been as well validated. Thus there are discrepancies between values of VO₂ max reported during treadmill exercise and those reported during swimming, with the swimming values usually lower (15, 65). This may reflect the difficulty of performing graded exercise in the swimming model and calls into question whether true VO₂ max can be measured with this method.
Respiratory exchange ratio and anaerobic threshold. There is much less known about murine indices of AT. Murine resting RER is similar to the human resting value of ~0.8 and reflects the predominant utilization of fat as an energy source at rest (5, 11, 38). With increasing workloads, the increases in murine RER are qualitatively similar to that in humans and larger mammals (Fig. 1, bottom) (11). When the RER increases above 1.0, anaerobic metabolism plays an increasing role in energy production (58). We have shown that serum lactate, measured at 5-min intervals during an incremental treadmill exercise protocol, begins to increase at ~50% of peak $\dot{V}O_2$ (10).

Exercise capacity. With swimming exercise, the most commonly used parameter of exercise capacity is duration of exercise before exhaustion (15, 31, 36, 61). With treadmill exercise, both duration of exercise and total distance have been utilized to determine exercise capacity (21, 22, 40, 42, 46, 56). With aid of a photometric detection device located at the end of the treadmill belt, the number of "beam breaks" has also been used to determine exercise capacity. Treadmill exercise can also be quantified using the maximal workload achieved (11, 56) or by estimating actual work performed (24).

Conditioning. The cardiovascular and metabolic adaptations to repeated exercise in the mouse are evidence that conditioning does occur. During chronic swimming exercise, Kaplan et al. (27) found increased heart weight-to-body weight ratios, decreased heart rates during exercise, and increased skeletal muscle glycolytic capacity after 4 wk of training. During chronic treadmill exercise, Ruwitch et al. (56) demonstrated an increase in exercise capacity and maximal $\dot{V}O_2$, as well as a lower serum lactate level at equivalent submaximal exercise workloads, after 12 wk of training (Fig. 2).

Significant confounding factors. There are many additional confounding factors besides those already mentioned. One of the most important is related to strain (see the excellent editorial by Hintze and Shesely (19)). There are substantial differences in exercise capacity between different inbred strains. This becomes a particular problem when transgenic or knockout mice are crossed to produce animals with multiple genetic alterations (such as double knockouts), with multiple strains contributing to the background. Lightfoot et al. (34) have shown fourfold differences in exercise capacity between different strains (Fig. 3) (34). As previously mentioned, there are differences in exercise capacity between forced treadmill and voluntary wheel running protocols. These are exaggerated when evaluating different strains: for example, C57BL/6J mice had one of the worst exercise capacities on the treadmill but one of the best on the voluntary wheel. DBA mice had the worst capacity on the wheel but a much better capacity on the treadmill (32). Strain differences also exist for other metabolic parameters such as $\dot{V}O_2$ and $\dot{V}CO_2$ (11) and for peripheral vascular responses mediated by endothelium-dependent and independent vasodilators (3).

Environment also has a substantial influence on murine cardiovascular parameters, although some of the influences that have the largest effect on resting parameters (e.g., state of arousal) are minimized during exercise. In fact, we have utilized low-level constant workload treadmill exercise as one mechanism for reducing the influence of activity level and arousal state. Attention should also be paid to maintaining a thermoneutral environment, to avoid excessive handling of animals, and to acclimatize animals to the exercise apparatus before actually doing the study. This latter can be accomplished by placing the mouse in the treadmill for 10–20 min before the exercise procedure; however, remember that exposure to acute treadmill running could result in some degree of conditioning (see above). Given that mice are normally nocturnal animals, studies should always be performed at the same time of day. Season may also influence cardiovascular performance, so that controls and experimental subjects should be studied in tandem rather than sequentially. Although sex differences have been described for other mammals during exercise, we have not been able to reliably reproduce these differences also exist for other metabolic parameters such as $\dot{V}O_2$ and $\dot{V}CO_2$ (11) and for peripheral vascular responses mediated by endothelium-dependent and independent vasodilators (3).

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Fig. 2. Effect of conditioning on exercise capacity in mice. Conditioned mice showed an increase in total distance run as well as in maximal $\dot{V}O_2$ compared with sedentary controls. Lactate levels at submaximal workload were also lower in conditioned mice.
changes in mice. Still, it remains prudent not to mix males and females within a single study.

Protocols for Exercising Mice

Swimming exercise. Forced swimming has been a commonly used tool in assessing exercise capacity in mice. Motivation is less of a problem than with treadmill exercise; however, assessment of cardiovascular variables remains difficult due to technical limitations of the aqueous environment. The recent miniaturization of fully implantable telemetric systems for measuring both heart rate and blood pressure should greatly enhance the utility of swimming protocols. Oxygen consumption can even be measured during swimming (65), albeit with some difficulty.

The advantages of swimming protocols include the relative ease with which it is possible to induce mice to swim compared with running on a treadmill and the simplicity and minimal expense of the equipment required. The disadvantages of swimming are related to the difficulty in measuring cardiovascular parameters, the difficulty in quantifying exercise intensity, and the lack of graded workload protocols. It should be noted that the exercise responses to swimming are quite different from those to treadmill running, complicated by factors such as the diving reflex, mental stress, and episodes of hypoxia associated with diving (10, 13). Whereas this does not discount swimming as a means of inducing exercise stress, the investigator should be cognizant of these features and choose between the two methodologies based on the question at hand.

Treadmill exercise. Treadmill exercise has long been the gold standard for inducing controlled cardiovascular stress in humans and other large mammals. In mice, Seeherman et al. (58) reported the first successful measurements of \( \dot{V}O_2 \) and \( \dot{V}CO_2 \) during treadmill exercise. A commonly used commercially available setup (Columbus Instruments) employs a moving belt encased in a sealed Plexiglas enclosure, allowing for measurement of oxygen consumption and carbon dioxide production. The speed of the belt can be varied from 0 to 99 m/min, and the slope can be varied from 0 to 45°. Stimulus devices consist of either a metal shock grid or forced air or cold water jets. Metabolic measurements are performed using an open circuit volumetric gas analysis system (Oxy max System; Columbus Instruments). The low dead space of this circuit allows quick gas equilibration with a \( t_{1/2} \) of 30 s, ideal for use during graded exercise protocols (11).

Both incremental and constant workload exercise protocols can be performed. The incremental exercise protocol we have used begins with a 45- to 60-min acclimation period followed by 2.5 m/min increases in running speed and 2° increases in slope every 3 min until exhaustion. However, with this protocol, \( \dot{V}O_2 \) does not always plateau, thus \( \dot{V}O_{2\text{max}} \) is not always achieved (11). We have shown that lactate levels increase significantly at this level of exercise, indicative of an increase in anaerobic metabolism (10). Several modifications of this protocol, using different increments of workload or higher starting workloads, have been described and may allow for the more reproducible measurement of \( \dot{V}O_{2\text{max}} \) (5, 30, 47).

Constant workload exercise allows the evaluation of cardiovascular responses in two phases. There is the initial dynamic phase, during which the cardiovascular system is rapidly adapting to the increased metabolic needs. The second, steady-state phase, allows evaluation of parameters of tissue metabolism, since an equilibrium between all systems has been reached. Constant workload protocols can be performed at any level of exercise, depending upon the parameter of interest (5, 58).

The major advantage of treadmill exercise is the ability to precisely regulate work intensity. Treadmill exercise also allows the application of uniform exercise workloads to all experimental groups and easier measurements of cardiovascular parameters and oxygen consumption. The major disadvantages of treadmill
exercise are the difficulty in motivating mice to exercise beyond a certain point and the complexity and expense of the equipment required.

Effect of telemetry implants. Telemetric units, implanted in the abdominal cavity or subcutaneously on the back (PhysioTel; Data Sciences International), have revolutionized the continuous measurements of heart rate and blood pressure in the mouse. However, the size and weight of these devices can influence exercise capacity. We implanted heart rate telemetry devices in mice and repeated measurements of exercise capacity after 2 wk of recovery (Fig. 4), finding a 33% reduction overall. Thus both controls and experimental animals must have similar devices implanted for similar time periods for valid comparisons to be made.

Use of Exercise Phenotyping in Genetically Altered Models of Human Heart Disease

β-Adrenergic receptor-deficient mice. The first murine knockout model to undergo full cardiovascular evaluation using treadmill exercise was the β1-adrenergic receptor knockout mouse. These mice lack chronotropic or inotropic responses to β-agonists (52). Despite an absent cardiac sympathetic response, β1-receptor knockout mice had exercise capacities similar to their wild-type littermates (Fig. 5) (53). Exercise-induced increases in \( VO_2 \) were identical to wild-type mice; however, there was a marked attenuation of the exercise-induced tachycardia. For these mice to maintain normal \( VO_2 \) during exercise, there must be some compensation for their relative bradycardia. Considering the Fick equation, this could be mediated either through an increase in left ventricular stroke volume, by an enhancement of oxygen extraction, or both. Measuring oxygen extraction directly, we demonstrated that it was equivalent in β1 knockout and wild-type mice, suggesting that the primary method of compensation for the lack of chronotropic response must be an increase in stroke volume. Since adrenergic mechanisms for increasing contractility are eliminated in this model, the increase in stroke volume is most likely mediated by adaptations in peripheral vascular resistance and venous return.

Exercise studies were also performed in mice with targeted deletions of the β2-receptor (7) and in mice with deletions of both β1- and β2-receptors (51). The β2 knockout mice have a greater exercise capacity than wild-type littermates. They also have a lower RER at comparable workloads, suggesting that they may use a greater ratio of fat to carbohydrate than wild types.
during exercise (7). Similar to β1 knockouts, β1/β2 double knockout mice were able to exercise to the same total workload as wild types, despite also having a blunted tachycardic response. However, in contrast to β1 knockouts, the double knockouts manifested decreased VO₂ and VCO₂ at all workloads (51).

Models of hypertrophic cardiomyopathy: α-myosin heavy chain (R403Q) mutation. Freeman et al. (14) produced transgenic mice carrying the R403Q mutation of the α-myosin heavy chain gene associated with human hypertrophic cardiomyopathy. These mice showed a marked increase in left ventricular wall thickness compared with nontransgenics, followed by dilation and ventricular failure with aging. Exercise capacity, measured by number of beam breaks or by total endurance, was markedly reduced in the transgenic animals compared with controls.

Effect of exercise on cardiac pathology in a murine model of muscular dystrophy. Nakamura et al. (44) developed mice with deletions of dystrophin (mdx knockout), a model for human muscular dystrophy. Exercise was used to demonstrate that exercise stress is cardiotoxic in dystrophin deficiency. Exercised mdx knockout mice showed increased myocardial fibrosis as well as markedly increased activity of p38 MAPK and ERK1/2 as well as upregulation of calcineurin compared with wild-type or sedentary mdx mice (44).

Phospholamban-deficient mice. Kranias and colleagues (37) ablated the gene for phospholamban, a sarcoplasmic reticulum protein that regulates cardiac contractility and relaxation. Ex vivo working heart preparations from these mice show increased levels of basal contractility and relaxation as well as a lack of further increase in response to β-adrenergic stimulation, suggesting that they are already working at maximal contractility. Treadmill exercise studies demonstrated normal exercise capacity (12) and normal peak VO₂ and peak HR values, despite the fact that these mice were unable to increase cardiac contractility via adrenergic mechanisms. These data suggest that the cardiovascular compensation for the phospholamban deletion may be an increase in stroke volume mediated via enhanced ventricular filling or reduced ventricular afterload during exercise, consistent with in vitro data (20).

Model of dilated cardiomyopathy: chronic activation of the inhibitory G protein (Gi). Conklin and colleagues (49) have produced transgenic mice which overexpress a mutant opioid receptor (OR1) resulting in controllably (tet transactivator system) increased stimulation of the inhibitory G protein, Gi. These mice developed clinical signs of congestive heart failure and echocardiographic evidence of poor ventricular function (49). In this case, exercise testing was utilized to detect preclinical abnormalities in cardiac function before the development of overt heart failure. OR1 mice had decreased exercise capacity, as determined by duration of exercise, workload achieved, and maximum VO₂ (10).

Future Directions

The microminiaturization of telemetry units has made possible continuous ECG and blood pressure recordings in the awake, unrestrained mouse. Further reduction in the size and filtering capabilities of these devices will enhance their utility in murine exercise studies. The miniaturization of transducer-tipped left ventricular pressure catheters (Millar Instruments, Houston, TX) may soon make possible measurement of left ventricular contractile indices such as dP/dt during exercise. Direct measurement of cardiac output remains difficult in exercising mice; however, we have successfully measured real-time cardiac output during treadmill exercise using a Doppler flow probe placed around the ascending aorta (Fig. 6). Fluorescent-labeled microspheres have been used to measure regional distribution of blood flow during exercise in the mouse (39), although these studies have to date been limited to measurement of relative changes rather than absolute blood flow. However, the combination of the two techniques, measurement of aortic blood flow with Doppler and regional blood flow with microspheres, should allow the calculation of absolute blood flows to various regional beds. If recent strides in miniaturizing physiological monitoring technology continue, then exercise testing will become a more routine part of physiological phenotyping of genetically altered murine models of human cardiovascular disease.

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