Selecting exercise regimens and strains to modify obesity and diabetes in rodents: an overview

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ABSTRACT

Exercise is part of a healthy lifestyle and frequently is an important component in combating chronic diseases, such as obesity and diabetes. Understanding the molecular events initiated by regular exercise is best studied in laboratory animals, with mice and rats being favoured for a number of reasons. However, the wide variety of rodent strains available for biomedical research often makes it challenging to select an animal strain suitable for studying specific disease outcomes. In the present review we focus on exercise as a management strategy for obesity and diabetes and we discuss: (i) exercise paradigms in humans shown to ameliorate signs and symptoms of obesity and diabetes; (ii) different rodent strains in terms of their advantages, disadvantages and limitations when using specific forms of exercise; (iii) the strengths and weaknesses of commonly used laboratory methods for rodent exercise; and (iv) the unintended consequences of exercise that are often manifested by increased hormonal and oxidative stress responses.

INTRODUCTION

Obesity and related metabolic diseases, such as T2D (Type 2 diabetes) have reached epidemic proportions across the globe. In North America, 55% of 97 million adults are either overweight or obese [with a BMI (body mass index) > 25]. It is estimated that nearly 246 million people worldwide and 27 million North Americans suffer from diabetes, with the majority of cases being T2D (according to the American and Canadian Diabetes Associations [1,2]). Increases in body weight largely result from an imbalance between energy intake and expenditure. Excess adipose tissue, in particular abdominal and visceral fat pads, is associated with the development of characteristic features of the metabolic syndrome including hypertension, insulin resistance, glucose intolerance and dyslipidaemia [3]. Prolonged exposure to these abnormalities leads to the development of atherosclerosis, diabetes, ischaemic heart disease and stroke. The advocacy of exercise as an interventional strategy against obesity and related metabolic diseases gains added importance from the realization that restriction of calories without exercise can lower resting metabolic rate and prevent weight loss [4].

Key words: cardiovascular system, diabetes, exercise, obesity, rodent, superoxide.

Abbreviations: ABP, arterial blood pressure; AMPK, AMP kinase; BMI, body mass index; GLUT, glucose transporter; HR, heart rate; PKB, protein kinase B; RER, respiratory exchange ratio; T1D, Type 1 diabetes; T2D, Type 2 diabetes; VO2max, maximal oxygen consumption; ZDF, Zucker diabetic fatty.

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EXERCISE: GENERAL CLASSIFICATIONS

According to the guidelines of the American College of Sports Medicine, exercise is defined as “Any and all activity involving generation of force by the activated muscle(s) that results in disruption of a homeostatic state” [5,6]. Exercise can be classified by the type, intensity and duration of activity [7]. Endurance exercise is characterized by prolonged and continuous periods of contractile activity (high repetition) against low resistance. Resistance exercise (also termed strength training) involves short periods of contractile activity (low repetition) against a high opposing resistance. Sprint exercise consists of short periods of maximal (intense) repetitive contractile activity with a low interval and against a low resistance, e.g. running an 100-m sprint race. However, sprint training can also be performed against high resistance resulting in a combination of resistance and endurance modalities, e.g. running with added weights.

Traditionally, attention has been focused on endurance training in the management of chronic metabolic diseases, such as diabetes and obesity, given the steady-state nature of endurance activities makes it easy to design well-controlled experiments. However, strength training has also been shown to be successful in obesity and diabetes management [8–14] and, as such, sprint training is rapidly gaining importance in the management of obesity/diabetes with the main advantages of having higher patient compliance and shorter protocols [15–18].

MOTOR UNIT RECRUITMENT WITH DIFFERENT FORMS OF EXERCISE

At the beginning of any exercise activity, the brain signals the muscle about the impending activity, following which specific fibres within a single muscle are recruited according to their relative size and ‘rate code’ [19]. Smaller motor units that have a lower activation threshold are recruited first. As force generation needs increase, larger units are recruited. Each motor unit functions within a specific range of firing frequencies that are described as either ‘slow’ (lower frequency) or ‘fast’ (higher frequency). As firing frequency increases, so does the force generated. Endurance training requires recruitment of both slow and fast fibres in an asynchronous manner with some fibres in the same muscle undergoing rest periods while others continue firing. In contrast, during sprint training, most of the fibres in a muscle are recruited synchronously to generate the explosive force needed to successfully complete the exercise. Resistance exercise improves recruitment of motor units and thus aids in the successful completion of both endurance and sprint training [20].

The most commonly used method to assess exercise capacity is derived from measuring VO$_{2\text{max}}$ (maximal oxygen consumption), which records the maximum volume of oxygen that is consumed by the body during intense exercise while breathing air at sea level [21]. Anaerobic threshold is the point at which anaerobic metabolism plays a dominant role in energy production and is estimated by using the RER (respiratory exchange ratio; the ratio of the net output of CO$_2$ to the simultaneous net uptake of O$_2$ [22]). For example, during low-intensity exercise for shorter durations, the RER is approx. 0.7 (denoting that fats are the primary energy source); however, during intense exercise, this value can exceed 1.0 because with rising metabolic demand aerobic metabolism shifts to anaerobic metabolism of carbohydrates, i.e. glycolysis [7]. Additional exhaled CO$_2$ is derived both from glycolysis and blood buffering of increasing lactate accumulation. Values of RER greater than one are an early indication that the subject is nearing exhaustion [23]. Other attributes of exercise that are manifested close to or at exhaustion includes a near-maximal HR (heart rate), hypercapnia, unsteady gait, facial flushing and sweating.

EXERCISE IN CHRONIC OBESITY/DIABETES

Insulin increases cellular glucose uptake by stimulating GLUTs (glucose transporters) to translocate from intracellular sites to the cell membrane, e.g. insulin stimulates GLUT4 translocation to the cell surface of cardiac, skeletal and adipose tissues [25]. After uptake by specific GLUTs, glucose is oxidized, via glycolysis, followed by entry into the tricarboxylic acid cycle; unused glucose is stored as glycogen via the action of glycogen synthase. During insulin resistance, there is reduced glucose uptake into peripheral adipose tissue and skeletal muscle, leading to excessive circulating glucose levels. To maintain normoglycaemia greater concentrations of insulin are required, which in insulin-resistant humans or animals causes the β-cells of the pancreas to hypersecrete insulin (hyperinsulinaemia) and normalize blood glucose, at least during the early stages. At the later stages of insulin resistance, pancreatic β-cell dysfunction occurs, leading to a loss of insulin release and the manifestation of overt T2D [26].

Both endurance and resistance training improves whole-body insulin sensitivity and glucose tolerance while also preventing or delaying the onset of T2D [27–29]. Insulin sensitivity is improved by increased GLUT4 and PKB (protein kinase B; a kinase involved in the insulin signalling pathway) content, as well as by increased glycogen synthase activity [11–14,30,31]. However, the causes of these benefits with resistance exercise remains unclear. Some studies suggest that the benefits of resistance exercise are largely due to muscular...
hypertrophy and/or loss of fat mass [8–10]; however, other studies report that such effects are independent of total fat content in the body [32,33].

Sprint training also leads to significant decreases in blood glucose concentrations. Glucose transport into skeletal muscle is increased through translocation of GLUT4 to the cell surface after an acute bout of exercise [34–36]. In T2D patients, exercise on a bicycle ergometer for 45 min at 70 % maximal workload reduces blood glucose (from 7.6 mM to 4.8 mM) [37]. Even in young patients with T1D (Type 1 diabetes), sprint training improves skeletal muscle metabolism [38]. However, the positive effects of sprint training and their equivalency to the more established forms of endurance exercise are, so far, limited to observations made in healthy young adults, who are better suited to undertake intense bouts of exercise [39].

From an interventional point of view, there are advantages of resistance and sprint training over endurance training. Unlike most T2D patients, euglycaemia in T1D is maintained by exogenous insulin, where the dose is adjusted according to diet and normal physical activity. Thus increased uptake of glucose in skeletal muscles during physical activity, without any corresponding reduction in insulin doses, increases the risk of hypoglycaemia [40,41]. Sprint exercise for shorter durations may be advisable when advising T1D patients on exercise regimens, given that sprint exercise would probably result in a lower risk of hypoglycaemia than endurance training [42–44]. On the other hand, during obesity and T2D, the benefits obtained with resistance exercise are often achieved with low-to-moderate intensity exercise, a setting better suited to obese pre-diabetic subjects who have difficulty in engaging in high-intensity endurance or sprint exercise regimens [45]. Recent studies suggest that high-intensity sprint and/or resistance training can lower adipose tissue mass and improve cardiovascular function in a manner that is comparable with that achieved by prolonged endurance training protocols [46–48]. In addition, gains in strength achieved from resistance exercise can improve endurance training performance in the elderly [49,50]. Sprint training involving intense short workouts is usually better suited to busy lifestyles; it incorporates some measures of endurance and resistance training and thus enjoins the beneficial effects of both aerobic and strength exercise routines.

**WHY STUDY EXERCISE IN ANIMALS?**

The causation of obesity in humans, as with other polygenic disorders, is complex and includes many variables that influence energy intake and expenditure [51]. In humans, these include, but are not limited to, the effects of genetic predisposition to hyperphagia and fat storage [52,53], substrate oxidation capabilities [54], integrity of the mitochondria [54,55], metabolic programming in *utero* [56], early childhood over-nutrition [57] and emotional stress [58]. The isolation of a causal relationship between some of these factors, e.g. emotional stress, can only be studied in humans. Moreover, it is challenging to undertake long-term randomized controlled exercise trials in humans due to issues related to subject compliance and the fact that observational population-based follow-ups may include genetic bias among individuals [59]. Additionally, metabolic diseases often develop over long periods (years) and, likewise, require lengthy periods of intervention. Another limitation is that studying organ-specific changes following interventions often requires invasive procedures or organ isolation, procedures that are best undertaken in a laboratory setting.

A pioneering study using targeted phenotypic selection of rats with low (14 min) and high (43 min) running capacity (from an original population with a maximum running capacity of 23 min) led to the identification of metabolic risk factors such as increased body weight, raised blood pressure, endothelial dysfunction, higher circulating triacylglycerols (triglycerides), insulin resistance and accumulation of non-esterified (‘free’) fatty acids in the low-capacity runners [60]. These results imply that, in the absence of any major genetic or dietary variations, the emergence of metabolic risk factors is directly dependent on the extent of physical activity. Of course, metabolic diseases in laboratory animals do not always mimic disease pathogenesis in humans, and clearly there are some limitations in results from laboratory animals. Importantly, differences in exercise protocols and strains of rats and mice can profoundly alter the outcome and interpretation of these studies. In the next section, we discuss different rodent strains and their specific advantages and disadvantages for exercise paradigms with an emphasis on obesity/diabetes research. There are complementary reviews related to research findings on laboratory animal models in diabetes and obesity [61–66].

**LABORATORY RODENTS: MODELS OF CHOICE FOR BIOMEDICAL RESEARCH ON EXERCISE**

Rodents remain popular for studying exercise physiology in laboratory settings for a number of reasons: their relatively small size allows for reduced animal housing costs and easier handling; rodents also have high fertility rates, short gestational times and mature fairly rapidly, features that increase their appeal for studying exercise physiology; and importantly, the easy availability of inbred strains of rats and mice makes them useful due to their genetic uniformity and predictable characteristics.
An inbred strain is derived from brother–sister mating for at least 20 generations, by which time 97.5% of their genetic loci are homozygous [67]. Although animals within a strain have virtually identical genetic traits, a comparison across inbred strains reveals a striking variation in many of the physiological and psychological variables, including several cardiovascular parameters [68–71], as shown in Figure 1, which summarizes the results reported by Desai et al. [72] on the cardiovascular indices in six strains of adult mice (8–12 weeks old; average weight 26.3 ± 0.4 g) at rest and during exercise. As is evident from those results, the HRs and ABP (arterial blood pressure) in response to submaximal exercise (15 m/min at a 10° inclination) varies significantly across mouse strains, even under similar workloads. CD-1 mice had a significantly higher basal HR than C3H and 129sv mice, whereas C3H mice had significantly lower ABP than FVB, SW and 129sv mice (Figure 1). It is important to note that the ABP values at submaximal exercise did not increase significantly in most mice strains. Of importance, the ABP response of mice during exercise is different from that in humans; in mice, the mean ABP increases with the onset of exercise but then plateaus despite further increases in workload [72], whereas, in humans, there is a linear increase in ABP with incremental exercise. Other important differences between different rodent strains that influence exercise outcomes includes learning behaviour [73], cardiac output [74], circadian rhythms [75], locomotor activity [76], the levels of blood glucose, circulating catecholamines [77] and haematocrit [78], the response to handling (fear response) [79] and avoidance.

Beyond these biological variables of efficiency and endurance, psychological factors, such as the desire to run and the perception of pain, also exist [82]. Morphological variables such as the presence of fast-twitch muscle fibres in the gastrocnemius and soleus muscles in different strains also influences wheel-running activity in rats [81].

**RODENT MODELS FOR OBESITY AND DIABETES: STRAIN DIFFERENCES**

Strain-related differences exist in commonly used laboratory mouse strains in terms of their sensitivities to glucose, diet and insulin [82]. Insulin secretion in response to an intravenous bolus of glucose in anaesthetized C57BL/6, DBA/2 and 129T2 mice fed on a high-fat diet for 6 weeks causes the poorest response in 129T2 mice, which was also accompanied by poor spontaneous or voluntary activity [82,83]. However, a key advantage of rodent models in obesity and diabetes research stems from the fact they possess several gene defects that are also present in humans [84]. In 1961, a spontaneous mutation that decreases the affinity of leptin for its receptor gave rise to the Zucker fatty (or fa/fa) rat model of obesity and insulin resistance [85,86] and, in 1966, inbreeding of fa/fa rats with diabetic traits gave rise to the ZDF (Zucker diabetic fatty) rats. As leptin effects in the central nervous system are responsible for satiety and eating behaviour, fa/fa rats become hyperphagic, which leads to obesity and insulin resistance with ZDF rats also demonstrating hyperglycaemia. In mice, a similar mutation, leading to a complete deficiency in leptin for its receptor gave rise to the Lepob (also known as ob) model of obesity in C57BL/6j mice [87]. Thus ob/ob mice have severe early-onset obesity, hyperphagia, hyperinsulinaemia and insulin resistance with modest hyperglycaemia. Although leptin deficiency causes the major phenotypic features of ob/ob mice, studies with ob/ob mice with different genetic backgrounds demonstrate the importance of the strain-related modifier genes [88], making it important to recognize strain differences when selecting animal models for obesity/diabetes-related research. Congenic ob/ob mice with the BLKS background are obese, but with increasing age they lose weight and die prematurely due to severe β-cell failure [89,90]. By comparison, ob/ob mice with the Balb/cJ genetic background have severe diabetes, but with only modest increases in white adipose tissue, and demonstrate an improved tolerance to cold and have improved insulin secretion over their lifetimes [91]. Besides strain-related variables, the decrease in adiposity, tolerance to cold and improved β-cell function in the Balb/cJ mice can be explained...
Treadmill performance in different mice strains

Common mouse strains listed in descending order of performance during treadmill exercise. Lerman et al. [80] used a 30 min treadmill run at 20 m/min, whereas Lightfoot et al. [217] used maximal treadmill running in mice until exhaustion with speeds up to 42 m/min. The order of performance from the study by Massett and Berk [68] is from untrained animals at baseline and the effect of training in these mice is depicted in Figure 2.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Order of performance</th>
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<tr>
<td>Lerman et al. [80]</td>
<td>FVB/NJ &gt; SW-&gt; DBA/2J &gt; Balb/c &gt; DBA/2J &gt; C57Bl/6J</td>
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<tr>
<td>Lightfoot et al. [217]</td>
<td>Balb/c &gt; SW/J &gt; CBA/J &gt; C57L/J &gt; C57BL/6J/Fj &gt; C57Bl/6J &gt; AKR/J &gt; DBA/2J &gt; A/J</td>
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<tr>
<td>Massett et al. [68]</td>
<td>FVB/NJ &gt; Balb/c &gt; C57Bl/6J</td>
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by the levels of spontaneous physical activity present in these two strains; between 6 and 10 weeks of age Balb/cj mice are approximately twice as spontaneously active as C57BL/6J mice in standard mouse cages (see http://phenome.jax.org/pub-cgi/phenome/mpdcgi?rtn=projects/details&sym=Mogil2). The spontaneously active nature of Balb/cj mice also makes them more suitable for forced exercise paradigms such as treadmill running, as shown in at least three studies (Table 1).

Table 1  Treadmill performance in different mice strains

Much like ZDF rats, which demonstrate spontaneous hyperglycaemia, the autosomal recessive diabetes (db) mutation of the leptin receptor (Lep<sup>db</sup>) in mice was discovered in 1966 at the Jackson Laboratories in an inbred C57BLKS/J strain. If homozygous for this mutation, mice become obese at 3–4 weeks, which is followed by frank diabetes within 2 months of age. However, the causative factors behind hyperglycaemia and its extent are also influenced by background strain.

On the original C57BLKS background, there is an uncontrolled rise in blood sugar due to a severe loss of β-cells in the pancreas, with death by 10 months. However, on C57BL/6 and FVB backgrounds, there is compensatory hyperplasia of the β-cells, and hyperinsulinaemia continues for its 18–20-month lifespan, despite ongoing diabetes (see http://jaxmice.jax.org/strain/000697.html). Thus, in the latter strains, diabetes is not due to hypoinsulinaemia but a lack of glucose uptake that results from insulin resistance. Therefore, when conducting experiments on exercise-induced interventions in insulin sensitivity, the latter strains are more desirable due to their ability to maintain insulin levels over the period of hyperglycaemia.

**COMMON EXERCISE REGIMENS USED IN ANIMAL MODELS OF OBESITY AND DIABETES**

**Treadmill running**

Treadmill exercise is considered the gold standard for exercise stress tests in humans and other mammals on the basis of the sheer volume of successful outcomes that have been reported. In treadmill protocols, both the duration and the intensity of exercise can be manipulated, and mice can be made to exercise at either submaximal or maximal workloads, thus allowing for the application of uniform exercise workloads for all experimental groups. Measuring individual running speeds of mice on a treadmill provides important information on training intensity without the need to measure VO<sub>2max</sub> as there is a correlation between VO<sub>2max</sub> and running speed in mice [94]. The disadvantages of treadmill exercise are frequent animal handling, the difficulty in motivating mice to exercise and constant vigilance by the investigator to ensure that the animals run for the entire duration of the exercise bout. Many treadmill exercise machines expose rodents to either a metal shock grid, forced air or cold-water jets, which aggravates an already stressful experience in these animals [95–97]. Several studies have examined strain-related differences in treadmill exercise, and three such studies are listed in Table 1. A consistent finding among these studies is that C57BL/6J mice are poor performers on a treadmill, with Lerman et al. [80] reporting that C57BL/6J mice have a maximal running capacity of 21 m/s over 30 min in a treadmill exercise protocol.

These findings raise concerns when studying the effects of exercise in diabetes/obesity research. Of the nearly 2000 genetically modified inbred mouse strains available from laboratory animal suppliers such as Jackson Laboratories, nearly 60% (including the db/db and ob/ob mouse, the most popular models for studying T2D and obesity respectively) are bred on a C57BL/6J background [98]. Many studies examining the effects of exercise in both ob/ob and db/db mice often utilize a 1 h of forced exercise at speeds of 20–23 m/s. Thus such protocols may actually exceed the ability of these mice to sustain training efforts on a regular basis and therefore probably induce stress responses. Recent studies demonstrate that treadmill exercise, even at 15 m/min for 30 min for 12 weeks is unable to improve blood glucose, body weight or cardiac oxytocin and natriuretic peptide systems, which are protective against diabetic cardiomyopathy, in db/db mice [99,100] and may even be related to elevated plasma markers of stress, such as cortisol levels [99]. Increased glucocorticoids induce insulin resistance in skeletal muscles and elevate blood glucose (see the Hormonal stress section below). However, we reported that exercising db/db mice made to run only 5.2 m/min for 60 min (treadmill exercise) prevented diabetes-related dysfunction in several vascular beds, such as coronary arteries, aorta and kidney [101–103]. It is of interest that these findings show that low levels of physical activity did not change either circulating glucose or insulin levels and caused a weight loss of less than 10%, but still attenuated many of the vascular abnormalities associated with hyperglycaemia [101–103].
Similar to findings made in leptin-resistant obese or diabetic mice, the beneficial effects of exercise have been also been demonstrated in fa/−fa rats. Cardiac insulin resistance in fa/−fa rats was reversed with chronic exercise training for 4 weeks due to increased cardiac GLUT4 expression and PKB phosphorylation [104]. Acute bouts of sprint training (60 min of treadmill running at 25 m/min at a 5% incline) increased metabolic activity in Zucker gastrocnemius muscles, whereas an 8-week exercise regimen prevented the development of diabetes altogether in ZDF rats [105]. In contrast with fa/−fa and ZDF rats, which are characterized by mutations in the leptin receptor, the cp (corpulent) mutation in rats is characterized by a complete loss of leptin receptor in homozygous cp/cp rats [106], such as in the JCR:LA-cp rat strain [107]. Just as in fa/−fa rats, homozygous cp/cp rats develop hyperphagia, obesity, hyperinsulinemia and dyslipidaemia [107]. Exercising JCR:LA-cp rats for 40 days on a voluntary wheel (average running 8 km/day) normalized body weight and plasma glucose to the levels seen in control rats.

Just as in mice, there are also considerable strain-dependent variations in exercise ability in rats. For example, Britton and colleagues compared 11 inbred strains of male rats for endurance and ranked their performance accordingly as DA>PVG>SR>AUG>ACI>LEW>WKY>F344>MNS>COP [70]; the DA strain of rats displayed the highest levels of performance, whereas the COP rat had the poorest performance. A significantly greater cardiac output in DA rats compared with COP rats was also demonstrated [70]. Later studies identified other factors, such as increased sympathetic and reduced parasympathetic tone at rest, a faster HR and increase in blood pressure during exercise, as well as differences in cardiac gene expression in DA rats [108,109]. Differences in endurance activity lead to a reduction in baseline abdominal fat in retroperitoneal, subcutaneous and visceral fat pads in DA rats compared with COP rats. Creating a hybrid from COP and DA strains led to the development of a phenotype with characteristics between pure COP and DA strains [110]. This led to the conclusion that baseline differences in performance and adiposity are due to genetic influences inherent to the strain. Thus using diverse strains in obesity/diabetes research would probably lead to outcomes that are largely dependent on background strain differences in both mice and rats.

Some factors affecting treadmill performance and outcome in obesity and diabetes

Age

The age of rodents is an important variable in determining the success of laboratory exercise protocols. Untrained C57BL/6j mice reach \( \dot{V}O_2 \) max during treadmill exercise when they perform at workloads of 25 m/min (for adults) and 20 m/min (for aged mice). Further increases in running speeds decreases \( \dot{V}O_2 \) in adult mice, but results in a refusal to run in aged mice [111]. Older mice have a much reduced aerobic capacity and increased fatigability in endurance tests [112,113].

Age-dependent outcomes of exercise are also evident in obese or diabetic mice, e.g. post-weaning access to running wheels prevents the development of obesity in pups fed a high-fat diet, an effect that is maintained for up to 10 weeks [114]. Such long-term effects of exercise do not occur in adult mice [115], suggesting that early-onset exercise may affect brain development in terms of energy regulation and metabolism. However, it should be stressed that exercise reduces metabolic risk factors in all ages of rats and mice [116–118].

Training

The effect of prior training can significantly alter exercise performance, an effect that can also be strain-dependent. Massett et al. [68] examined pre-training and post-training exercise capacities in three commonly used
laboratory mouse strains and demonstrated significant variance in training effects (Figure 2). The exercise regimen consisted of 4 weeks of treadmill running for 5 days/week for 60 min/day at a final intensity equivalent to approx. 60% of the maximal workload. The FVB strain of mice by far exceeded both Balb/cj and C57/BL6 mice in terms of a training effect on endurance performance; these results are in agreement with those presented in Table 1 and suggest that FVB is a very active mouse strain, whereas C57/BL6 may well be the least active, even after training. It is tempting to speculate that such increases in activity post-training may be linked to up-regulation of the metabolic capacity in FVB mice, but not in C57/BL6 mice. This assumption gains strength from studies demonstrating that the FVB strain is more resistant to obesity than C57/Bl6 mice even under similar high-fat-feeding protocols [119,120]. The mechanisms underlying increased treadmill performance, as well as resistance to obesity in FVB mice may be associated with changes in AMPK (AMP kinase) activity, a mediator of insulin signalling. In the FVB strain of mice, AMPK activity is directly controlled by the actions of leptin [121]. Perturbation of such signalling, upon superimposition of the ob mutation, worsens insulin resistance and diabetes in the FVB compared with the C57/BL6 mice [88], resulting in significantly greater insulin resistance and postprandial triacylglycerol clearance in the FVB strain. Such augmented activity is not limited to metabolism, but can also extend to enhanced aggressiveness, with C57/BL6 mice being more timid [122] and increased requirements for hypnotic drugs for anaesthesia in FVB mice [123].

**Ambient temperature**

Hyperthermia decreases exercise tolerability in many mammalian species, including rodents. Rats with body temperatures kept at approx. 40°C ran for only half the duration compared with rats with body temperatures clamped below 38°C [124]. Deterioration in endurance performance under such conditions is due to the achievement of a critical internal temperature. Human volunteers in the laboratory also become exhausted and stop exercising when their core temperatures approached 39.5°C [125]. Fuller et al. [126] evaluated the effect of a hot environment on voluntarily exercise in three groups of rats. The first group was rested at 23°C and exercised at 33°C, the second group was rested at 23°C and exercised at 38°C, and the third group was rested at 38°C and exercised at 38°C. Running time to fatigue was 29.4 ± 5.9, 22.1 ± 3.7 and 14.3 ± 2.9 min (means ± S.D.) for the three trials respectively [126]. Consistent with these findings, Kim et al. [127] also reported on the effects of a single bout of submaximal exercise on a treadmill at varying ambient temperatures (11°C, 23°C and 44°C). The running times were 102.0 ± 39.5 min in the control group (23°C), 44.1 ± 18.0 min in the hot-exercise group (44°C) and 55.4 ± 11.9 min in the cool-exercise group (11°C). Moreover, ambient temperature may also affect metabolism, growth and development, food and water consumption, immune responses and cardiovascular function, all of which can indirectly affect aerobic capacity and metabolic disease outcomes in rodents [128]. The temperature of animal housing facilities also influences aerobic exercise capacity. When placed in a temperature gradient, permitting selection from a wide range of ambient temperatures, individual mice prefer temperatures near 30–31°C [129]. Animal housing facilities and testing facilities are generally maintained at a much cooler temperature of near 22°C. However, mice are often housed in groups of three or more where they huddle and maintain close body contact and core internal temperature.

**Socialization**

Rats are gregarious animals that in the wild live in social groups. Several decades ago, individual housing of laboratory rats was shown to have an impact upon their health and behaviour [130,131]. Since then, individual housing of rats has been shown to affect blood pressure and HR [132], circadian rhythms [133], body weight and lifespan [134,135], and their immune systems [136]. Likewise, mice are also social creatures and the choice of housing, either singly or in groups, will also alter their physiology and behaviour and thus affect their ability to perform exercise [129]. In one study, female CD-1 mice housed either individually or in groups of five demonstrated stark variations in 24-h motor activities. When housed individually, young (2 month) and aged (11 month) mice demonstrated motor activity rates of 15.7 ± 1.9 and 9.7 ± 1.4 units of activity. This level of activity increased sharply when mice were housed in groups of five, with mean activity rates reaching 36.6 ± 1.2 and 23.8 ± 1 in young and the aged groups respectively [129]. It is possible that individually housed mice have increased HRs but not an increased activity level, possibly due increased release of catecholamines and stress hormones, factors known to have an impact upon exercise performance [137]. Thus maintaining a comfortable ambient temperature and encouraging social engagement when housing mice or rats is desirable during metabolic experiments.

**Circadian rhythms**

It is important to remember that unlike humans, most rodents are nocturnal animals and thereby have heightened activities during the dark when they tend to be more naturally active, inquisitive and more responsive. Figure 3 shows the circadian rhythms of wild-type C57/Bl6 and diabetic db/db mice, which are derived from the same mouse strain. By far, nocturnal activity, as measured by distance ran in a voluntary exercise
Six wild-type C57/Bl6 mice (upper panel) and six db/db mice (lower panel; mice are derived from a C57/Bl6 background) were given free access to a voluntary running wheel for 9 days (total 204 h). The duration of daytime activity (grey) is much lower in every mouse than activity during the nocturnal period (black); each bar represents the total daily activity achieved by each mouse (M1 to M6). Significant intra-group variations were also observed among mice from both the wild-type and diabetic mice groups. The results represent the means ± S.D. in activity from each mouse in each period per day, as recorded over 9 days. The average activity calculated was significantly different in at least three mice (50%) within either group. db/db mice demonstrated greater variability, and were much less active (by approx. 25%) than wild-type mice.

Voluntary wheel running

Although treadmill exercise generally measures maximal exercise capacity [141], voluntary exercise is analogous to the average level of physical activity normally undertaken [142]. This has led to the preference by some investigators for voluntary wheel running systems for exercising laboratory rodents. This is especially true for metabolic research where this will likely reduce stress levels, which in rodents can offset many of the benefits of exercise. Wheel running provides a stable, reliable marker of circadian activities (i.e. in the dark cycle) under constant environmental conditions, with reduced animal handling and stress-inducing exercise motivators [13]. This form of exercise in normal rats does not lead to hypertrophy of the adrenal gland or increase cardiac catecholamine levels, indicating reduced levels of stress compared with swimming or treadmill exercise protocols [143]. During voluntary wheel running exercises, animals are able to self-select the time, duration and intensity of exercise. However, it involves a higher cost for the researcher as these rodents are often singly caged, with access to a running wheel for each animal for accurate quantification of aerobic activity. Most of the commercially available voluntary exercise equipment allows for automated computer recording of several running parameters including distance, speed and total running duration at various known time periods [144]. The equipment also often enables the researcher to automatically ‘lock’ the wheels at a particular speed and distance to maintain uniformity [145]. An important advantage of voluntary exercise is that there is no need for continuous monitoring, unlike the continued investigator presence required during treadmill or swimming protocols; this makes voluntary exercise ideal for conducting long-term intervention studies of obesity and diabetes. In addition, mice (and other mammals) exhibit an intermittent,
Exercise regimens and strains modifying obesity and diabetes in rodents

Figure 4 Voluntary running distance and time spent on exercise wheels in common strains of mice

The duration of voluntary running (min; upper panel) and the distance run (km; lower panel) are shown. Five-to-six-month-old mice were housed individually in lower standard cages (47 cm × 26 cm × 14.5 cm) containing a running wheel and the activities were recorded over a 2-week period. Results are modified from Lerman et al. [80].

'stop-and-go' locomotion, and intermittency of locomotion may improve wheel running performance and so lead to higher endurance levels [146]. The voluntary activity of several commonly used strains, as estimated by Lerman et al. [80], are shown in Figure 4. It is interesting to note that although C57/Bl6 mice are poor performers on treadmill exercises they perform much better on voluntary wheels in this and other studies [80,82].

Wheel running, much like treadmill running, can be affected by the gender and age of mice. Female mice perform better in wheel running exercise both in terms of distance and duration compared with their male counterparts [147]. It is likely that oestrogen could play a role in this improved performance as ovarioctomized female rats demonstrate blunted voluntary exercise responses [96]. Moreover, the loss of body weight with exercise in mice is more consistent in males than in females [148]. As can be expected, older mice tend to be less active [147], but this can also vary across different inbred species of mice [149]. Some mice strains do not show an age-related decline in running activity, whereas other strains, e.g. SWR/J, demonstrate increased activity levels with age [150].

Forced and voluntary exercise often produces different outcomes in mice, possibly due to differences in exercise patterns and stress levels in mice. Voluntary exercise in mice often involves mice running at high speeds for brief periods (sprint-like activity), whereas treadmill running requires sustained work patterns over longer periods (endurance training). A recent study reported that voluntary exercise, but not forced exercise, improves blood glucose levels and decreases body weight in $db/db$ mice, a frequently used animal model of T2D that is derived from a C57/BL6J background [99]. In that study, even though mice ran a shorter distance in the voluntary regimen compared with the forced exercise regimen (a total of 4.2 km compared with 11.8 km over 12 weeks), it led to lowering of body weight and amelioration of plasma glucose, which may underscore the importance of these very different types of training modes in mice regarding the experimental outcome. On the other hand, as with voluntary exercise, animals are often singly caged to record locomotor activity from each animal, and as described above this lack of socialization can lead to decreased activity in rodents.

Some have argued that treadmill running, lasting for restricted periods of time, mimics human exercise better than voluntary wheel running does [151]. However, rodents naturally exhibit intermittent locomotion [152], so that sustained running may be ‘un-natural’ and can induce stress responses [153]. Thus it is perhaps better stated that treadmill running mimics only those humans who are highly motivated to engage in frequent sustained physical activity (such as endurance athletes), instead of brief periods of everyday activity normally undertaken by most people [145].

Swimming

Swimming has a number of advantages compared with other types of exercise modalities: the procedure is relatively simple and requires inexpensive equipment with no animal training required as typical laboratory rodents have a natural swimming ability. Rats can swim continuously or stay afloat in calm water for as long as 2.5 days [154]. During the initial moments after water immersion, rats create a lot of turbulence, thus trapping air bubbles in their fur and increasing their buoyancy [155]. Adding a small amount of detergent to the animal’s fur, shaving the animals or agitating the water continuously makes floating difficult for the rat and so ensures proper exercise. Swimming behaviours are affected by diverse factors, such as the diving reflex, mental stress and episodes of hypoxia associated with diving [141]. Importantly, continuous investigator presence is required as is a high-degree of vigilance in order to prevent drowning. To ensure reduction of cold-induced stress, rats can be dried with a towel and placed under a heat lamp for 30 min after each training session [156].

Three laboratory procedures for rodent swimming tests have been described: (i) swimming to exhaustion (the most common procedure); (ii) speed swimming in
a water channel; and (iii) maze swimming. Swimming tests require the animal be placed into a tank of water and allowed to swim until they sink and are unable to return to the surface [157]. Different strains of mice have been used in swimming studies but there appears to be no systematic inter-strain comparison; however, it seems that in all cases, mice can tolerate up to 3 h of continuous swimming without much difficulty.

Effect of age, gender and strain

The relationship between gender and age on swimming performance in mice is not well-established. Some studies report that young mice perform better in maze swimming tests [157], whereas older mice swim for shorter periods, possibly because of their reduced physical endurance and increased muscle fatigue [112]. Female rats appear to perform better than males in both speed swimming and exhaustion tests [158]. The importance of strain related differences on the performance of various mouse strains in swimming exercise protocols has not been reported.

Other environmental factors

Clearly, water temperature can directly affect the swimming behaviour of mice. Typically mice show improved performance when the water temperature is between 32 and 36 °C, which is slightly below their normal core body temperatures [157]. Temperatures above or below this range markedly reduce the duration of swimming activities in rodents. Animals demonstrating stress during repeated swimming bouts can also lead to hormonal stress responses (see the section on Hormonal stress below).

Using swimming as a means of exercise training has largely been superseded by running exercises due to the difficulty in measuring cardiovascular parameters, quantifying exercise intensity and the lack of graded workload protocols [141]. However, some studies have utilized acute or chronic swimming bouts in obesity/diabetes research [159,160].

Resistance and sprint exercise

Most studies of lifestyle modifications, such as exercise in animal models of obesity and diabetes intervention, have utilized endurance-type training. However, some, but not all [161], animal studies demonstrate similar effects on insulin sensitivity and body weight when the effects of sprint and resistance training are compared [162–164]. An acute bout of sprint-type swimming in healthy Wistar rats, consisting of eight 20-s periods with an added weight equal to 18% of their respective body weight, increases skeletal muscle glucose uptake; these effects are similar to results obtained in rats that underwent 3 h of continuous swimming without any added weights [163,164]. When a similar intermittent high-intensity swimming exercise was continued for eight consecutive days, there was an increase in the GLUT4 content in skeletal muscle [165]. Thus it seems likely that sprint training against resistance, e.g. running on a treadmill with a higher inclination or with added weights, can increase insulin sensitivity in the skeletal muscle and may benefit obese individuals who are at an increased risk of developing metabolic syndrome. Furthermore, male Sprague–Dawley rats subjected to 10 days of intense intermittent swimming bouts (14 repeats of 20-s high-intensity swimming with an added load of 14–16% body weight) develop increases in skeletal muscle fatty acid oxidation enzymes and citrate synthase activity [166], which are similar to effects observed when rats are exercised for 5 days with 6-h prolonged slow swimming activities. Both endurance and sprint training stimulate insulin-induced glycogen synthesis in the skeletal muscle in rats [167].

Resistance exercise training in rodents is frequently used to study regenerative phenomena in the context of the response to injury or paralysis, with an end goal of inducing muscle hypertrophy. The use of a loaded running wheel [168] is similar to that of a free spinning wheel but with known resistances that can be graded during the experimental protocol. In this method, although the pattern of activity differs, progressive resistance loading does not affect the total running distance or duration, in comparison with free spinning wheels [169]. Using loaded running wheels leads to enhanced muscle hypertrophy [170]. Electrical stimulation performed in an unconscious animal can also simulate the effects of exercise in the stimulated muscle. With this method, involuntary muscle contractions are evoked by electrical stimulation, which has been shown to induce gastrocnemius muscle hypertrophy and increased protein content after 16 weeks [171]. Implantation of electrodes at the sciatic nerve can directly isolate the effect of muscular contractions without the confounding effects of the metabolic changes that occur during with normal exercise [172]. As the muscles are unilaterally activated, one advantage of this method is that the contralateral leg acts as an unstimulated control in the same animal. The use of the above exercise paradigms, such as loaded running wheels or electrical stimulation of isolated muscle bundles, remains largely unexplored in laboratory animal models of diabetes/obesity.

HORMONAL STRESS IN EXERCISE

Exercise usually leads to the activation of the sympathetic nervous system [173], the hypothalamic–pituitary–adrenal axis, and the associated ‘flight-or-fight’ responses [174]. These adaptive changes in homoeostatic regulation lead to increased blood pressure, diversion of blood to activated muscles, reduced blood flow to the gastrointestinal tract and increased modulation of immune responses. The adrenal medulla is stimulated
via sympathetic nerves from the celiac ganglion and, in humans, injection of a local anaesthetic around the celiac ganglion blocks exercise-induced increases in plasma adrenaline by up to 90% [175]. Repeated stimulation of adrenaline secretion in humans undergoing training can lead to ‘sports adrenal medulla’ [176]. Endurance athletes exhibit higher baseline adrenaline levels than sedentary individuals [177–179]. Sprint bicycling for 3–4 months also leads to similar outcomes [180]. In animals, chronic stimulation of this pathway can adversely affect the well-being of the animal and can confound outcome variables of diabetes and obesity [153]. In Wistar rats, 10 weeks of a swimming regimen induced heavier adrenal glands, higher catecholamine content in the glands and higher adrenal medulla volumes in both sexes [156], as depicted in Figure 5. Continuous stimulation of the adrenal gland leads to the release of glucocorticoids, such as corticosterone. Excess glucocorticoid impairs insulin sensitivity, contributing to generation of the metabolic syndrome, including obesity and hypertension [181,182]. Glucocorticoids also induce hyperphagia [183] and have been reported to reduce peripheral glucose uptake in skeletal muscle and adipose tissue [184]. Glucocorticoid responses to forced exercise (of 16.6 m/min) in laboratory house mice (Mus domesticus) were evaluated by Coleman et al. [185], who reported that resting average serum corticosterone levels of between 11.6 and 29.5 ng/ml were increased to 621 ng/ml within 25 min of treadmill running in females and to 332 ng/ml within 40 min in males. Not only does prolonged exercise elevate plasma markers of stress hormones, but even short-term treadmill running for 10 days can elevate corticosterone levels in rats [186]. Such stress can, over time, manifest as ulcers [187], suppression of the immune system [188], neural damage [189] and the stiffening of blood vessels [190]. At a cellular level, such changes are accompanied by elevated levels of catecholamines [191] and cortisol [192], alterations in levels of IL-6 (interleukin-6) [193,194] and Hsps (heat-shock proteins) [195,196], and the activity of lymphocytes [153,197].

**OXIDATIVE STRESS IN EXERCISE**

Free radicals cause widespread damage following excessive levels of exercise [198,199]. Free radicals, which are a subset of ROS (reactive oxygen species), are physiological products of aerobic metabolism [198]. The free radical content is increased in limb muscles stimulated by a single series of repetitive contractions [200], as well as in rat skeletal muscles after intense exhaustive running [201]. However, a mild oxidative stress can also act as a ‘stimulant’ of physiological antioxidant systems and as a trigger for various physiological adaptations [202]. This has led to our current understanding of the free-radical-mediated effects of exercise as a phenomenon of ‘hormesis’ [203], according to which there may be a bell-shaped curve of oxidative stress in response to exercise, with none and excessive exercise being considered harmful and moderate levels being of most beneficial [204,205]. In a series of recent studies, our laboratory described the beneficial effects of moderate levels of treadmill exercise in db/db mice, where multiple organs demonstrated up-regulation of antioxidant systems [101–103]. As a further testament to the hormesis theory, it has been demonstrated by others that administration of antioxidants, such as resveratrol to ob/ob mice [206], as well as vitamin E and vitamin C [207] to humans, can reduce the beneficial effects of exercise in attenuating metabolic risk factors. These results point towards mild oxidative stress as inducers of protective response against chronic metabolic diseases.

**LIMITATIONS OF LABORATORY RODENTS FOR OBESITY/DIABETES RESEARCH**

Genetically uniform, inbred strains of laboratory mice and rats do not always accurately represent the diverse nature of humans. Several studies have noted that, unlike most humans, body weight changes in some strains of mice are unrelated to the distance run or average velocity of exercise over chronic study periods [150,208], implying a lack of exercise-induced responses towards reduction of body weight. Others report no differences in the body weights of sedentary control mice and physically activity mice [209]. Moreover, there are
considerable inter-individual variations within the same groups of animals when commonly used mouse models of obesity and diabetes are compared (Figure 3). This poorly defined relationship between exercise and body weight may result from the long-term domestication of these mice, which may have inadvertently led to a dissociation of aerobic activity from the control of body weight in some instances. It can be argued that domestication and subsequent adaptation to a human-defined environment may have induced genetic changes over multiple generations due to the environmentally induced developmental events re-occurring during each generation [210]. For instance, compared with a more natural environment, animals housed in well-defined laboratory conditions, with a constant supply of food and water and the absence of natural predators, may actually limit self-selection for high-grade locomotor performers [210]. Domestication may, therefore, have resulted in subtle but significant genetic changes compared with their wild counterparts [211] in turn leading to a more relaxed selection for locomotor and exercise excellence and producing lowered exercise abilities in laboratory mice [212]. The ancestors of many laboratory Mus strains (e.g. Swiss Webster) were derived from wild European stocks of M. domesticus approx. 100 years ago [213,214]. Assuming that 3–4 generations are bred per year, laboratory mice have then possibly been under domestication for nearly 300–400 generations, thus providing ample opportunity for a relaxed genetic selection for exercise performance.

Following similar arguments, van den Brandt et al. [215] compared the metabolic traits of inbred rat strains (DA, BN, LEW, WKY and F344) with wild rats (Rattus norvegicus). Compared with wild rats, serum cholesterol values were significantly increased in all inbred rats. Body weight and BMI were used as estimates of body fat in all strains and were significantly higher than in wild rats (except in the DA strain). In addition, serum insulin and leptin levels were substantially elevated in three (F344, LEW and WKY) of the five inbred rat strains used in the study. Thus with phenotypes in control inbred rat strains demonstrating a ‘metabolic syndrome-like’ tendency, it is unclear which of the inbred rat strains best represents a ‘healthy’ control rat strain for metabolic research.

**CONCLUSIONS**

Exercise is frequently used as a tool to modify disease progression, lifespan and the morphological and physiological responses in both humans and rodent models of diabetes and obesity. Correct design of an exercise protocol is imperative in achieving desired outcomes in exercise-based laboratory research using rodents [216]. A useful resource to guide animal experimentation protocols on laboratory exercise is provided on by the American Physiological Society handbook (http://www.the-aps.org/pa/action/exercise/book.pdf). The available results using laboratory rodents suggests that selection of the appropriate mouse strains is an important criterion that may also determine the outcome of laboratory research in obesity and diabetes. It is important to note that, regarding preferred exercise regimens, voluntary running exercise is gaining importance as, under these conditions, animals are able to run at will while demonstrating fewer stress markers compared with animals undergoing forced exercise. However, it is still unclear what the benefits of such exercise in diabetes and obesity research are and how to compare these results with decades of reports on the benefits demonstrated by treadmill-based exercise regimens. Importantly, it is not known whether long-term exercise (voluntary compared with forced exercise) actually prolongs the life-span of diabetic or obese rodents. For metabolic benefits, both endurance exercise and sprint exercise can confer beneficial effects during obesity and diabetes, as demonstrated by numerous human trials and animal studies. Important considerations in any type of exercise protocol include monitoring rodent housing conditions, such as ambient temperatures and socialization, as well as timings of exercise intervention, with the dark cycle preferred as this is in harmony with rodent’s natural activities. Significant variations in glucose and energy metabolism exist within strains of common laboratory mice and rats and selection of an appropriate strain should be given priority based on the goal, nature and predicted outcome of the experiment.

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**REFERENCES**


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211 Connor, J. L. (1975) Genetic mechanisms controlling the domestication of a wild house mouse population (Mus musculus L.). J. Comp. Physiol. Psychol. 89, 118–130

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