Am J Cancer Res 2019;9(12):2813-2820
www.ajcr.us /ISSN:2156-6976/ajcr0102866

Commentary

Ethical consideration and feasibility demonstration of high-intensity interval training without the use of electrical shocks in mice with and without doxorubicin exposition

Maxime Caru[1,2,3*], François Lalonde[1,4,5*], Elise Legault[4,5], Daniel Curnier[1,3,4], David H St-Pierre[3,5], Alain Steve Comtois[5], François Tournoux[4]

[1]Laboratory of Pathophysiology of EXercise (LPEX), School of Kinesiology and Physical Activity Sciences, Faculty of Medicine, University of Montreal, Montreal, Quebec, Canada; [2]Laboratoire EA 4430 - Clinique Psychanalyse Developpement (CliPsyD), Department of Psychology, University of Paris Nanterre, Nanterre, Ile-de-France, France; [3]Sainte-Justine University Health Center, Research Center, Montreal, Canada; [4]University Hospital of Montreal, Research Center, Montreal, Canada; [5]Department of Exercise Sciences, Faculty of Sciences, Université du Québec à Montréal, Montréal, Canada. [*]Equal contributors.

Received September 26, 2019; Accepted September 30, 2019; Epub December 1, 2019; Published December 15, 2019

Abstract: Introduction: Most protocols intended to stimulate cardiovascular training in mice use electrical shocks that cause psychological stress and interfere with running performance. The aim of this study was to: 1) demonstrate the feasibility of a two-week high-intensity interval training (HIIT) program without the use of electric shocks in mice and 2) show that HIIT without electric shocks is feasible in the specific context of mice exposed to chemotherapy (i.e., doxorubicin). Methods: Ten C57bl/6 6-week-old female mice underwent a maximal exercise capacity test before and after two weeks of HIIT (five sessions per week) to measure their maximum running speed. The electrical stimulus was substituted by gently lifting the hind legs of the training mice using a tongue depressor. A second sample of ten C57bl/6 10-week-old female mice receiving a single intravenous injection of 20 mg/kg of doxorubicin underwent a single session of HIIT post-DOX using the same gentle stimulation method. Results: After two weeks of HIIT without the use of electric shocks, non-treated mice had a significant increase in their maximal speed (4.4 $m \cdot min^{-1}$; P = 0.019). In DOX-treated mice, the compliance rate to run went from 100% during the acclimation period prior to doxorubicin treatment to 100% when HIIT was performed after the DOX treatment. Doxorubicin treatment seemed to affect exercise compliance in DOX-treated mice. Our study demonstrated that a two-week HIIT program in non-treated mice and a single HIIT session in DOX-treated mice are feasible. Conclusion: The use of electric shocks was not required to obtain acceptable exercise compliance and a significant change in mice physical capacity. Our technique to perform a treadmill maximal exercise capacity test was shown to be feasible, even in specific pathological conditions like chemotherapy infusion, and could become a reference for future research protocols aimed at reducing the impact of psychological stress caused by electric shocks in mice. This model of exercise training in mice introduces an alternative to ethical conduct standards in animal research.

Keywords: Exercise, high-intensity interval training, exercise testing, animal ethics, doxorubicin

Introduction

High intensity interval training (HIIT) is characterized by brief and repeated bouts of high-intensity exercise, with intensities equal or superior to the ventilatory threshold, followed by low-intensity exercise or recovery periods below the ventilatory threshold [1, 2]. Its benefits are similar to moderate-intensity continuous training (MICT), which is recommended to improve cardiorespiratory fitness in the general population [3, 4]. The American College of Sports Medicine qualified HIIT as the most popular type of exercise because it is perceived to be more enjoyable, time-saving and better achieved by participants than MICT [5, 6]. This is even more interesting considering that previous studies [7-11] suggested that HIIT exerts

favorable effects on risk factors associated with cardiometabolic diseases by improving insulin sensitivity, lipid profiles, mitochondrial biogenesis and glucose control, and promoting weight loss. Consistent with these results, HIIT has been shown to be associated with cardiovascular [12] and metabolic adaptations [13].

Animal models are used to provide a better understanding of mechanistic adaptations that translate into physiological benefits after exercise. Indeed, the laboratory environment allows the accurate investigation of physiological and performance adaptations and their cellular mechanisms, with better methodological control. Recent studies in both mice and rats have shown that HIIT was efficient to improve cardiometabolic health [14] and physical performance [15, 16]. In mice, HIIT has been shown to exert beneficial effects on glucose metabolism and insulin resistance [17, 18], metabolic dysfunctions [19], skeletal muscle Glut4 content [17] and grip strength as well as treadmill endurance and gait speed [15]. Unfortunately, many exercise studies using animal models do not use exercise doses that are relevant to humans. According to a recent review by Melo and Hagar [20], most pre-clinical rodent trials in exercise oncology have a lack of precision when it comes to describing exercise stimuli (high or low intensity). This is particularly accentuated by the use of exercise interventions that no human can endure. This methodological issue deserves to be addressed since there is a need to find an exercise dose for pre-clinical mice trials that is comparable to exercise doses applicable to humans.

Furthermore, many animal-based exercise studies stimulate the mice after running cessation with an electric shock grid. According to Conner [21], the use of electric shocks to stimulate running in mice induces psychological stress, such as anxiety and aversion to training that can interfere with the mice's running performance. Importantly, the use of this empiric electrical shock method does not meet the guidelines for ethical conduct in the care and use of animals provided by American Association of Psychologists. In fact, it appears that no research has studied the feasibility of a high-intensity exercise program without the use of electric shocks that takes into consideration ethical issues in animal experimentation. Thus, the aim of this study was to 1) demonstrate the feasibility of a two-week high-intensity interval training (HIIT) program without the use of electric shocks in mice and 2) show that HIIT without electric shocks is feasible in the specific context of mice exposed to chemotherapy (i.e., doxorubicin).

Methods

Animals experiments

All animal experiments were approved by the institutional committee for the protection of animal (CIPA, protocol number: C15051JFc and CM18001FTs) of the University of Montreal Hospital (CRCHUM), Research Center (Montreal, Canada), and all animals received care in compliance with NIH standards. We used thirteen 6-week-old C57bl/6 female mice that were provided by the Charles River Laboratories (Saint-Constant, Québec, Canada). A second sample of ten 10-week-old C57bl/6 female mice from Charles River Laboratories (Saint-Constant, Québec, Canada) received a single intravenous injection of 20 mg/kg of doxorubicin (Cayman Chemical, CO). All animals were acclimated to a 12:12 hour artificial light-dark cycles, were fed on a regular chow diet and had access to water ad libitum. Animals were housed 3 to 5 mice per cage, and did not have any restrictions for voluntary activity, though they did not have access to a running wheel.

Acclimation period

Mice had one week of acclimation to the treadmill (Accusan, Omnitech Electronics, Columbus, Ohio, USA) [22, 23]. Two types of acclimation protocols were used according to the mice's status (i.e., non-treated mice and DOX-treated mice). The non-treated mice were acclimated to running on the treadmill on five consecutive days by increasing the time from 15 to 35 min followed by two days of rest. On the first day, mice ran at a speed of 15 $m \cdot min^{-1}$ at a 0 degree slope. On the second day, in order to simulate HIIT, mice warmed-up for 10 min and continued with 5 sets of 1 min at 17 $m \cdot min^{-1}$ (2 degrees slope) with active recovery at 15 $m \cdot min^{-1}$ at a 0 degree slope and a 10 min cool-down. According to Conner [21], mice that refuse to run are identified very quickly (~5 min) (i.e., three successive stops, refusal to resume running despite gentle encouragement, physical signs of exhaustion, splayed posture and la-

bored breathing). Those mice (n = 3) were excluded from the HIIT protocol. The DOX-treated mice were acclimated using the Petrosino [24] protocol that obtained a high exercise compliance rate in C57bl/6 mice. Furthermore, exercise preconditioning has a cardioprotective effect in the animal model [25] and can become a confounding factor when studying the effect of exercise during doxorubicin treatments. Thus, a low dose acclimation protocol is preferred in this context. DOX-treated mice were acclimated to running on the treadmill for 3 min prior to exercise. Initial running speed was set at 6 $m \cdot min^{-1}$ for 5 min, followed by 9 $m \cdot min^{-1}$ for 2 min and 12 $m \cdot min^{-1}$ for the last 2 min. For the entire exercise session, the slope was set to 0 degree. Acclimation was performed three times with 48 to 60 hours of recovery between training sessions. All of the DOX-treated mice accepted to run during the acclimation period according to the criteria by Conner [21].

Maximal exercise capacity test

After the acclimation period, non-treated mice performed a maximal exercise capacity test on a treadmill (Accusan, Omnitech Electronics, Columbus, Ohio, USA) and were reassessed after a two-week HIIT training period (Table S1). The maximal exercise capacity test, inspired by de Moraes [26], consisted of a standard incremental procedure starting at 10 $m \cdot min^{-1}$ at a 5 degree slope for 3 min followed by a first stage at a speed of 13 $m \cdot min^{-1}$ at a 10 degree slope for 1 min and a second stage at a speed of 16.6 $m \cdot min^{-1}$ at a 10 degree slope for 1 min. Thereafter, the running speed increased by 3.3 $m \cdot min^{-1}$ every minute until the mice were exhausted. For DOX-treated mice, the maximal exercise capacity test was slightly different and based on the Petrosino [24] protocol. Exhaustion was defined as the point where mice refused to run, stopped three times in a row or remained more than 10 seconds on a small resting area at the extremity of the running belt. Also, mice that showed signs of physical fatigue such as poor posture or labored breathing, were stopped.

High intensity interval training

The HIIT program for the non-treated mice was composed of 5 sessions (of 30 min) per week for two weeks. Each session started with a 10 min warm-up (< 50% of the maximum running speed) followed by 5 sets of 1 min at high intensity (over 75% of the maximum running speed) and a 10 min cool-down. Since mice rarely run for more than 1 to 2 min bouts on voluntary wheels, interval times were assigned according to Meijer and Robbers [27]. Each set was interrupted by 2 min of active recovery period at low intensity (< 50% of the maximum running speed) qualifying the training session in a 1:2 ratio. Mice were allowed 2 days of rest at the end of the week to promote their recovery. To increase mice compliance, the training program was performed in a reverse day/night cycle in order for the mice to run during their active part of the day. For the DOX-treated mice, a single HIIT session was performed 30 to 78 h following the doxorubicin injection. The exercise session started with a 3 min warm-up (< 50% of the mouse's maximum running speed) followed by 8 sets of 1 min at high intensity (85-90% of the maximum running speed). Each set was interrupted by 1 min of active recovery period at low intensity (< 50% of the maximum running speed). The 8 sets were repeated a second time following 5 min of passive recovery period for a total of 16 high intensity exercise bouts. The exercise session lasted 40 min and was performed at a fixed slope of 15 degrees. The electrical stimulus was substituted by a gentle encouragement by a human operator using a tongue depressor to slightly lift the mice's hind legs [21].

Statistical analysis

Statistical analyses were performed using IBM SPSS statistics, version 24.0 (IBM Corp., Armonk, NY, USA) and graphs were created using GraphPad Prims 8.0.2 (GraphPad Software, San Diego, CA, USA). All variables were reported as mean ± standard deviation (SD). Descriptive analyses were performed to characterize our cohort of mice. To compare the maximum running speed before and after the HIIT program, a two-tailed paired Student *t*-test was performed with a significance level of 5%. Effect sizes were calculated from Cohen's d analysis where d = 0.20 was considered a small effect, d = 0.50 as a medium effect and d = 0.85 as a large effect.

Results

Out of the initial sample of thirteen non-treated mice, ten successfully completed the HIIT program and the maximal exercise capacity test

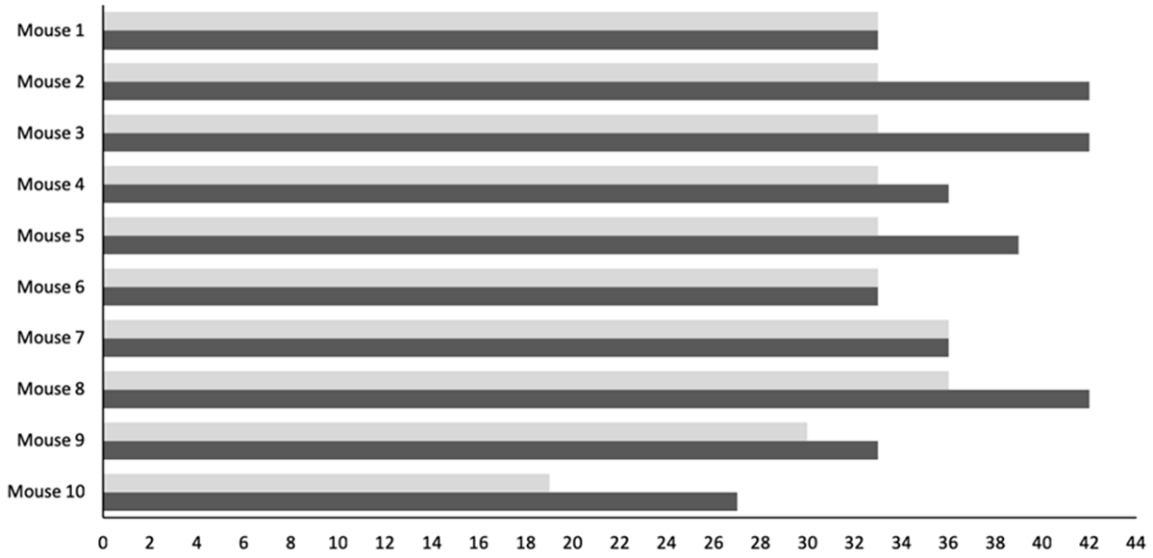

Figure 1. Maximum speed (m•min^{-1}) recorded before and after two-week of HIIT in mice. Maximum speed recorded before the training program is represented in grey, while maximum running speed recorded after the training program is represented in black.

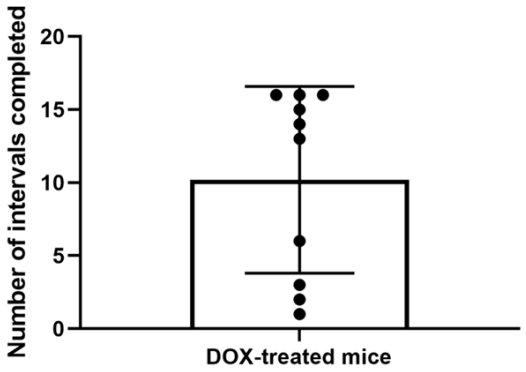

Figure 2. Compliance of a single HIIT session in DOX-treated mice. Number (n) of high intensity bouts completed during the single HIIT session performed 30-78 h after doxorubicin injection. Animals were removed from the treadmill if they stopped three times in a row or showed signs of physical fatigue such as poor posture or labored breathing and vocalization.

before and after the intervention. Three mice refused to run during the acclimation period and were excluded from the study. During the first maximal exercise capacity test, before the HIIT program, the mean maximum running speed recorded was of 31.9 ± 4.8 m•min^{-1}, while the second maximum running speed recorded after the HIIT program was of 36.3 ± 5.0 m•min^{-1}. Analyses showed that mice significantly improved their maximum running speed by 4.4 m•min^{-1} (P = 0.019; d = 0.89) after two weeks of HIIT without the use of electric shocks (**Figure 1**). Also, we observed a non-significant (P = 0.09; d = 1.91) increase in weight between the beginning (18.1 ± 1.2 g) and the end (20.3 ± 1.1 g) of the HIIT program. In DOX-treated mice, the compliance rate to run during the acclimation period prior to doxorubicin treatment was 100% and the mean running speed recorded was 31.4 ± 4.1 m•min^{-1}. After the doxorubicin injection, the compliance rate for HIIT was 60% (high-intensity bouts completed ≥ 8). The exercise compliance in DOX-treated mice is presented in **Figure 2**.

Discussion

This is the first study that investigates the effect of a HIIT program (1:2 ratio) without the use of electric shocks in mice. Our main findings support the hypothesis that two weeks of HIIT without the use of electric shocks in non-treated mice is feasible in improving maximal running speed while also being reliable and safe. Contrary to previous studies [15, 17-19], the HIIT protocol was shorter and despite that, there was a significant increase of maximum running speed. Furthermore, we investigated the feasibility of a single HIIT session 30-78 h following an intravenous injection of doxorubicin. Treated mice showed an acceptable compliance to the single HIIT session shortly after the doxorubicin injection.

Feasibility of two weeks of HIIT without the use of electric shocks in non-treated mice

Our findings were consistent with others using animals to show that HIIT enhanced physical performance in both rats and mice [15-17]. In fact, after two weeks of HIIT, we observed a 13.8% increase in the maximum running speed. A study using aged mice, reported a 32.6% increase in treadmill endurance and 107% in gait speed after 16 weeks of HIIT [15]. This difference can be explained in part by the duration of the training program that was 8 times longer than the one in our study.

Compliance to exercise in DOX and non-treated mice

During the acclimation period, compliance to exercise was different between our two study samples (non-treated and DOX-treated mice): three non-treated mice and zero DOX-treated mice refused to run. In that perspective, it is plausible that the acclimation protocol influenced the mice's willingness to exercise on the treadmill. The use of only 3 exercise sessions rather than 5 over a 7-days period may be preferable in training mice to be more compliant to exercise. Similar acclimation compliance success rates were reported in Petrosino's study, in which no mice refused to run during the 3 exercise sessions lasting 12 minutes each [24]. Furthermore, a small dose of exercise at constant load during the acclimation period such as tested in the DOX-treated mice would be preferable if the researcher wants to avoid an increase in the animal's physical function to increase prior to the intervention (e.g., avoiding a preconditioning confounding factor). However, gradually increasing the exercise session duration over the acclimation period as tested in the non-treated mice may be preferable to a constant and small load (duration and frequency) to promote further adaptation and to prepare animals for the higher exercise intervention dose.

Exercise compliance rates during the intervention period (single HIIT session and HIIT program) were different between our two samples since we observed a 100% compliance in non-treated mice, while we reported a 60% compliance (high-intensity bouts completed $\geq$ 8) in DOX-treated mice. This may be related to the DOX-induced treatment side effects affecting the animal's willingness to exercise and physical capacity. Indeed, it has been reported that doxorubicin side effects in rodents include asthenia, diarrhea, cachexia and cardiotoxicity that affect exercise capacity [28, 29]. According to one study, C57bl/6 female mice treated with a combination of cyclophosphamide, doxorubicin and 5-fluorouracil experienced a significant reduction in voluntary wheel running parameters such as: time, distance, average speed and peak speed post-treatment compared to pretreatment activity [29].

Ethical considerations

Finally, it is important to discuss the ethical aspects regarding the present study. It appears that the use of motorized treadmills with electric shock grids to force mice to run is not an ethically viable approach. This is an important issue in animal experimentation that deserves to be discussed. Impairments in some experimental parameters (i.e., psychological stress, anxiety as well as food-seeking/avoidance behaviors and aversion to training) are caused by electric shocks [30-32]. It is therefore imperative to develop innovative strategies to reduce pain in laboratory animals. Our study indicates that it is possible to establish better standardized methods that more reliable experimental results. Thus, without the use of electric shocks, the present exercise training protocol that we implemented in mice brings a new solution to the reduction of their psychological stress and eliminates an important bias as a confounding factor to their physiological performance [21, 33, 34]. For ethical reasons, we invite researchers to reconsider the use of electrical stimulation and substitute it with a more "humane" approach such as using a tongue depressor. In accordance with our results, this technique was shown to be effective even for high-intensity training and may also be useful for cardiotoxic chemotherapy pre-clinical exercise trials using rodent models.

Clinical application

In exercise oncology, some methodological issues deserve to be addressed. Finding an exercise dosage for pre-clinical mice trials that is comparable to exercise dosages applicable to humans is one of them. This is a challenge since exercise physiologists do not necessarily have access to a complete cardiometabolic lab

set-up to propose the best follow-up to their patients. Thus, field tests (e.g., six-minute walk test, incremental shuttle walk test) are used to quantify training intensity based on the participant's speed [35, 36]. The same aspect is observed in mice since the execution of a maximal exercise capacity test is rarely done in a metabolic chamber [37]. In order to get closer to clinical oncology reality, the same approach was applied to our mice sample. Thus, like a field test in humans, our maximal exercise capacity test in mice contributed to prescribing the running intensity from the speed parameters with a normalized slope during the training session. Further studies are needed to explore the best prescription training dose. One of the first steps would be to compare different training protocols (e.g.: continuous vs HITT or different work-rest ratio in HIIT). Some studies have begun to explore this aspect in breast cancer patients [38, 39]. However, some breast cancer patients wait between two to four weeks before starting chemotherapy. Therefore, there is a need to offer appropriate care for patients regarding physical activity as soon as they are diagnosed. A two-weeks HIIT program could be a viable solution, as our study proposes. Training mice should receive the same basic exercise prescription as found in human training, as follows: Frequency, Intensity, Time and Type (FITT) [40].

Conclusion

Our study demonstrated that a two-weeks HIIT program is feasible in increasing the maximal running speed with gentle lifts to the hindquarter in the absence of electric shocks to stimulate mice to reach high levels of exercise intensity. We also developed an experimental mouse model exercise intervention that is feasible during doxorubicin treatments and that is humanely applicable. Moreover, our treadmill maximal exercise capacity test was shown to be feasible, humane and easily translated into running speed for the training program. Our study could become a reference for future research aimed at studying mice running performance, or the effect of high intensity exercise on mice with acute toxicity induced by chemotherapy, without the use of electric shocks.

Acknowledgements

We appreciate the assistance of Ariane Levesque (McGill University) for her review of the article in the English language.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. François Tournoux, Centre Cardiovasculaire Du Centre Hospitalier De l'Université De Montréal (CHUM), 900 Rue Saint Denis, Montréal QC H2X 0A9, Canada. E-mail: francois.tournoux@umontreal.ca

References

[1] Buchheit M and Laursen PB. High-intensity interval training, solutions to the programming puzzle. Part II: anaerobic energy, neuromuscular load and practical applications. Sports Med 2013; 43: 927-954.

[2] Billat LV. Interval training for performance: a scientific and empirical practice. Special recommendations for middle- and long-distance running. Part II: anaerobic interval training. Sports Med 2001; 31: 75-90.

[3] Gillen JB, Martin BJ, MacInnis MJ, Skelly LE, Tarnopolsky MA and Gibala MJ. Twelve weeks of sprint interval training improves indices of cardiometabolic health similar to traditional endurance training despite a five-fold lower exercise volume and time commitment. PLoS One 2016; 11: e0154075.

[4] Hannan AL, Hing W, Simas V, Climstein M, Coombes JS, Jayasinghe R, Byrnes J and Furness J. High-intensity interval training versus moderate-intensity continuous training within cardiac rehabilitation: a systematic review and meta-analysis. Open Access J Sports Med 2018; 9: 1-17.

[5] Gaesser GA and Angadi SS. High-intensity interval training for health and fitness: can less be more? J Appl Physiol (1985) 2011; 111: 1540-1541.

[6] Kravitz L. High-intensity interval training. Indianapolis, In: American College of Sports Medicine; 2011.

[7] Kessler HS, Sisson SB and Short KR. The potential for high-intensity interval training to reduce cardiometabolic disease risk. Sports Med 2012; 42: 489-509.

[8] Gibala MJ, Little JP, Macdonald MJ and Hawley JA. Physiological adaptations to low-volume, high-intensity interval training in health and disease. J Physiol 2012; 590: 1077-1084.

[9] Boutcher SH. High-intensity intermittent exercise and fat loss. J Obes 2011; 2011: 868305.

[10] Donnelly JE, Blair SN, Jakicic JM, Manore MM, Rankin JW, Smith BK; American College of Sports Medicine. American college of sports medicine position stand. Appropriate physical activity intervention strategies for weight loss and prevention of weight regain for adults. Med Sci Sports Exerc 2009; 41: 459-471.

[11] Whyte LJ, Gill JM and Cathcart AJ. Effect of 2 weeks of sprint interval training on health-related outcomes in sedentary overweight/obese men. Metabolism 2010; 59: 1421-1428.
[12] Munk PS, Butt N and Larsen AI. High-intensity interval exercise training improves heart rate variability in patients following percutaneous coronary intervention for angina pectoris. Int J Cardiol 2010; 145: 312-314.
[13] Mangiamarchi P, Caniuqueo A, Ramirez-Campillo R, Cardenas P, Morales S, Cano-Montoya J, Bresciani G and Álvarez C. [Effects of high-intensity interval training and nutritional education in patients with type 2 diabetes]. Rev Med Chil 2017; 145: 845-853.
[14] Batacan RB Jr, Duncan MJ, Dalbo VJ, Connolly KJ and Fenning AS. Light-intensity and high-intensity interval training improve cardiometabolic health in rats. Appl Physiol Nutr Metab 2016; 41: 945-952.
[15] Seldeen KL, Lasky G, Leiker MM, Pang M, Personius KE and Troen BR. High intensity interval training improves physical performance and frailty in aged mice. J Gerontol A Biol Sci Med Sci 2018; 73: 429-437.
[16] Li FH, Li T, Ai JY, Sun L, Min Z, Duan R, Zhu L, Liu YY and Liu TC. Beneficial autophagic activities, mitochondrial function, and metabolic phenotype adaptations promoted by high-intensity interval training in a rat model. Front Physiol 2018; 9: 571.
[17] Chavanelle V, Boisseau N, Otero YF, Combaret L, Dardevet D, Montaurier C, Delcros G, Peltier SL and Sirvent P. Effects of high-intensity interval training and moderate-intensity continuous training on glycaemic control and skeletal muscle mitochondrial function in db/db mice. Sci Rep 2017; 7: 204.
[18] Marcinko K, Sikkema SR, Samaan MC, Kemp BE, Fullerton MD and Steinberg GR. High intensity interval training improves liver and adipose tissue insulin sensitivity. Mol Metab 2015; 4: 903-915.
[19] Wang N, Liu Y, Ma Y and Wen D. High-intensity interval versus moderate-intensity continuous training: superior metabolic benefits in diet-induced obesity mice. Life Sci 2017; 191: 122-131.
[20] Melo L and Hagar A. How to train a mouse-methodological issues in pre-clinical exercise oncology. Am J Cancer Res 2019; 9: 1246-1253.
[21] Conner JD, Wolden-Hanson T and Quinn LS. Assessment of murine exercise endurance without the use of a shock grid: an alternative to forced exercise. J Vis Exp 2014; e51846.
[22] Ashraf J and Roshan VD. Is short-term exercise a therapeutic tool for improvement of cardio-protection against DOX-induced cardiotoxicity? Asian Pac J Cancer Prev 2012; 13: 4025-4030.
[23] Parry TL, Hydock DS, Jensen BT, Lien CY, Schneider CM and Hayward R. Endurance exercise attenuates cardiotoxicity induced by androgen deprivation and doxorubicin. Can J Physiol Pharmacol 2014; 92: 356-362.
[24] Petrosino JM, Heiss VJ, Maurya SK, Kalyanasundaram A, Periasamy M, LaFountain RA, Wilson JM, Simonetti OP and Ziouzenkova O. Graded maximal exercise testing to assess mouse cardio-metabolic phenotypes. PLoS One 2016; 11: e0148010.
[25] Scott JM, Khakoo A, Mackey JR, Haykowsky MJ, Douglas PS and Jones LW. Modulation of anthracycline-induced cardiotoxicity by aerobic exercise in breast cancer: current evidence and underlying mechanisms. Circulation 2011; 124: 642-650.
[26] de Moraes WM, Melara TP, de Souza PR, Guimarães Fde S, Bozi LH, Brum PC and Medeiros A. Impact of leucine supplementation on exercise training induced anti-cardiac remodeling effect in heart failure mice. Nutrients 2015; 7: 3751-3766.
[27] Meijer JH and Robbers Y. Wheel running in the wild. Proc Biol Sci 2014; 281: 20140210.
[28] Milano G, Raucci A, Scopece A, Daniele R, Guerrini U, Sironi L, Cardinale D, Capogrossi MC and Pompilio G. Doxorubicin and trastuzumab regimen induces biventricular failure in mice. J Am Soc Echocardiogr 2014; 27: 568-579.
[29] Elsea CR, Kneiss JA and Wood LJ. Induction of IL-6 by cytotoxic chemotherapy is associated with loss of lean body and fat mass in tumor-free female mice. Biol Res Nurs 2015; 17: 549-557.
[30] Ghasemi M and Dehpour AR. Ethical considerations in animal studies. J Med Ethics Hist Med 2009; 2: 12.
[31] Baertschi B and Gyger M. Ethical considerations in mouse experiments. Curr Protoc Mouse Biol 2011; 1: 155-67.
[32] Dunbar G, Higa J, Jones T, Kaminski B and Panicker S. Guidelines for ethical conduct in the care and use of nonhuman animals in research. American Psychological Association Committee on Animal Rights Research and Ethics 2012; 1-9.
[33] Knab AM, Bowen RS, Moore-Harrison T, Hamilton AT, Turner MJ and Lightfoot JT. Repeatability of exercise behaviors in mice. Physiol Behav 2009; 98: 433-40.
[34] Cook MD, Martin SA, Williams C, Whitlock K, Wallig MA, Pence BD and Woods JA. Forced treadmill exercise training exacerbates inflammation and causes mortality while voluntary

wheel training is protective in a mouse model of colitis. Brain Behav Immun 2013; 33: 46-56.
[35] Lanza Fde C, Zagatto Edo P, Silva JC, Selman JP, Imperatori TB, Zanatta DJ, de Carvalho LN, Reimberg MM and Dal Corso S. Reference equation for the incremental shuttle walk test in children and adolescents. J Pediatr 2015; 167: 1057-1061.
[36] Schumacher AN, Shackelford DY, Brown JM and Hayward R. Validation of the six-minute walk test for predicting peak VO2 in cancer survivors. Med Sci Sports Exerc 2019; 51: 271-277.
[37] Speakman JR. Measuring energy metabolism in the mouse - theoretical, practical, and analytical considerations. Front Physiol 2013; 4: 34.
[38] Mijwel S, Backman M, Bolam KA, Jervaeus A, Sundberg CJ, Margolin S, Browall M, Rundqvist H and Wengström Y. Adding high-intensity interval training to conventional training modalities: optimizing health-related outcomes during chemotherapy for breast cancer: the OptiTrain randomized controlled trial. Breast Cancer Res Treat 2018; 168: 79-93.
[39] Dolan LB, Campbell K, Gelmon K, Neil-Sztramko S, Holmes D and McKenzie DC. Interval versus continuous aerobic exercise training in breast cancer survivors--a pilot RCT. Support Care Cancer 2016; 24: 119-127.
[40] Wasfy MM and Baggish AL. Exercise dose in clinical practice. Circulation 2016; 133: 2297-313.

Table S1. Maximal exercise capacity protocol

Stage	Speed ($m \cdot min^{-1}$)	Incline (degree slope)	Duration (min)
Warm-up	10	5	3
1	13	10	1
2	16.6	10	1
3	19.9	10	1
4	23.2	10	1
5	26.5	10	1
6	29.8	10	1
*	...	10	1
Cool-down	< 10	< 5	3

*Exhaustion was defined as the point where mice refused to run, stopped three times in a row or remained more than 10 seconds on a small resting area at the extremity of the running belt.