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A systematic comparison of exercise training protocols on animal models of cardiovascular capacity

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Abstract

Cardiovascular disease (CVD) is a major global cause of mortality, which has prompted numerous studies seeking to reduce the risk of heart failure and sudden cardiac death. While regular physical activity is known to improve CVD associated morbidity and mortality, the optimal duration, frequency, and intensity of exercise remains unclear. To address this uncertainty, various animal models have been used to study the cardioprotective effects of exercise and related molecular mechanism such as the mice training models significantly decrease size of myocardial infarct by affecting Kir6.1, VSMC sarc-K_{ATP} channels, and pulmonary eNOS. Although these findings cement the importance of animal models in studying exercise induced cardioprotection, the vast assortment of exercise protocols makes comparison across studies difficult. To address this issue, we review and break down the existent exercise models into categories based on exercise modality,

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Author contributions

XHX conceived the study. XHX, FR, LYW, ZGL and GGK prepared the manuscript. FR, LYW, RY, YTS, YL, QXY, GGK, YPS, YJW, WZ, XZ, MMX, JJM, WI and XHX edited the manuscript. All authors read and approved the final manuscript.

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Conflict of interest

The authors declare that they have no conflict of interest.

Data availability

All data generated or analyzed during this study are included in this published article.

intensity, frequency, and duration. The timing of sample collection is also compared and sorted into four distinct phases: pre-exercise (Phase I), mid-exercise (Phase II), exercise recovery (Phase III), and post-exercise (Phase IV). Finally, because the life-span of animals so are limited, small changes in animal exercise duration can corresponded to untenable amounts of human exercise. To address this limitation, we introduce the Life-Span Relative Exercise Time ($RET_{life\ span}$) as a method of accurately defining short-term, medium-term and long-term exercise relative to the animal's life expectancy. Systematic organization of existent protocols and this new system of defining exercise duration will allow for a more solid framework from which researchers can extrapolate animal model data to clinical application.

Keywords

Animal model; Exercise training procedure; Exercise stress testing; Exercise-promoted cardio-protection

1. Introduction

Cardiovascular disease (CVD) such as heart ischemic or coronary artery disease (CAD) continues to be one of the major causes of death worldwide. Genetic features combined with age and factors such as dyslipidemia, atherosclerosis, and hypertension are considered risk factors of CVD. The severity of these diseases is greatly impacted by excess caloric intake and a lack of physical activity (PA) in a patient's daily life [1]. With high morbidity and high mortality rates associated with the disease, it is imperative to discover innovative and efficient strategies to protect the heart against CVD and, at the very least, reduce this life-threatening risk.

Low-levels of physical activity are considered a strong predictor of mortality in CVD patients. On the other hand, high levels of physical activity lead to 30–41% reduction in death (2457 in 7681) with 600 to 1499 kcal/wk., 2400 in 5853 with ≥1500 kcal/wk. risk of CVD [2,3].

Exercise as a category of physical activity is frequently regarded as an effective intervention to improve cardiovascular function. Regular exercise has been considered an effective method to improve heart function and reduce mortality of CVD. Several animal models and human epidemiological studies support the cardio-protective effect of exercise [4–6]. Several protective mechanisms demonstrated that exercise increased VO_{2max} (maximal oxygen consumption), improved cardiorespiratory fitness (CRF) [7], lipid profile [8], endothelial function [9], increased number of capillaries [10], and mitochondria anti-oxidant activity in the cardiovascular system [11]. However, while exercise typically results if beneficial effects on heart function, there are occasions when it could result in unexpected death during exercise especially for the CVD cohort [12–14]. These conflicting results discourage patients from necessary daily physical activity. Therefore, using animal models with exercise is critical for determining exercise components and phases including intensity, timing, and duration which will be discussed below.

In order to provide effective life style intervention to avoid the risk of CVD, exercise animal models were introduced to demonstrate safety and evaluate the clinical efficiency of exercise training (EX) [6,15–17]. We researched both animal models and a publication which proved that exercise reduces the incidence of myocardial ischemia (MI), ischemia/reperfusion (I/R) injury, and atherosclerosis [18]. This information provided a valuable reference for the patient with CVD or an individual at risk of CVD to choose a reasonable exercise program. Of course, to design an appropriate program for individual patients, multiple components and phases of exercise training need to be determined. Furthermore, many different researchers utilized diverse standards in their animal model EX. It appears necessary to classify and standardize a protocol of animal EX. The standardized strategy will benefit our urgent need to determine the criteria for selecting an effective exercise regimen to improve cardiovascular function and avoid the occurrence of cardiovascular accidents during EX for both healthy and cardiovascular risk individuals [19].

In this summarization, we first discuss that clinical exercise training can be used as a testing stress to evaluate cardiovascular function, and furthermore to inspect severity and risk assessment of CVD. Second, we summarize current procedures on experimental exercise models applied in research on the cardiovascular system and distinguish their function improvement compared to similar clinical practices. We categorized the current protocols on animal model as the short-term exercise approach, the medium-term exercise approach, and the long-term exercise approach. We characterized the different functions on improving cardiovascular function. Eventually, we concluded that a reasonable effective exercise routine should be arranged, and suggested that the patients with CVD should carry out their exercise routine from short-term low-strength exercise to medium-term medium-strength exercise according to individual severity of their CVD.

2. Exercise-induced stress tests for patient CVD risk assessment

Although the importance of physical activity has been recognized, there are different viewpoints as to what intensity of exercise should be proposed for patients with the CVD or regular individuals with the risk of CVD. Early quantitative diagnosis and risk assessment of cardiovascular disease should be carried out before making a personal exercise program, which is becoming more and more recognized by both physicians and individuals (or potential) patients in extending life expectancy.

Exercise Stress Testing (EST) (also known as Exercise Testing (ST) is an inexpensive and noninvasive tool that was primarily introduced by the American College of Cardiology (ACC)/American Heart Association (AHA) in 1997. For > 20 years, EST has provided valuable cardiopulmonary data for both healthy and diseased peoples. The comprehensive understanding of the EST on cardiopulmonary function attracted the attention of the best cardiologists soon after. It has been 10 years that physician and exercise related physiologists have applied EST in their clinical inspection to diagnose CVD [20–23]. Effective protocols became a regular approach to assess the risk of CVD. For instance, for heart failure, symptom-limited cardiopulmonary exercise testing (CPET) is used in middle-aged male patients with heart failure (HF) to screen candidates for heart transplantation. For coronary artery disease, Electrocardiographic exercise test (ETT) is used. For pulmonary

hypertension, incremental shuttle walk exercise testing is used with no or minimal symptomatic limitation. For peripheral arterial disease (PAD), treadmill testing containing continuous (C) and graded (G) protocols is the main assessment method to evaluate walking ability in patients with PAD in clinical studies [20–26]. These protocols are widely used in both private clinic stations and hospitalized centers to determine further treatments and before making personal exercise programs.

The EST programs are set based on the patient's safety as the need for high intensity aerobic exercise for cardiopulmonary function are reliant upon the patient's diagnostic results for CVD. The effective EST is routinely made up of three main types:

a. Submaximal EST is set patients younger than 40 years of age. A heart rate (HR) limit of 140 beats/min and a metabolic equivalent (MET) level of seven are often used. For the patients over 40 years of age, a HR limit of 130 beats/min and a MET level of five are often used. The submaximal test is fit for hospitalized patients and maximal utilizing gas exchange is used to evaluate congestive heart failure and timing for heart transplantation [27–30].

b. Maximal EST is Continues exercising as workload, which is increased until volitional fatigue, or until the desired testing criteria reached. The intensity should be > 85% of the participants' age-predicted maximal HR or an HR equivalent to 85% of 220 minus age. The maximal utilizing normally requires gas exchange to fit appropriate individuals in different cardiopulmonary pathological conditions. The maximal test is most commonly used and appropriate for general individual. Both Submaximal and Maximal EST are generally performed on a treadmill [27–30].

c. Bruce protocol is an incremental workload treadmill exercise. Heart rate and rating of perceived exertion are taken every minute for patients, and blood pressure is taken at the end of every three minutes [31–33]. The EST is the most commonly used in North America.

3. Principle of animal exercise training protocols and sample collection

3.1. Characteristics of common animal exercise training protocols

Exercise training procedures should take into account intensity, modality of exercise, frequency, and duration. Based on the involvement of the tension and the length of working muscles, exercise can be classified as dynamic, static, and resistive exercise [34–36]. Dynamic exercise requires a large number of muscle groups to participate and this type of exercise produces an increase of cardiac output and oxygen uptake. Dynamic exercise is fit for the prevention of CVD, while the static and resistive exercise is more suitable for the patients who are already conditioned [34,37–39]. Depending on modality of exercise, the intensity and frequency of the certain modality along with duration of the exercise are a critical factor for a successful outcome.

Exercise training procedures can be divided into short-term and long-term exercise depending on the exercise cycle [4,40,41]. Based on the level of exercise intensity, exercise

training procedures could be categorized as low, moderate, or high intensity exercise. A well-established standard for inspecting exercise intensity is performed by live recording of oxygen uptake (VO_2) and maximal oxygen uptake (VO_{2max}), which is most commonly used in different animal models including pigs, mice, and rats [42–44].

As the most commonly used animal models, data collection on rats and mice benefit from their short gestation periods and numerous off-spring. A number of modalities of exercise training such as treadmill running, voluntary wheel running, and swim training have been utilized on rat and mice models of various genetic backgrounds [9,45–48].

As discussed above, many categories affect the successful outcome of exercises and final benefit for both healthy and at risk of CVD individuals. The intensity, modality, frequency, and duration of exercise illustrate the fundamental property of exercise. The level of exercise intensity characterizes exercise as low, moderate, or high intensity exercise. The length of time the exercise lasted (days or week in animals, months or years in human) is defined as short-term, medium-term, or long-term exercise (Fig. 1). All of these categories should be considered major features of the standard procedure on animal models in order to support the comprehensive understanding from these models to how they can benefit human beings.

3.2. Phases of exercise training and timing of sample collection in animal models

For all kind of animals used to assess and strengthen cardiovascular or cardiopulmonary function, exercise training should use the following phases. The animals designed to be used in experiments all need pre-exercise training (Phase I) before any exercise design should be carried out. The designed exercise procedure with diverse intensity, modality of exercise, frequency, and duration will then be performed on the animal (Phase II). In any daily exercises, resting after each cycle of exercise duration is necessary (Phase III). For exhausting experiments on animal models, an extra phase has to be carried out under strong stress conditions (Phase IV) (Fig. 2).

In Phase I, the animals need to learn how to adapt their behavior in a certain modality of exercise under physical stimulation forces such as sounds, lighting, and electronic shock. The adaption training should fulfil two aims; *i.e.* through phase I (Ph-I), the animals should know how to run and should be ready to run at the same intensity if the treadmill is utilized as the modality in any experimental approach. In Phase II (Ph-II), the animals will perform their exercise according to the designed procedure at a desired frequency and duration. In Phase III (Ph-III), resting follows the exercise in Phase II. Resting is essential as it allows the animals to accomplish the next cycle of the P-II exercise-and-P-III resting. Phase IV (Ph-IV) is the terminal measurement that is typically for inspection of limited cardiovascular function under strong stress conditions.

For every experiment, timing scale sampling is critical for these samples to express their properties that will be representative for signed experiments. Regularly, control samples for the sedentary group are collected after Phase I or even without adaption. Measured data during Phase II with a telemetry recording device illustrate the cardiac function during exercise. The measurement on EKG (Elektrokardiogramm or Electrocardiography) and blood pressure (BP) recordings should be performed at the end of Phase II and before Phase

III. These measurements completed after Phase III will be the resting status. Data harvested between Phase II and Phase III presented as $EKG_{pII\text{-}III}$ and $BP_{pII\text{-}III}$ elucidate different characteristics than that of $EKG_{p\text{-}III}$ and $BP_{p\ III}$. Especially, if sudden cardiac death (SCD) occurs after Phase II, the collected data in Phase III will be end-status, and the BPP-III $EKG_{p\text{-}III}$ will not the cause of the SCD, but the result after the death [49–52].

4. Classification of long-term exercise models and exercise-induced cardio-protection against CVD

Large scale cohort studies and meta-analyses showed that people who accomplished maximum physical activity levels had several-fold significant reduction in the risk of ischemic heart diseases and ischemic stroke than the people who were recommended the minimum level [53–55]. For people who suffered a CVD event, exercise capacity seriously affected the patients' quality of life. Investigations revealed that adults with congenital heart disease have a lower exercise capacity than the general population. Long-term lower exercise capacity is also found in a cohort with higher CVD risk factors such as patients suffering from hyperlipidemia, hypertension, and hyperglycemia. Currently, the major concern from patients with CVD or from those who are at risk of CVD is whether exercise itself can possibly trigger unexpected accidents such as SCD [56–58]. These concerns need to be investigated. The evaluation system of animal model plus physical activity, mainly developing a training exercise strategy, could be the best system to overcome the unidentified concerns and provide the best guidelines. In the following discussion, we summarized the protocols of long term-term and short-term training, which defines by the preceding time of the full-scale for animal exercise-training, and characteristics of their function on cardiovascular protection.

4.1. Long-term moderate-intensity training prevents cardiac injury

Long-term exercise models are the most common procedure to carry out animal exercise training from weeks to months at 45 min-1 h/day and 5 days/week [59–61]. The animal treadmill or voluntary wheels are normally used as the modality to perform dynamic exercise and static & resistive exercise respectively. Moderate-intensity continuous training (MICT) is normally utilized within long-term exercise and the procedure is generally maintained at 75% Vo_{2max}. The long-term MICT model demonstrated its positive effect on CVD [62]. Long-term exercise models normally can be carried the exercise condition up to 10–12 week total training time (Table 1).

4.1.1. Systemic cardio-protective effects of long-term MICT exercise—Long-term MICT have several protective aspects on CVD. Long-term MICT induces favorable adaptations to the cardiovascular system including physiological remodeling of the heart representative with increased O_2 consumption, improvement of cardiac contractile function, and calcium handling. Long-term MICT plays an effective role in preventing injury caused by HF myocardial ischemia [74]. The result provides the best comprehensive understanding on the dramatic decrease of mortality and quality improvement of the patients' life after cardiac injury when the long-term MICT procedure is applied [75,76]. It is certain that the long-term MICT procedure using an animal model will gain deeper insights into the

molecular mechanism of CVD and this mechanism could help us to accomplish a more effective program to benefit both healthy and cardiovascular at-risk individuals.

4.1.2. Long-term MICT exercise significantly reduces the size of myocardial ischemia infarct—In the late 19th century, utilizing regular exercise with 5 weeks of swimming training (1 h/day, 5 days/week), McElroy et al. demonstrated the cardio-protective effect against MI before the occurrence of induced left coronary occlusion. For the MI male Sprague-Dawley rats, the infart size in exercised rats decreased by 30% compared to controls [77].

Combining MICT exercise with doxorubicin (DOX) chemotherapy can prevent cardiotoxicity of the resveratrol supplementation from the DOX-induced cardiac injury. Using female C57BL6 mouse models with chronic DOX exposure, long-term MICT exercise was performed on the animals with treadmill running at 5 days/week for 45 min/day. This MICT was carried out on mice at a speed of 18 m/min for the 8-week treadmill running which reduced the DOX-induced oxidative stress [78], and the 20-week treadmill exercise significantly improved both diastolic function and left ventricle pressure on the MI female rats. Meanwhile, the infarct size on these rats was reduced by 24% after 1 h of myocardial ischemia generated by 2 h of reperfusion [79]. These data revealed that both MICT and resveratrol supplementation prevent cardiotoxicity in female C57BL6 mice undergoing DOX chemotherapy [78].

4.1.3. Preventative effects of long-term MICT exercise involve Kir6.1, VSMC sarc-K_{ATP} channels, and pulmonary eNOS—The long-term MICT exercise offers not only protection against regional ischemia, but also prevention on the decrease of heart oxygen consumption subsequently after I/R injury. The researchers found that the ability of the C57BL/6 mice to prevent I/R was promoted after the 8 weeks MICT exercise. Furthermore, the exercise up-regulated Kir6.1 protein, increased the stiffness, and reduced the relaxation capacity in the vasculature of male Sprague-Dawley rats [80].

The MICT exercise improvements on CVD are universal targets on multiple signaling pathways. It has been proven that the VSMC sarc-K_{ATP} channels perform an important role in exercise-induced cardio-protective mechanism within the 8-week (15 m/min, 45 min/day, 5 days/week) MICT exercise program [80]. Interestingly, another study reported that the 10-week MICT exercise program (75–80% VO_{2max}, 4 days/week) performed on female Sprague-Dawley rats expressed a higher systolic blood pressures after I/R-reperfusion compared with the sedentary [62]. Research from the Mary Beth Brown group claimed that MICT with 75% Vo_{2max} promptly activated pulmonary eNOS in lung tissue, and induced transiently normalized pulmonary pressure through telemetric recordings during running (75% VO_{2max}, 45 min/day, 5 days/week) [81].

As the most complicated issue, physiological function and disorder of heart are determined by multiple factors including EC coupling pathway in cardiomyocytes [82], voltage-dependent gate and connexins pathway in cardiac conduction system [83], macrophage associated regulation of cardiac conduction [82,84], and nutrient delivery involved epithelial vascular system. It is rational to believe that the molecular mechanism of long-term MICT

exercise on improvement and protection of cardiac function should be involved in the most pathways related to daily cardiac physiology.

4.2. Long-term MICT exercise prevents coronary artery disease (CAD) and sudden cardiac death (SCD)

Over 50% of CVD is CAD which accounts for approximately 50% of all CVD related deaths [85,86]. CAD complications lead to HF, arrhythmia, and SCD [4]. Nearly 230 to 350 thousand people suffered SCD per year in the United States alone during the past 20–30 years [87]. Approximately 6.5 million Americans were affected by HF and health care expenditures reached nearly $30 billion per year [58]. This circumstance requires an urgent medical solution and it is obvious that the situation needs more basic comprehension on behalf of the research society.

It is certain that long-term MICT exercise impaired endothelium-dependent vasorelaxation and reduced muscle perfusion in the HF animal model [88–91]. The MICT exercise caused endothelial dysfunction in C57Bl/6 mice HF through activating nitric oxide (NO) production [88]. The research group from University of Missouri-Columbia demonstrated that chronic MICT attenuated the impaired cardiac diastolic function and prevented the imbalance of myocardial oxygen consumption in miniature male Yucatan miniature swine with left ventricular (LV) hypertrophy [89]. After 15 weeks MICT with increasing intensity training, the animal presented decreased fibrosis, strengthened myocardial mitochondrial function and coronary artery function. Their data revealed that the MICT exercise preserved extracellular matrix regulatory mechanisms. The physiological molecular hypertrophic signaling pathway including JNK/SAPK signal pathway were activated against HF while the animals performed the MICT exercise. [89].

In addition, metabolic syndrome, including obesity and insulin resistance, are chartered by adipose inflammation which are cardiovascular risk factors. An 8-week MICT (15 m/min, 40 min/day, 5 day/week) decreased high-fat diet (HFD)-induced metabolic disorders of male C57BL/6 mice abdominal fat contents (from total abdominal mass to visceral fat and subcutaneous fat). The inflammation, enhanced glucose tolerance, and insulin resistance were approaching normal physiologic conditions with improvement of vascular function [92].

5. Suggested medium-term exercise procedure required more inspection

Little researches output considerably attention on the preceding time of the full-scale for animal exercise-training recently. When projects were conceived, > 56% (25 out of 45) of the projects carried out their experiments under for weeks based on published references that we categorized as Shor-term procedure. The function of the exercise was inspected on four week training animal model. About 47% (21 out of 45) of the projects performed their study between eight to twelve weeks, which we defined as Long-term procedure. Therefore, 10% (10 out of 45) of projects accordingly left within the preceding time of the full-scale among five to ten weeks. Based on our continuous recording EKG of mouse model for up to twelve weeks, RR interval extremely increased along with decrease of heart beat compared to their date from the beginning to end of 12-weed documentation.

Perceptibly, to comprehend the molecular mechanism of exercise on human health, we should not left such large time-gap on the animal training model. Otherwise, massive information that we can benefit from animal model would be abandoned without conspicuous.

6. Classification of short-term exercise models and their application in CVD

The most common short-term exercise models are exercise preconditioning (EP) models. This model is one of the short-term exercise models for studying the protective effect of exercise against cardiac I/R injury in rat and dog [93–97]. The procedures in short-term exercise models can be cycled from one session up to total 4 weeks exercise training time after 3-day to 2-week adaption training period (Table 1). The speed and intensity are different depending on animal species. The EP protocols maintain short-term and repeated high intensity exercise which cause transient ischemic and hypoxia in the heart, and induce endogenous cardio-protection against heart injury. Many evidences prove that EP can attenuate heart injury with two distinct phases of heart protection by EP [98–100]. The first phase happens immediately after the exercise and another phase occurs 24 h post-exercise (dogs) [101,102].

6.1. Short-term exercise mimics ischemic preconditioning and directly promotes cardio-protection mechanism

Short-term exercise could render the heart more tolerant and prevent the subsequent ischemic injury. After two weeks of adaption at 20–25 m/min for 30 min/day performing treadmill exercise, at 27–30 m/min for 25–30 min before I/R, the Male Wistar rat exhibited a significant reduction of the myocardial infarct area. This momentous reduction was accompanied with the increase of reactive oxygen species and endogenous activation of antioxidant system [103].

In another study, under the condition of five cycles of 5 min at 6 km/h with intervening 5-min rests, the early-induced exercise and late preconditioning of the infarct size was mediated through mitochondrial ATP-sensitive potassium channels on mongrel dogs [95,101]. When Shen et al. investigated the early cardioprotective effect on the exhaustive exercise-induced myocardial injury in rats, the high expression level of PKCδ and p-PKCδThr507 was significantly reduced in order for the short-term exercise to initiate an effect on cardio-protection [104].

6.2. Short-term low intensity aerobic exercise is associated with cardiac allograft success and protection against cardiopathology of obesity

Many studies have reported that short-term low-intensity aerobic exercise models show protective effects on the heart [105–108]. Uchiyama et al. reported that one-week postoperative treadmill exercise may induce hypo-responsiveness to cardiac allograft. In their study, the CBA (H2k) mice were set on 1-h treadmill exercise per day at speeds of 9.6 and 12.8 m/min. Mortality decreased significantly when CBA allograft recipients were subjected to the exercise for one week before and after transplantation. They claimed that the

short-term exercise increased the regulatory T cells population with $CD4^+CD25^+Foxp3^+$ antigens, changing the balance of Th-1 cytokines (IL-2 and IFN-gamma) and Th-2 cytokines (IL-4 and IL-10), and further inducing temporary immunosuppression [107].

Obesity related cardiac syndrome is usually accompanied with dysregulation of kinase signaling pathways in myocardium infarction. Four-week short-term treadmill exercise (6–18 m/min, 60 min/day, 5 days/week, 4° slope) increased the ability to protect the myocardium against ischemia with exercise increased kinase phosphorylation. The decreased levels of phosphatases and increased resistance of mPTP opening promoted the ability of the exercise to protect the myocardium against ischemia on obese (ob/ob) mice [108].

In addition, the exercise intensity is 4 to 8 min high-intensity treadmill running bouts/session/cycles at 85–90% of VO_{2max}. The running protocol is achieved by speeds of > 30 m/min on a 25° inclined treadmill for 4 weeks. The 2-min low-intensity intervals (about 50% of VO_{2max}) are dispersed in the exercise training. These short-term high-intensity interval exercise models can induce physiological hypertrophy of the heart and enhanced heart function in female Sprague Dawley rats [43].

6.3. Prolonged and exhaustive exercise as estimates of exercise capacity and intolerance

Evaluating physical activity on pathological status of the patients who are at risk of CVD, MI, and even SCD is necessary, but experimentally difficult. The biomarkers of cardiac injury in healthy persons and risky patients can be obtained from animal models using prolonged and exhaustive exercise. To reach this study goal, experimental animals have been sacrificed for human health.

The animal exhaustive exercise procedures in the experiment are not only used to estimate exercise capacity and intolerance, to develop biomarkers in these diseases, but also utilized to diagnose metabolic abnormalities and muscle dystrophy related diseases [109–111]. In these procedures, animals first are obtained from a few days to 2-week adaption training and followed by exhaustive session.

Two exercise procedures are set to carry on the above research aims. One is voluntary wheel running recording 60 min of activity for 3 days to mimic a 6-min walk test in humans [112–114]. The other is a treadmill exercise test with speeds increased from 6 to 18 m/min, accelerated at 1 m/min^2 until fatigue or after 2.5 h of running [112].

Using the regular exercise procedures, the patients with peripheral artery disease had an exercise intolerance indicated by muscle pain [26,115–117]. However, the HFD mice with AMPK activation augmented exercise performance and peripheral vascular insufficiency [112,118,119]. These interesting data show that focusing AMPK signaling and connected pathway may develop a biomarker for early diagnostics.

Utilizing exhaustive exercise plus epinephrine treatment, research group in Columbia University mimicked emotion stress on FKBP12.6 deficient mouse model and successful linked calcium release channel Ryanodine Receptor (RyR) to exercise-induced sudden cardiac death [49] and further approved that protein calstabin2 functions as cardiac

protection from cardiac arrhythmia by through stabilizing cardiac RyR [50–52]. However, intense endurance exercise suspiciously resulted in atrial arrhythmia caused by cardiac arrhythmogenic remodeling [65,66,70] accompanying with fibrosis pathology [71]. Longstanding support from China government has granted the excellent accomplishment in Olympics each year and recently athletes' injuries on their hearts caught researchers' attention. Using animal models, long-term intensity endurance exercise can generate cardiac arrhythmia and cardiac sudden death associated with myocardial remodeling. Extracellular matrix proteins including collagen I, Col-III, MMP-1/TIMP-1 along with CTGF play critical role in endurance exercise-associated remodeling [120–124]. The endurance exercise induced cardiac arrhythmia are related with the involvement of TGF-b1/miR-21 [122] and experience the autophagy pathologic pathway marked with expression alternation of Beclin1 and LC3 [123,124].

7. Challenges of translating animal exercise models to clinical practice

While translating the data obtained from animal exercise models to clinical applications, multiple subjects still require further investigation. Several subjects may seem too minute to pay any attention, but additional characterization is indeed necessary for translation.

7.1. Data conflicting challenges the translation from animal to human health

Regardless of the short-term or long-term exercise models, it is critical to determine how long the acquired cardio-protection is maintained after the exercise detraining. Lennon et al. reported that the short-term exercised model (30 m/min, 60 min/day, and 3 days) maintained the improved cardioprotection at 1, 3, and 9 days post-exercise. At these three timing-points plus18 days after exercise cessation, the exercise-promoted cardioprotection were detectable in global I/R Sprague-Dawley rats [125]. In the long-term exercise model, the cardioprotection maintained for much longer. For instance, that 10-week exercise could preserve the exercise-promoted cardioprotection for 4 weeks after detraining [126]. Up to date, only actual limited studies can be referenced with no detailed molecular mechanism.

As discussed above, the high-intensity interval exercise models induced cardiac physiological hypertrophy of the heart in C57BL/6J mice [127]. The six weeks of treadmill MICT induced marked hypertrophy in the wild type mice involved with p21-activated kinase-1 (Pak1) regulated exercise-induced cardiac hypertrophy [128]. The others disputed that the five-day treadmill running per week for 40 min at a speed of 24 m/min for eight weeks did not induce cardiac hypertrophy in male 129 SvJ/C57BL6 mice [129]. Several explanations could be used to explain the conflicting results among these researchers including animal species and strain differences, exercise protocol alterations, and life-span.

7.2. Comprehending life-span of different animals and age index

Considering different animals normally used for exercise training procedures includes mouse, rat, dog and swine, we equitably introduce a concept of Age Index for comprehensive understanding the information obtained from animals. Age Index of different species (Table 2) could be another important factor when applying the animal exercise models to clinics because the week number in difference animal species means different

fraction of their life span. If using life-span relative exercise time ($RET_{life\text{-}span}$), short-term exercise and long-term exercise can be categorized as the $RET_{life\text{-}span}$ smaller than ≤4, and the $RET_{life\text{-}span}$ larger than ≥6. The medium-term exercise can be set the $RET_{life\text{-}span}$ between 4 and 6. Therefore, protection function of the long-term exercise for large animals such as swine with life-pan of 624 weeks should be considered equal to small animal such as mouse with life-pan of 78 weeks. For swine, 15-week exercise should be considered in the category of short-term exercise according to its life-span relative exercise time (1.78).

7.3. Genetic background along with race, aging and gender on exercise-promoted cardioprotection

Apparently, since Africa athletes owning prestigious talents on sport are well recognized circumstance, it reasonable to consider that this difference of genomic background on human [131,132] should be replicated in animals. Many animals were employed to investigate exercise-promoted cardioprotection as we described above, but few publications discussed the intra-species diversity on exercise training. The only source of this intra-species can be recognized from gene modified mice.

It is reasonable that animal strain difference can exhibit dramatic characteristics on phenotype such as FKBP12.6 convenient knockout mice generated from mouse 129/SvEv/C57BL strain in Vanderbilt University [130] and mouse DBA/1lacJ/C57BL strain (brown coat color) in Columbia University [49]. It is most likely that the genomic background could be the root cause of the two dramatically different cardiac phenotypes [49,130]. Concerning from the results, we would like to speculate that, based on this phenomenon, the exercise data obtained from 129/SvEv/C57BL strain and DBA/1lacJ/C57BL would somehow reflect the possible difference from intra-species in human community. Currently, circulating cell-free DNA seems lighting-up an imperturbable approach to link genomic inspection to physical activity [133] but it need further investigation.

Interestingly, the FKBP12.6 knockout mice with 129/SvEv/C57BL background also exhibited sex-associated cardiac hypertrophy defect only in male mice but not in females, and this sex associated also can be protected by Oestrogen in FKBP12.6 null mice [130]. This interesting result encourages the research subject by utilizing animal exercise training model to investigate the related molecular mechanism of the exercise-promoted cardiovascular protection involved with sex associated cardiac diseases.

There is no doubt about that exercise can benefit health including aging population. Impropriate physical training could induce large spectrum of ventricular fibrillation [134] and the modest exercise could not promote protection on stress-induced tachycardia [135]. Therefore, what exercise program composed of appropriate combination of intensity, modality, frequency, and duration of exercise should be chosen as the intrinsic part of aging cohort still remain unknown largely. Recently, aged spontaneous hypertensive rats at age of 80 weeks old (corresponding to 76 years old patient according to the Age Index showed in Table 2) were used to inspect the effect of long-term exercise on ventricular tachycardia (VT) and ventricular fibrillation (VF). The data approved that the exercise were able to decrease VF incidence with increase exercise intensity by compensating action potential [136]. As one of the most critical properties of electrical propagation dynamics in cardio-

physiology, this compensation promoted by long-term exercise protected aged rats from lethal VT and VF. It is the matter of time to catch on an efficient program for aging population using animal exercise model.

8. Conclusions and prospects

With high morbidity and high mortality characteristics of CVD such as heart ischemia or CAD, physical activity such as exercise training is commonly regarded as an effective intervention to improve cardiovascular function. However, inappropriate exercises could lead to unexpected death during exercise especially for CVD at risk individuals and even healthy people. Thus, this situation makes animal exercise models powerful tools to evaluate various exercise programs or procedures to find the molecular mechanism behind designing individualized exercise programs based on their risk assessment of CVD.

Exercise training procedures normally take into account intensity, modality, frequency, and duration of exercise through four categories as shown in Fig. 1. Modality of exercise, the intensity and frequency of the certain modality, along with duration of the exercise, are all critical factors for a successful program. Among many animal exercise programs, long-term MICT maintained at 75% Vo_{2max} is an effective program revealing that the exercise function decreased the infarct size of myocardial ischemia, preventing CAD and SCD, and unveiling the molecular mechanism of the prevention associate with Kir6.1, VSMC sarc-K_{ATP} channels and pulmonary eNOS. The short-term low intensity aerobic exercise plays an incredible role in recovering cardiac allograft and obese cardiopathology accompanied with dysregulation of kinase signaling pathways in myocardium infarction. Short-term exercise also can be used to mimic ischemic preconditioning and further to investigate molecular mechanism exercise promoted cardio-protection. The animal exhaustive exercise procedures are used to estimate capacity and intolerance of physical activity; however, this data is rarely collected from patient samples.

To translate data from animal exercise models to clinical applications, many molecular mechanisms still need to be investigated further. Researchers still face significant challenges in how they will match data gained from animal exercise to patients, how they will synchronize the data obtained from different animals, and how they will finalize the most efficient programs for both healthy and cardiovascular at-risk individuals. Animal exercise models will be the most potential technique for their molecular mechanism.

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Abbreviations

ACC	American College of Cardiology
AHA	American Heart Association

BP	blood pressure
CAD	coronary artery disease
CPET	cardiopulmonary exercise testing
CRF	cardiorespiratory fitness
CVD	cardiovascular disease
DOX	doxorubicin
EKG or ECG	Elektrokardiogramm or Electrocardiography
EP	exercise preconditioning
EST or ST	Exercise stress testing
ETT	Electrocardiographic exercise test
EX	exercise training
HF	heart failure
HFD	high-fat diet
HR	heart rate
I/R	ischemia/reperfusion
LV	left ventricular
MET	metabolic equivalent
MI	myocardial ischemia
MICT	moderate-intensity continuous training
NO	nitric oxide
PA	physical activity
PAD	peripheral arterial disease
$\mathbf{RET_{life\ span}}$	Life-Span Relative Exercise Time
RyR	Ryanodine Receptor
SCD	sudden death
VF	ventricular fibrillation
VT	ventricular tachycardia
$\boldsymbol{VO_2}$	oxygen uptake

VO_{2max}	maximal oxygen consumption, maximal oxygen uptake, peak oxygen uptake or maximal aerobic capacity

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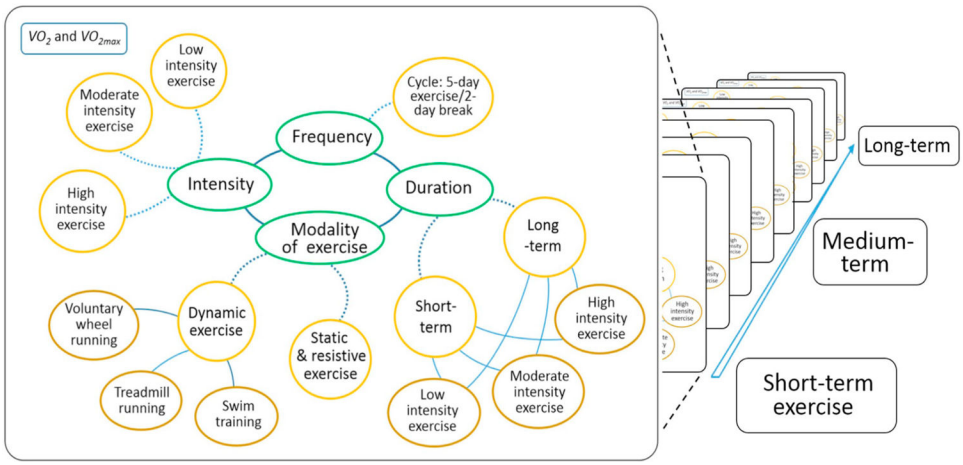

Fig. 1.

Web of exercise modality classifications.

Fundamental elements of an applied exercise protocol should include intensity, modality of exercise, frequency and duration. According to different intensity of exercise, exercise can be divided into high, moderate, and low intensity exercise. According to the modality of exercise, exercise can be divided into dynamic, static, and resistive exercise. According to the duration of exercise, exercise can be divided into short, medium, and long term exercise. All of these elements interact with each other and form a training protocol.

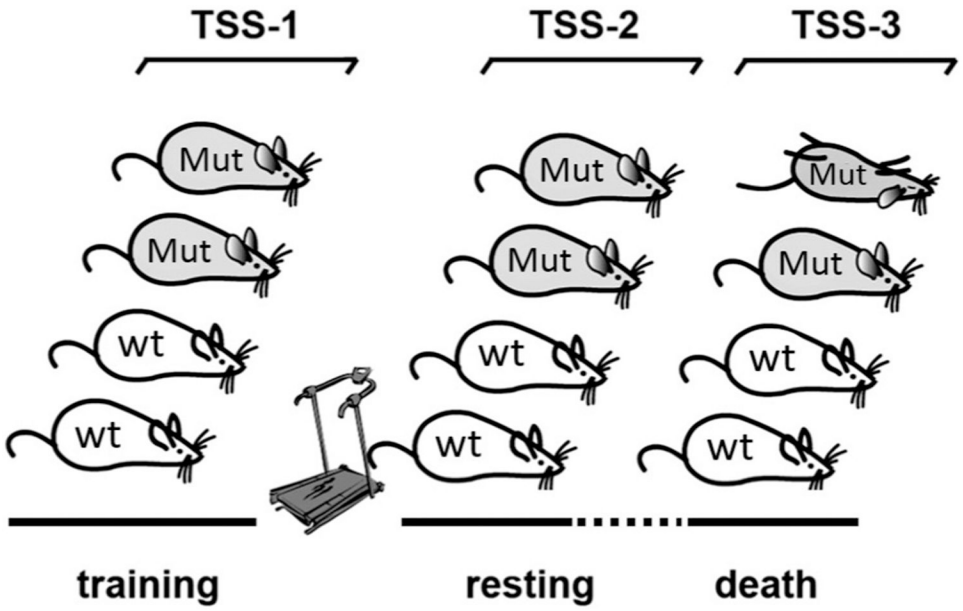

Fig. 2.

Phases of exercise training.

Exercise training should include the following phases. In Phase I, the animals should learn to adapt to their exercise environment and learn to run on the treadmill. In Phase II, the animals finish their daily training at designed intensity. In Phase III, the animals need to restore physical energy after daily training. For exhaustive exercise, one needs to design an extra Phase IV. The Phase IV includes a high intensity exercise protocol. TSS is short for Timing Scale Sampling. TSS-1, TSS-2 and TSS-3 are the three stages for sample harvesting corresponding to training stage, resting stage and death occurring stage for some gene modification animal models.

Table 1

The characteristics of various exercise animal models applied in protection mechanism of cardiovascular diseases.

Types	Species	Adaption*	Cycle	Modality	Speed/intensity	Inclination	Stimulation	Material collection	References
Short-term (<4 weeks)	Dog (Mongrel)	Yes	One session	Treadmill	5 periods, 5 min of 6 km/h with intervening 5 min periods of rest	dnp	dnp	dnp	Parra et al. [84] Domenech et al. [90] Parra et al. [91]
	Rat (Wistar)	Yes	One session	Treadmill	25–30 min 27–30 m/min	dnp	dnp	48 h after reperfusion	Yamashita et al. [92]
	Rat (Sprague-Dawley)	Yes	One session	Treadmill	4 periods of 10 min each at 30 m/min with intervening 10 min periods of rest	0%	dnp	dnp	Hao et al. [89] Shen et al. [93]
	Rat (Sprague-Dawley)	Yes	One session 45 min	Treadmill	75% VO_{2max}	dnp	dnp	dnp	Brown et al. [70]
	Rat (Sprague-Dawley)	Yes	One session 3 h	Swimming	Flexible	dnp	dnp	2 h and 24 h after exercise	Nie et al. [99]
	Rat (Sprague-Dawley)	Yes	60 min/day 3 days	Treadmill	30 m/min, 3 spaced 2 or 3 min rest	0%	Electric	dnp	Lennon et al. [109]
	Rat (Sprague-Dawley)	Yes	60 min/day 10 days	Treadmill	30 m/min	0%	dnp	dnp	Morton et al. [11]
	Rat (Sprague-Dawley)	Yes	60 min/day 10 days	Treadmill	15 m/min for 15 min, 30 m/min for 30 min, 15 m/min for 15 min	6%	dnp	24 h after exercise	Frasier et al. [85] Frasier et al. [86]
	Rat (Wistar)	Yes	30 min/day 3 weeks	Treadmill	10–20 m/min	0–5%	dnp	dnp	Scalzo et al. [45]
	Rat (Sprague-Dawley)	Yes	30 min/day 6 days/ week 3 weeks	Treadmill	15 m/min	0%	electric	dnp	Zhu et al. [83]
	Rat (Wistar albino)	dnp	90 min/day 4 weeks	Swimming	Flexible	dnp	dnp	dnp	Sharma et al. [82]
	Rat (Sprague-Dawley)	Yes	120 min/day 5 days/week 4 weeks	Treadmill	8 and 2 min intervals at 85–90% and 50–60% of VO_{2max}	25%	dnp	dnp	Wisloff et al. [43]
	Mouse (DBA/1lacJ)	dnp	One session	Treadmill	Exhaustive exercise	dnp	dnp	dnp	Wehrens et al. [49] [50]
	Mouse (C57BL/6 J)	Yes	One session	Treadmill	5 m/min for 5 min, 10 m/min for 5 min; Speed was	0%	Electric	dnp	Ito etal. [100]

Types	Species	Adaption*	Cycle	Modality	Speed/intensity	Inclination	Stimulation	Material collection	References
					increased 1 m/min every 30 s				
	Mouse (C57BL/6)	Yes	75–80 min/day 3 days	Voluntary running wheel	Flexible	dnp	dnp	dnp	Baltgalvis et al. [101]
	Mouse (C57BL/6)	dnp	1 week	Voluntary running wheel	Flexible	dnp	dnp	dnp	Baum et al. [10]
	Mouse (C57BL/6 J and CBA)	Yes	60 min/day one week	Treadmill	9.6 and 12.8m/min	5%	no	dnp	Uchiyama et al. [96]
	Mouse (C57BL/6 J)	Yes	90 min/day twice daily 3 weeks	Swimming	Flexible	dnp	dnp	dnp	Bellinger et al. [51]
	Mouse (129 and C57BL6)	dnp	4 weeks	Voluntary running wheel	Flexible	dnp	dnp	dnp	Do et al. [47]
	Mouse (C57BL/6 J)	Yes	60 min/day 5 days/ week 4 weeks	Treadmill	6–18m/min	4%	dnp	24 h after exercise	Pons et al. [97]
Suggested Medium-term (4–8 weeks)	Rat (Sprague-Dawley)	dnp	60 min/day 5 days/ week 5 weeks	Swimming	Flexible	dnp	dnp	dnp	McElroy et al. [66]
	Rat (Sprague-Dawley)	Yes	30 min/day 5days/week 6 weeks	Treadmill	10m/min, 20m/ min, 25 m/min, 30 m/min, 35 m/min, 40 m/min with the increasing week	0	dnp	48 h after exercise	Wang Xueqin et al. [63]
	Rat (Sprague-Dawley)	Yes	60 min/day 6 weeks	Treadmill	75% VO_{2max}	dnp	dnp	after the 45 min run/ rest period	Brown et al. [17]
	Mouse (C57BL/6)	dnp	6 weeks	Voluntary running wheel	Flexible	dnp	dnp	dnp	Baum et al. [10]
	Mouse (C57BL/6)	Yes	30 min/day 5days/week 6 weeks	Treadmill	15m/min	dnp	dnp	dnp	Meilhac et al. [18]
	Mouse	Yes	60 min/day 5days/week 6 weeks	Treadmill	55–60% VO_{2max}	0.15	dnp	dnp	Davis et al. [112]
	Mouse (C57BL/6)	Yes	90 min/day 5days/week 6 weeks	Treadmill	85–90% VO_{2max}	0.25	dnp	24 h after exercise	Kemi et al. [111]
	Mouse (ICR)	dnp	40 min/day 6 days/ week 6 weeks	Treadmill	15m/min, 22m/ min, 27 m/min, 31 m/min, 38 m/min with the increasing week	dnp	dnp	24 h after exercise	Cui et al. [64]

Types	Species	Adaption*	Cycle	Modality	Speed/intensity	Inclination	Stimulation	Material collection	References
	Mouse (C57BL)	dnp	30–120 min/day 6 weeks	Treadmill	21 m/min	30%	Electric for the first 3–4 sessions	24 h after exercise	Aschar-Sobbi R et al. [65]
	Mouse (C57BL)	dnp	30–90 min/day twice daily 6 weeks	Swimming	Swimming training began with 30 min swimming sessions that were increased to 90 min by adding 10 min per day	dnp	dnp	24 h after exercise	Aschar-Sobbi R et al. [65]
Long-term (> 8 weeks)	Swine (Yucatan miniature)	dnp	55 min/day 3 days/wk. 15 weeks	Treadmil	5 min at 3 mph 4 min at 4 mph	dnp	dnp	dnp	Marshall et al. [78]
	Swine (Yucatan miniature)	Yes	60 min/day 16–20 weeks	Treadmill	4–5 mph	0%	dnp	dnp	Hinken et al. [44]
	Rat (Wistar Kyoto)	dnp	60 min/day 5 days/ week 4 weeks	Treadmill	36 m/min	dnp	Electric	72 h after exercise	Benito B et al. [66]
	Rat (Sprague-Dawley)	Yes	60 min/day 5 times/ week 8 weeks	Swimming	a load attached to the body	dnp	dnp	24 h after exercise	Li jun [67]
	Rat (Sprague-Dawley)	Yes	60 min/day 5 days/ week 8 weeks	Treadmill	7 and 3 min intervals at 85–90% and 50–60% of VO_{2max}	dnp	dnp	24 h after exercise	Jia dandan et al. [68]
	Rat (Wistar Kyoto)	dnp	60 min/day 5 days/ week 8 weeks	Treadmill	16–18 m/min (55%–65% VO_{2max}	dnp	dnp	dnp	Chen Yu et al. [69]
	Rat (Wistar Kyoto)	dnp	60 min/day 5 days/ week 8 weeks	Treadmill	28 m/min	dnp	dnp	dnp	Guasch et al. [70]
	Rat (Wistar Kyoto)	dnp	60 min/day 5 days/ week 8 weeks	Treadmill	36 m/min	dnp	Electric	72 h after exercise	Benito B et al. [66]
	Rat (Wistar)	Yes	5 days/ week 25 min/day 7 weeks	Treadmill	27 m/min	5%	dnp	dnp	Gorres-Martens et al. [16]
Long-term (> 9 weeks)	Rat (Wistar)	Yes	60 min/day 5 days/	Treadmill	60% VO_{2max}	0%	dnp	48 h after exercise	Rodrigues et al. [60]

Types	Species	Adaption*	Cycle	Modality	Speed/intensity	Inclination	Stimulation	Material collection	References
			week 8 weeks						
	Rat (Sprague-Dawley)	Yes	60 min/day 3 times/ week 10 weeks	Treadmill	30 m/min	0%	dnp	24 h after exercise	Esposito et al. [110]
	Rat (Sprague-Dawley)	Yes	4 days/ week 10 weeks	Treadmill	75–80% VO_{2max}	dnp	Electric	42–48h after exercise	Powers et al. [62]
	Rat (Wistar Kyoto)	Yes	60 min/day 5 days/ week 16 weeks	Wheel, Nonvoluntary running	1 rev/min for 30 s, speed increased every 30 s by 2 rev/min until exhausted	dnp	dnp	dnp	Zhang et al. [9]
	Rat (Wistar Kyoto)	dnp	60 min/day 5 days/ week 16 weeks	Treadmill	28 m/min	dnp	dnp	dnp	Guasch et al. [70]
	Rat (Wistar Kyoto)	dnp	60 min/day 5 days/ week 16 weeks	Treadmill	36 m/min	dnp	dnp	72 h after exercise	Gay-Jordi G et al. [71]
	Rat (Wistar Kyoto)	dnp	60 min/day 5 days/ week 16 weeks	Treadmill	36 m/min	dnp	Electric	72 h after exercise	Benito B et al. [66]
	Rat (Sprague-Dawley)	Yes	60 min/day 5 days/ week 20 weeks	Treadmill	20–35 m/min	10%	dnp	dnp	Brown et al. [68]
	Mouse (129, C57BL6 and FVB)	dnp	8 weeks	Voluntary running wheel	Flexible	dnp	dnp	dnp	Grassi et al. [46] Do et al. [47] van Deel et al. [77]
	Mouse (B6/SJL)	dnp	180 min/day 8 weeks	Voluntary running wheel	Flexible	dnp	dnp	dnp	Choi SH et al. [72]
	Mouse (C57/B6)	Yes	40 min/day 5 days/ week 8 weeks	Treadmill	15 m/min	dnp	dnp	48 h after exercise	Xu et al. [81]
	Mouse (129 SvJ and C57BL6)	Yes	40 min/day 5 days/ week 8 weeks	Treadmill	24 m/min	dnp	dnp	dnp	Han GS [113]

Types	Species	Adaption*	Cycle	Modality	Speed/intensity	Inclination	Stimulation	Material collection	References
	Mouse (C57/B6)	Yes	45 min/day 5 days/ week 8 weeks	Treadmill	18m/min	0%	dnp	48 h after exercise	Dolinsky et al. [67]
	Mouse (C57/B6)	Yes	50 min/day 5 days/ week 8 weeks	Treadmill	17m/min	0%	dnp	dnp	He et al. [61]
	Mouse (C57BL/6 J)	Yes	60 min/day 5 days/ week 8 weeks	Treadmill	60% VO_{2max}	0%	dnp	48 h after exercise	Rodrigues et al. [59]
	Mouse (C57BL/6 J)	Yes	120 min/day 5 days/week 8 weeks	Treadmill	8 min at 85–90% VO2max; 2 min at 50–60% VO_{2max}	dnp	Electric	dnp	Kemi et al. [42]
	Mouse (C57/B6)	Yes	40 min/day 3 times/ week 12 weeks	Treadmill	15m/min	0%	dnp	dnp	Li et al. [69]
	Mouse (SAMP8)	Yes	40 min/day 3 times/ week 12 weeks	Treadmill	10 m/min for 10 min; 15 m/min for 30 min	dnp	no	24 h after exercise	Li Xin et al. [73]
	Mouse (C57BL/6)	Yes	30 min/day 5 days/ week 12 weeks	Treadmill	15m/min	dnp	dnp	dnp	Meilhac et al. [18]

Notes: “dnp” represents that the data is not provided in the article.

“*” indicates 3-day to 2-week adaption training period based on competency of animals.

“VO_{2max}” represents maximal oxygen uptake.

Table 2

Short-, medium- and long-term exercises in various animal models categorised by age index.

	Animal life span (weeks)	Age index (weeks/weeks)	Short-term exercise		Medium-term exercise		Long-term exercise	
			$RET_{life\text{-}span}$ ($\times 10^2$)	Exercise weeks	$RET_{life\text{-}span}$ ($\times 10^2$)	Exercise weeks	$RET_{life\text{-}span}$ ($\times 10^2$)	Exercise weeks
Swine (Yucatan miniature)	624	0.16	≤0.25	≤4 weeks	> 0.25 to ≤0.49	> 4 to ≤8	> 0.49	> 8 weeks
Dog (Mongrel)	624	0.16	≤0.25	≤4 weeks	> 0.25 to ≤0.49	> 4 to ≤8	> 0.49	> 8 weeks
Rat (Sprague-Dawley)	78	0.02	≤1.97	≤4 weeks	> 1.97 to ≤3.95	> 4 to ≤8	> 3.95	> 8 weeks
Mouse (DBA/1lacJ)	78	0.02	≤1.97	≤4 weeks	> 1.97 to ≤3.95	> 4 to ≤8	> 3.95	> 8 weeks

Notes: "Life-Span Relative Exercise Time" short for $RET_{life\ span}$ represents the duration of training time in the life cycle of humans. The human life is calculated as 74 years corresponding to 3848 weeks. Age Index = Animal Life spam (weeks) / Human life (3848 weeks); $RET_{life\ span}$ = Exercise time (weeks) / Age Index.