EXERCISE PHYSIOLOGY AND ITS ROLE IN CHRONIC DISEASE PREVENTION AND TREATMENT - MECHANISMS AND INSIGHTS

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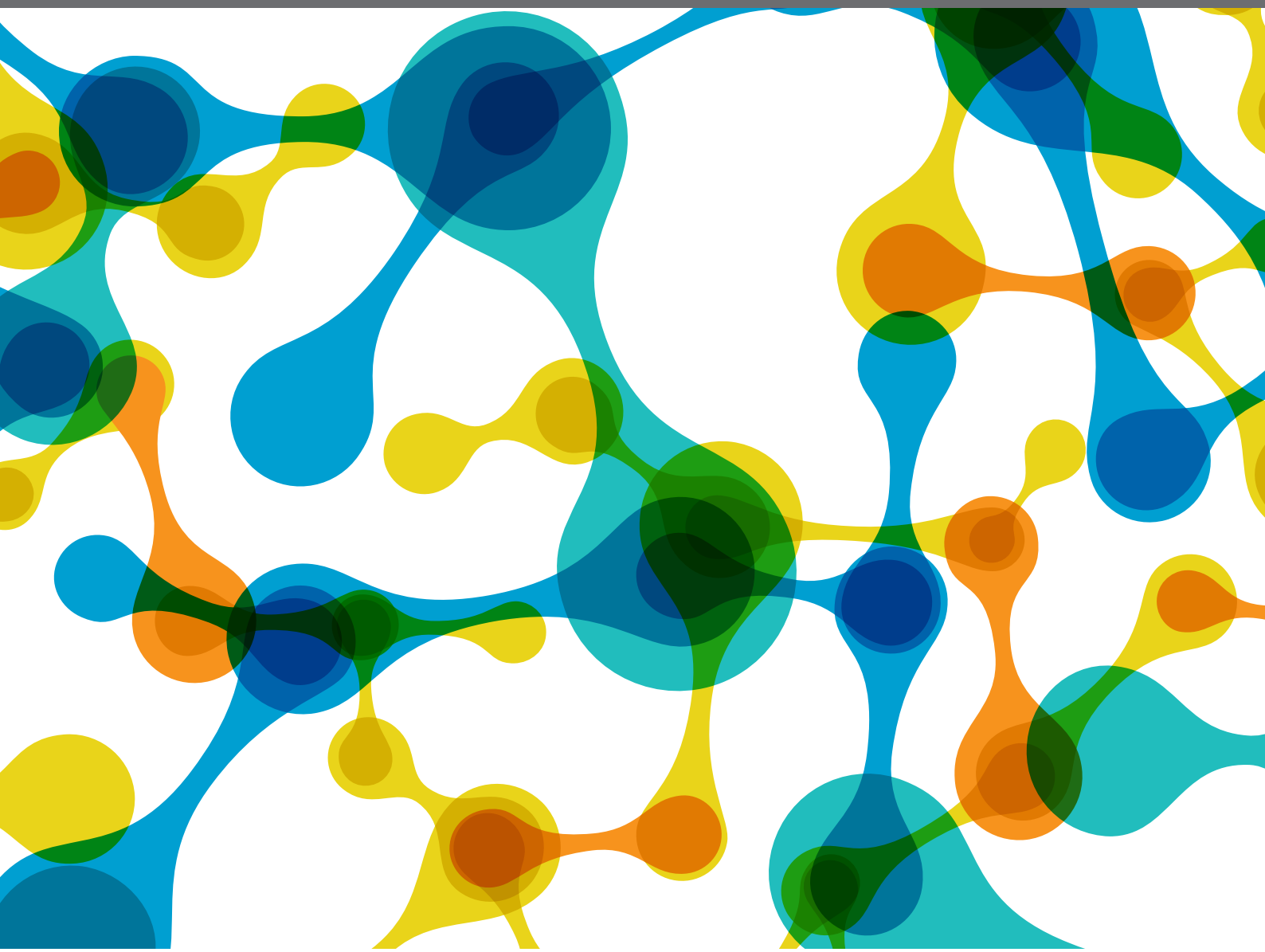

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EXERCISE PHYSIOLOGY AND ITS ROLE IN CHRONIC DISEASE PREVENTION AND TREATMENT - MECHANISMS AND INSIGHTS

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Editorial: Exercise physiology and its role in chronic disease prevention and treatment —mechanisms and insights

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physical activity, exercise, training, chronic diseases, health

Editorial on the Research Topic
Exercise physiology and its role in chronic disease prevention and treatment –mechanisms and insights

Introduction

The performance of regular physical exercise offers protection against all-cause morbidity and mortality, and prevents t atherosclerosis, type 2 diabetes, colon, and breast cancer (Eyre et al., 2004). In addition, exercise training is effective in the treatment of patients with ischemic heart disease, heart failure, and chronic obstructive pulmonary disease (Borghi-Silva et al., 2021). Moreover, sufficient physical activity according to the World Health Organization (150-300 min per week) has several benefits on psychiatric conditions/diseases (depression, anxiety, stress, schizophrenia) and neurological diseases (dementia, Parkinson's disease, multiple sclerosis) (Pedersen & Saltin, 2015). Evidence-based treatment is the preferred therapeutic approach and provides the most effective management strategy entailing the fewest side effects or risks. Hence, physical exercise is considered a part of a healthy lifestyle, and an important extension of medical treatment and health care (Warburton et al., 2006).

Little is known about the psycho-physiological mechanisms involved in the health benefits of physical exercise, including a lack of knowledge on the effective dosage of programming parameters according to the FITT principle (frequency, intensity, time and type of exercise) (Pedersen & Saltin, 2015; Saeidi et al., 2021; Zouhal et al., 2021;

Hortobágyi et al., 2022). As such, further research is needed to determine which exercise type is more effective for the treatment of various chronic diseases and to explore the underlying physiological mechanisms of exercise in chronic diseases such as psychiatric diseases (depression, anxiety, stress, schizophrenia), neurological diseases (dementia, Parkinson's disease, multiple sclerosis), metabolic diseases (obesity, hyperlipidemia, metabolic syndrome, polycystic ovarian syndrome, type 2 diabetes, type 1 diabetes), cardiovascular diseases (hypertension, coronary heart disease, heart failure, cerebral apoplexy, and claudication intermittent), pulmonary diseases (chronic obstructive pulmonary disease, asthma, cystic fibrosis), musculoskeletal disorders (osteoarthritis, osteoporosis, back pain, rheumatoid arthritis), and several types of cancers. In an attempt to clarify the effects of physical exercise on chronic diseases, the Frontiers Research Topic entitled "Exercise Physiology and its Role in Chronic Disease Prevention and Treatment - Mechanisms and Insights" was launched. Accordingly, the aim of this Research Topic was to provide evidence in the form of original research and or review articles on the protective effects of physical exercise on the treatment of chronic diseases in humans and animals.

Summary of selected articles from this research topic

Twenty-nine manuscripts were received for this Frontiers Research Topic. After rigorous review, 16 articles were finally accepted for publication. The contributing 80 authors were from 13 countries and four continents, including Canada, China, France, Germany, India, Iran, Japan, Lebanon, Portugal, Tunisia, Turkey, United Kingdom, United States of America. This Research Topic received more than 38,000 views and downloads as of August 2022. The key contents and findings of each paper are as follows:

The study by Gomarasca and colleagues examined the benefits of Nordic exercise (also known as pole walking), which combines moderate levels of cardiovascular exercise with increased muscle activity of the arms, legs, and upper body, on whole body inflammation in elderly women (mean age: 68 years old). Blood levels of inflammatory markers were measured using RT-qPCR before and after 12 weeks of Nordic exercise. Nordic exercise caused modest reductions in the resting levels of the expression of inflammatory markers in both normal and overweight elderly women.

The literature review by Wu et al. summarizes the potential benefits of exercise on cardiovascular, renal, neurological, and pulmonary function in sepsis. The findings, largely based on experimental models of sepsis, suggest that exercise improves outcomes in sepsis by augmenting mitochondrial quality and biogenesis, attenuating inflammation, recovering redox balance, and restoring the health of the gut microbiome.

The potential effectiveness of yoga therapy in managing type 2 diabetes was explored in a systematic review of 13 studies (1,335 patients) by Chen et al. There were significant benefits of short-term yoga (10-24 weeks) on HbA1c, fasting blood glucose, postprandial blood glucose, total cholesterol, triglyceride, and BMI levels, suggesting that yoga may be a useful adjuvant therapy in the usual clinical management of uncomplicated type 2 diabetes.

Wang and colleagues examined the potential benefits of low-intensity aerobic exercise in high-fat diet-associated pulmonary fibrosis in C57BL/6 mice. Aerobic exercise improved obesity-related pulmonary fibrosis, chronic inflammation, and insulin resistance following 16 weeks of a high-fat or chow diet followed by 8 weeks of exercise training.

The narrative review by Zeng et al. summarized symptoms related to knee osteoarthritis and the therapeutic benefits of different exercise types (e.g., aerobic, resistance, neuromuscular training) on patients with knee osteoarthritis. The reported outcomes are specific to the exercise type under investigation. Accordingly, individualized exercise prescriptions are needed.

The study by Li and colleagues investigated the dosage effects of single bouts of high-intensity interval training on brain-derived neurotrophic factor (BDNF) and vascular endothelial growth factor-A (VEGF-A) and cognitive function in healthy young men. Twenty minutes of exercise improved BDNF and VEGF-A as well as cognitive function.

Saedi and colleagues examined the differential effects of high-intensity interval training (HIIT), circuit resistance training (CRT), and moderate-intensity continuous training (MICT) on neuregulin 4 in young men with obesity. HIIT and CRT protocols had greater benefits on blood neuregulin 4 levels, metabolic and cardiovascular risk factors, and body composition.

The systematic review and meta-analysis by Zouhal and colleagues evaluated the influence of exercise training on bone health indices in obese individuals. Their findings, based on 10 studies (889 initial records, 8 countries, 263 participants), suggest that physical exercise has little to no effect on the whole-body bone mineral density in individuals with overweight/obesity.

The narrative review by Razi and colleagues summarized the neuro-invasive properties of SARS-CoV-2 and the possible pathways for the entry of the virus into the central nervous system, and discussed the multimodal effects of exercise on peripheral and central inflammation, blood-brain barrier integrity, glial and neural cells, and remyelination. Moderate exercise training produced health benefits in patients with multiple sclerosis, prior to or after infection with SARS-CoV-2.

Yu et al. examined the effects of exercise on hepatic ApoA5 expression of ApoA5 and TLR4-mediated pathway in mice with high-fat diet (HFD)-induced non-alcoholic steatohepatitis (NASH). Their results demonstrated that exercise improved HFD-induced NASH by triggering the

inhibitory effects of the ApoA5 on the TLR4-mediated NF-κB pathway.

A meta-analysis by You et al. reported the effects of different intensities and durations of aerobic exercise on vascular endothelial function of middle-aged and elderly people. Nine studies involving 221 participants were utilized. Vigorous-intensity aerobic exercise (≥8 weeks) improved endothelial function in healthy middle-aged and elderly people.

The study by Feng et al. explored the effects of 4 weeks of hypoxic training on β-aminoisobutyric acid (BAIBA) secretion and white fat browning in inguinal fat in obese rats. Their results showed that this kind of training reduced body weight, Lee's index, and regulated blood lipid profile. Moreover, hypoxic training up-regulated BAIBA concentrations in gastrocnemius muscle and the circulation, with increased expression of PPARα and UCP-1 in inguinal fat of obese rats and greater white fat browning. The authors concluded that BAIBA could improve the blood lipid profile and stimulate white fat browning by modulating PPARα and UCP-1 expression.

Paillard examined the potential role of using percutaneous electrical stimulation for reconditioning functional capabilities in older subjects utilizing two modalities of electrical stimulation: neuromuscular electrical stimulation (NMES) and sensory electrical stimulation (SES). SES was particularly useful for maintaining or even improving muscle function, control movement, and postural balance in older subjects, provided that their basal functional capabilities are not reduced and their risk of falling is low. In turn, in frail older subjects with diminished basal functional capabilities and at high risk of falling, NMES can potentially boost their neuromuscular system to recondition lower-limb muscle strength/power and thus limit the risk of falling.

The literature search of original data and review by Lefferts et al. summarized the physiological effects of acute exercise on the brain (cognitive, brain-blood-barrier), cardiovascular, neuroendocrine, inflammation/oxidative stress, metabolic, and musculoskeletal systems and then aligned those observations with literature describing changes seen with aging and age-related chronic disease. These authors concluded that regular exercise protects against aging and age-related chronic disease exercise acts as an aging mimetic.

The meta-analysis by Ma et al. evaluated the effect of regular aerobic exercise on renal function in patients with chronic kidney disease. Regular aerobic exercise has significant effects on the estimated glomerular filtration rate, serum creatinine, 24-h urine protein amount, and blood urea nitrogen in patients with chronic kidney disease, and aerobic exercise with a single exercise duration longer than 30 min has a more significant effect on the estimated glomerular filtration rates, and aerobic exercise by walking or running can more effectively improve serum creatinine in patients with chronic kidney disease.

The review by Krüger et al. described the effects of exercise on the treatment of dyslipidemia and discussed possible immunological-related mechanisms. The authors concluded that if statin therapy is indicated, it can be combined with activity and sports programs without any concerns. If patients follow the activity recommendations in the long term, effects on blood lipids, especially on HDL and triglycerides, can occur after a few weeks. However, most patients with lipometabolic disorders have multiple morbidities that are influenced by physical activity. Here, it is particularly important to clarify the patients' fitness for sports by means of a sports medicine stress examination.

We as editors of this volume are extremely grateful to the authors for their contributions and hard work in bringing forth their scientific research. We hope the readers of these papers can gain insight from them and utilize the information herein to advance their scientific pursuits.

Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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SYSTEMATIC REVIEW
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Benefits and Mechanisms of Exercise Training for Knee Osteoarthritis

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Knee osteoarthritis is a chronic degenerative disease. Cartilage and subchondral bone degeneration, as well as synovitis, are the main pathological changes associated with knee osteoarthritis. Mechanical overload, inflammation, metabolic factors, hormonal changes, and aging play a vital role in aggravating the progression of knee osteoarthritis. The main treatments for knee osteoarthritis include pharmacotherapy, physiotherapy, and surgery. However, pharmacotherapy has many side effects, and surgery is only suitable for patients with end-stage knee osteoarthritis. Exercise training, as a complementary and adjunctive physiotherapy, can prevent cartilage degeneration, inhibit inflammation, and prevent loss of the subchondral bone and metaphyseal bone trabeculae. Increasing evidence indicates that exercise training can improve pain, stiffness, joint dysfunction, and muscle weakness in patients with knee osteoarthritis. There are several exercise trainings options for the treatment of knee osteoarthritis, including aerobic exercise, strength training, neuromuscular exercise, balance training, proprioception training, aquatic exercise, and traditional exercise. For Knee osteoarthritis (KOA) experimental animals, those exercise trainings can reduce inflammation, delay cartilage and bone degeneration, change tendon, and muscle structure. In this review, we summarize the main symptoms of knee osteoarthritis, the mechanisms of exercise training, and the therapeutic effects of different exercise training methods on patients with knee osteoarthritis. We hope this review will allow patients in different situations to receive appropriate exercise therapy for knee osteoarthritis, and provide a reference for further research and clinical application of exercise training for knee osteoarthritis.

Keywords: knee osteoarthritis, exercise training, mechanisms, inflammatory, pain, strength training, traditional exercise

INTRODUCTION

Knee osteoarthritis (KOA) is a chronic degenerative disease which often causes disability and pain (Madry et al., 2016); especially in people over 50 years of age (Abbassy et al., 2020). Genetic mutations, obesity, trauma, aging local biomechanical factors, and hormones are vital risk factors of KOA (Martel-Pelletier et al., 2016), which can damage in any joint tissue, but mainly cause

cartilage destruction, subchondral bone alteration, and synovial inflammation. Moreover, KOA always reduces patients' ability to perform activities of daily living and work, which causes a severe economic burden on society (Mesa-Castrillon et al., 2021). Therefore, a treatment that can effectively against the degeneration associated with KOA will significantly benefit both patients and society.

Currently, multiple therapy options, including pharmacotherapy, physiotherapy, surgery, and rehabilitation, are available to treat KOA in clinic (Michael et al., 2010). However, pharmacotherapy has many side effects, such as congestive heart failure, hypertension, and renal toxicity (Kan et al., 2019). Physiotherapy has its limitations, which should combine with surgery. Appropriate physiotherapy preoperatively and postoperatively can restore quadriceps strength and improve proprioception of patients with KOA after surgery (Henderson et al., 2018). Surgery is not suitable for patients with early-stage KOA. It is very necessary to find a non-surgical treatment to effectively relieve the symptoms of patients with KOA.

Exercise training aims to improve any part of body functions through the patient's own strength or the assisted operation of the therapist or with the aid of equipment (Sheikh and Vissing, 2019). A systematic review of randomized trials of therapeutic exercise in patients with KOA indicated that exercise can significantly reduce pain, improve physical function and quality of life (Fransen et al., 2015). Furthermore, exercise training may improve cardiorespiratory function, increase muscle strength, stabilize posture, and ameliorate psychological health (Garber et al., 2011). Thus, exercise training is an effective complementary therapy and plays an important role in the treatment for patients with KOA. However, there are few review articles on exercise training for KOA, and lack the exploration of their mechanisms.

In this review, we summarize the related mechanisms and the therapeutic effects of regular exercise training for the treatment of KOA, describes the main clinical symptoms of KOA, so as to provide a reference for further research and clinical application.

We used "osteoarthritis," "knee osteoarthritis," "exercise training," "mechanism," "inflammation," and "rat" as key words. Then we searched PubMed CINAHL, and web of science for methodological papers on articles from July 2001 to July 2021, especially recently 5 years. Having examined 723 full articles, we finally selected 185 articles for this review.

MECHANISMS AND SYMPTOMS OF KOA

Mechanism of KOA

According to pathogenic progression, osteoarthritis contains primary osteoarthritis and secondary osteoarthritis. Primary osteoarthritis can be classified into three types according to pathophysiological mechanisms, type I, genetically determined; type II, oestrogen hormone dependent; and type III, age related osteoarthritis (Castaneda et al., 2014). The incidence rate of age related osteoarthritis was the highest.

Not simply articular cartilage damage, KOA is a disease of whole knee joint, involving subchondral bone, capsule, ligaments, synovial membrane, and periarticular muscles (Hunter and Bierma-Zeinstra, 2019). Normal wear and tear, abnormal mechanical loading, injury, and aging are common causes to damage articular cartilage (Xia et al., 2014).

At the early stages of KOA, articular cartilage is still intact. But the molecular composition and organization in the extracellular matrix has altered first (Goldring and Goldring, 2010), which causes a change in water-binding capacity with a reduced mechanical strength (chondromalacia), and leading to a higher deformation of the cartilage under load (Ryd et al., 2015). In the regions of cartilage damage, subchondral plate and subarticular spongiosa thickness progressive increase (Madry et al., 2016), which diminish its biomechanical properties. In addition, Hoffa fat pad will suffer from inflammation, quadriceps femoris become weakness, and knee joint ligament will be laxity (Mahmoudian et al., 2021).

During the progressive stages of KOA, the material properties and structural integrity of the articular surface and underlying hyaline cartilage deteriorate gradually (Goldring and Goldring, 2010). In subchondral bone, the changes include progressive increase in subchondral plate thickness, modification in the architecture of subchondral trabecular bone, and formation of new bone at the joint margins (Goldring and Goldring, 2010). Mechanical and structural changes in meniscal entheses may contribute to meniscal tear, avulsion, and extrusion (Abraham et al., 2014). In infrapatellar fat pad, inflammation enhanced, IL-1β increased, and macrophages raise, which can aggravate cartilage degeneration (Bastiaansen-Jenniskens et al., 2012).

Furthermore, changes in the composition and structure of the articular cartilage further stimulate chondrocytes to produce more catabolic factors involved in cartilage degradation (Xia et al., 2014). The most important catabolic factors are two families member of metalloproteases: the matrix metalloproteinases (MMPs) and the ADAMTSs (a disintegrin and a metalloprotease with thrombospondin motifs). They are key molecules in the extracellular matrix produced by activated chondrocytes of osteoarthritic joints, which are responsible for the degradation of the major components of articular cartilage (Mort and Billington, 2001). As the process continues, increased catabolic activity is related to enhanced production of degradative proteinase genes, which could result in gradual loss of proteoglycans followed by type II collagen degradation (Goldring and Goldring, 2010). Then cartilage integrity is disrupted, and the water content of hyaline cartilage is increased (Adatia et al., 2012). The articular chondrocytes undergo apoptosis and the articular cartilage eventually be completely lost (Loeser et al., 2016). The cartilage serves to decrease friction and distributes the force exerted by loads evenly onto the underlying bone. The total loss of cartilage will reduce joint space, followed by friction between bones (Loeser et al., 2016). At the end stage of KOA, patients appear pain, stiffness, swelling, and limited joint mobility and other symptoms.

A study showed that injection of senescent chondrocyte in a healthy joint is sufficient to promote cartilage damage in an osteoarthritis-like fashion in rat (Xu et al., 2017). Aging decreases the responsiveness of the chondrocytes to growth factors, which influence the catalytic and anabolic metabolism of chondrocytes (Rahmati et al., 2017). Composition of chondrocyte and the extracellular matrix will change with age, which will lead to chondrocyte gradual loss the ability to repair the damage (Rahmati et al., 2017).

Higher mid-stance transverse plane moments could be contributing to higher shear forces in the joint, which can lead to degenerative processes in osteoarthritis cartilage (Astephen Wilson and Kobsar, 2021). Obesity and metabolic syndrome as risk factors, *via* a cumulative influence of the metabolic disorders, accelerate the structural degenerative progress of osteoarthritis (Courties et al., 2019). In addition, in early-stage of KOA, innervation of the articular cartilage and osteophytes is associated with their invasion by blood vessels and nerves (Suri et al., 2007). In addition, estrogen *via* estrogen receptor mediates osteoarthritis cartilage degradation and accelerates the progression of cartilage loss (Tang et al., 2021).

In short, inflammation plays a central role in the development of KOA. Under the stimulation of inflammatory molecules and cytokines, the microenvironment within the joints is gradually destroyed. Which leads to injury of cartilage, subchondral bone, meniscus, and even infrapatellar fat pad, finally patients suffer from KOA (**Figure 1**). At the same time, mechanobiology, aging, metabolic disorders, hormonal alterations, and vascular and neural invasion also made outstanding contributions to the development of KOA.

Symptoms of KOA

Patients with KOA usually experience a variety of symptoms, which disturb their daily activities. Four main signs are predominant, including pain, stiffness, reduced joint motion, and muscle weakness (Sharma, 2021).

Pain

Pain is a leading feature and one of the most severe disabling symptoms of KOA. The pain of KOA is intermittent and associated with weight-bearing (Hawker et al., 2008; Hunter and Bierma-Zeinstra, 2019). When the affected knee is put in motion, the pain usually becomes worse, but improves when the knee is at rest (Michael et al., 2010). As KOA aggravate, pain will appear at rest and at night, even interfere with sleep (Sharma, 2021). Trouvin et al. (2019) found that in lower limb OA, pain is mostly stable in a long time, and pain is better accepted when stable.

In the synovial fluid of patients with KOA who experience pain, inflammatory molecules, such as interleukin (IL)-6, tumor necrosis factor (TNF)-α, and MMP-13 are constantly elevated (Runhaar et al., 2019). After treatment, the inflammatory molecules significantly reduced (Schell et al., 2017). Therefore, inflammatory molecules in synovial fluid play a vital role in the occurrence of KOA-associated pain.

When tissue damaged during joint degeneration, these nerve fibers distributed in the deep layers of cartilage and subchondral bone will transmit sensation to the brain and crate pain (Adatia et al., 2012). Furthermore, bone attrition considered as flattening or depression of the articular cortex is associated with pain (O'Neill and Felson, 2018). Osteoarthritis patients reporting severe osteoarthritis pain have neuropathic pain-like symptoms, and develop central sensitization. Development of novel compounds targeting molecular pathways implicated in central sensitization may provide improved pain management in advanced osteoarthritis patients (Havelin et al., 2016). In osteoarthritis joint, indirect

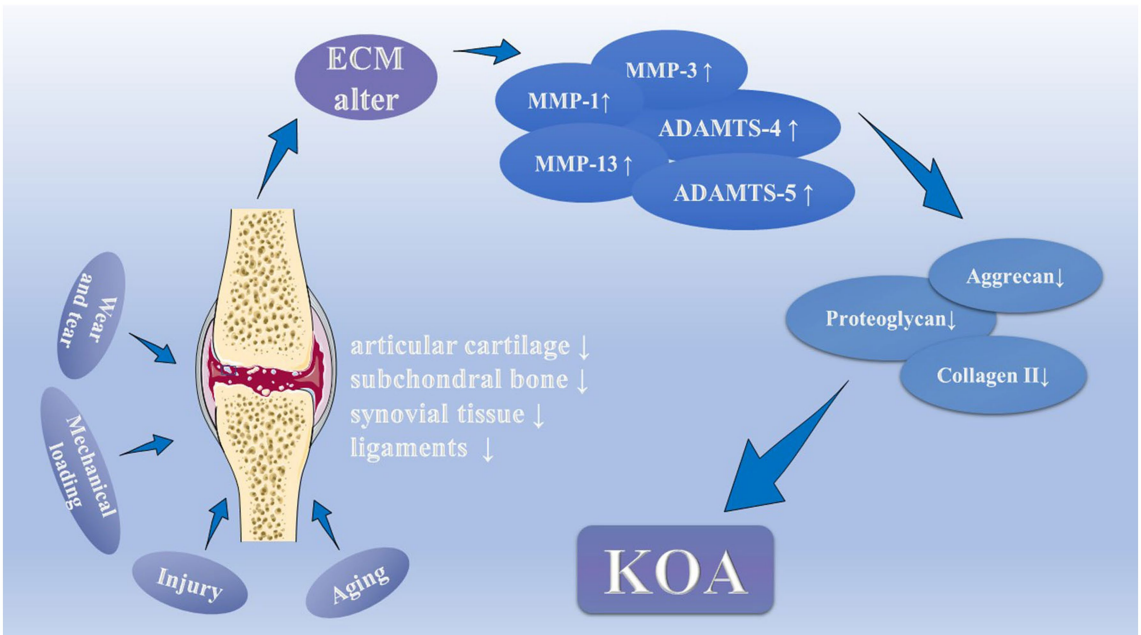

FIGURE 1 | The pathology and pathophysiology of Knee osteoarthritis (KOA). Normal wear and tear, abnormal mechanical loading, injury, and aging are common causes to damage articular cartilage, as well as subchondral bone, synovial tissue and ligaments, which could change the molecular composition and organization in the extracellular matrix. Under the stimulation, injured chondrocytes produce the matrix metalloproteinases (MMP-1, MMP-3, and MMP-13) and the ADAMTSs (ADAMTS-4 and ADAMTS-5). They thus contribute to declining levels of proteoglycans, aggrecan, and type II collagen in the cartilage matrix by inhibiting the synthesis of key components of the extracellular matrix, which eventually leads to cartilage degeneration.

neuro-immune signaling may occur when innate immune cells produce algogenic factors, such as chemokines and cytokines, which act on the pain pathway (Miller et al., 2020). The transient receptor potential vanilloid (TRPV)-4 ion channel transduces mechanical loading of articular cartilage *via* the generation of intracellular calcium ion transients. Moreover, loss of TRPV4-mediated cartilage mechanotransduction in adulthood could delay the progress of aging-associated osteoarthritis (O'Conor et al., 2016).

Many patients with KOA stop participating in sports and leisure activities because of pain (Gay et al., 2016). Due to the pain, they are afraid to flex and stretch the knee joint, and gradually develop joint mobility disorders, which will diminish their quality of life, and even leads to physical disability (Focht, 2006). Patients with KOA develop neuropathic pain experiencing higher levels of pain, which are significant predictors of risk for all-cause death (Syx et al., 2018). Intense pain may reflect a high degree of joint degeneration (Perrot, 2015), and requiring adequate treatment urgently.

Some studies found that increasing muscle strength and decreasing weight-bearing indexes of the quadriceps could relieve joint pain efficiently (Muraki et al., 2015; Alkhawajah and Alshami, 2019). In addition, KOA patients with pain usually appear long-term depression. Therefore, improving psychological symptoms may be an essential treatment to alleviate pain of KOA (Rathbun et al., 2018).

In summary, pain is intermittent and stable, which is mainly associated with inflammatory stimulation and nerve conduction. The level of inflammatory molecules is related to pain relief. Mechanosensory ion channels, neuro-immune signaling, and central sensitization play a vital important role of neuropathic pain. As the most significant symptoms that disrupted KOA patient's life, pain relief is their primary demand for seeking treatment for KOA. Muscle strength and psychology improvements could be critical directions for the treatment of KOA-associated pain.

Stiffness

Stiffness is a common complaint of patients with KOA (Oatis et al., 2006). It usually appears in the morning on first waking and lasts less than 30 min. Long-term synovitis leads to cell proliferation and increase synthesis of matrix proteins (collagen types I, III, and VI), resulting in mutual adhesion, arthrofibrosis, and gradual joint dysfunction (Mayr and Hochrein, 2015). In the gait weight-bearing phase, changes in knee stiffness during walking are mainly due to decreased knee flexion in patients with KOA, while shifts in external knee flexion account for less than internal knee flexion (Dixon et al., 2010). Gustafson et al. (2016) believe that knee stiffness is a self-compensatory and protective effect when patients with KOA develop joint instability. There is a significant inverse relationship between the symptoms of knee instability and passive mid-range knee stiffness (Creaby et al., 2013), while the relationship between active stiffness and knee joint stability requires further research. Further, Fukutani et al. (2016) found that when the cutoff was K/L grade 2, varus thrust is significantly associated with pain and stiffness.

In summary, KOA-associated stiffness aggravate gradually, and this stiffness is a self-compensatory and protective effect of joint instability.

Reduced Joint Motion

Reduced joint motion of the knee is primary and essential manifestation of knee instability symptoms (Chaudhari et al., 2019). Predicting radiographic KOA from range of motion may be possible in the future by further studies of larger sample size conducted in different populations (Ersoz and Ergun, 2003). KOA patients' knee range of motion is usually limited to different phases (van Dijk et al., 2009). In the early stage of KOA, the range of motion limitation always appears in the end-range motion. As KOA worsens, the joint range of motion becomes smaller (Taylor et al., 2014). Patients with KOA often complain of knee extension and flexion disorders when visiting the doctor (Ersoz and Ergun, 2003). Leon et al. (2005) found that KOA patients with stenosis of the intercondylar notch mixed type have significantly limited joint mobility. van der Esch et al. (2014) found that joint activity limitations are always complimented by muscle weakness; long-term joint activity limitations can lead to muscle weakness and thus exacerbate joint activity limitations. Strengthening the quadriceps can improve joint activity to some extent (Alkhawajah and Alshami, 2019). Furthermore, patients with early-stage KOA have decreased axial tibial rotation excursion, while patients with end-stage KOA have increased knee adduction (Nagano et al., 2012). Campbell et al. (2020) found that knee flexion contractures are associated with worse pain, stiffness, and dysfunction in a severity-dependent manner in patients with KOA.

In summary, reduced joint motion is associated with joint instability, and it is mainly a manifestation of the narrowing of the inner and outer gaps of the knee joint. Patients with KOA often suffer extension and flexion disorders. Pain, stiffness, and muscle weakness are all related to reduce joint mobility. Increasing muscle strength can be a method to alleviate reduced joint mobility.

Muscle Weakness

Muscle weakness is a characteristic of patients with KOA (de Zwart et al., 2015), and is a better predictor of disability than pain or joint space narrowing (Roos et al., 2011). Most adults attain their peak muscle strength in their mid-20s and maintain this level until their 60s, but in their 80s, their muscle strength drops to only half of their peak (Latham and Liu, 2010). Muscle weakness may be caused by muscle dysfunction and may be a risk factor for the progression of KOA (Coudeyre et al., 2016). The most apparent muscle weakness is the decrease in extension and flexion strength (Heiden et al., 2009). Extensor weakness is common in patients with KOA, especially quadriceps weakness, which could lead to an increased risk of functional limitation and disability (Latham and Liu, 2010; Jegu et al., 2014). There is an atrophy of the type I and type II fibers of the vastus medialis muscle in patients with end-stage KOA who underwent total knee replacement (Fink et al., 2007). Ikeda et al. (2005) found decreases in the muscle cross-sectional area in patients with early-stage KOA. Decreased muscle strength can increase the risk of falls by decreasing knee stabilizers and proprioceptors (de Zwart et al., 2015). Moreover, knee extensor muscle

weakness is related to an increased risk of developing KOA in both men and women (Oiestad et al., 2015; Dell'isola et al., 2018). Long-term weakness of the quadriceps can accelerate the progression of degenerative KOA (Roos et al., 2011). A 6-year cohort study showed that increasing knee muscle strength can prevent the development of KOA-related dysfunction (Latham and Liu, 2010). Increasing the strength of the quadriceps and resisting muscle weakness can relieve the degeneration associated with KOA (Segal et al., 2012).

Muscle weakness is associated with aging and muscle dysfunction, and it can decrease the stability of the knee and accelerate the progression of KOA. Improving muscle weakness, especially in the quadriceps, is significant for the treatment of KOA.

Pain, stiffness, joint dysfunction, and muscle weakness are the essential symptoms of KOA, and they are interrelated, not independent (**Figure 2**). Clinically, our primary treatment goal is to delay the degeneration associated with KOA, reduce the four symptoms, and maximize the function of the knee. Excepting pharmacotherapy and surgery, exercise training may be one of the ways to improve the above symptoms based on their root causes.

EXERCISE TRAINING AND KOA

Exercise Training in Animal Experiments

In recent years, a growing number of people have begun to study the mechanisms of exercise training in KOA. Exercise training could effectively increase muscle cross-sectional area and decrease muscle fiber density in experimental animals with KOA (Assis et al., 2015). Increasing the ultimate load supported during the exercise training, the biomechanical characteristics and the structure of the tendon in experimental animals can be improved (Bezerra et al., 2012). Four weeks of regular exercise training can alleviate cartilage degeneration in model rats with KOA (Fallah Mohammadi et al., 2013). There is a biological and biomechanical link between the cartilage and subchondral bone, and that gentle short-term treadmill walking can through inhibiting the increase in osteocyte death to protect the chondrocytes in rat model (Iijima et al., 2015). Four-week treadmill training could alleviate the subchondral bone loss and remodeling, and reprogram the cartilage-subchondral unit (Hao et al., 2021).

Furthermore, aerobic exercise can reduce the expression in IL-1β, caspase-3, and MMP-13, and prevent the degeneration of cartilage caused by KOA in model rats (Assis et al., 2016). Resistance training can decrease MMP-2 activity in quadriceps tendon in a rat model of osteoarthritis (Vasilceac et al., 2021). Treadmills and wheel exercise can decrease the levels of IL-1β, IL-6, and TNF-α, and regulate JNK/NF-KB signaling to prevent inflammation in model rats with KOA (Chen et al., 2020). Moderate physical exercise can prevent type B synovial cell dysfunction in rats with early-stage osteoarthritis, and delay the progress of the disease (Castrogiovanni et al., 2019). An animal experiment indicated that, at very early stages of cartilage damage, early intervention by swimming provides better effects than delayed intervention when post-traumatic osteoarthritis already developed (Hsieh and Yang, 2018).

In addition, Allen et al. (2017) found that 4 weeks of treadmill exercise can reverse tactile hypersensitivity and weight asymmetry, and persistent pain in KOA model rats caused by monosodium iodoacetate. Moreover, exercise training has a potential bone stabilizing effect of the osteoarthritis joint (Allen et al., 2017). Cormier et al. (2017) found that voluntary exercise may protect against OA pain, the effect varies as a function of prior exercise duration, and is associated with distinct trabecular bone modifications.

In short, various animal experiments of the exercise training to treating KOA have suggested that exercise training can increase muscle cross-sectional area, decrease muscle fiber density, change the tendon structure, delay musculoskeletal atrophy, stabilize the osteoarthritis joint, inhibit inflammation, rescue synovial cell dysfunction, and prevent cartilage degeneration and the loss of subchondral bone (**Figure 3**). These studies provide an experimental foundation for the application of exercise training in the treatment of KOA, which may be beneficial for patients with this disease.

Different Types of Exercise Training

In clinical, there are several exercise trainings options for the treatment of KOA, including aerobic exercise, strength training, neuromuscular exercise, balance training, proprioception training, aquatic exercise, and traditional exercise (**Figure 4**). Each kind of exercise training therapy has corresponding therapeutic mechanism and special therapeutic effect on KOA.

Aerobic Exercise and KOA

Aerobic exercise is the most convenient exercise training, including walking, jogging, cycling, skating, rhythmic exercises, aerobics, ball games, and rowing (Bartels et al., 2016; Brosseau et al., 2017; Wellsandt and Golightly, 2018). Aerobic exercise has many benefits, such as increasing cardiopulmonary activity, reducing oxidative stress, promoting adipose tissue metabolism, and preventing muscle disuse atrophy in patients with KOA (Ferreira et al., 2015; Gay et al., 2016). Chua et al. (2008) found that aerobic exercise can significantly increase the cartilage oligomeric protein and accelerate the growth of damaged cartilage in patients with KOA. Walking not only can activate T lymphocytes and enhance the body's immunity in older women with KOA, but also can improve their quality of life and physical performance (Gomes et al., 2016). Moderate supervised aerobic exercise in patients with KOA can improve knee cartilage glycosaminoglycan content, as well as improve pain and function parallel structural (Roos and Dahlberg, 2005). Moreover, Kilic and Tanaka et al. proved that aerobic exercise has a specific therapeutic effect on relieving the pain and dysfunction associated with KOA (Tanaka et al., 2013; Kilic et al., 2020). Retrograde walking can significantly reduce pain, dysfunction, and improve quadriceps strength and performance (Alghadir et al., 2019). Although the results of high-intensity and low-intensity aerobic exercise are consistent, low-intensity aerobic exercise is better for patients with severe

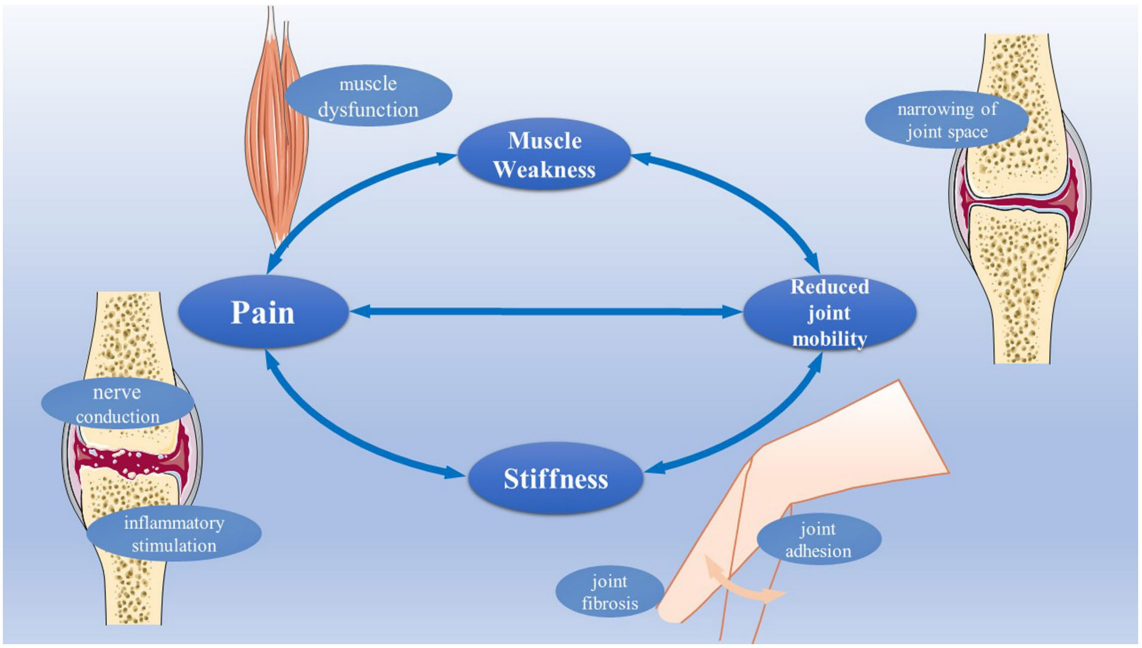

FIGURE 2 | The main symptoms of patients with KOA. The main symptoms of patients with KOA include pain, stiffness, muscle weakness, and reduced joint mobility. Pain is always associated with inflammatory stimulation, and never conduction. Joint fibrosis and joint adhesion are related to stiffness. Muscle dysfunction can cause muscle weakness. Joint space narrowing is related to reduced joint mobility.

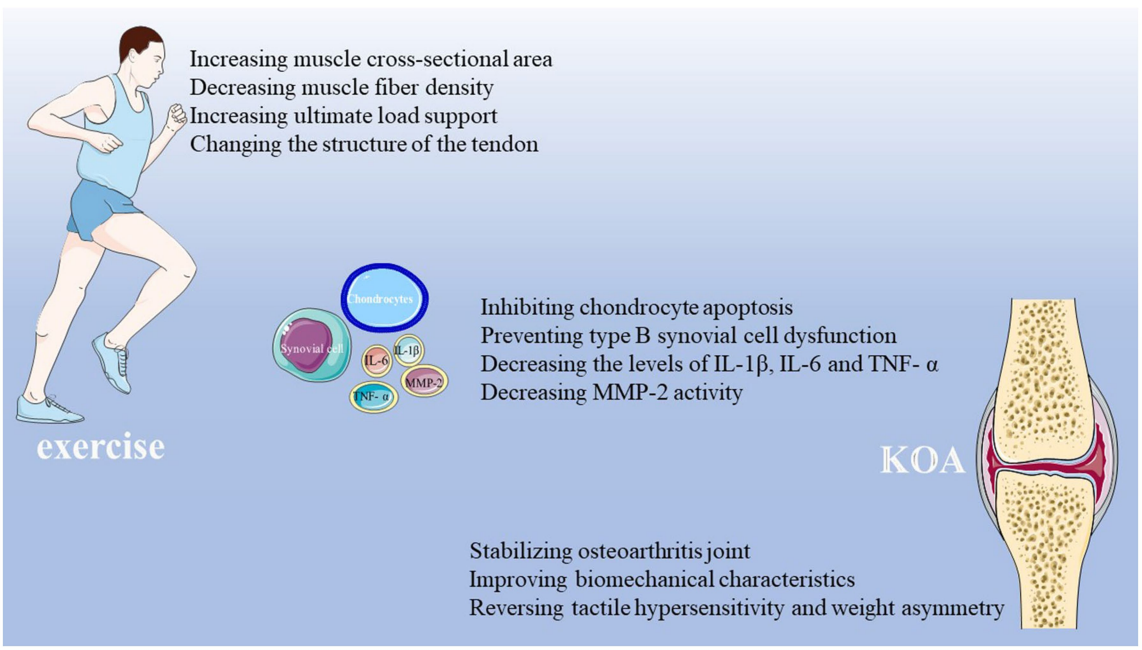

FIGURE 3 | The involved mechanisms for exercise training in treatment of KOA. Existing experimental studies have proved that exercise training has a therapeutic effect on KOA. Exercise training can increase muscle cross-sectional area, decrease muscle fiber density, increase the ultimate load support, change tendon structure, delay musculoskeletal atrophy, stabilize osteoarthritis joint, inhibit inflammation, decrease MMP-2 activity, rescue synovial cell dysfunction, and prevent cartilage degeneration and the loss of subchondral bone of osteoarthritis joint.

KOA (Messier et al., 2021). High-intensity aerobic exercise is effective for patients with mild KOA. In contrast, high-intensity aerobic exercise can lead to more severe damage to cartilage in patients with severe KOA (Multanen et al., 2017).

FIGURE 4 | Different exercise training types of KOA. There are several exercise trainings options for treating KOA in the clinic, including aerobic exercise, strength training, neuromuscular exercise, balance training, proprioception training, aquatic exercise, and traditional exercise. Strength training includes isokinetic exercise, isometric exercise, and isotonic exercise. Traditional exercise includes Ba Duanjin, Tai Chi, Wuqinxi, and Yoga.

Furthermore, high-intensity interval training is more effective than moderate-intensity training in improving health, body composition, and muscle function in those with chronic disease (Keogh et al., 2017).

Aerobic exercise can promote the metabolism of adipose tissue, prevent muscle atrophy, accelerate the recovery of damaged cartilage, enhance the body's immunity, and relieve pain. Different intensities of aerobic exercise have different therapeutic effects. Low-intensity aerobic exercise is better for patients with severe KOA, and high-intensity aerobic exercise is more suitable for patients with mild KOA. Furthermore, for mild KOA patients with chronic diseases, high-intensity interval training is better. Doctors should choose the most appropriate treatment for different patients.

Strength Training and KOA

Strength training is indispensable for patients with KOA to restore muscle strength. The main functions of strength training are relieving pain, alleviating stiffness, enhancing muscle strength, improving physical function, and increasing the shock absorption ability of the lower extremity muscles during walking (Li et al., 2016; DeVita et al., 2018; Chen et al., 2019; Messier et al., 2021). The types of strength training mainly include isokinetic exercise, isometric exercise, and isotonic exercise (Malas et al., 2013; Sharma, 2021). We will summary the different strength exercise and their effects as follow (**Table 1**).

Isokinetic Exercise and KOA

Isokinetic exercise refers to exercise training in which muscle strength changes but movement speed does not change (Coudeyre et al., 2016). Isokinetic muscle strengthening is an effective way to promote dynamic muscle strengthening for KOA rehabilitation and has a significant effect on disability and pain (Coudeyre et al., 2016). Samut et al. (2015) found that 6 weeks of isokinetic exercise in patients with KOA can decrease TNF-α, IL-6, and C-reactive protein in patients' serum, as well as relieve pain, increase functional capacity, and improve muscle strength. A randomized controlled clinical study showed that isokinetic exercise can enhance muscle strength, increase walking distance, and improve quality of life in patients with KOA (Akyol et al., 2010). Moreover, a randomized trial proved that isokinetic eccentric exercise is better than isokinetic concentric exercise for patients with KOA in improving gait, enhancing static equilibrium, and relieving pain (Jegu et al., 2014). By combining isokinetic exercise with a variety of treatment methods, Cetin et al. (2008) tested 100 patients with bilateral KOA and found that KOA patients in the isokinetic exercise group alone experienced the most significant pain alleviation, with a maximum in walking speed and function at 60 and 180 degrees per second of speed, as well as increased muscle strength.

In summary, isokinetic exercise can decrease IL-6 and TNF-α levels in patients' serum, inhibit inflammation, relieve pain, and increase muscle strength. Further, for the patients with bilateral KOA, the therapeutic effect of isokinetic eccentric exercise is better than that of isokinetic centripetal exercise.

Isometric Exercise and KOA

Isometric exercise (also known as static exercise) involves the isometric contraction of the muscle. During muscle

TABLE 1 | Summary of strength training included in this review.

Study author	Study design	Number of studies/ subjects	Intervention studied	Relevant outcome	Main finding
Jegu et al., 2014	RCT	*N* = 80	Eccentric isokinetic strengthening	WOMAC, static postural balance, walking speed, range of knee motion, temporospatial gait parameters, isokinetic tests, and parameters of walking	Increase muscle strength
Ojoawo et al., 2016	RCT	*N* = 45	Proprioceptive and isometric exercises	WOMAC	Reduce pain intensity, enhance physical function, and improve joint stiffness
Malas et al., 2013	RCT	*N* = 61	Strength training	VAS, WOMAC, fascicle length, isokinetic muscle testing, muscle thickness, gait velocity, and function, static balance function	Increase knee extensor strength, increase fascicle length, increase muscle thickness, and influence muscle architecture
Akyol et al., 2010	RCT	*N* = 40	Isokinetic exercise, short-wave	VAS, WOMAC, 6-MWT, isokinetic muscle testing, SF-36, and beck depression index	Relieve pain, reduce disability, increase walking distance, increase muscle strength, increase quality of life, and reduce depression
Samut et al., 2015	Prospective	*N* = 42	Isokinetic and aerobic exercise	VAS, WOMAC, ROM, 6-MWT, functional activity status, isokinetic testing, serum biomarker, and 30 s sit to stand test	Reduce pain, improve physical function, increase muscle strength, and decrease TNF-α, IL-6, and CRP
Cetin et al., 2008	RCT	*N* = 100	Hot pack, short-wave diathermy, TENS, Ultrasound, and Isokinetic muscle-strengthening exercise	VAS, ISK, Ambulation time, and Isokinetic test	Reduce pain, improve walking ability, increase walking speed and function, increase muscle strength, and improve knee extension and flexion
Miyaguchi et al., 2003	Prospective	*N* = 17	Isometric quadriceps exercise	VAS, circumference of the thigh, maximum isometric quadriceps and hamstring forces at 30 and 60° knee flexion, and joint fluid biomarker	Relieve pain, increase muscle strength, increase molecular weight of hyaluronan, increase viscosity of joint fluid, and decrease chondroitin 4-, 6-sulfate concentration in joint fluid
Tok et al., 2011	RCT	*N* = 40	Electrical stimulation, continuous passive motion vs. isometric exercise	VAS, WOMAC, SF-36, knee and thigh circle measurements, isokinetic tests, and dynamic and static balance tests	Increase dynamic and static balance, increase muscle strength, improve pain, and improve quality of life
Anwer et al., 2013	Preliminary	*N* = 43	Isometric exercise, electromyographic biofeedback	Isometric strength of quadriceps	Increase muscle strength
Onigbinde et al., 2017	Prospective	*N* = 21	Isometric quadriceps strengthening training	Quadriceps strength	Increase quadriceps strength
Huang et al., 2003	Prospective	*N* = 135	Isokinetic, isotonic, and isometric muscle-strengthening exercise	VAS, muscle power of leg flexion and extension, and ambulation speed	Relieve pain, decrease disability, increase muscle strength, improve joint stability, improve walking endurance, and increase walking speed
Burrows et al., 2014	RCT	*N* = 33	Acute resistance exercise	Pressure pain threshold, pressure pain tolerance	Increase pain sensitivity, increase pressure pain thresholds
Chang et al., 2012	Prospective	*N* = 41	Elastic-band exercise	VAS, WOMAC, 30s CST, and walking function (10 m walk test, TUG test, and going up-and-down 13-stair test)	Improve lower-extremity function
León-Ballesteros et al., 2020	RCT	*N* = 32	Kinesiotape and quadriceps strengthening with elastic band	WOMAC (pain, stiffness and functionality), VAS	Relief pain, improve functionality, and decrease stiffness
Ferraz et al., 2018	Prospective	*N* = 48	Resistance training with blood flow restriction	WOMAC (Pain, stiffness, and physical function), SF-36, 1-RM test, TST, TUG tests, and Quadriceps cross-sectional area	Improve pain, induce less joint stress, increase muscle strength, increase quadriceps muscle mass, and improve functionality
Segal et al., 2014	RCT	*N* = 45	Blood flow restricted low-load resistance training	Isotonic knee extensor strength, stair climb muscle power, quadriceps volume, and KOOS	Increase muscle strength and volume, increase knee extensor and leg press strength

BMI, body mass index; CRP, C-reactive protein; IL-6, Interleukin-6 ISK; the index of severity for knee osteoarthritis; KOOS, Knee Injury and Osteoarthritis Outcome Score; RCT, randomized controlled trial; SF-36, Medical Outcomes Study Short-Form Health Survey; TNF-α,Tumor Necrosis Factor-α; TST, timed-stands test; TUG test, Timed Up and Go test; VAS, Visual Analog Scale; WOMAC, Western Ontario and McMaster Universities Osteoarthritis Index; 1-RM test, one repetition maximum test; 6-MWT, 6-min walk test; and 30s CST, 30 s Chair Stand Test.

TABLE 2 | Summary of traditional exercise included in this review.

Study author	Study design	Number of studies/subjects	Intervention studied	Relevant outcome	Main finding
An et al., 2008	Prospective	*N* = 28	Baduanjin	WOMAC, SF-36, 6-MWT, Isokinetic Strength of the Knee Extensors (ISKE) and BMI	Relieve pain, reduce stiffness, improve general and emotion health, decrease disability, enhance knee extensors and flexors strength, improve aerobic ability, and lose weight
An et al., 2013	Prospective	*N* = 28	Baduanjin	WOMAC, SF-36, 6-MWT, Isokinetic Strength of the Knee Extensors and Flexors (ISKEF), and BMI	Relieve pain, reduce stiffness, improve general and emotion health, decrease disability, enhance knee extensors and flexors strength, improve aerobic ability, and lose weight
Chen et al., 2019; Liu et al., 2019a	Multiple mode MRI study	*N* = 140	Tai Chi, Baduanjin, stationary cycling, health education	KOOS, functional and structural MRI, and serum biomarkers	Reduce pain, decrease BDNF, IFN-γ, PD-1, and TIM-3, and modulate brain areas known to be involved in the opioidergic and dopaminergic neurotransmitter systems
Liu et al., 2019b	Multiple mode MRI study	*N* = 140	Tai Chi, Baduanjin, stationary cycling, and health education	KOOS, functional MRI, serum biomarkers	Relieve pain, decrease BDNF, INF-γ, PD-1, and TIM-3, and decreased the rsFC between the bilateral DLPFC and bilateral
Lee et al., 2018	Prospective	*N* = 182	Tai Chi, physical therapy exercise	WOMAC, VAS, SF-36, Kellgren and Lawrence grade	Relieve pain, improve physical function
Zhang et al., 2020	Prospective	N = 46	Tai Chi	Plantar load assessment (peak pressure and maximum force)	Increase plantar loads in forefoot
You et al., 2021	Systematic review and meta-analysis	11 studies	Tai Chi	6-MWT, TUG test, and WOMAC	Improve dynamic stability and walking capacity
Ghandali et al., 2017	Prospective	*N* = 20	Tai Chi	Area and mean velocity of CoP, Postural stability and control	Improve motor control and postural stability
Brismee et al., 2007	RCT	*N* = 41	Group and home-based tai chi	VAS, WOMAC, active range of motion for flexion and extension	Reduce pain, improve physical function
Hu et al., 2020	Prospective	*N* = 52	Tai Chi	VAS, WOMAC, and knee and ankle proprioception	Reduce pain, improve ankle and knee proprioception
Tsai et al., 2013	Pilot cluster-randomized trial	*N* = 55	Tai Chi	WOMAC, Get Up and Go test, Sit-to-Stand test, and Mini-Mental State Examination	Reduce pain, improve stiffness, improve physical function, and improve cognitive function
Hu et al., 2021	Systematic review and meta-analysis	16 studies (*N* = 986)	Tai Chi	WOMAC, 6-MWT, dynamic balance, and physiological and psychological health	Reduce pain, maintain mobility, enhance muscle strength, enhance range of joint motion, and ameliorate physical and mental health
Xiao et al., 2021	RCT	*N* = 68	Wuqinxi	WOMAC, Berg Balance Scale, TUG Test, 6-MWT, 30sCST, and isokinetic muscle strength testing of knee flexion and extension	Decline pain, increase knee extensor strength and Knee flexor strength
Xiao et al., 2020	RCT	*N* = 98	Wuqinxi	Berg Balance Scale, TUG Test, 6-MWT, 30sCST, WOMAC, knee extension strength, and knee flexion strength	Decline pain, increase knee extensor strength, and Knee flexor strength
Xiao and Li, 2021	Prospective	*N* = 284	Wuqinxi	Limits of stability tests, static posture stability tests, dynamic fall index tests, WOMAC, and SF-36	Reduce pain, improve balance function, and improve subjective quality of life
Cheung et al., 2014	RCT	*N* = 36	Yoga	WOMAC, QoS, QoL, repeated chair stands, balance, and timed 8 foot walk	Reduce pain, decrease stiffness, improve sleep, and improve physical function
Moonaz et al., 2015	RCT	*N* = 75	Yoga	SF-36, HRQoL	Increase physical activity, improve physical and mental health, and improve quality of life
Longpre et al., 2015	Prospective	*N* = 30	Yoga-based knee strengthening exercises	Muscle Activation, Knee Adduction Moment	Improve leg strength
Kuntz et al., 2018	RCT	*N* = 31	Biomechanically-based yoga	KOOS, ICOAP, self-reported physical function, 6-MWT,30sCST, 40 m W, TUG test, stair ascent test, muscle strength, CESD, and HRQoL	Reduce pain, improve physical function, improve quality of life, increase muscle strength, and improve mobility

BDNF, Brain-derived neurotrophic factor; BMI, body mass index; CoP, Center of Pressure; DLPFC, dorsolateral prefrontal cortex; HRQoL: health-related quality of life; INF-γ, interferon-γ; KOOS, Knee Injury and Osteoarthritis Outcome Score; MRI, magnetic resonance imaging; PD-1, programmed cell death protein 1; QoL, quality of life; QoS, quality of sleep; RCT, randomized controlled trial; SF-36, Medical Outcomes Study Short-Form Health Survey; TIM-3, T-cell Ig-and mucin-domain–containing molecule–3; TUG test, Timed Up and Go test; VAS, Visual Analog Scale; WOMAC, Western Ontario and McMaster Universities Osteoarthritis Index; 6-MWT, 6-min walk test; 30sCST, 30 s Chair Stand Test; 40 m W, 40-meter walk; and CESD, Center for Epidemiological Studies Depression Scale.

contraction, muscle tension increases significantly, while muscle length does not change (Huang et al., 2018). Miyaguchi et al. (2003) found that 12 weeks of exercise of the quadriceps of patients with KOA, through increasing the molecular weight of the hyaluronan and the viscosity of the joint fluid in the knee joint to improve the symptoms of KOA (Miyaguchi et al., 2003). Isometric resistance exercise of the quadriceps of patients with KOA can significantly increase the sensitivity and coordination of proprioceptors in the quadriceps (Topp et al., 2002). Tok et al. (2011) demonstrated that isometric exercise of the quadriceps can improve the dynamic and static balance of patients with KOA. Furthermore, maximum or secondary isometric exercise is benefit in restoring neuromuscular function in patients with KOA (Mau-Moeller et al., 2017). Ojoawo et al. (2016) found that isometric exercise can effectively improve the joint stiffness and physical difficulties associated with KOA. Anwer et al. (2013) demonstrated that isometric exercise can significantly increase muscle strength in both female and male patients. When only the ipsilateral homologous muscle is strengthened, there is a cross-training effect on the contralateral quadriceps in patients with KOA (Onigbinde et al., 2017).

In summary, isometric exercise can increase the hyaluronic acid levels and viscosity of the joint fluid in the joint capsule in patients with KOA. Moreover, isometric exercise has an excellent therapeutic effect on proprioception and muscle strength recovery in patients with KOA. There is no difference in the training effect between different sexes.

Isotonic Exercise and KOA

Isotonic exercise (also known as dynamic contraction) refers to exercise training with isotonic contraction. During muscle contraction, muscle tension remains unchanged, but the length of the muscle fibers is shortened or prolonged, resulting in visible movement of the joints. The change in muscle fiber length during muscle contraction can be categorized based on isotonic centripetal exercise (such as jumping) or isotonic centrifugal exercise (such as squatting and walking down stairs). Compared with isometric exercise and isokinetic exercise, isotonic exercise has the most significant effects to relieve pain for patients with KOA (Huang et al., 2003). Furthermore, a clinical trial of 61 patients with KOA showed that isotonic exercise can alleviate pain, stiffness, and improve knee joint function effectively, but it cannot increase quadriceps strength significantly (Malas et al., 2013). In addition, Tanaka et al. (2018) found that the muscle strength of patients with KOA could be effectively improved through low-load isotonic resistance exercise. Moreover, isotonic-centripetal exercise and isotonic-eccentric exercise has the same effect on increasing knee extension and knee flexion muscle strength, as well as relieving pain in patients with KOA (Vincent et al., 2019).

In summary, isotonic exercise is prevalent in people's daily life. Isotonic exercise can alleviate pain; enhance muscle strength significantly in patients with KOA. The therapeutic effects of different isotonic exercises are almost similar. People could choose different training modes based on their preferences, goals, physical tolerance levels, and equipment availability.

Other Strength Exercise and KOA

Apart from above strength exercise training options, many other strength exercise training options are available in hospital. Resistance training is one of the most common rehabilitation training options for patients with KOA, and it is often combined with aerobic exercise, strength training, or aquatic exercise (Kristensen and Franklyn-Miller, 2012; Coudeyre et al., 2016; Munukka et al., 2020). Following resistance exercise, the pain thresholds of patients with KOA changed, and pain sensitivity tolerance decreased (Burrows et al., 2014). There is a significant improvement in function, strength, and mobility after 8 weeks of resistance exercise in patients with KOA (Pazit et al., 2018). Elastic-band exercise is flexible and convenient, an 8 weeks of leg press exercise using elastic bands has been shown to significantly improve lower-extremity function in females with KOA (Chang et al., 2012). However, the existing research does not prove that elastic-band training of the quadriceps femoris results in better pain relief than quadriceps strengthening exercise in patients with KOA (León-Ballesteros et al., 2020). Recently, resistance training with blood flow restriction has attracted the attention of physical therapists and has been applied in patients with KOA. Using a pressure cuff with continuous compression of the proximal portion of the extremity, the cuff occludes venous return from the muscle, and maintains partial arterial flow in the muscle (Cuyul-Vasquez et al., 2020). Resistance training with blood flow restriction can induce less joint stress, relieve pain, increase muscle strength, increase quadriceps muscle mass, and increase functionality in patients with KOA (Ferraz et al., 2018). Moreover, blood flow-restricted low-load resistance training is effective in increasing knee extensor strength and leg press ability in women at risk for KOA (Segal et al., 2014).

In summary, strength training is an essential part of exercise training for patients with KOA. Different strength training options have different effects, but the common feature is increasing muscle strength. Different strength training options may be combined to treat patients with KOA of different stages. This may be an important development direction for the future treatment of KOA.

Neuromuscular Exercise and KOA

Neuromuscular exercise can improve balance, muscle activation, functional alignment, and joint stability. The primary purpose of neuromuscular exercise is to achieve compensatory functional stability and improve sensorimotor control (Ageberg et al., 2013). A randomized, single-blind, controlled trial found that neuromuscular exercise can significantly improve cartilage matrix quality and reduce knee-joint loads in patients with mild KOA (Holsgaard-Larsen et al., 2017). Individualized and gradual neuromuscular exercise improves patient-reported outcomes and physical function (such as the ability to move independently and knee extensor strength), even in older patients with severe primary KOA (Ageberg et al., 2013). A randomized controlled trial found that 8 weeks of supervised neuromuscular exercise before total knee replacement can effectively improve the quality of life of KOA patients after operation (Fernandes et al., 2017). Neuromuscular exercise may be the best choice of exercise

training for pain relief in KOA patients with inverted thrust (Ageberg and Roos, 2015). Increased medial knee neuromuscular activity is prevalent for exhibiting medial knee joint laxity and varus alignment patients. Increasing neuromuscular exercise can increase the co-contraction, amplitude, and duration of the lateral knee muscles in patients with KOA (Mills et al., 2013). Moreover, neuromuscular exercise has a better therapeutic effect on knee joint loads, pain, and physical function in patients with intra-KOA and varus malalignment than quadriceps strengthening exercise (Bennell et al., 2011). Holsgaard-Larsen et al. (2018) showed that comparing to pharmacotherapy, neuromuscular exercise might be a better choice to relieve long-term symptoms such as swelling and stiffness, dealing with mechanical problems, and avoiding the potential side effects of analgesics and anti-inflammatory drugs. Clausen et al. (2017) first reported that neuromuscular exercise is therapeutic for patients with KOA in the early and mid-stage, but it cannot improve patients' ability to jump.

Clinically, neuromuscular exercise has a good therapeutic effect on patients with KOA with inverted thrust or varus malalignment. For end-stage KOA patients who require knee arthroplasty, neuromuscular exercise before surgery can effectively relieve postoperative pain. Currently, neuromuscular exercise focuses on patient's post-knee joint replacement, and more research on neuromuscular exercise for early KOA is needed.

Balance Training and KOA

Balance training challenges people to regain their center of gravity during destabilizing movements and to reduce the size of their support base, which requires feedforward and feedback postural control instances and gait tasks (Schlenstedt et al., 2015). There are many forms of balance training, including static balance training, dynamic balance training, balance instrument training, and Virtual Reality (VR) training (Diracoglu et al., 2005; Duque et al., 2013; Takacs et al., 2017). Balance training is necessary for KOA patients with a higher risk of falling; it can reduce the risk of falls in patients with KOA (Levinger et al., 2017; Anderson et al., 2019). The clinical trial showed that preoperative balance training can improve the early postoperative balance but not the perceived functionality of patients with KOA (Blasco et al., 2020). Balance training can improve walking ability and balance performance, alleviate pain, as well as enhance physical function (Doma et al., 2018). Furthermore, progressive and dynamic balance training has a better effect than conventional physiotherapy on improving physical function, range of motion, and balance for patients with KOA (Lee et al., 2021). Biodex balance training is better than traditional exercise programs to improve functional performance, stability, and body sensation, and to reduce swaying and pain in patients with KOA (Javed et al., 2021). A randomized controlled trial proved that dynamic balance training based on visual feedback can alleviate knee pain and joint stiffness, by preventing asymmetric joint alignment, improving the motion of the knee joint, and reducing mechanical friction of the knee (Lee et al., 2020).

In short, balance training is better than traditional physical training to improve the physical function of patients with KOA. Enhancing balance ability, stabilizing motor function, and reducing fall risk are a characteristic effect of balance training.

Proprioceptive Training and KOA

In patients with KOA, weakening and damage of the knee muscles, tendons, ligaments, and articular capsule is related to body's proprioception decreases (Relph and Herrington, 2015). The weakening and damage of proprioceptors make patients' pain and perception abnormal could lead to the severe consequence of KOA (Bennell et al., 2003). Weak patients with poor proprioceptors have limited joint functional ability (van der Esch et al., 2007). Proprioceptive training takes more significant improvement in proprioception recovery, walking time, and knee extension strength in patients with KOA (Lin et al., 2009). At the same time, proprioceptive training can delay the progression of KOA, such as reducing pain, improving joint and muscle health, and improving the functional quality of patients with early-stage KOA (John Prabhakar et al., 2020). Furthermore, enhancing patients' walking ability can effectively reduce the basic risk of falls in patients with end-stage KOA (Aljehani et al., 2021). Duman et al. (2012) proprioceptive training has a great effect on improving the accuracy of static balance and proprioception.

In summary, proprioceptive training through activation of proprioceptors improves the condition of KOA. For end-stage KOA, increasing exercise precisely for proprioception and balance dysfunction is necessary. The effect is more pronounced in patients who experience pain when bearing weight.

Aquatic Exercise and KOA

Aquatic exercise *via* temperature stimulation and buoyancy of water improve patients' motor dysfunction (Hinman et al., 2007). Especially weight-bearing loss caused by the buoyancy of water, which play a therapeutic role for patients (Lu et al., 2015). There is a faster effect to decrease knee stiffness in a short period therapy with aquatic exercise than routine rehabilitation training (Munukka et al., 2020). Aquatic exercise with progressive resistance can increase the thickness of the posterior region of interest of the medial femoral cartilage, and improve cardiopulmonary function (Munukka et al., 2016). Further studies revealed that regular swimming can reduce joint pain and stiffness, improve muscle strength and function in middle-aged and older adults with KOA (Alkatan et al., 2016). Compared to land-based exercise, aquatic exercise has fewer side effects and better therapeutic effects (Lund et al., 2008; Lu et al., 2015). Aquatic exercise has a better therapeutic effect for obese postmenopausal women with KOA, not only can alleviate pain, dysfunction, and improves quality of life, but also decrease fat mass (Lim et al., 2010; Yazigi et al., 2013; Waller et al., 2017; Rewald et al., 2020). A randomized controlled trial showed that dance-based aquatic exercise can significantly improve physical function and cardiorespiratory capacity, as well as decrease postexercise heart rate and fatigue in obese postmenopausal women with KOA (Casilda-Lopez et al., 2017). Roper et al. (2013) found that acute aquatic treadmill exercise can be a conservative treatment to improve joint angular velocity and arthritis-related joint pain.

Aquatic exercise can significantly improve knee flexibility, strength, and aerobic fitness, and do not worsen the joint condition associated with KOA (Wang et al., 2007). Kunduracilar et al. (2018) found that adding upper extremity and trunk exercises to lower extremity exercises during aquatic exercise training is effective for improving physical function, balance, and pain.

In summary, water temperature stimulation and buoyancy are the advantages of aquatic exercise. Aquatic exercise has a recognized effect on physical function and quality of life of patients with KOA. Moreover, due to the weight-bearing reduction effect of water, aquatic exercise is a good treatment option for KOA patients with a high body mass index.

Traditional Exercise and KOA

Ba Duanjin and KOA

Ba Duanjin, a traditional Chinese exercise, is described as a mind–body practice that integrates spirit and meditation with slow and gentle postures, as well as musculoskeletal stretching and deep breathing (Zeng et al., 2020). It can alleviate morning stiffness, spinal pain, and fatigue, and it can relieve musculoskeletal pain in elderly people in particular (Li et al., 2019; Xie et al., 2019). An et al. (2008) found that Ba Duanjin is a safe and feasible treatment option for elderly with KOA, as it offers reductions in pain, stiffness, and disability, which can improve patients' quadriceps strength and aerobic ability. Long-term Ba Duanjin may be viable and safe exercise training for KOA patients to relieve pain and stiffness, and improve physical function (An et al., 2013). A study showed that Ba Duanjin can increase the resting-state functional connectivity of the right lingual gyrus and the right cerebellum/occipital fusiform gyrus/thalamus, as well as decrease the resting-state functional connectivity of the right medial orbital prefrontal cortex, left superior parietal lobule, and left superior temporal gyrus in patients with KOA (Liu et al., 2019a). Liu et al. (2019b) found that patients with KOA have higher dorsolateral prefrontal cortex resting-state functional connectivity on the left side, as well as increase dorsolateral prefrontal cortex resting-state functional connectivity in the left-supplementary motor area and left-temporoparietal junction after participating in Ba Duanjin.

In summary, Ba Duanjin regulates both the downstream opioid energy pathway and the dorsolateral prefrontal cortex (cognitive control) pathway, altering inflammatory blood markers through resting-state functional connectivity and the dorsolateral prefrontal cortex-supplementary motor area resting-state functional connectivity of the reward/incentive system. Furthermore, it has a particular curative effect on patients with KOA, which is associated with decreased joint mobility, and is thus worthy of continued clinical research.

Tai Chi and KOA

Tai Chi, a gentle aerobic exercise, is derived from ancient Chinese martial arts that can relax the body and mind (Chen et al., 2016). Compared with other conventional physical therapy, Tai Chi has a better treatment effect on reducing depression (Li et al., 2020). The shortest adequate treatment time of Tai Chi for patients with KOA is 2–5 weeks (Lee et al., 2018). A clinical experiment showed that Tai Chi can change KOA patients' gait and plantar pressure load pattern during walking (Zhang et al., 2020). You et al. (2021) found that Tai Chi can be an excellent physical training strategy for improving postural control and walking function in older individuals with KOA. Furthermore, Tai Chi has positive effects on muscular activities and proprioception of the leg and ankle, and it can improve balance on both rigid and foam surfaces in older patients with KOA (Ghandali et al., 2017; Hu et al., 2020). Tsai et al. (2013) found that Tai Chi might have a therapeutic effect on elderly people with cognitive impairment and KOA. Hu and Brismee et al. found that Tai Chi can significantly reduce pain and dysfunction, improve KOA patients' physical and mental health, which can be an alternative to non-drug therapies in rehabilitation programs (Brismee et al., 2007; Hu et al., 2021).

In summary, Tai Chi is a popular mind-body exercise, which can relieve pain, reduce dysfunction of KOA, and it has significant effects on improving depression, exercise gait, and postural stability. However, the effects of Tai Chi are slowly, it always takes more than 2 weeks to get an effect.

Wuqinxi and KOA

Wuqinxi is a traditional Chinese exercise that was designed by Hua Tuo at the end of the Eastern Han Dynasty (Guo et al., 2018). It can release muscle tone and increase blood flow, thereby relieving pain. Long-term Wuqinxi can significantly enhance the physical function of chronically ill patients, improving their strength, bone density, balance, joint flexibility, mental vitality, and psychological confidence (Wei et al., 2015). A randomized controlled trial showed that from pretest to follow-up, KOA patients in the Wuqinxi group showed significantly improved, isokinetic knee flexion, and extension strength, timed up and go test, 6-min walk test, 30-s chair stand test, and their pain was much relieved (Xiao et al., 2021). Kang et al. showed that Wuqinxi promotes balance and pain relief in KOA patients more effectively than traditional physiotherapy exercises (Xiao et al., 2020). In addition, Xiao et al. found that the stability test, the static postural stability test, and the dynamic fall index test results of elderly, female KOA patients improved after 24 weeks of Wuqinxi (Xiao and Li, 2021).

In short, Wuqinxi is a very suitable exercise for elderly people, which can enhance the balance of KOA patients, reduce pain, and increase muscle strength. However, there is not enough research on Wuqinxi. More profound and relevant studies are needed.

Yoga and KOA

Yoga, a traditional Indian exercise, has apparent effects on psychological and physical health (Field, 2016). Yoga can stimulate baroreceptors, increase vagus nerve activity and serotonin levels, slow cortisol and substance P production, and relieve pain (Melzack and Wall, 1965). A randomized controlled study showed that after 8 weeks of yoga training, the pain, stiffness, and sleep disturbance of elderly female KOA patients were significantly reduced at 20 weeks (Cheung et al., 2014). A randomized controlled trial by Moonaz et al. (2015) found that yoga can safely increase

physical activity, improve physical and mental health, and improve health-related quality of life in sedentary patients with arthritis. Lomgpre et al. showed that the yoga postures of squatting and lunging can improve quadriceps strength and minimize exposure to high adduction torque of the knee joint in patients with KOA (Longpre et al., 2015). Compared with traditional exercise-based program, biomechanically-based yoga is better for female patient with KOA to reduce pain, improve physical function and mobility (Kuntz et al., 2018).

In summary, the therapeutic effect of yoga on KOA is noticeable. Breaking down the various postures of yoga and designing more appropriate exercise training programs based on effective yoga postures may be a new direction for the treatment of KOA in the future.

All in all, traditional exercise has been proven to have a certain therapeutic effect on KOA, such as reducing pain, improving joint mobility, improving quality of life, and enhancing physical function (**Table 2**). However, the evidence and mechanism are not sufficient; we hope there will be more and more studies on traditional exercise.

CONCLUSION

Various exercise training options have been reported to have therapeutic effects on KOA. Among the different exercise interventions, aerobic exercise, which alleviates pain and improves physical function, is the most widely used. Strength training is the most effective exercise therapy against muscle weakness. Neuromuscular exercise and balance training are the best exercise training options to improve proprioception, sensorimotor control, and functional stability. Aquatic exercise has fewer side effects than other exercise training, more and more people are trying aquatic exercise. In addition, many traditional exercises, such as Ba Duanjin, Tai Chi, Wuqinxi, and Yoga are gradually being used to treat KOA. Their effects on the psychology of patients with KOA are noticeable. On the premise of ensuring patient safety, we should provide more individualized exercise prescriptions for patients with different stages of KOA.

However, the current research on exercise training for KOA is not satisfactory. We need more extensive and more profound clinical studies (involving education, traditional exercise, etc.) to confirm the effectiveness of exercise therapy in treating patients with KOA. In addition, combining various exercise therapies to make an optimal treatment plan for different patients would be an important development direction for the treatment of KOA in the future. At present, the mechanisms of exercise training for KOA treatment are poorly understood. We urgently need more animal experiments to prove the principle of efficient treatment, and to promote the development of exercise therapy for KOA treatment in humans.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material; further inquiries can be directed to the corresponding authors.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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Exercise Alleviates the Apolipoprotein A5-Toll-Like Receptor 4 Axis Impairment in Mice With High-Fat Diet-Induced Non-alcoholic Steatohepatitis

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Background: Apolipoprotein A5 (ApoA5), an important modulator of plasma and hepatic triglyceride metabolism, has been found to be downregulated by metformin to improve non-alcoholic fatty liver disease. Meanwhile, exercise has been recommended as a therapeutic strategy for non-alcoholic steatohepatitis (NASH). However, no study has yet determined whether exercise affects hepatic ApoA5 expression or the inhibition of ApoA5 to toll-like receptor 4 (TLR4). We herein examined the effects of exercise on hepatic ApoA5 expression and the relevance of ApoA5 and TLR4-mediated pathway in mice with high-fat diet (HFD)-induced NASH.

Methods: Male C57BL/6J mice were built NASH model with high-fat diet for 12 weeks, and following mice were subjected to exercise for 12 weeks on a treadmill. Microscopy and enzyme-linked immunosorbent assay were used to measure histological analysis of liver and hepatic lipids, respectively. Quantitative real-time PCR and western blot were used to determined mRNA and protein levels of ApoA5 and TLR4-mediated nuclear factor kappa B (NF-κB) pathway components, respectively. ApoA5 overexpression plasmids transfected into mice to investigate the relevance of ApoA5 and TLR4.

Results: 12 weeks of exercise remarkably alleviated HFD-induced hepatic lipid accumulation, inflammation, and fibrosis, as well as reduced serum lipopolysaccharide (LPS), hepatic TLR4, myeloid differentiation factor 88 (MyD88), and NF-κBp65 expression. Importantly, exercise did not reduce ApoA5 expression but instead enhanced its ability to suppress TLR4-mediated NF-κB pathway components by decreasing circulating LPS in our experiments involving transfection of ApoA5 overexpression plasmids and LPS interventions.

Conclusion: The results demonstrated that exercise improved HFD-induced NASH by triggering the inhibitory effects of ApoA5 on the TLR4-mediated NF-κ B pathway.

Keywords: exercise, ApoA5, TLR4, LPS, non-alcoholic steatohepatitis (NASH)

INTRODUCTION

Non-alcoholic steatohepatitis (NASH) is a severe manifestation of non-alcoholic fatty liver disease (NAFLD) and has been considered the main cause of liver failure, cirrhosis, and cancer (Todoric et al., 2020). The lipid metabolic disorder is an important pathogenesis factor of NASH (Yu Y. et al., 2019). Reports have shown that more than 20% of patients with NASH will have developed cirrhosis during their lifetime (Younossi et al., 2018). Regrettably, no specific drugs have yet been approved for NASH, making liver transplantation the leading treatment method in recent years (Noureddin et al., 2018). At present, lifestyle modifications, such as exercise, have been primarily recommended for the prevention and treatment of NASH in the United States, Europe, and China (Fan and Farrell, 2009; Chalasani et al., 2012; European Association for the Study of the Liver et al., 2016). However, the mechanisms responsible for the protective effects of exercise against NASH have remained unclear.

Apolipoprotein A5 (ApoA5), a member of the apolipoprotein family specifically expressed in the liver (O'Brien et al., 2005), has been considered as an important modulator of plasma and hepatic triglyceride (TG) metabolism in earlier studies (Pennacchio et al., 2001; Priore Oliva et al., 2005; Shu et al., 2007; Shu et al., 2010; Sharma et al., 2013). Shu et al. (2010) and Blade et al. (2011) showed that *APOA5* transgenic mice and hepatoma cells transfected with ApoA5 expression plasmids exhibited increased hepatic TG accumulation. Furthermore, recent data have indicated that hepatic ApoA5 mRNA and protein is overexpressed in patients and mice with NAFLD. The hepatic steatosis and other phenotypes of NAFLD may be alleviate with decrease of ApoA5 mRNA expression or with down-regulation of ApoA5 involving signaling pathway (Ress et al., 2011; Feng et al., 2015; Lin et al., 2017). But there was an opposite result. van den Berg et al. (2013) showed that *APOA5* (−/−) mice fed high fat diet manifest greater hepatic steatosis, and ApoA5 overexpression prevented ectopic lipid accumulation rather than increasing it. These findings implicated that ApoA5 may be as a potential therapeutic target for NASH, whereas the mechanisms need to be clarified. Additionally, ApoA5 acts as a predictor for remnant liver growth after preoperative portal vein embolization and liver surgery (Hoekstra et al., 2012). Evidence has shown that hepatic ApoA5 overexpression inhibited the protein expressions of toll-like receptor 4 (TLR4) and TLR4-mediated signaling pathway, thereby alleviating fulminant liver failure in mice (Tao et al., 2019). However, the inhibitory effects would weaken with increasing concentrations of lipopolysaccharide (LPS) despite ApoA5 overexpression (Tao et al., 2019).

Lipopolysaccharide is part of the outer membranes of gram-negative bacteria, with its circulating concentrations significantly increasing in mice with high-fat diet (HFD)-induced NASH (Aron-Wisnewsky et al., 2020). However, LPS was partially reduced after exercise (Yu C. et al., 2019). Moreover, mRNA and protein expression of ApoA5 was remarkably increased in mice with HFD-induced NASH (Ress et al., 2011; Feng et al., 2015; Lin et al., 2017), implying that the inhibitory effects of ApoA5 on TLR4-mediated signaling pathway depended on the reduction of circulating LPS concentrations in mice with HFD-induced NASH. This study therefore established a HFD-induced NASH model to investigate the effects of exercise on hepatic ApoA5 expression and determine the relevant of ApoA5 and TLR4-mediated signaling pathway.

MATERIALS AND METHODS

Animal Model

Male C57BL/6J mice aged 6 weeks were purchased from the Experimental Animal Center of Guangdong Province (Guangzhou, China) and acclimated for 1 week. The mice were housed on a 12-h light–dark cycle at 22–24°C and were provided free access to food and water. All animal care and lab experimental procedures were conducted in accordance with the Chinese Guidelines for Animal Welfare and Experimental Protocols and were approved by the Animal Experiment Administration Committee of Guangzhou Sport University (2020DWLL-005). All mice were randomly divided into three groups: low-fat diet control group (LFD, n = 24), a high-fat diet group (HFD, n = 24), and a high-fat diet plus exercise group (HFD + EXE, n = 24). The LFD group received a low-fat diet containing 10% kcal from fat (D12450J, Research Diets Inc.), whereas the HFD and HFD + EXE groups were fed a HFD containing 60% kcal from fat (D12492, Research Diets Inc.) for 24 weeks, and after 12 weeks of HFD feeding, mice in HFD + EXE group were subjected to exercise training for 12 weeks. A day after the final training session, mice were killed under anesthesia (sodium pentobarbital 50 μg/g) for collection of serum and liver.

Training Procedures

The exercise group was trained at 0% grade 5 days per week for 12 weeks on a treadmill. After a 5 min of warm up period at 6 m/min, mice performed 20 min of main exercise at 10 m/min and 5 min of cool down at 6 m/min were performed during the first week for adaptation. From the 2nd week to the 12th week, mice performed 5 min of warm up at 6 m/min, 50 min of main exercise at 12 m/min (75% maximum oxygen consumption) (Fernando et al., 1993), and 5 min of cool down at 6 m/min were performed.

Hepatic Triglyceride and Total Cholesterol Analysis

Hepatic TG and TC levels were measured using commercial kits (Jiancheng Bioengineering Institute, Nanjing, China), according to the manufacturer's instructions.

Histological Analysis of Liver

Fresh liver tissues were fixed with 4% paraformaldehyde solution for 24 h, embedded in paraffin, and sliced into 4-μm sections for hematoxylin-eosin (H&E) staining and Sirius Red staining. The NAFLD activity score (NAS) was calculated according to the guidance provided by the Pathology Committee of the NASH Clinical Research Network (Kleiner et al., 2005): steatosis (<5% = 0, 5–33% = 1, 33–66% = 2, >66% = 3), lobular

inflammation (none = 0, <2 foci = 1, 2–4 foci = 2, >4 foci = 3), and hepatocellular ballooning (none = 0, few = 1, prominent = 2). All features were scored in a blinded manner based on six fields of view per sample. Individual scores for each field of view were summed to calculate the NAS for each animal. Histological assessments were performed by a pathologist who was blinded to the treatment.

Blood Analysis

Serum lipopolysaccharide (LPS) levels was measured using ELISA kits (CUSABIO Technology LLC.), according to the manufacturer's instructions.

Examining the Effects of Apolipoprotein A5 on Toll-Like Receptor 4-Mediated Nuclear Factor Kappa B Pathway

To analyze the effects of ApoA5 on TLR4-mediated signaling pathway, LFD mice were randomly divided into three groups (n = 4/group) and injected with an ApoA5 overexpression plasmid (pEGF-N1-ApoA5, 10 μg), negative control empty vector (pEGF-N1 vector, 10 μg), and normal saline through the tail vein, respectively. The mice were killed, after which serum and liver samples were harvested and stored for analysis after treatment for 3 days. The ApoA5 overexpression plasmid (pEGF-N1-ApoA5) and negative control empty vector (pEGF-N1 vector) were designed and purchased from Heyuan Biotechnology (OBIO, China).

Examining the Effect of Lipopolysaccharide on the Ability of Apolipoprotein A5 to Inhibit Toll-Like Receptor 4-Mediated Nuclear Factor Kappa B Pathway

To investigate the ability of ApoA5 to inhibit TLR4-mediated NF-κB pathway within a certain LPS concentration, HFD + EXE mice were randomly divided into three groups (n = 4/group) that subsequently received intraperitoneal injections of normal saline, 5 μg/kg·wt LPS and 10 μg/kg·wt LPS, respectively. After the mice were killed, serum and liver samples were harvested and stored for analysis after treatment for 12 h. LPS (*Escherichia coli*, 0111:B4) was purchased from Sigma (St. Louis, MO).

Quantitative Real-Time PCR

The primer sequences used herein are detailed in **Table 1**. Expression levels were normalized to those of the housekeeping gene GAPDH.

TABLE 1 | Primer sequences used for qRT-PCR.

Gene	Forward primer (5′–3′)	Reverse primer (5′–3′)
ApoA5	AGTTGGAGCAAAGGCGTGAT	TTTCCGAATGCCTTCTGGGT
TLR4	TGAGGACTGGGTGGAAATG AGC	CTGCCATGTTTGAGCAATCTCAT
MyD88	TGACCCCACTCGCAGTTTGT	TTTGTTTGTGGGACACTGCTTTC
NF-κB	CGAGTCTCCATGCAGCTACG	TTTCGGGTAGGCACAGCAATA
Collagen I	GCCCGAACCCCAAGGAAG AAGC	CTGGGAGGCCTCGGTGACA TTAG
GAPDH	CCTCGTCCCGTAGACAAAATG	TGAGGTCAATGAAGGGGTCGT

Western Blot Analysis

Protein was extracted from mouse livers. Total protein concentrations were measured using a BCA protein assay kit (Thermo Fisher Scientific). Equal amounts of total protein were separated using sodium dodecyl-sulfate polyacrylamide gel electrophoresis and transferred to polyvinylidene fluoride membranes. These membranes were then blocked and incubated with primary antibodies against ApoA5 (Abcam, Cambridge, MA, United States, ab239579), TLR4, MyD88, NF-κBp65, and β-actin (Cell Signaling Technology, Beverly, MA, United States, #14358, #4283, #8242 and #4970). Membranes were incubated for 1 h with the following secondary antibodies: goat anti-mouse IgG-HRP and mouse anti-rabbit IgG-HRP (Cell Signaling Technology, Beverly, MA, United States, #43593 and #58802). Signal detection was performed using SuperSignal Dura Substrate (Pierce, Biotechnology, United States), after which immunoblot signals were quantified using Quantity One software.

Statistical Analysis

All data were expressed as mean ± standard error of the mean. Statistical significance was evaluated using one-way analysis of variance with the Bonferroni test for multiple comparisons. All analyses were performed using GraphPad Prism 5.0, with a P value of $\leq$0.05 indicating statistical significance.

RESULTS

Exercise Ameliorates High-Fat Diet-Induced Body and Liver Weight Gain, Hepatic Lipid Accumulation, Inflammation, and Fibrosis

Non-alcoholic steatohepatitis is characterized by hepatic steatosis, inflammation, and fibrosis (Tiniakos et al., 2010; Liu et al., 2016). Mice with HFD-induced NASH had increased body weight and liver weight, which reduced significantly after 12 weeks of exercise training (**Figures 1A,B**). The HFD + EXE group had significantly lower hepatic lipid accumulation, total cholesterol (TC), and TG levels compared to the HFD group (**Figures 1E,F**). Exercise significantly reduced histological parameters reflecting hepatic steatosis and inflammation, such as the steatosis score, lobular inflammation score, ballooning score, and total NAFLD activity score (NAS) (**Figures 1C,D**). Sirius Red staining showed that exercise suppressed HFD-induced collagen accumulation (**Figures 1G,H**). The HFD + EXE group had lower collagen I mRNA levels compared to the HFD group (**Figure 1H**). The aforementioned results indicated that 12 weeks of exercise training remarkably ameliorated HFD-induced NASH.

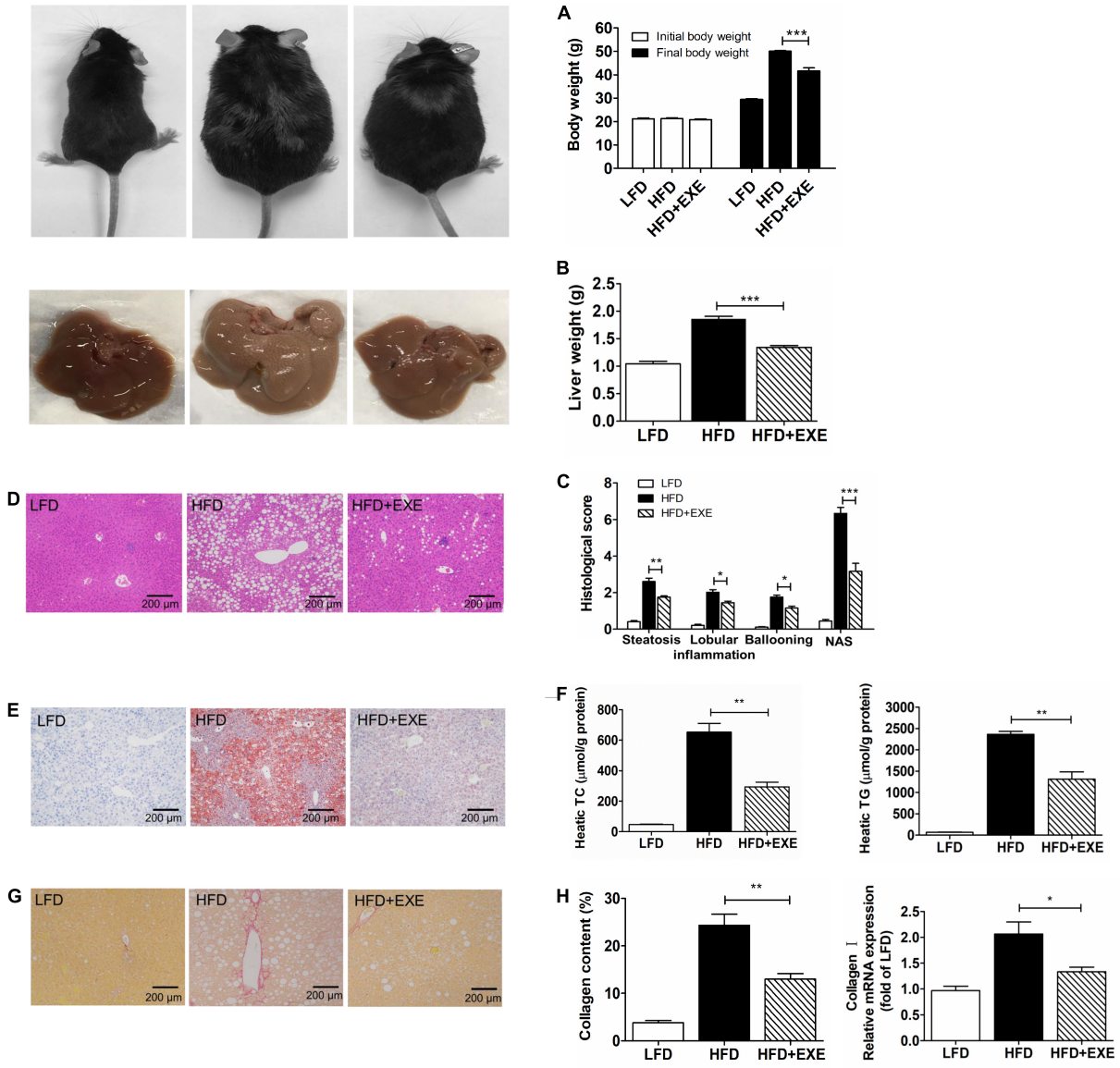

FIGURE 1 | Exercise reduced HFD-induced body weight, liver weight, hepatic steatosis, inflammation, and fibrosis. The body weight **(A)** and liver weight **(B)** decreased after 12 weeks of exercise training. Liver sections stained with H&E (×100) **(C)**. Hepatic histological analysis of steatosis, inflammation, ballooning, and NAFLD activity score (NAS) **(D)**. Hepatic lipid accumulation as determined by Oil Red O staining (×100) **(E)**. Hepatic TC levels and TG levels **(F)**. Liver sections stained with Sirius Red (×100) **(G)**. Collagen content determined by counting Sirius Red positive areas in six randomly selected fields using Image Pro Plus 6.0 software and hepatic mRNA levels of Collagen I **(H)**. Results are presented as the mean ± SEM, n = 6–8 per group. * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$.

Exercise Does Not Significantly Decrease Hepatic Apolipoprotein A5 Expression but Reduces Circulating Lipopolysaccharide Concentrations and Inhibits Hepatic Toll-Like Receptor 4-Mediated Signaling Pathway

Compared to the LFD group, the HFD group exhibited significantly higher circulating LPS concentrations (609.42 ± 42.21 vs. 71.11 ± 14.15 ng/mL), which sharply declined after exercise (230.88 ± 35.03 vs. 609.42 ± 42.21 ng/mL) (**Figure 2A**). Although exercise slightly decreased ApoA5 mRNA and protein expression in the liver, no significant difference in hepatic mRNA and protein expression of ApoA5 was observed between the HFD and HFD + EXE groups (**Figures 2B,C**). Compared to the LFD group, HFD group exhibited higher levels of TLR4 (**Figures 2B,D**). Exercise reduced both mRNA and protein levels of TLR4 (**Figures 2B,D**). Moreover, MyD88 and NF-κBp65 mRNA and protein levels were also markedly lowered by exercise (**Figures 2E,F**). Supporting such findings, one study showed that high expression of ApoA5 can inhibit TLR4 expression (18). The aforementioned results implied that exercise

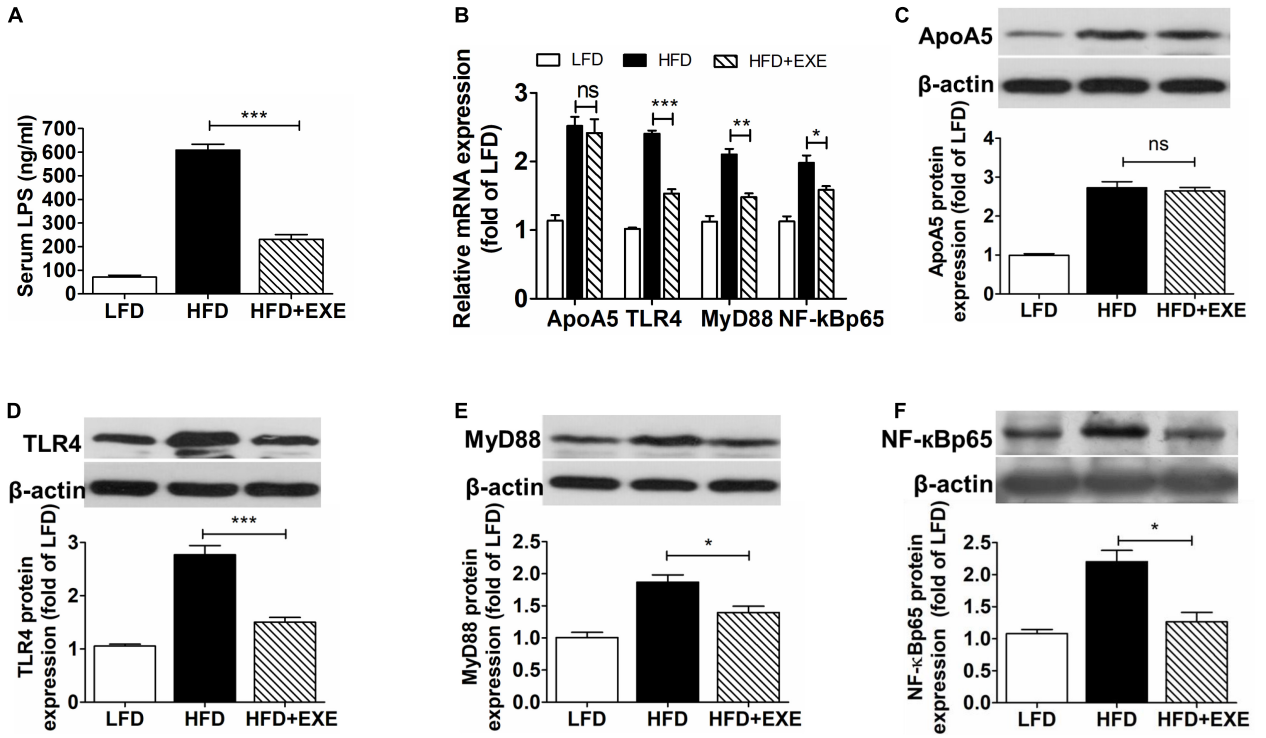

FIGURE 2 | Exercise did not affect hepatic ApoA5 expression but reduced hepatic TLR4-mediated pathway components and circulating LPS concentrations. Circulating LPS concentrations **(A)**. Hepatic mRNA levels of ApoA5, TLR4, MyD88, and NF-κBp65 **(B)**. Western blot analyses of ApoA5 **(C)**, TLR4 **(D)**, MyD88 **(E)**, and NF-κBp65 **(F)**. Results are presented as the mean ± SEM, n = 4–6 per group. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, and ^{ns} $P > 0.05$ (no difference).

enhanced the ability of ApoA5 to inhibit the TLR4-mediated signaling pathway at certain LPS concentrations.

Exercise Enhances the Ability of Apolipoprotein A5 to Inhibit the Toll-Like Receptor 4-Mediated Nuclear Factor Kappa B Pathway by Lowering Lipopolysaccharide Concentrations

To investigate whether ApoA5 could inhibit the TLR4-mediated signaling pathway, we assessed the expression of TLR4, MyD88, and NF-κBp65 after transfection with the ApoA5 overexpression plasmid (pEGF-N1-ApoA5) in LFD mice. Accordingly, LFD mice transfected with pEGF-N1-ApoA5 demonstrated remarkably higher mRNA and protein expression of ApoA5 and distinctly lower mRNA and protein expression of TLR4, MyD88, and NF-κBp65 compared to untransfected mice (**Figures 3A–C**), indicating that high ApoA5 expression can inhibit the TLR4-mediated NF-κB pathway. However, ApoA5 overexpressed mice unchanged in body weight, liver weight and serum LPS concentrations compared to the untransfected mice (**Figures 3H–J**).

However, the inhibitory effects of ApoA5 on TLR4 were limited by circulating LPS concentrations. HFD + EXE mice had lower LPS concentrations, which were obviously enhanced after injection of 5 and 10 μg/kg·wt of LPS, respectively (**Figure 3D**). HFD + EXE mice treated with 10 μg/kg·wt of LPS had equal LPS concentrations as mice with HFD-induced NASH (614.27 ± 36.21 vs. 609.42 ± 42.21 ng/mL) (**Figure 3D**). Nevertheless, ApoA5 expression remained unchanged and showed high expression in treated mice (**Figures 3E–G**). The mRNA and protein levels of TLR4, MyD88, and NF-κBp65 remained unaffected after treatment with 5 μg/kg·wt of LPS but were distinctly increased after treatment with 10 μg/kg·wt LPS (**Figures 3E–G**). These results revealed that exercise enhanced the ability of ApoA5 to inhibit TLR4-mediated NF-κB pathway by lowering LPS concentrations.

DISCUSSION

Non-alcoholic steatohepatitis has been associated with hepatic disease progression, development of cirrhosis, and hepatocellular carcinoma (Ibrahim et al., 2018). The ideal therapy for NASH is one that involves effectively reversing liver injury and fibrosis and improving or at least having no negative effects on other metabolic parameters or cardiovascular comorbidities (Sheka et al., 2020). Lifestyle modifications, such as diet control and exercise, have been the primary treatment for NASH (Goncalves et al., 2013; Neuschwander-Tetri, 2020). Exercise is an important strategy for preventing and treating NASH given its ability to decrease hepatic fat content and insulin resistance, as well as modify *de novo* synthesis of free fatty acids, all of which have an effect on NASH (van der Windt et al., 2018). The

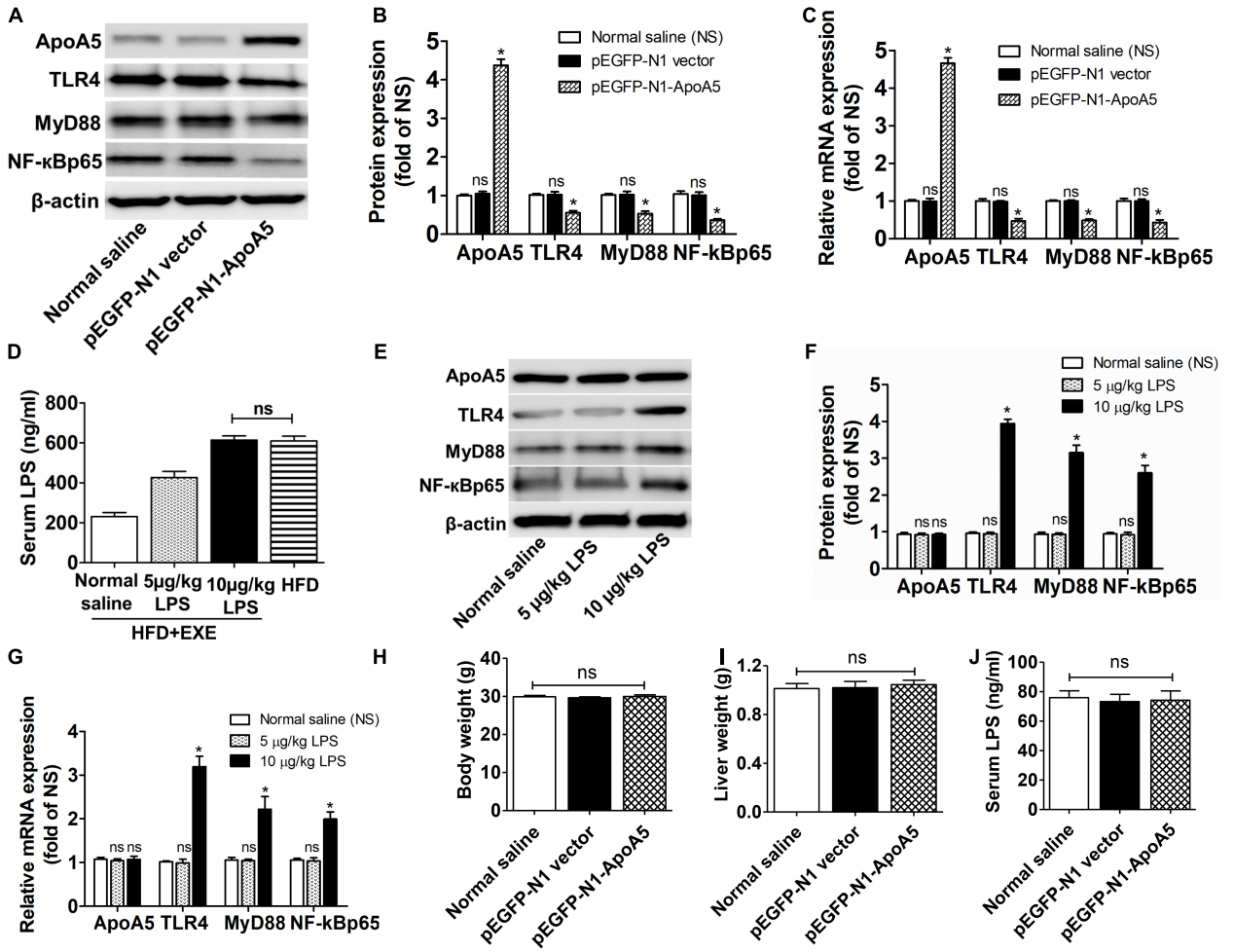

FIGURE 3 | Exercise enhanced the ability of ApoA5 to inhibit TLR4 signaling pathway by lowering LPS concentrations. The hepatic protein **(A,B)** and relative mRNA **(C)** expression levels of ApoA5, TLR4, MyD88, and NF-κBp65 in LFD mice transfected with pEGF-N1-ApoA5. The LPS levels of serum in HFD + EXE mice after injection of different concentrations of LPS **(D)**. The hepatic protein **(E,F)** and relative mRNA **(G)** expression levels of ApoA5, TLR4, MyD88, and NF-κBp65 in HFD + EXE mice after injection of different concentrations of LPS. The body weight **(H)**, liver weight **(I)**, and circulating LPS concentrations **(J)** in LFD mice transfected with pEGF-N1-ApoA5. Results are presented as the mean ± SEM, $n = 4$ per group. $^{*}P < 0.05$ for different from the pEGF-N1 vector group or 5 μg/kg·wt group; $^{ns}P > 0.05$ (no difference) for different from the normal saline group.

present study also confirmed that exercise not only reduced body weight, liver weight, and hepatic lipid accumulation but also attenuated hepatic inflammation and fibrosis in mice with HFD-induced NASH.

Evidence has shown that ApoA5 plays an important role in maintaining plasma TG levels and in the pathogenesis of NAFLD given the association between ApoA5 and storage of TG in intrahepatic lipid droplets (Forte and Ryan, 2015). Similarly, simultaneously increased hepatic TG contents and ApoA5 expression had been detected in our mice with HFD-induced NASH. Studies have observed that patients and mice with NAFLD have elevated levels of hepatic ApoA5 mRNA and protein, which were markedly downregulated after amelioration of hepatosteatosis (Ress et al., 2011; Feng et al., 2015; Lin et al., 2017). Our study also demonstrated that mice with HFD-induced NASH had higher hepatic ApoA5 expression, which remained unchanged after exercise intervention. Interestingly, exercise resulted in a considerable reduction in hepatic TLR4, MyD88, and NF-κBp65 expression and circulating LPS concentrations.

Toll-Like Receptor 4, the main receptor for the recognition of LPS, is upregulated in endotoxin-induced liver injury (Takayashiki et al., 2004). In response to LPS, TLR4 activates the NF-κB pathway to release NF-κBp65 (Medvedev et al., 2000; Byun et al., 2015), which subsequently translocates to the nucleus and stimulates the transcription of inflammatory genes (Li et al., 2014). Therefore, targeting the TLR4-mediated NF-κB pathway may be one method of alleviating hepatic inflammation in NASH. Tao et al. observed that increased ApoA5 expression could attenuate liver injury by inhibiting the TLR4-mediated NF-κB pathway, although the inhibitory effects weakened with increasing LPS concentrations (Tao et al., 2019). Serum LPS levels significantly increased after HFD administration, which

were partially reduced after exercise (Yu C. et al., 2019). Based on the results of previous studies and our own, we hypothesized that exercise enhanced the ability of ApoA5 to inhibit the TLR4-mediated NF-κB pathway by lowering LPS concentrations.

To test this hypothesis, we performed the ApoA5 transfection and LPS intervention studies, which showed that increased ApoA5 expression indeed inhibited the expression of TLR4, MyD88, and NF-κBp65—crucial cytokines involved in the TLR4-mediated NF-κB pathway (**Figures 2**, **3**). However, the inhibitory effects of ApoA5 declined when circulating LPS concentrations increased to match those observed in HFD mice (**Figure 3D**). The current study found that exercise could remarkably reduce serum LPS concentrations while maintaining increased hepatic ApoA5 expression, which triggered the inhibitory effects on the TLR4-mediated NF-κB pathway.

In conclusion, exercise alleviated HFD diet-induced hepatic steatosis, inflammation and fibrosis, all of which were characteristics of NASH. Notably, exercise did not reduce ApoA5 expression but instead increased its ability to suppress TLR4-mediated NF-κB pathway by lowering LPS concentration in mice with HFD-induced NASH. Taken together, our results suggested that exercise may target the ApoA5-TLR4 pathway to improve NASH.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding authors.

ETHICS STATEMENT

The animal study was reviewed and approved by the Animal Experiment Administration Committee of Guangzhou Sport University (2020DWLL-005).

AUTHOR CONTRIBUTIONS

YY and LZ performed study concept and design. YY, LY, XL, and CF performed development of methodology and writing, review, and revision of the manuscript. YY, LY, and SL provided acquisition, analysis and interpretation of data, and statistical analysis. LY provided technical and material support. NC performed supplementary experiments and data analysis. All authors read and approved the final manuscript.

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frontiers in Physiology

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Neuromuscular or Sensory Electrical Stimulation for Reconditioning Motor Output and Postural Balance in Older Subjects?

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Percutaneous electrical stimulation is used for reconditioning functional capabilities in older subjects. However, its optimal application depends on the specific physiological needs of the individual. Depending on whether his/her needs are related to motor function or sensory and central functions, the relevant modality of electrical stimulation differs significantly. In fact, there are two main modalities of electrical stimulation, that is, neuromuscular electrical stimulation (NMES) and sensory electrical stimulation (SES). NMES involves high-intensity currents (above the motor threshold) and provokes involuntary visible direct muscle contractions. With chronic application, the induced adaptations occur mainly at the neuromuscular function level and thus enhance muscle strength/power and motor output. SES involves low-intensity currents (below, at or only just above the sensory threshold), does not induce any visible muscle contraction and provides only sensory information. With chronic application, the induced adaptations occur at the level of potentiation and transmission of proprioceptive afferents and thus facilitate sensorimotor activity (movement and balance). Overall, SES is interesting for the improvement/maintenance of sensorimotor capabilities in non-frail older subjects while NMES is relevant to develop muscle strength/power and thus reduce the risk of falls due to a lack of muscle strength/power in frail older subjects.

Keywords: aging, electrical stimulation, muscle electrical stimulation, somatosensory electrical stimulation, muscle strength, balance, fall, elderly

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INTRODUCTION

Advancing age engenders progressive structural and functional alterations of different organs and systems linked to the motor and postural functions. These alterations naturally and chronologically are likely to lead to motor and postural disturbances related, first, to functional capabilities as part of maximal/intense physical activities, second, to basal functional capabilities as part of activities of daily living, and third, to frailty and an increased risk of falling as part of different body displacements and domestic motor actions – activities at home (ANSES, 2016). A sedentary or inactive life accentuates and accelerates these motor and postural alterations. Although regular physical activity and exercise are the best way of preventing, slowing down

or limiting these progressive alterations and maintaining the whole functional capabilities (Intiso et al., 2012; Hafström et al., 2016), older subjects are often unable or unwilling to engage in conventional physical activity and exercise or to undertake whole-body physical activity (Paillard, 2018). Hence, in older subjects, in order to limit motor and postural alterations, the optimal (only) solution seems to be the use of artificial techniques, such as the percutaneous peripheral electrical stimulation (Paillard, 2018). This type of stimulation enables the artificial activation of the motor pathway (peripheral and/or central stimulation) and/or the sensory pathway that ensure command, control and execution of movements (Paillard, 2018, 2020, 2021).

However, there are two types of percutaneous peripheral electrical stimulation which, through surface electrodes placed over the bellies or motor points of one (or more) superficial skeletal muscle(s), allow either direct activation of the muscle fibres (i.e., excito-motor stimulation that activates not only the motor and sensory nerve fibres but also the muscle fibres directly) or activation of the sensory nerve fibres only (i.e., sensory stimulation that does not activate muscle fibres and motor nerve fibres; Collins, 2007). Stimulation of sensory nerve fibres can also be applied directly to a nerve – for example, femoral or tibial nerve – or to a joint – for example, hip, knee and ankle (Yoshida et al., 2015; Saadat et al., 2017). Excito-motor stimulation is named neuromuscular electrical stimulation (NMES) while sensory stimulation is called sensory (or somatosensory) electrical stimulation (SES). In fact, the type of a stimulation depends on the intensity of the current applied (cf. paragraphs Application modalities and Motor and postural adaptations; **Figure 1**). Each stimulation induces specific physiological effects on the sensory, central and motor functions related to movement and postural balance with chronic applications (Schröder et al., 2018; Paillard, 2020, 2021). The choice of the stimulation should be based on the specific physiological needs of the older subjects under consideration.

The aim is to propose the type of electrical stimulation best suited to the different physiological profiles of older subjects while specifying the effects induced for each technique and suggesting the underlying mechanistic explanations.

PHYSIOLOGICAL PROFILES AND SPECIFIC RECONDITIONING NEEDS IN OLDER SUBJECTS

Depending on the physiological profile of older subjects, there are different reconditioning needs for movement and

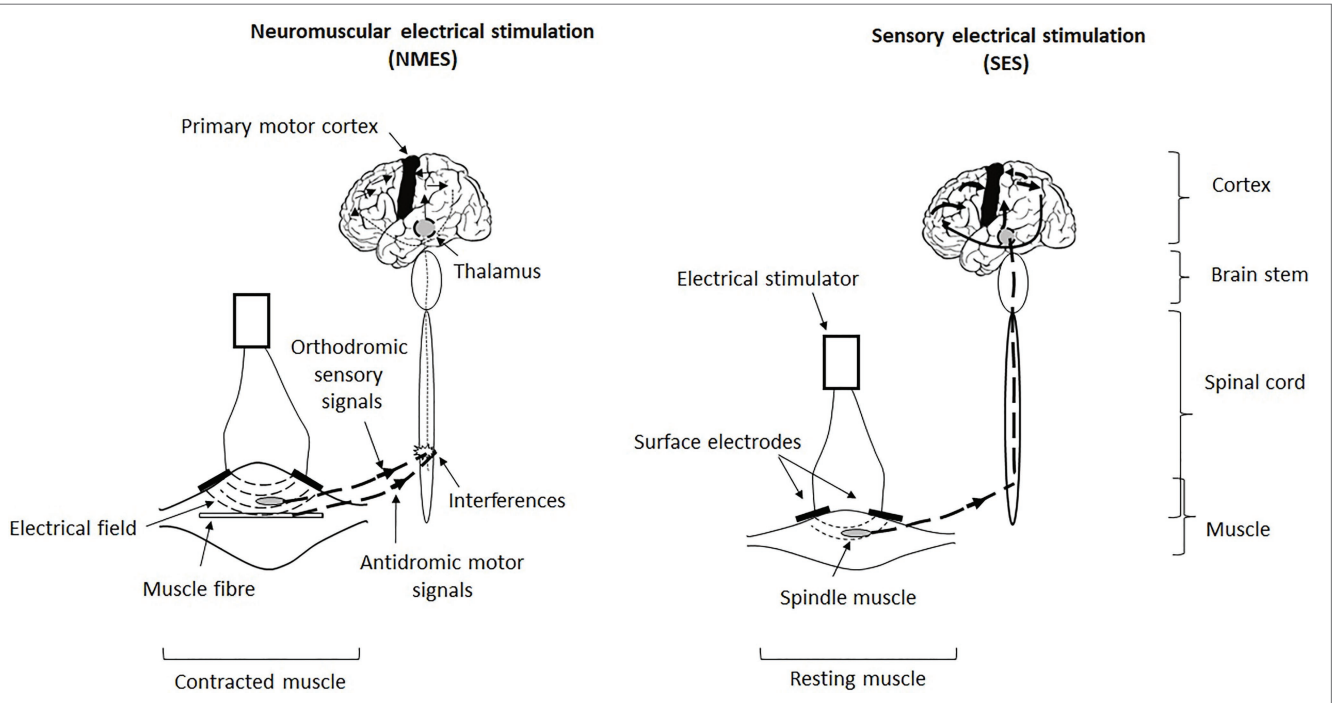

FIGURE 1 | Illustration of the triggering, transmission and central integration of signals generated by neuromuscular electrical stimulation (NMES) and sensory electrical stimulation (SES). NMES involves an excito-motor current, that is, it is strong enough to directly contract the muscle (contracted muscle), whereas SES involves a current below the motor threshold and thus does not induce muscle contraction (resting muscle). In fact, NMES simultaneously activates both sensory and motor neurons and causes a conflict between antidromic motor action potentials and orthodromic sensory action potentials at the spinal level, which interferes with the central integration of the induced afferents (indicated in the diagram of the spinal cord) and thus reduces the ascending sensory volley at the supraspinal level (thin ascending dotted line and thin arrows at cortical level). Hence, NMES generates little exploitable sensory information by the central nervous system to optimise and refine motor and postural skills. In return, SES stimulates only (or almost only) sensory neurons linked to mechanoreceptors which triggers proprioceptive afferents that go up *via* the thalamus to the postcentral and parietal cortices (primary somatosensory cortex and posterior parietal cortex) to precentral cortices (primary motor cortex) in the stimulated hemisphere (thick ascending dotted line and thick arrows at cortical level). Hence, SES generates enhanced sensorimotor activity that is likely to improve motor and postural capabilities.

balance. The first physiological profile of advancing age (from a chronological viewpoint) mentioned above (physiological alteration) can be characterised by impaired proprioceptive (myotendinous and articular cues), vestibular (otolithic and semicircular cues) and exteroceptive (visual and skin cues) input and/or integration and/or decreased motor output (Maitre et al., 2013). With this physiological profile, the needs are mainly sensorimotor. Moreover, since the absence of physical activity accentuates all the impairments mentioned above, the functional capabilities are primarily and clearly impeded due to excessive degradation of motor output (Paillard, 2017b; Trajkov et al., 2018). This corresponds to the second physiological profile described above and its needs are primarily based on the development of muscle strength/power (pure motor output) at least in order to partially recondition the basal functional capabilities more easily (Paillard, 2017b). For the third physiological profile corresponding to an increased fall risk, evidence suggests that the needs are the same but are still much more pronounced than those in the second physiological profile related to the absolute needs to develop muscle strength/power (Paillard, 2017b). Whether it is a question of developing sensorimotor function or only motor function (e.g., muscle strength/power), the technique of electrical stimulation constitutes an excellent means for reconditioning older subjects corresponding to the second and third physiological profiles, but it can also be appropriately used with subjects of the first physiological profile, especially when they are sedentary or inactive. However, the type of electrical stimulation required depends on whether the aim is to develop sensorimotor function or only motor function.

MOTOR AND POSTURAL ADAPTATIONS INDUCED BY ELECTRICAL STIMULATION

Neuromuscular Electrical Stimulation

Application Modalities

NMES is excito-motor which means that it directly activates muscle fibres by bypassing motor neurons (even if they are simultaneously activated as well as sensory neurons). To this end, it must be clearly above the motor threshold, that is, the minimal intensity of stimulation that produces a direct motor response and generates involuntary non-controlled segmental movements. It turns out that the higher the intensity, the greater the extent of the electrical field and the greater the number of recruited muscle fibres – that is, both I and II fibre types (Collins, 2007). In the context of regular or chronic application, it appears that the higher the current intensity, the greater the physiological effects/benefits induced (Paillard, 2018). NMES produces strong muscle contractions and provokes pain through surface electrodes placed over the bellies or motor points of muscle(s) targeted (Lake, 1992; Vanderthommen and Duchateau, 2007). In order to recondition the basal functional capabilities in older (frail) subjects, NMES should be applied specifically to the muscles of their lower limbs with high intensities (maximal tolerable by subject), high frequencies (>30 Hz and rather 50–80 Hz), optimal width pulses (matching to the chronaxy of the stimulated muscle that is, for instance 300–450 μs for quadriceps femoris) short contractions interspersed with long recovery times – for example, 3–10 s/10–30 s on/off – for 10–15 min, 20 min maximum (Paillard, 2018).

Motor and Postural Adaptations

Evidence suggests that NMES regularly applied on quadriceps femoris or dorsi/plantarflexor muscles (e.g., tibialis anterior, and soleus) in older subjects (>60 years old) improves lower-limb muscle strength (Caggiano et al., 1994; Paillard et al., 2004; Bezerra et al., 2011; Caulfield et al., 2013; Kern et al., 2014; Mignardot et al., 2015; Von Stengel et al., 2015; Mani et al., 2018; Acaröz Candan et al., 2019; Langeard et al., 2021) and postural balance (Amiridis et al., 2005; Paillard et al., 2005a,b; Nejc et al., 2013; Mignardot et al., 2015; Alptekin et al., 2016; Bondi et al., 2021).

In this context, the functional improvements of motor and postural functions in older subjects are mainly linked to enhancements of motor output through muscle structural (mass) and functional (neural networks) adaptations – **Figure 2** (Paillard, 2018). The naturally irreversible atrophy of lower-limb muscle in inactive or sedentary individuals can be reversed with the chronic application of NMES (Boncompagni et al., 2007; Carraro et al., 2015). NMES stimulates not only anabolic pathways (e.g., secretion of insulin-like growth factor-1), but also negatively modulates muscle catabolism, which increases protein synthesis and reduces protein degradation and activates satellite cells in aged individuals (Barberi et al., 2015; Mancinelli et al., 2019). Hence, NMES induces an increase in the size of muscle fibres especially in the type II muscle fibres which are particularly affected by the effects of advancing age (Zampieri et al., 2015; Mancinelli et al., 2019). From a functional viewpoint, NMES results in greater contribution of muscles regularly stimulated (i.e., electromyographic activity of tibialis anterior and medial gastrocnemius) in the postural regulation (Amiridis et al., 2005). It also entails increased musculotendinous stiffness of the muscles regularly stimulated (Mignardot et al., 2015).

However, even if one cannot ignore that NMES applied on selected muscle groups engenders sensory information likely to be used as part of the command and control of voluntary movement – for example, during walking – Paillard (2020) inferred that it generates little sensory information likely to be exploited by the central nervous system to optimise and refine perceptual and motor skills as part of the postural balance regulation (**Figure 2**). In fact, NMES simultaneously activates both sensory and motor neurons and would provoke conflict between the antidromic motor action potentials and the orthodromic sensory action potentials at the spinal level (Bergquist et al., 2011), thus causing interference in the central integration of induced afferents (although the presence of central adaptations cannot be totally excluded).

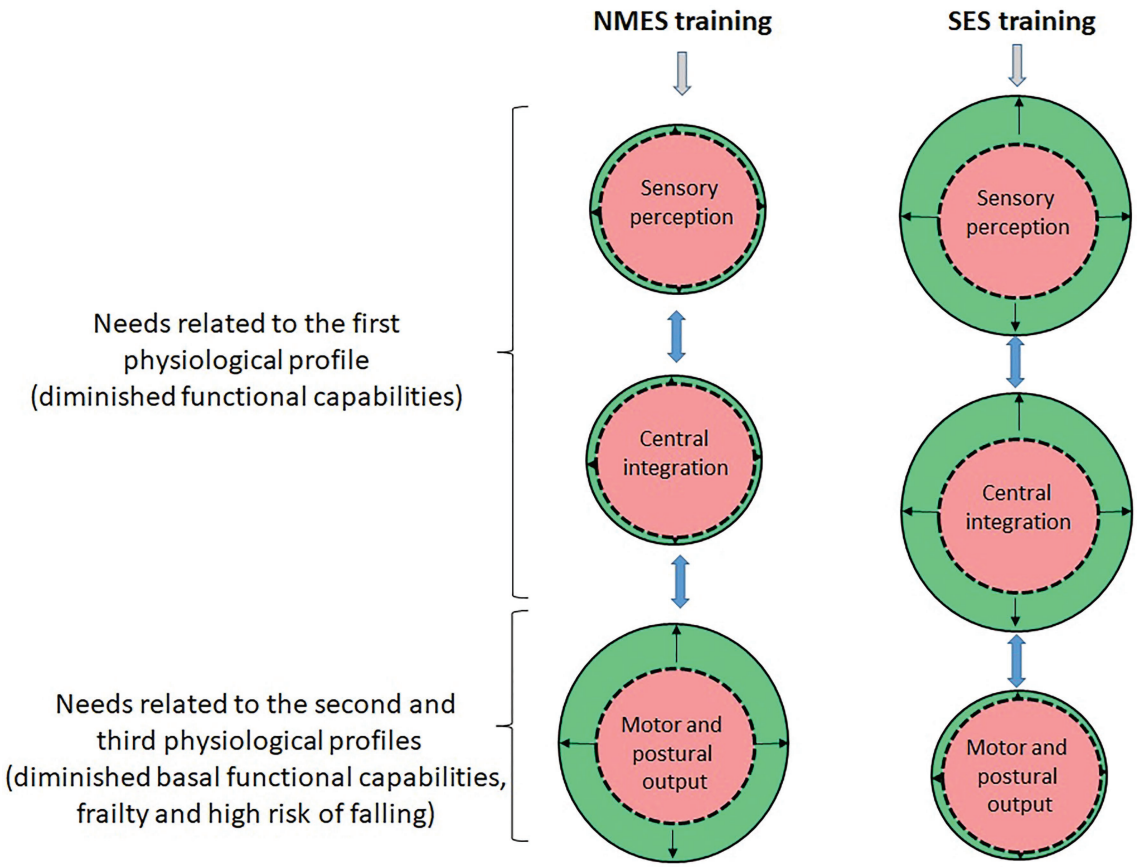

FIGURE 2 | Illustration of the chronic adaptations related to the sensory, central and motor functions induced by NMES and SES (the red/central circles on the figure correspond to the initial condition – that is, pre-training – and the green/peripheral circles correspond to the final condition – that is, post-training; the difference between the red/central and green/peripheral circles corresponds to the amplitude of adaptations induced by NMES or SES). NMES mainly stimulates the neuromuscular system and especially induces muscle structural and nerve functional adaptations that enhance muscle strength/power as well as motor output related to movement and postural balance. Since the induced ascending sensory volleys are reduced due to interference with antidromic motor signals, NMES generate little adaptation at the sensory and central levels. SES alters the sensibility threshold of mechanoreceptors in the long term that optimises the potentiation and transmission of proprioceptive afferents and thus enhances the sensory contribution which facilitates sensorimotor activity and motor cortex excitability related to movement and postural balance. In older subjects in the first physiological profile, their need to improve their diminished functional capabilities is more related to the implementation of SES, while in frail older subjects in the second and third physiological profiles, their need to reduce/reverse their frailty and risk of falling is more related to the implementation of NMES.

In order to improve the functional capabilities, NMES would be particularly interesting since it is likely to enhance motor output especially muscle strength/power in older frail subjects with a high risk of falling precisely due to a lack of muscle strength/power.

Sensory Electrical Stimulation

Application Modalities

SES is not excito-motor (it cannot directly activate the muscle fibres) and only (or almost only) activates the sensory pathways (this is the principle of this stimulation). To this end, it must be well below the motor threshold and close to the sensory threshold, that is, the minimum intensity of stimulation that can be perceived by individual. Indeed, the current intensity must be around the sensory threshold without exceeding it or only slightly, since a non-excito-motor current (low intensity) is already likely to lead to recruitment of type I muscle fibres from reflex pathways – mediated at spinal level, that is, homolateral monosynaptic connections of the Ia fibres with the α-motoneurons generate depolarisation of the latter and induce the contraction of the muscle fibres they innervate (Zeronian et al., 2021). SES is painless (although there are possible sensations) and applied to a peripheral nerve, belly muscles (motor points) and/or joints at current intensities below, at or slightly above the sensory threshold (Schröder et al., 2018; Paillard, 2021). In order to recondition the motor and postural functions in older subjects, SES should be applied with large pulses (e.g., 1 ms) and high frequencies (80–100 Hz) to facilitate potentiation and central integration of emitted signals (Collins, 2007; Bergquist et al., 2011) for several tens of minutes in a uninterrupted way.

Motor and Postural Adaptations

Evidence suggests that SES regularly applied to lower-limb muscle (motor points), peripheral nerve or joints in older subjects

(>60 years old) improves sensorimotor function (Park et al., 2014; Ng et al., 2016; Paillard, 2021). However, since SES generates no muscle deformation or contraction, it thus cannot stimulate synthesis of contractile proteins responsible for muscle structural adaptations (Coffey and Hawley, 2007). The adaptations induced cannot occur at the level of the motor output of muscle. In fact, SES generates sensory cues that are detected by sensory sensors which transmit signals throughout sensory pathways to cortical areas (**Figure 1**). With chronic application, SES induces adaptations at different stages (sites) throughout sensory pathways – sensory sensors and spinal and supraspinal structures (**Figure 2**).

At the peripheral level (i.e., sensory sensors), SES would modify the sensitivity threshold of mechanoreceptors that result from altering the ion permeability of their membrane (Gravelle et al., 2002; Ross et al., 2007). This sensory adaptation would optimise the potentiation and transmission of proprioceptive afferents and thus enhance the sensory contribution in the motor and postural regulation (Ross et al., 2007; Severini and Delahunt, 2018; Paillard, 2021). At the spinal level, SES is likely to engender a reduction or increase in the amplitude of the induced H-reflex (Goulet et al., 1997; Hardy et al., 2002, respectively) that, in both cases, can improve motor and postural abilities (Paillard, 2017a). The reduction of the Ia-afferent excitation to the α-motoneuron pool can attenuate destabilising joint movements while the enhancement of the activation of the α-motoneuron pool can facilitate instantaneous segmental reactions to the demands of the motor or postural task (Paillard, 2017a). At the cortical level, SES is likely to induce durable changes in motor cortex excitability that can be assimilated to enhance sensorimotor activity and connectivity (Kaelin-Lang et al., 2002; Schröder et al., 2018; Insausti-Delgado et al., 2021). Enhanced sensorimotor activity could result from favourable impact of SES over postcentral and parietal cortices (primary somatosensory cortex and posterior parietal cortex) to precentral cortices (primary motor cortex) in the stimulated hemisphere (Paillard, 2021).

SES would be of particular interest as it is likely to improve sensorimotor output in older subjects who are not at high risk of falling due to lack of muscle strength/power or very weak motor output.

WHICH ELECTRICAL STIMULATION FOR WHICH PHYSIOLOGICAL PROFILE IN OLDER SUBJECTS?

On the basis of an older subject's needs, belonging to the first physiological profile, SES would turn out to be particularly interesting since it especially facilitates the sensorimotor reconditioning and thus optimises functional capabilities (**Figure 2**). For older subjects in the second physiological profile who exhibit diminished basal functional capabilities, NMES would turn out to be relevant since it can initiate/trigger structural and functional muscle adaptations that can reverse the process of reduction of functional capabilities in activities of daily living (**Figure 2**). Regarding older subjects in the third physiological profile who are at high risk of falling due to extreme frailty and a notable lack of muscle strength/power in the lower-limb, NMES would reverse the process of frailty and muscle involution, thus reducing the risk of falling.

CONCLUSION

In older subjects, as long as their basal functional capabilities are not clearly limited/reduced as part of activities of daily living and their risk of falling is low, SES is particularly useful for maintaining or even improving their capabilities to command and control movement and postural balance. In turn, in frail older subjects with diminished basal functional capabilities and at high risk of falling, NMES can potentially boost their neuromuscular system in order to recondition the lower-limb muscle strength/power thus limiting their risk of falling.

AUTHOR CONTRIBUTIONS

The author confirms being the sole contributor of this work and has approved it for publication.

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Aerobic Exercise Improves Pulmonary Fibrosis by Improving Insulin Resistance and Inflammation in Obese Mice

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Background: Previous studies have demonstrated that obesity is associated with pulmonary fibrosis. We attempted to identify whether regular aerobic exercise (AE) can protect against high-fat diet (HFD)-associated pulmonary fibrosis.

Methods: Forty-eight C57BL/6 mice were randomly assigned to four groups: chow group (Ch), chow plus exercise group (CE), obesity group (Ob), and obesity plus exercise group (OE). The mice were fed either an HFD or a chow diet for 16 weeks, and low-intensity aerobic exercise (AE) was performed in the last 8 weeks. We measured the degree of pulmonary fibrosis; pulmonary inflammation; oxidative stress parameters; insulin resistance-related indicators; the number of inflammatory cells in bronchoalveolar lavage fluid (BALF); the mRNA expression levels of IL-10, IL-1β, TGF-β, TNF-α, CXCL-1, IL-17, MMP-9, MPO, NE, and sirt-1; and the BALF levels of CXCL-1, IL-17, TGF-β, IL-10, IL-1β, and TNF-α in lung tissue.

Results: AE in obese mice protected against obesity-associated pulmonary fibrosis, chronic inflammation, pro-oxidative/antioxidative imbalance, and insulin resistance. AE ameliorated the HFD-induced inflammatory response and neutrophil infiltration in the lung. AE downregulated BALF levels of CXCL-1, IL-1β, TNF-α IL-17, and TGF-β but upregulated BALF levels of IL-10. AE decreased IL-1β, TGF-β, TNF-α, CXCL-1, IL-17, MMP-9, MPO, and NE mRNA expression levels but upregulated IL-10 and sirt-1 mRNA expression levels in the lung.

Conclusions: AE protects against HFD-induced pulmonary fibrosis by improving obesity-associated insulin resistance, chronic low-grade inflammation, and pro-oxidative/antioxidative imbalance. AE improved HFD-induced pulmonary fibrosis by suppressing IL-17, TGF-β, NE, and MMP-9 expression and activating IL-10 and sirt-1 expression.

Keywords: obesity, pulmonary fibrosis, inflammation, oxidative stress, insulin resistance, exercise

INTRODUCTION

The prevalence of obesity is increasing drastically and its prevalence markedly upregulates the incidence of many complications such as chronic obstructive pulmonary disease (COPD), asthma, and pulmonary fibrosis (Bianco et al., 2017; Liu et al., 2017; Murtha et al., 2017; Rathinasabapathy et al., 2018; Bluher, 2019). At present, pulmonary fibrosis is a deadly chronic interstitial disease and a substantial cause of morbidity worldwide.

Obesity results in insulin resistance, vitamin D deficiency, and increased expression of fibrogenic factors including TGF-β, and proinflammatory cytokines such as TNF-α and C-reactive protein; these changes cause pulmonary fibrosis (Wortsman et al., 2000; Ford et al., 2005; Botella-Carretero et al., 2007; Vimaleswaran et al., 2013; Han et al., 2021). The vitamin D receptor (VDR) exists in bronchial epithelial cells and is an important cause of pulmonary fibrosis partly because vitamin D deficiency can regulate TGF-β signalling by suppressing phosphorylated Smad-2/3 and activating the renin-angiotensin system (RAS) activity (Li et al., 2004; Adav et al., 2011; Zerr et al., 2015; Shi et al., 2017; Park et al., 2018; Tzilas et al., 2019). Insulin resistance mediates pulmonary fibrosis *via* the TGF-β pathway (Park et al., 2019). *In vitro* experiments have demonstrated that bronchial cells and cells from insulin-resistant subjects were associated with increased TGF-β activation and collagen deposition (Spencer et al., 2010; Mayer et al., 2012). Insulin resistance is closely associated with proinflammatory reactions involving inflammatory cells and inflammatory cytokines (Asghar and Sheikh, 2017). Obesity is known to lead to changes in inflammatory parameters and impaired oxidative capacity, which are important risk factors for and pathogenic mechanisms in pulmonary fibrosis (Nishiyama et al., 2005; Jackson et al., 2010, 2014). Inflammatory cytokines, including TNF-α and IL-1β, and immune cells, including neutrophils, are important causes of pulmonary fibrosis (Stockley, 2002; Thannickal et al., 2004). Neutrophil elastase (NE) and matrix metalloproteinase 9 (MMP-9) are released by neutrophils and are important profibrotic factors (Bellaye et al., 2018; Matin et al., 2018). Recent studies have found that IL-17 is an important profibrotic factor (Zhang et al., 2019).

Exercise is a tool to treat many autoimmune diseases, including COPD, asthma and pulmonary fibrosis, because AE has immunomodulatory effects and modulates the redox balance (Holland and Hill, 2008; Nishiyama et al., 2008; Vainshelboim et al., 2014; Otoupalova et al., 2020). Here, we attempt to demonstrate whether an 8-week AE programme can improve HFD-induced pulmonary fibrosis in obese mice and to explore the related mechanisms: insulin resistance, inflammation, and oxidative stress.

The lung-protective effects of sirt-1 are well documented (Nakamaru et al., 2009; Rahman et al., 2012). Sirt-1 can regulate the transcriptional control of multiple genes related to anti-inflammatory action, antioxidation, and energetic metabolism (Rajendrasozhan et al., 2008; Yao et al., 2012). Sirt-1, which can be activated by exercise, improves pulmonary inflammation and modulates the pro-oxidative/antioxidative balance in the lung (Cantó et al., 2009). Sirt-1 was significantly associated with lung protection. AE may activate Sirt-1 expression in obese mice and therefore counter obesity-associated pulmonary fibrosis.

AE provides insight into pulmonary fibrosis, while the underlying molecular mechanisms through which AE protects against HFD-induced pulmonary fibrosis require more research.

MATERIALS AND METHODS

Animal and Experimental Groups

All protocols used in this study were approved the Animal Experimental Welfare of the Institute of Animal Science, Chinese Academy of Agricultural Sciences (Beijing, China). All experiments were performed in accordance with the Animal Experimental Welfare of the Institute of Animal Science, Chinese Academy of Agricultural Sciences and the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health. The mice were anaesthetized *via* intraperitoneal injection of pentobarbital (50 mg/kg).

Forty-eight male C57BL/6 mice were randomly divided into four groups: (1) chow (Ch) group, where the mice were fed a chow diet for 16 weeks; (2) chow diet plus exercise (CE) group, where the mice were fed a chow diet for 16 weeks and forced to train in the last 8 weeks; (3) the obesity (Ob) group, where the mice were fed a HFD for 16 weeks; and (4) the obesity plus exercise (OE) group, where the mice were fed a HFD for 16 weeks and forced to train in the last 8 weeks. Each group included 12 mice.

Exercise Protocol

OE mice were forced to train using a treadmill. Treadmill aerobic training lasted for 8 weeks and was performed once a day for 60 min per session (0° incline, 18 m/min).

Sample Collection

Blood samples were collected from the mice and placed into blood collection vessels. After centrifugation, the upper serum layer was harvested and frozen at −20°C immediately. The remaining lung tissues were frozen with liquid nitrogen.

Two millilitres of 0.9% normal saline was utilised for whole-lung lavage. The whole lung was flushed five times. The BALF was centrifuged at 3,500 r/min for 20 min. The BALF was immediately stored at −80°C.

Histopathology

Tissue samples were fixed with 4% paraformaldehyde. Five-micrometre thick sections were obtained using the paraffin section method. Haematoxylin and eosin staining were utilised to detect the degree of inflammation in the lung. Masson and Sirius Red staining were utilised to detect collagen fibre deposition in the lung. Images were captured with an inverted microscope.

Determination of Pulmonary Inflammation

BALF levels of CXCL-1, IL-1β, IL-10, IL-17, TNF-α, and TGF-β were detected with commercial kits (Multi sciences (Lianke) Biotech, CO., LTD, Hangzhou, China).

TABLE 1 | PCR primer sequence information.

Gene name	Primer sequence
CXCL-1	ACCTTCATCGGAAACTCCAAAG
	CTGTTAGGCTGGGAAAAGTTAGG
IL-1β	CTGCCGTCCGATTGAGACC
	CCCCTCCTTGTACCACTGTC
IL-10	CAAACCTCAATGTGTCTCTTTGC
	AGAGTAAAGCCTATCTCGCTGT
IL-17	CGAAGCGTGTGAAGGCAAC
	TTGTACGGGCCTGACATTTCC
MMP-9	ACATCGACCCGTCCACAGTAT
	CAGAGGGGTAGGCTTGTCTC
Sirt-1	CTCCCAACAGA CCTGTCTATAC
	CCATTGCACAACTCTTTTCTCA
TGF-β	AAACAGATGAAGTGCTCCTTCCAGG
	TGGAGAACACCACTTGTTGCTCCA
TNF-α	ATGTCTCAGCCTCTTCTCATTC
	GCTTGTCACTCGAATTTTGAGA
GAPDH	AGGTCGGTGTGAACGGATTTG
	TGTAGACCATGTAGTTGAGGTCA

CXCL, C-X-C motif chemokine ligand; IL, interleukin; MMP, matrix metalloproteinase; PCR, polymerase chain reaction; Sirt, sirtuin; TGF, transforming growth factor; TNF, tumour necrosis factor.

Determination of Oxidative Stress Index in Lung Homogenates

Following the manufacturer's specifications, superoxide dismutase (SOD), malondialdehyde (MDA), myeloperoxidase (MPO), and glutathione (GSH) levels in the mice were detected using spectrophotometry as previously described (Bio-Tek Instruments Inc., software KC4 v3.0) (Feng et al., 2016; Mai et al., 2021). We measured the conjugation of 1-chloro-2,4-dinitrobenzene (CDNB) to evaluate GST activity. The Bradford Method was used to evaluate MDA activity.

Determination of mRNA Expression Levels

Total RNA was extracted from DNase I-treated cells using TRIzol as previously described (Broderick et al., 2017). Total RNA (2.0 μg) was used as a template for cDNA synthesis. Real-time fluorescence quantitative PCR was used to measure gene expression levels. GAPDH served as the housekeeping gene. All primers were designed and synthesised by Shanghai Sangon Biotech Company. **Table 1** shows the murine PCR primer sequence information.

Detection of Insulin Resistance

According to the reagent manufacturer's specifications, plasma glucose levels were detected with a glucometer (Lifescan Benelux, Beerse, Belgium). The plasma insulin and adiponectin levels were detected using commercial kits (Shino Test Corporation, Tokyo, Japan).

Data Processing

All data were expressed as the mean ± SEM. Two-way ANOVA and Tukey's *post hoc* test was utilised to analyse the data in this study. The significance threshold was set at $P < 0.05$. GraphPad Prism 9 software was utilised to analyse the data and draw figures.

RESULTS

Effects of Aerobic Exercise on High-Fat Diet-Induced Adiposity

As **Table 2** shows, untrained obese mice had higher body weights ($P < 0.001$), net body weight gains ($P < 0.001$), abdominal fat weights ($P < 0.001$), and subcutaneous fat weights ($P < 0.001$) than did Ch mice. OE mice had lower body weights ($P < 0.001$), net body weight gains ($P < 0.001$), abdominal fat weights ($P < 0.001$), and subcutaneous fat weights ($P < 0.001$) than did Ob mice.

Detection of Pulmonary Inflammation

In the Ch group and the CE group, no inflammatory cell infiltration was observed in the lung. HFD markedly increased the number of neutrophils in the lung, while AE markedly decreased the number of neutrophils in obese mice (**Figure 1**).

As shown in **Table 3**, after HFD feeding for 16 weeks, the number of neutrophils ($P < 0.001$) in BALF was markedly upregulated. Regular AE markedly downregulated the number of neutrophils in obese mice ($P = 0.0301$).

As shown in **Table 4**, untrained obese mice had significantly higher levels of the neutrophil chemokines TNF-α ($P < 0.001$) and CXCL-1 ($P < 0.001$), profibrogenic factors TGF-β ($P < 0.001$) and IL-17 ($P < 0.001$), and proinflammatory factor IL-1β ($P < 0.001$) but significantly lower levels of the anti-inflammatory factor IL-10 ($P < 0.001$) in BALF than did Ch mice. AE markedly decreased BALF levels of IL-1β ($P = 0.033$), IL-17 ($P = 0.009$), TGF-β ($P = 0.013$), and TNF-α ($P = 0.025$) but markedly increased BALF levels of IL-10 ($P = 0.004$) compared with Ob mice.

TABLE 2 | Effects of HFD and exercise on body mass and body composition.

Group	Con	Ex	Ob	OE
Initial body weight (g)	20.62 ± 2.28	20.33 ± 2.81	20.24 ± 2.03	20.55 ± 2.94
Final body weight (g)	30.43 ± 5.68	26.11 ± 3.68	44.4 ± 6.28*	35.49 ± 4.97#
Body weight gain (g)	9.81 ± 1.08	5.78 ± 0.62	24.16 ± 3.28*	14.94 ± 1.37#
Subcutaneous fat weight (g)	0.28 ± 0.04	0.2 ± 0.03	1.88 ± 0.31*	0.95 ± 0.15#
Subcutaneous fat/body weight ratio (%)	0.93 ± 0.13	0.76 ± 0.08	4.23 ± 0.68*	2.68 ± 0.43#
Abdominal fat weight (g)	1.35 ± 0.24	0.95 ± 0.16	4.62 ± 0.86*	2.58 ± 0.51#
Abdominal/body weight ratio (%)	4.45 ± 0.52	3.64 ± 0.33	10.42 ± 1.34*	7.26 ± 0.97#

#*P < 0.05 compared with the Ch group. *P < 0.05 compared with the Ob group.*

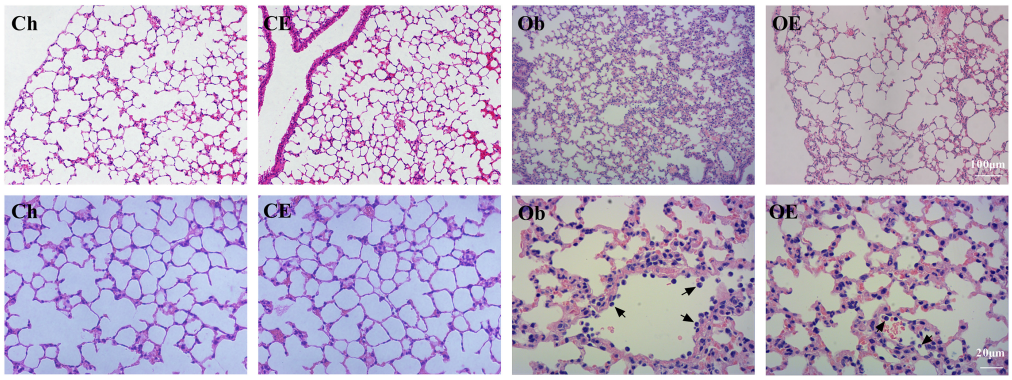

FIGURE 1 | Morphological analysis of lung tissue. Haematoxylin-eosin staining revealed evidence of the degree of inflammatory cell infiltration. Black arrows indicate neutrophils.

Detection of Pulmonary Fibrosis

Masson staining showed the degree of pulmonary fibrosis. Compared with those in the Ch group and the CE group, the magnitude of pulmonary fibrosis increased after 16 weeks of HFD administration, but AE decreased the magnitude of pulmonary fibrosis in obese mice (**Figure 2**).

Sirius Red staining showed collagen fibre deposition in lung tissue. In the Ch group and the CE group, no obvious collagen fibre deposition was noted in the airway wall, while collagen fibre deposition in the airway wall increased after 16 weeks of HFD administration. AE decreased collagen fibre deposition in the airway wall in obese mice (**Figure 3**).

As shown in **Table 4**, HFD administration markedly upregulated the profibrogenic factors TGF-β (P = 0.024) and IL-17 (P = 0.0031) in BALF. Regular AE downregulated BALF levels of TGF-β (P = 0.013) and IL-17 (P = 0.003) in obese mice.

As **Supplementary Material 1** showed, HFD administration markedly upregulated hydroxyproline levels ($P < 0.001$), Ashcroft fibrosis ($P < 0.001$), lung fibrotic score ($P < 0.001$), and airway collagen ($P < 0.001$) in the lung, whereas AE markedly downregulated hydroxyproline levels (P = 0.019), Ashcroft fibrosis (P = 0.024), lung fibrotic score (P = 0.031), and airway collagen (P = 0.005) in obese mice.

TABLE 3 | Total and differential cell counts in BALF (cells/ml).

Group	Con	Ex	Ob	OE
Total cells	1.29 ± 0.86	1.18 ± 0.75	3.54 ± 0.41*	2.14 ± 0.34#
Neutrophils	0.04 ± 0.01	0.04 ± 0.01	0.55 ± 0.093*	0.34 ± 0.088#
Lymphocytes	0.03 ± 0.05	0.02 ± 0.04	0.04 ± 0.14	0.03 ± 0.06
Macrophages	1.09 ± 0.38	0.98 ± 0.22	2.57 ± 0.77*	1.56 ± 0.57#
Eosinophils	0.01 ± 0.002	0.01 ± 0.003	0.02 ± 0.01	0.02 ± 0.006

The number of total cells, neutrophils, eosinophils, and macrophages in BALF were detected.
**P < 0.05 for difference from Ch; #P < 0.05 for difference from Ob.*

TABLE 4 | The levels of inflammatory factors in BALF (pg/ml).

Group	Con	Ex	Ob	OE
CXCL-1	8.56 ± 1.76	8.01 ± 1.13	43.68 ± 10.62*	25.34 ± 5.94#
IL-17	8.23 ± 1.97	7.88 ± 0.84	53.56 ± 13.89*	31.56 ± 7.82#
TGF-β	20.34 ± 4.86	18.11 ± 2.43	64.42 ± 15.45*	41.32 ± 10.58#
IL-10	15.34 ± 6.72	35.49 ± 4.29*	5.12 ± 2.45*	26.45 ± 4.83#
IL-1β	17.26 ± 2.14	15.65 ± 0.18	46.89 ± 10.67*	24.36 ± 5.84#
TNF-α	16.54 ± 4.81	14.22 ± 1.59	59.34 ± 16.47*	46.89 ± 13.56#

The BALF levels of CXCL-1, IL-17, TGF-β, IL-10, IL-1β, and TNF-α were detected.
*#P < 0.05 compared with the Ch group. *P < 0.05 compared with the Ob group.*
CXCL, C-X-C motif chemokine ligand; IL, interleukin; TGF, transforming growth factor; TNF, tumour necrosis factor.

Effects of Aerobic Exercise on mRNA Expression Levels in the Lung

The mRNA expression levels of CXCL-1 (**Figure 4A**), IL-1β (**Figure 4B**), IL-10 (**Figure 4C**), IL-17 (**Figure 4D**), MMP-9 (**Figure 4E**), MPO (**Figure 4F**), NE (**Figure 4G**), sirt-1 (**Figure 4H**), TGF-β (**Figure 4I**), and TNF-α (**Figure 4J**) in the lung were detected. HFD feeding upregulated CXCL-1 ($P < 0.001$), IL-1β ($P < 0.001$), IL-17 (P = 0.0034), MMP-9 (P = 0.0089), MPO ($P < 0.001$), NE ($P < 0.001$), TGF-β ($P < 0.001$), and TNF-α (P = 0.0072) mRNA expression levels but decreased IL-10 (P = 0.013) and sirt-1 (P = 0.011) mRNA expression levels compared with the Ch group. AE for 8 weeks markedly downregulated CXCL-1 ($P < 0.001$), IL-1β ($P < 0.001$), IL-17 (P = 0.024), MMP-9 (P = 0.0029), MPO (P = 0.013), NE (P = 0.011), TGF-β (P = 0.0125), and TNF-α ($P < 0.001$) mRNA expression levels in obese mice.

Detection of Oxidative Stress Index in the Lung

The levels of GSH (**Figure 5A**), MDA (**Figure 5B**), MPO (**Figure 5C**), and SOD (**Figure 5D**) in the lung were detected. HFD feeding resulted in decreased expression levels of GSH (P = 0.0063) and SOD (P = 0.0047) and increased expression levels of MDA (P = 0.0092) and MPO (P = 0.0025) in lung tissue compared with the Ch group. Significant downregulation of MPO (P = 0.0396) and MDA (P = 0.0412) expression and significant upregulation of GSH (P = 0.0037) and SOD (P = 0.0268) expression were detected after 8 weeks of AE compared with the Ob group.

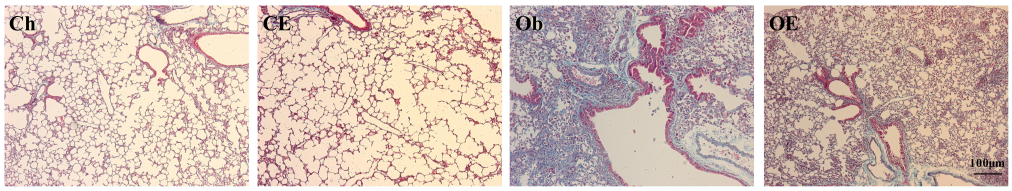

FIGURE 2 | Collagen deposition was detected by Masson staining. Masson staining revealed evidence of the degree of pulmonary fibrosis in the lung.

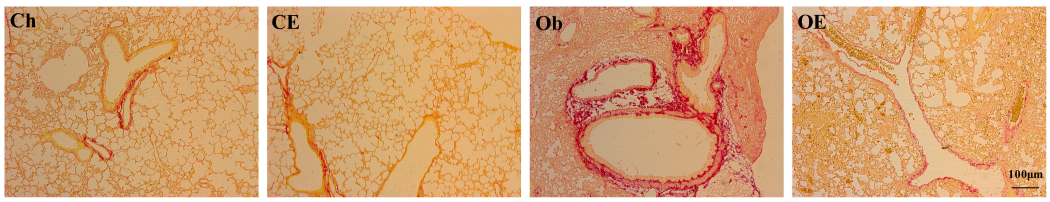

FIGURE 3 | Collagen deposition was detected by Sirius Red staining. Sirius Red staining revealed evidence of collagen fibre deposition in the lung.

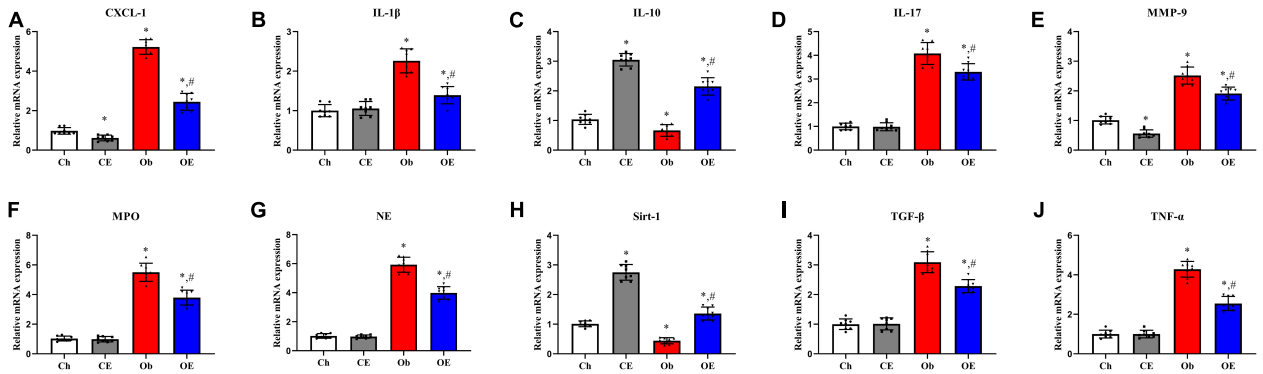

FIGURE 4 | Detection of mRNA expression levels in lung tissue. Detection of mRNA expression levels of CXCL-1 **(A)**, IL-1β **(B)**, IL-10 **(C)**, IL-17 **(D)**, MMP-9 **(E)**, MPO **(F)**, NE **(G)**, sirt-1 **(H)**, TGF-β **(I)**, and TNF-α **(J)** in lung tissue. $^{\#}P < 0.05$ compared with the Ch group. $^{*}P < 0.05$ compared with the Ob group. CXCL, C-X-C motif chemokine ligand; IL, interleukin; MMP, matrix metalloproteinase; NE, neutrophil elastase; Sirt, sirtuin; TGF, transforming growth factor; TNF, tumour necrosis factor.

Effects of Aerobic Exercise on High-Fat Diet-Induced Insulin Resistance

HFD feeding markedly downregulated plasma adiponectin levels (**Figure 6A**; $P < 0.001$) and plasma insulin levels (**Figure 6B**; $P < 0.001$) but markedly upregulated blood glucose levels (**Figure 6C**; $P < 0.001$) compared with Ch treatment. Significant downregulation of blood glucose levels ($P = 0.0164$) and significant upregulation of plasma adiponectin levels ($P = 0.0386$) and plasma insulin levels ($P = 0.0441$) were detected after AE for 8 weeks. The results of the GTT (**Figure 6D**), ITT (**Figure 6E**), and IRT (**Figure 6F**) demonstrated that an 8-week AE programme in obese mice markedly improved insulin resistance.

DISCUSSION

Previous studies demonstrate that obesity was closely associated with pulmonary fibrosis (Murtha et al., 2017; Rathinasabapathy et al., 2018; Han et al., 2021). However, the protective effects of AE on pulmonary fibrosis were unclear. Our data demonstrated that AE in obese mice had an antifibrotic effect and significantly decreased the magnitude of pulmonary fibrosis.

Morphological analysis demonstrated that AE in obese mice reduced hydroxyproline levels and collagen fibre deposition in lung tissue. Our data demonstrated that HFD administration dramatically increased the levels of the profibrogenic factors NE, MMP-9, TGF-β, and IL-17 in BALF. Similarly, HFD administration dramatically increased NE, MMP-9, TGF-β, and IL-17 mRNA expression levels in lung tissue, which was reversed by the end of the 8-week AE programme. Thus, AE protected against pulmonary fibrosis by repressing the expression of NE, MMP-9, TGF-β, and IL-17. In addition, sirt-1 was a negative regulator of MMP-9 (Nakamaru et al., 2009). Therefore, AE may suppress MMP-9 levels in part by activating sirt-1 levels in the lung.

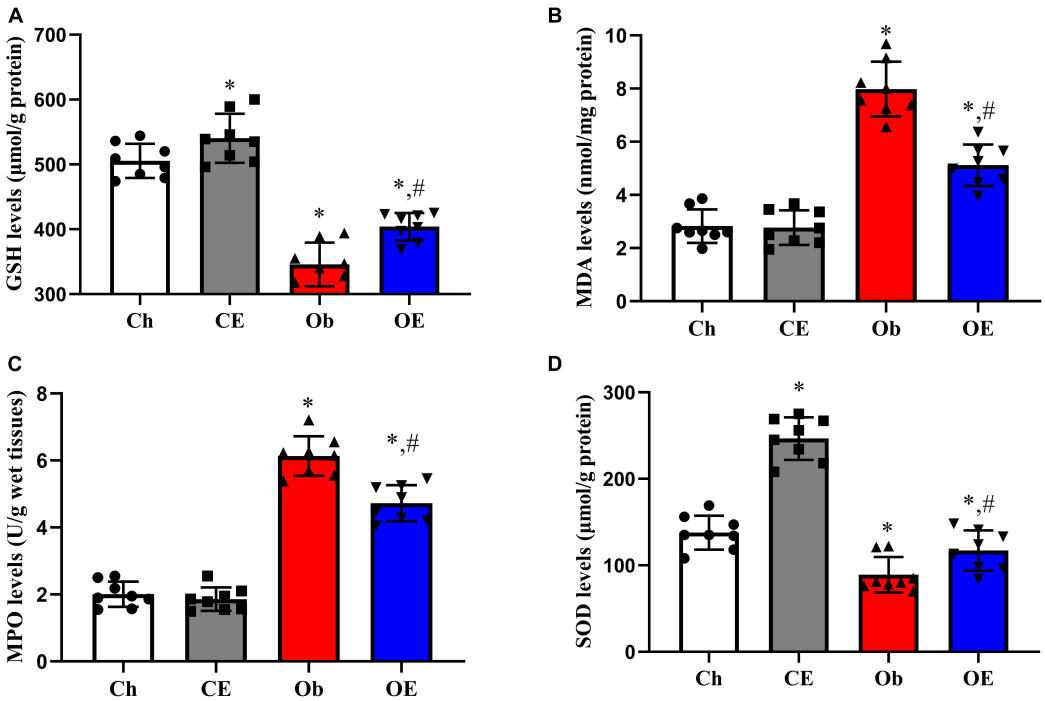

FIGURE 5 | Detection of oxidative stress-related genes in lung tissue. The levels of GSH **(A)**, MDA **(B)**, MPO **(C)**, and SOD **(D)** in lung tissue were detected. $^{\#}P < 0.05$ compared with the Ch group. $^{*}P < 0.05$ compared with the Ob group. MDA, malondialdehyde; MPO, myeloperoxidase; GSH, glutathione; SOD, superoxide dismutase.

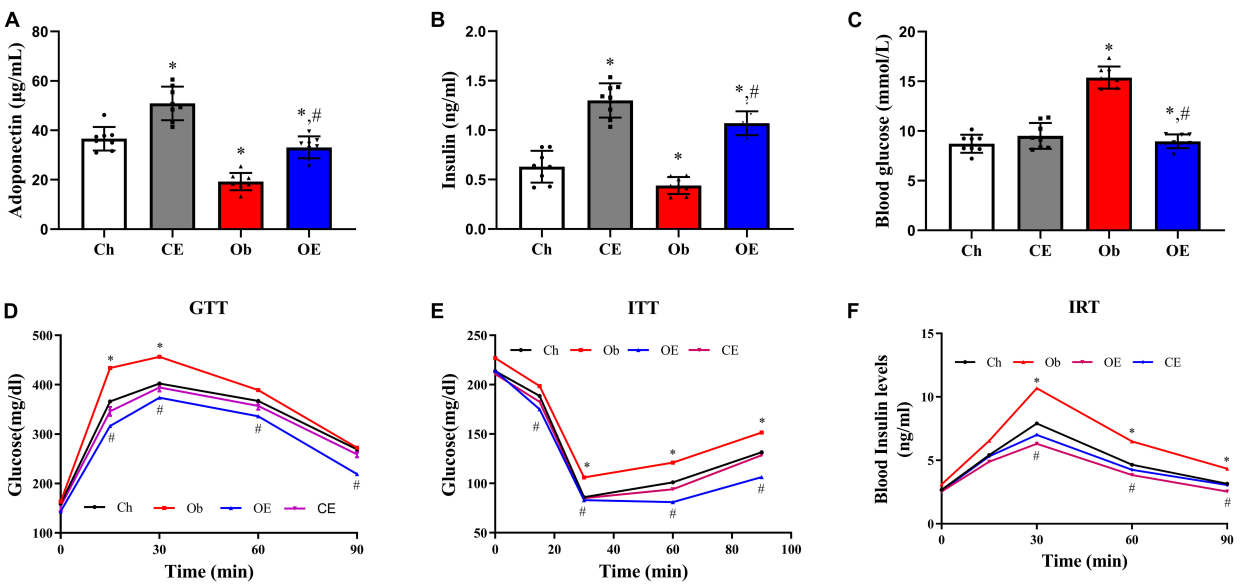

FIGURE 6 | Detection of insulin resistance. **(A)** Plasma adiponectin levels, **(B)** plasma insulin levels, **(C)** blood glucose levels, **(D)** GTT, **(E)** ITT, **(F)** IRT. $^{\#}P < 0.05$ compared with the Ch group. $^{*}P < 0.05$ compared with the Ob group. GTT, glucose tolerance test; ITT, insulin tolerance test; IRT, insulin release test.

Neutrophils play an important role in the pathogenesis of pulmonary fibrosis (Stockley, 2002; Jackson et al., 2014). We found that regular AE decreased the number of neutrophils in BALF in obese mice. Thus, AE reduced pulmonary fibrosis in part by reducing the neutrophil content in the lung. Previous studies have demonstrated that CXCL-1 and TNF-α are chemotactic for neutrophils (Thannickal et al., 2004; Matin et al., 2018).

Our data demonstrated that regular AE in obese mice markedly suppressed BALF levels of CXCL-1 and TNF-α. Our data showed that regular AE in obese mice markedly suppressed the mRNA expression levels of TNF-α and CXCL-1 in the lung. Hence, AE inhibited neutrophil infiltration in part by suppressing CXCL-1 and TNF-α expression.

HFD administration led to pro-oxidative/antioxidative imbalance, and the antioxidant effects of exercise were identified in this study. Our data identified that regular AE in obese mice significantly downregulated MDA and MPO levels but significantly upregulated SOD and GSH levels in the lung. AE in obese mice modulated the oxidative/antioxidative balance in lung tissue. Previous studies have demonstrated that sirt-1 has therapeutic effects in lung diseases because sirt-1 modulates the pro-oxidative/antioxidative balance (Rajendrasozhan et al., 2008; Nakamaru et al., 2009; Rahman et al., 2012). Our data showed that AE modulated the oxidation/antioxidation balance in lung tissues partly because AE increased sirt-1 expression. Sirt-1 exerts anti-inflammatory and antioxidant effects and protects against lung diseases, including COPD, asthma, and pulmonary fibrosis (Cantó et al., 2009; Yao et al., 2012). Thus, obesity increases the probability of lung diseases, including COPD and pulmonary fibrosis, in part by suppressing sirt-1 production. Therefore, targetted improvement of sirt-1 production in obese patients with pulmonary fibrosis may be a novel therapeutic strategy.

Previous studies identified that obesity-associated insulin resistance enhanced TGF-β expression, which played an important role in the pathogenesis of pulmonary fibrosis (Spencer et al., 2010; Mayer et al., 2012; Asghar and Sheikh, 2017; Park et al., 2019). We found that AE improved HFD-induced insulin resistance and decreased TGF-β mRNA expression in the lung and BALF levels of TGF-β. Hence, AE alleviated pulmonary fibrosis by improving insulin resistance and suppressing TGF-β expression. Exercise is Medicine (Li and Laher, 2015, 2020; Li et al., 2019).

Perspective

The results of this study provide insight into pulmonary fibrosis and indicate that AE is a novel tool to treat pulmonary fibrosis. HFD administration leads to pulmonary fibrosis by causing insulin resistance, a chronic inflammatory response, pro-oxidative/antioxidative imbalance, and increased levels of profibrogenic factors, including TGF-β, IL-17, NE, and MMP-9. AE improves HFD-induced pulmonary fibrosis by counteracting obesity-associated insulin resistance, chronic inflammatory responses, and pro-oxidative/antioxidative imbalance and shows an antifibrogenic effect by suppressing TGF-β, IL-17, NE, and MMP-9 production in obese mice.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding authors.

ETHICS STATEMENT

The animal study was reviewed and approved by Animal Experimental Welfare and Ethical Inspection Form of Institute of Animal Science, Chinese Academy of Agricultural Sciences.

AUTHOR CONTRIBUTIONS

XW: conceptualisation, methodology, software, validation, writing—original draft preparation, and project administration. XY and DT: formal analysis, investigation, resources, supervision, and funding acquisition. XW, XY, and DT: writing—review and editing. All authors have read and agreed to the published version of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2021.785117/full#supplementary-material

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Effects of Different Intensities and Durations of Aerobic Exercise on Vascular Endothelial Function in Middle-Aged and Elderly People: A Meta-analysis

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Background: Previous studies have found that aerobic exercise was more effective in improving vascular endothelial function than resistance training, high-intensity interval training (HIIT), and other types of exercise, while the effects between different intensities and durations of aerobic exercise were unclear. Therefore, we performed this meta-analysis to investigate the effects of different intensities and durations of aerobic exercise on the vascular endothelial function of middle-aged and elderly people.

Methods: Databases were searched up to April 2021 for studies evaluating the influences of different intensities and durations of aerobic exercise on endothelial function assessed by flow-mediated dilation (FMD) among healthy middle-aged and elderly people. Data were pooled using random-effects models to obtain the weighted mean difference (WMD) and 95% confidence intervals (CIs).

Results: A total of 9 studies involving 221 participants fulfilled the inclusion criteria. Aerobic exercise improved the overall FMD of healthy middle-aged and elderly people [WMD, 1.33 (95% CI, 0.37–2.28), $P < 0.05$]. Specifically, vigorous-intensity exercise increased FMD significantly in healthy middle-aged and elderly people [WMD, 1.10 (95% CI, 0.27–1.93), $P < 0.05$], while moderate-intensity exercise had no significant association with FMD [WMD, 1.49 (95% CI, −0.62 to 3.60), $P = 0.17$]. In addition, long-term (8 weeks or above) aerobic exercise increased the FMD in healthy middle-aged and elderly people [WMD, 1.63 (95% CI, 0.61–2.66), $P < 0.05$], while one-time acute aerobic exercise had no significant association with FMD of healthy middle-aged and elderly people [WMD, 0.89 (95% CI, −1.47 to 3.24), $P = 0.46$]. Specifically, 8 weeks or above of vigorous-intensity exercise increased FMD significantly in healthy middle-aged and elderly people [WMD, 1.48 (95% CI, 1.06–1.90), $P < 0.01$], while 8 weeks or above of moderate aerobic exercise had no significant association with FMD [WMD, 1.49 (95% CI, −0.62 to 3.60), $P = 0.17$].

Conclusion: Aerobic exercise, especially 8 weeks or above of vigorous-intensity aerobic exercise, improved the endothelial function in healthy middle-aged and elderly people.

Keywords: aerobic exercise, endothelial function, flow-mediated dilation, middle-aged people, elderly people

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INTRODUCTION

Aging is an inevitable cardiovascular risk factor, and the increase in age will make the body more susceptible to pathological stress (Tian and Li, 2014) and increase the prevalence of various cardiovascular diseases (CVD) include arteriosclerosis, hypertension, stroke, and so on (North and Sinclair, 2012). Vascular endothelial cells are a single layer of cells adjacent to the lumen of blood vessels and play an important role in the regulation of vascular tone and the maintenance of hemodynamics (Jia et al., 2019), which can directly act on various cardiovascular and peripheral vascular diseases (Rajendran et al., 2013). Endothelium dysfunction, especially impaired endothelium-dependent vasodilation, has been linked to arterial stiffness, atherosclerosis, coronary artery disease, and so on (Ungvari et al., 2018). Flow-mediated dilation (FMD) of the brachial artery is currently used as a parameter in evaluating vasodilation (Thijssen et al., 2019) and is used to predict the risk of cardiovascular diseases in clinic studies, independently, which provides us with a non-invasive method to assess vascular endothelial function. In addition, the risk of CVD will increase 13% following every 1% reduction in brachial artery FMD (Inaba et al., 2010; Ras et al., 2013; Xu et al., 2014; Matsuzawa et al., 2015; Thijssen et al., 2019).

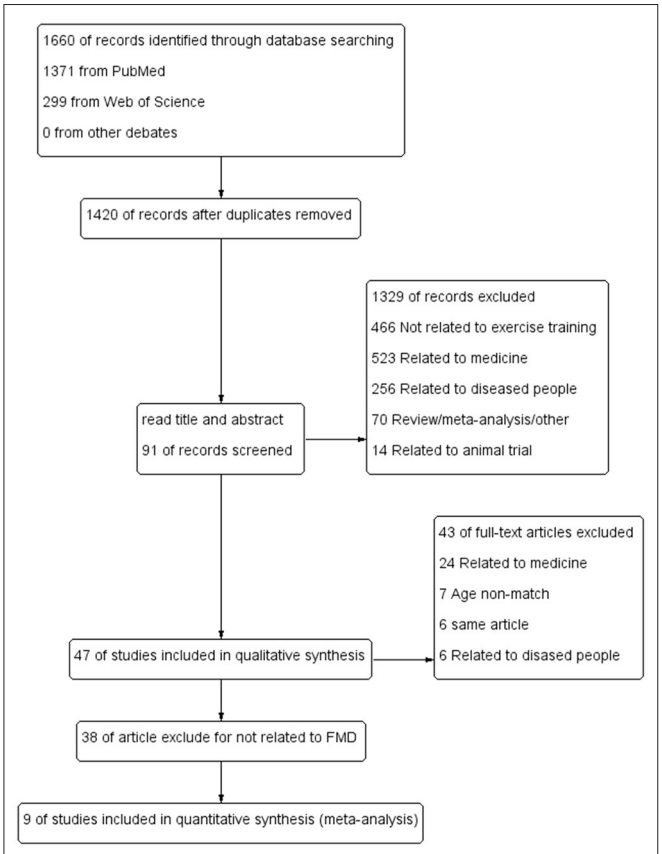

FIGURE 1 | PRISMA flowchart of study selection.

It is well-known that endothelial function is age-dependent (Ungvari et al., 2018). The epidemiologic study has shown that regular exercise can prevent CVD and reduce cardiovascular morbidity and mortality in the general population, especially healthy subjects (Eckel et al., 2014; Arnett et al., 2019; Seals et al., 2019). Therefore, this study pays more attention to the impact of aerobic exercise on improving vascular endothelial function in middle-aged and elderly people. Preliminary research suggested that aerobic exercise had a better effect than other types of exercise in improving vascular endothelial function and reducing the risk of CVD (Zhao et al., 2014; Boeno et al., 2020). The main mechanism was that aerobic exercise can improve the bioavailability of nitric oxide and reduce oxidative stress (Seals et al., 2019). However, some studies found that FMD does not always increase with the continuous training of 8–12 weeks (Tinken et al., 2010; Birk et al., 2012; Green et al., 2014; Green and Smith, 2018), which prompted that time characteristic was a key role. In addition, different aerobic exercise intensities have different effects on vascular endothelial function (Yoo et al., 2017; Green and Smith, 2018). In conclusion, there was not a unanimous result on the influence of different intensities and different durations of aerobic exercise on vascular endothelial function (Goto et al., 2003; Man et al., 2020). Therefore, we performed this meta-analysis to explore the effects of different intensities and durations of aerobic exercise on vascular endothelial function in healthy middle-aged and elderly people.

METHODS

Design

This meta-analysis was reported following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Moher et al., 2015).

Search Strategy

All the studies before April 2021 on aerobic exercise to improve the vascular endothelial function in middle-aged and elderly people were searched in PubMed and Web of Science, using the following MESH terms and text words: aerobic exercise, middle-aged, elderly people, and vascular endothelial function. We also hand-searched reference lists of all identified studies. All studies used for meta-analysis need to meet the following criteria: (1) the participants were healthy middle-aged and elderly people; (2) the intervention used in the study was aerobic exercise; (3)FMD was used for evaluating vascular endothelial function. Articles were excluded if the language was non-English or using an animal model. Reviews and conference articles were also excluded from the analysis. As Li et al. (2021) reported, middle-aged and elderly people was defined as people $\geq$45 years old.

Data Extraction and Quality Assessment

The documental information of all qualified studies includes author information, participant characteristics (including sex distribution), age, training type, training intervention duration,

TABLE 1 | Characteristics of studies included in this meta-analysis.

Included studies	Sample	Age	Type	Intensity	Duration	Frequency	Times	Results	Conclusion
Nishiwaki et al. (2011)	Normoxic group (n = 8); hypoxic group (n = 8)	Total 56 ± 1	AE	50% VO_{2max}	8 weeks	4 days/week	30 min/day	FMD	+
Pierce et al. (2011)	Intervention study (n = 44); exercise (n = 22)	63 ± 1	AE	70–75% HR_{max}	8 weeks	6–7 days/week	40–50 min/day	FMD	+
Schaun et al. (2011)	Male volunteers (n = 20)	54 ± 4	AE	65% HR_{max}	12 weeks	3 days/week	30 min/day	FMD	+
Serviente et al. (2016)	Menopausal women (n = 15)	58.9 ± 1.4	AE	60–64% VO_{2max}	30 min	one time	30 min/day	FMD	+
Hunter et al. (2018)	23 °C yoga (n = 14); 40.5 °C yoga (n = 19)	23 °C Yoga 49 ± 5; 40.5 °C Yoga 47 ± 5	AE	Moderate-intensity	12 weeks	3 days/week	90 min/day	FMD	+
Bouaziz et al. (2019)	Sedentary volunteers (n = 30)	79 ± 2.5	AE	88% HR_{max}	9.5 weeks	2 days/week	30 min/day	FMD	+
Klonizakis et al. (2020)	Aqua (n = 20); land (n = 20); mixed (n = 20)	Aqua: 63.7 ± 7; land: 65 ± 6; mixed: 66 ± 6	AE	3 METs	≥ 6 moths	≥ 2 days/week	60 min/day	FMD	+
Akazawa et al. (2012)	Postmenopausal women (n = 11)	59 ± 5	AE	60–75% HR_{max}	8 weeks	3 days/week	30–60 min/day	FMD	+
Swift et al. (2014)	African American (n = 8); Caucasian (n = 16)	55.8 ± 1.7	AE	RPE: 10–12; RPE: 15-17	12 weeks	4 days/week	Unclear	FMD	+

AE, aerobic exercise; FMD, flow-mediated dilation; VO_{2max}, maximal oxygen consumption; HR_{max}, maximum heart rate; MET, metabolic equivalent; RPE, rating of perceived exertion; "+" represents a positive result.

training intensity, training frequency, time of one training, and research result indicators (FMD). Two reviewers (QY and LY) independently reviewed the titles, abstracts, and full texts of all citations to identify studies reporting the effects of aerobic exercise on vascular endothelial function in healthy middle-aged and elderly people. When the data could not be extracted or there was a dispute, two authors negotiated or contacted the author of the article to resolve it. Otherwise, the platform was used to extract the information (WebPlotDigitizer., 2021).

The Cochrane collaboration bias tool, which includes items on selection bias, performance bias, detection bias, attrition bias, and reporting bias, was used to evaluate the quality of eligible studies.

Data Synthesis and Analysis

Data were pooled using random-effects models to obtain the weighted mean difference (WMD) and 95% confidence intervals (CIs). When analyzing whether aerobic exercise could improve the vascular endothelial function of healthy elderly people, the Chi-square (χ^2) test was used. If there was a high level of heterogeneity in the test ($I^2 > 60\%$), we used subgroup analysis or sensitivity analysis to explain the results (Moher et al., 2015; Shamseer et al., 2015). In the subgroup analysis, we tried to use different intensities and durations of aerobic exercise to explore the impact on vascular endothelial function. The analysis result, funnel plot, and forest chart were generated using the software RevMan.5. In terms of overall impact, $P < 0.05$ was considered statistically significant.

RESULTS

Studies Retrieved and Characteristics

The literature search results and research selection process were shown in **Figure 1**. Among the 1,426 articles identified, after reading the titles and abstracts, and then reading the full texts, 9 studies were considered eligible for meta-analysis (Nishiwaki et al., 2011; Pierce et al., 2011; Schaun et al., 2011; Akazawa et al., 2012; Swift et al., 2014; Serviente et al., 2016; Hunter et al., 2018; Bouaziz et al., 2019; Klonizakis et al., 2020).

The main characteristics of participants and exercise interventions were shown in **Table 1**. Nine studies involved 221 participants, of which 5 studies directly explored the effects of aerobic exercise on vascular endothelial function (Schaun et al., 2011; Akazawa et al., 2012; Swift et al., 2014; Serviente et al., 2016; Bouaziz et al., 2019), 4 studies explored factors related to aerobic exercise and endothelial function (Nishiwaki et al., 2011; Pierce et al., 2011; Schaun et al., 2011; Serviente et al., 2016), these articles contained perimenopausal women and postmenopausal women (Nishiwaki et al., 2011; Pierce et al., 2011; Akazawa et al., 2012; Swift et al., 2014; Serviente et al., 2016; Hunter et al., 2018; Bouaziz et al., 2019; Klonizakis et al., 2020), only one article discussed all men (Schaun et al., 2011). According to the position statement of physical activity and training intensity (Norton et al., 2010), we adjusted the intensity classification of aerobic exercise according to the included research situation: 1.6 < METs < 3, 20% < maximal oxygen uptake (VO_{2max}) < 40%, 40% < maximal heart rate (HR_{max}) < 55%, or 8 < RPE < 10 were determined as light-intensity; 3 < METs < 6, 40% < VO_{2max} <

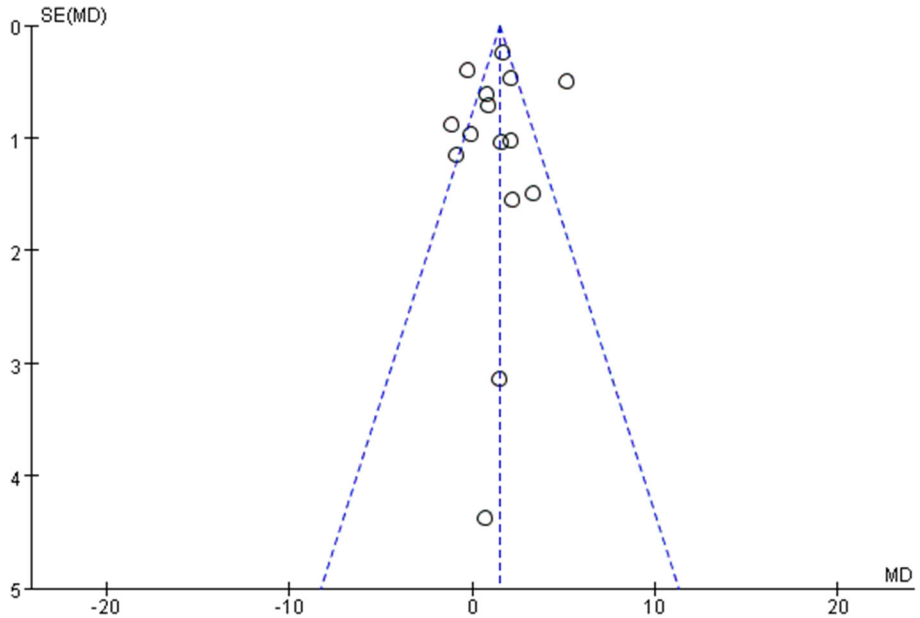

FIGURE 2 | Funnel plot.

60%, 55% < HR_{max} < 70%, or 11 < RPE < 13 were determined as moderate-intensity; 6 < METs < 9, 60% < VO_{2max} < 85%, 70% < HR_{max} < 90%, or 14 < RPE < 16 were determined as vigorous intensity.

Risk of Bias

Cochrane risk assessment tool was used to evaluate the methodological quality of the included literature, mainly from six aspects: selection bias, performance bias, detection bias, attrition bias, reporting bias, and other bias (**Figure 2**). The quality score was made according to three levels (low risk, high risk, and unclear). The quality of the included literature was divided into three levels from high to low: high quality, medium quality, and low quality (**Figure 2**). Publication bias was assessed visually by inspecting the funnel plot (**Figure 3**).

Effects of Aerobic Exercise on the FMD

After analyzing the data of all included studies, we found that aerobic exercise could increase the FMD of healthy middle-aged and elderly people. However, this increase did not distinguish between exercise intensities and durations. As shown in **Figure 4**, aerobic exercise increased the FMD significantly [WMD, 1.33 (95% CI, 0.37–2.28), $P < 0.05$], while there was a significant heterogeneity ($I^2 = 85\%$, **Figure 4**).

Subgroup Analysis: Effects of Different Intensities of Aerobic Exercise on the FMD

Different results were shown when considering exercises intensities. Specifically, vigorous-intensity exercise increased the FMD significantly [WMD, 1.10 (95% CI, 0.27–1.93), $P < 0.05$], while moderate-intensity exercise had no significant association with FMD in healthy middle-aged and elderly people [WMD, 1.49 (95% CI, −0.62 to 3.60), $P = 0.17$]. However, both subgroups had significant heterogeneity ($I^2 = 77\%$ and $I^2 = 87\%$, respectively; **Figure 5**).

Subgroup Analysis: Effects of Different Durations of Aerobic Exercise on the FMD

The subgroup analysis of different durations of aerobic exercise showed that long-term (8 weeks or above) aerobic exercise increased the FMD significantly [WMD, 1.63 (95% CI, 0.61–2.66], $P < 0.01$], which had a significant heterogeneity ($I^2 = 80\%$, **Figure 6**). However, one-time acute exercise had no significant associations with FMD in healthy middle-aged and elderly people [WMD, 0.89 (95% CI, −1.47 to 3.24), $P = 0.46$], which had a significant heterogeneity ($I^2 = 93\%$, **Figure 6**).

Effects of Different Intensities of 8 Weeks or Above of Aerobic Exercise on the FMD

In the study, we compared the effects of 8 weeks or above of moderate-intensity aerobic exercise and vigorous-intensity aerobic exercise on the FMD, and our results showed that 8 weeks or above of moderate-intensity aerobic exercise had no effect on the FMD in healthy middle-aged and elderly people [WMD, 1.49 (95% CI, −0.62 to 3.60), $P = 0.17$], which had a significant heterogeneity ($I^2 = 87\%$, **Figure 7**). However, 8 weeks or above of vigorous-intensity aerobic exercise increased the FMD significantly in healthy middle-aged and elderly people [WMD, 1.48 (95% CI, 1.06–1.90), $P < 0.01$}, with no evidence of heterogeneity ($I^2 = 0\%$, **Figure 7**).

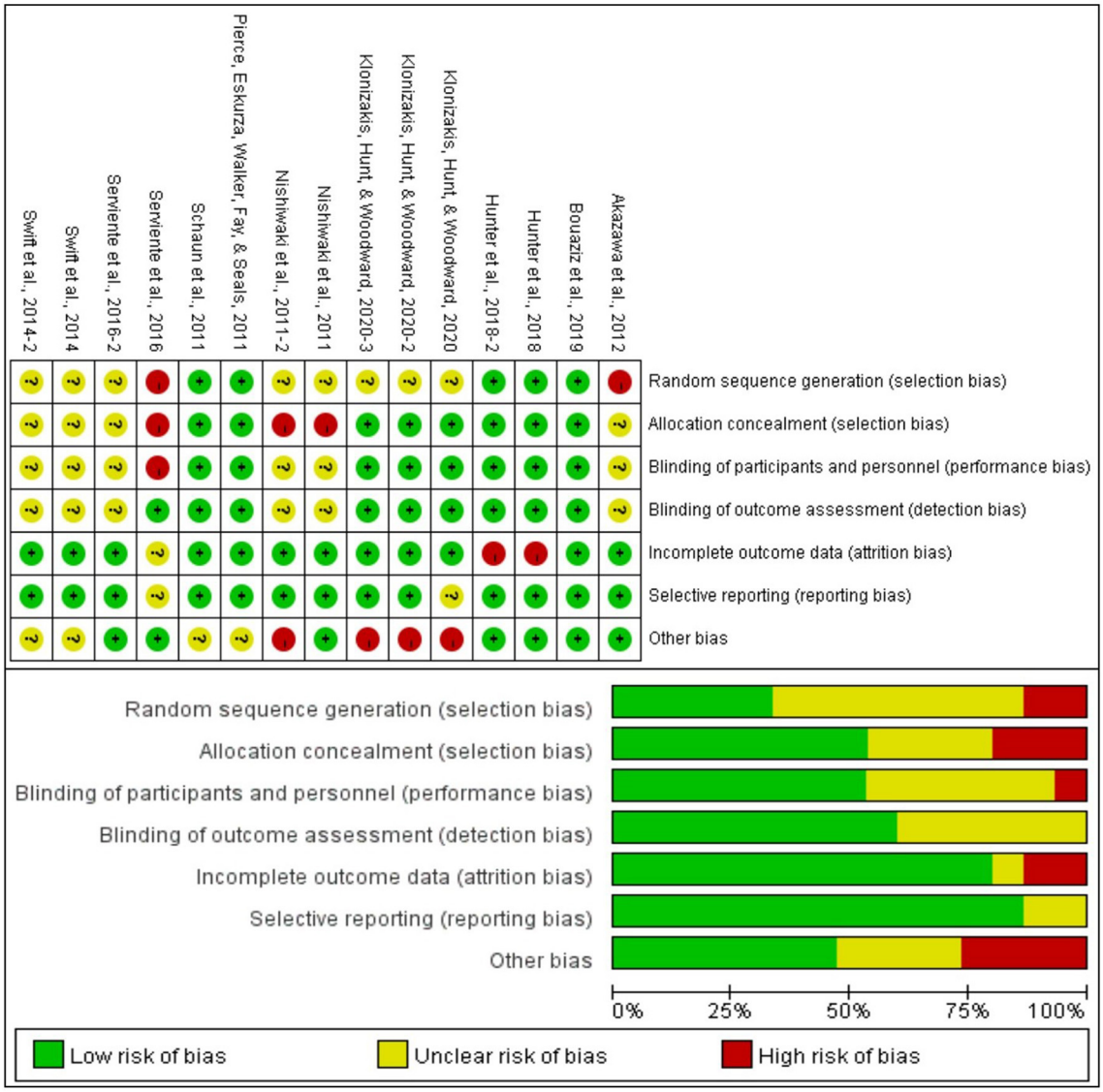

FIGURE 3 | Results of Cochrane risk of bias tool.

DISCUSSION

Our meta-analysis showed that aerobic exercise, especially different intensities and durations, significantly improved vascular endothelial function in healthy middle-aged and elderly people, as expressed by increased FMD. Noteworthy, 8 weeks or above of vigorous-intensity aerobic exercise significantly increased FMD in healthy middle-aged and elderly people. However, our meta-analysis did not provide adequate evidence on which exercise intensity was superior in improving vascular endothelial function, since subgroup analysis failed to show significant difference between vigorous-intensity and moderate-intensity. At the same time, it was obvious that aerobic exercise improved vascular endothelial function by increasing the FMD, requiring more proper exercise intensity and longer exercise duration.

Our study showed that aerobic exercise contributed to an overall improvement in the FMD by 1.33%, which was of clinical importance for healthy middle-aged and elderly people. According to previous studies, the increase of the FMD was positively correlated with the reduction of the risk of CVD, which will decrease 13% following every 1% increase in brachial artery FMD (Inaba et al., 2010; Ras et al., 2013; Xu et al., 2014; Matsuzawa et al., 2015; Thijssen et al., 2019). Therefore, we could conclude that people who participated in aerobic exercise for a longer time (8 weeks or above) had better effects on vascular function than those who did not participate in aerobic exercise regularly. The lifestyle of long-term aerobic exercise might provide a protective mechanism for middle-aged and

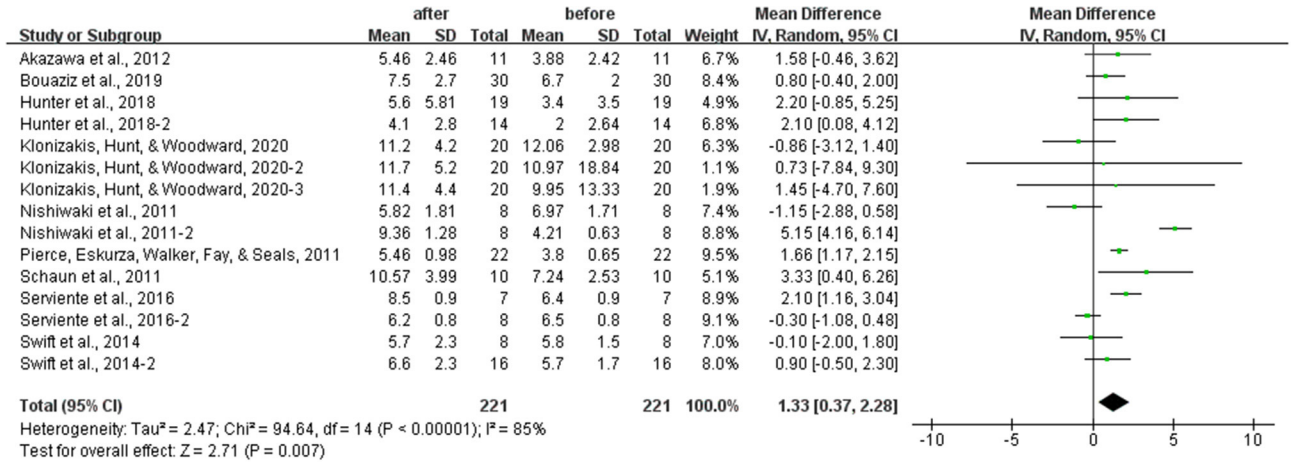

FIGURE 4 | Meta-analysis results of the effects of aerobic exercise on the FMD in healthy middle-aged and elderly people. The pooled estimates were obtained from random effects analysis. Diamonds indicated the effect size of each study summarized as WMD. The size of the shaded squares was proportional to the percentage weight of each study. Horizontal lines represented the 95% CI and the vertical line represented the overall effect. Aerobic exercise improved FMD significantly [WMD, 1.33 (95% CI, 0.37–2.28), $P < 0.05$].

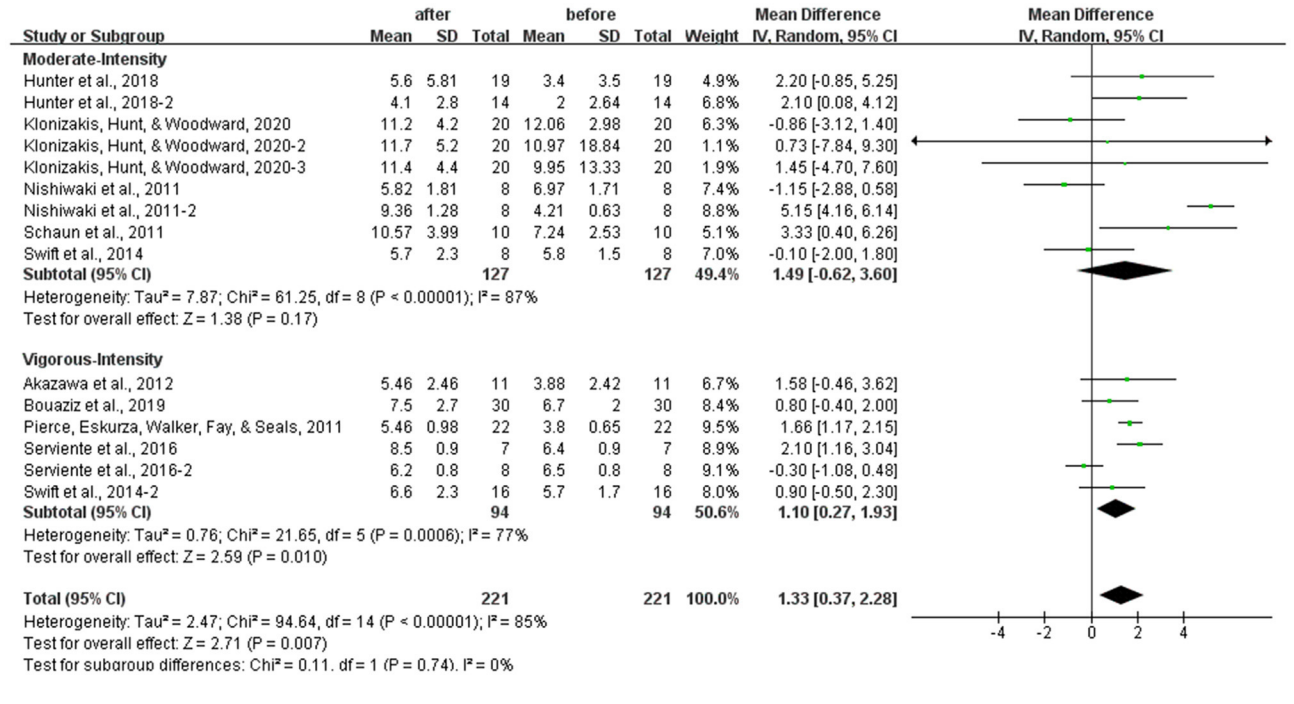

FIGURE 5 | Meta-analysis of the effects of different intensities of aerobic exercise on the FMD in healthy middle-aged and elderly people. The pooled estimates were obtained from random effects analysis. Diamonds indicated the effect size of each study summarized as WMD. The size of the shaded squares was proportional to the percentage weight of each study. Horizontal lines represented the 95% CI and the vertical line represented the overall effect. Moderate-intensity aerobic exercise had no significant relationship with FMD in the middle-aged and elderly people [WMD, 1.49 (95% CI, −0.62 to 3.60), $P = 0.17$], while vigorous-intensity aerobic exercise improved FMD significantly [WMD, 1.10 (95% CI, 0.27–1.93), $P < 0.05$].

elderly people to slow down the rate of vascular degeneration. Although the mechanism of aerobic exercise improving vascular endothelial function had not been fully revealed, it was speculated that the beneficial effect of exercise on endothelial function might be strengthened through the following mechanisms.

Firstly, there might be a dose-response relationship between vascular endothelial function and aerobic exercise intensity and duration in healthy middle-aged and elderly people. Our results showed that vigorous-intensity exercise increased the FMD significantly, while moderate-intensity exercise had no

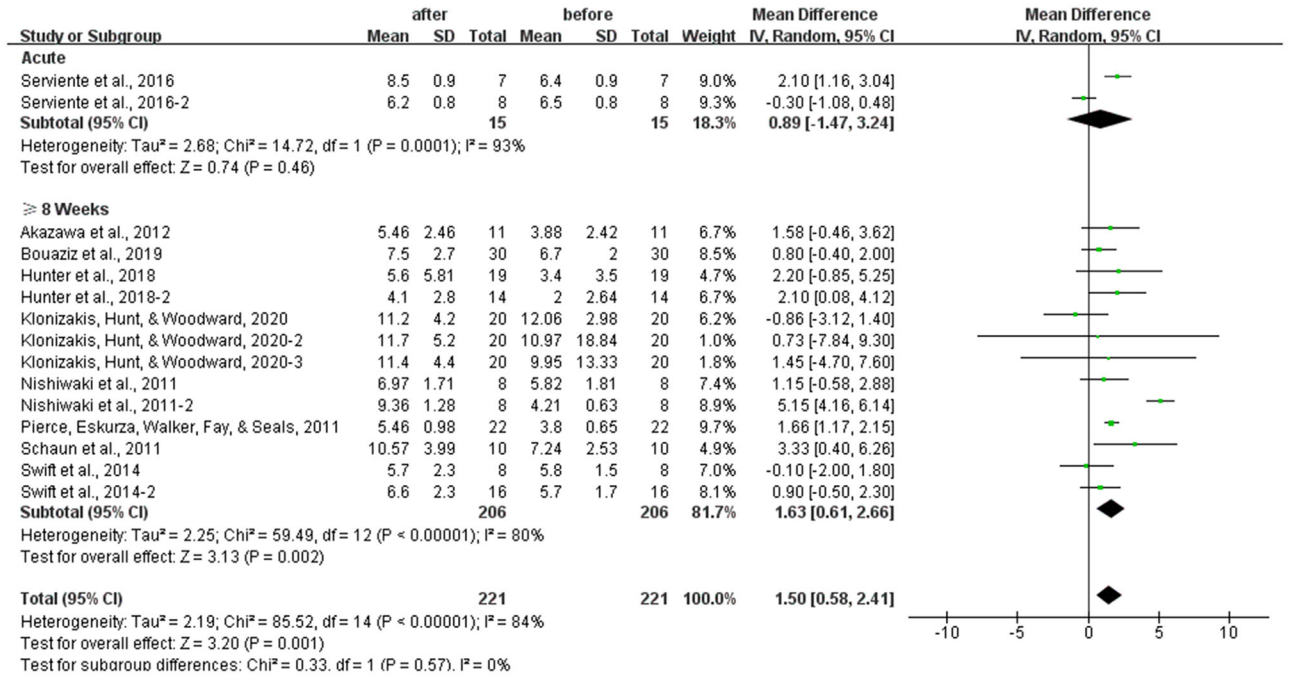

FIGURE 6 | Meta-analysis of the effects of different durations of aerobic exercise on the FMD in healthy middle-aged and elderly people. The pooled estimates were obtained from random effects analysis. Diamonds indicated the effect size of each study summarized as WMD. The size of the shaded squares was proportional to the percentage weight of each study. Horizontal lines represented the 95% CI and the vertical broken line represented the overall effect. One-time acute aerobic exercise had no significant associations with FMD in healthy middle-aged and elderly people [WMD, 0.89 (95% CI, −1.47 to 3.24), $P = 0.46$], while 8 weeks or above of aerobic exercise improved FMD significantly in the middle-aged and elderly people [WMD, 1.63 (95% CI, 0.61–2.66), $P < 0.05$].

significant association with FMD in healthy middle-aged and elderly people, which suggested that higher exercise intensity was more effective in improving FMD. Aerobic exercise could produce an increase in blood flow, thus increasing the shear stress on the endothelium to increase the synthesis and bioavailability of nitric oxide (NO) (Simmons et al., 2011; Reynolds et al., 2021), as different intensities of aerobic exercise could produce different arterial pressure, blood flow, and shear stress patterns (Green and Smith, 2018), the improvements of vascular function and vascular remodeling were more obvious in higher intensity aerobic exercise (Tinken et al., 2010; Birk et al., 2012). Ramos et al. (2015) showed that higher intensity aerobic exercise had a greater effect on the endothelial function in adults than moderate-intensity continuous aerobic exercise, which was consistent with our study. In addition, Rakobowchuk et al. (2008) observed the FMD was significantly improved after 6 weeks of 65% VO_{2peak} (vigorous) intensity aerobic exercise, which was also consistent with our study. However, another study reported that both moderate-intensity and vigorous-intensity had a beneficial effect on improving vascular endothelial function (Islam et al., 2021). Therefore, further prospective and intervention studies were needed.

Furthermore, exercise intensity could not explain the improved vascular endothelial function alone, as the acute aerobic exercise of both moderate-intensity and vigorous-intensity did not affect improving the FMD. Harris et al. (2008) found that acute low-intensity (25% VO_{2max}), moderate-intensity (50% VO_{2max}), and vigorous-intensity (75% VO_{2max}) did not influence the FMD response, which suggested that the dose-response relationship of aerobic exercise and vascular endothelial function was dependent not only on the intensity of exercise but also on the duration of exercise.

Secondly, aerobic exercise could reduce the expression of oxidative stress and pro-inflammatory molecules (Teixeira-Lemos et al., 2011). It was reported that regular aerobic exercise could enhance men's vascular endothelial function by reducing oxidative stress and maintaining the bioavailability of NO, which were the factors of endothelial dysfunction (Yoo et al., 2017; Seals et al., 2019). Aerobic exercise could help to restore the function of endothelial progenitor cells, promote endothelial repair, and then promote angiogenesis (Koutroumpi et al., 2012). However, these reactions required a certain exercise intensity and duration, which provided a new idea for increasing the FMD through aerobic exercise to improve vascular endothelial function and prevent CVD. Our results showed that 8 weeks or above of vigorous-intensity aerobic exercise improved the FMD significantly, which suggested that exercise duration was an important factor in improving vascular endothelial function in middle-aged and elderly people. However, this improvement was not observed in acute vigorous-intensity aerobic, and it might be caused by the following aspects. On the one hand,

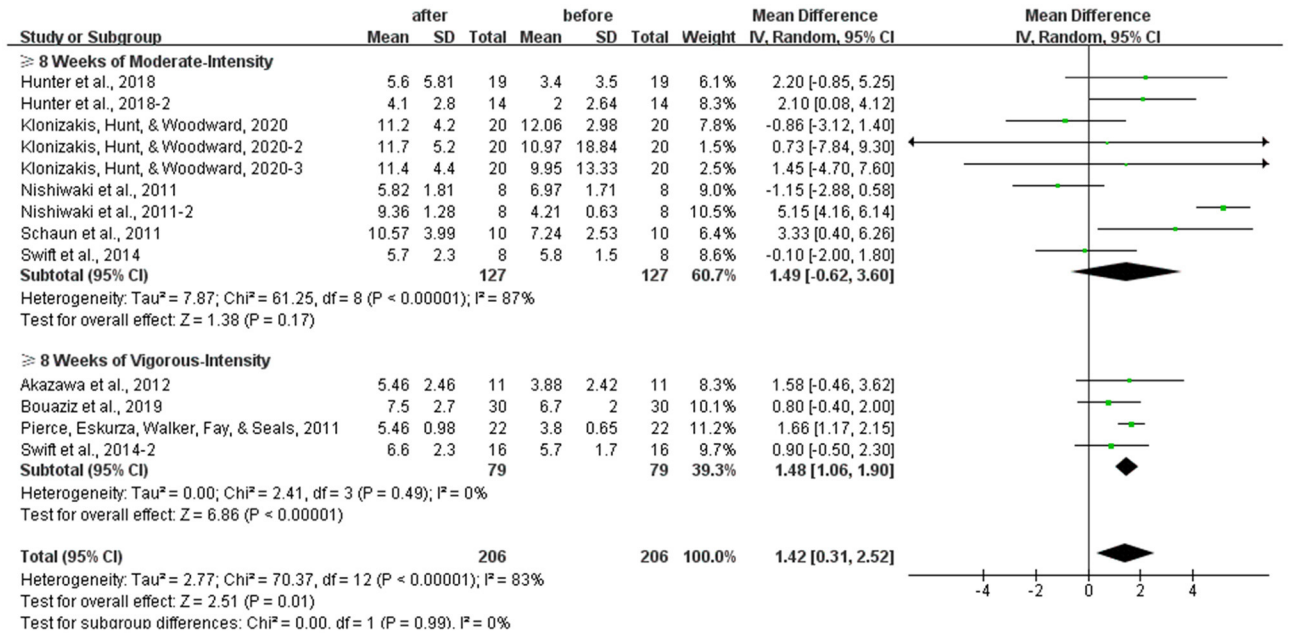

FIGURE 7 | Meta-analysis of the effects of different intensities of 8 weeks or above of aerobic exercise on the FMD in healthy middle-aged and elderly people. The pooled estimates were obtained from random effects analysis. Diamonds indicated the effect size of each study summarized as WMD. The size of the shaded squares was proportional to the percentage weight of each study. Horizontal lines represented the 95% CI and the vertical broken line represented the overall effect. Eight weeks or above of moderate-intensity aerobic exercise had no association with the FMD in healthy middle-aged and elderly people [WMD, 1.49 (95% CI, −0.62 to 3.60), $P = 0.17$], while 8 weeks or above of vigorous-intensity aerobic exercise significantly improved FMD in middle-aged and elderly people [WMD, 1.48 (95% CI, 1.06–1.90), $P < 0.01$].

age-related endothelial damage could be prevented by long-time aerobic exercise through improving vascular endothelial function. And the mechanisms included changes in blood flow conditions, increases in blood flow, blood flow speed, and shear stress, and reduces in reactive oxygen species (ROS) production (DeSouza et al., 2000; Taddei et al., 2000), thereby increasing the bioavailability of NO in the vascular endothelium, maintaining vascular homeostasis (Simmons et al., 2011; Reynolds et al., 2021), delaying the rate of vascular degeneration (DeSouza et al., 2000), and finally having an effect on the prevention of age-induced endothelial dysfunction. On the other hand, the role of NO in the endothelium was unlikely to produce a response in a single skeletal muscle exercise (Gilligan et al., 1994), which was consistent with our results.

Third, according to previous studies, the FMD returned to normal after 60 min of acute exercise in elderly men, while postmenopausal women were not affected by vigorous-intensity exercise (Yoo et al., 2017; Seals et al., 2019), which suggested that sex affected the response of the FMD to aerobic exercise in middle-aged and elderly people. Reviewing our study, the heterogeneities in the subgroup might be related to the impaired vascular endothelial function in women perimenopausal or postmenopausal, since the impaired vascular endothelial function was more pronounced in perimenopausal women, which was more common in postmenopausal women with estrogen deficiency (Moreau et al., 2012). Previous study showed that compared with postmenopausal women, only perimenopausal women had improved vascular endothelial function under acute aerobic exercise (Serviente et al., 2016), which might be due to severe vascular endothelial damage caused by estrogen deficiency in postmenopausal women. Moreau et al. (2013) found that 12 weeks of moderate-intensity aerobic exercise could improve the FMD of postmenopausal women who received estradiol hormone therapy during exercise intervention, while FMD improvement was not seen in the postmenopausal women who did not receive estradiol hormone therapy during exercise intervention. The result of moderate-intensity aerobic exercise could not improve vascular endothelial function in postmenopausal women with estrogen deficiency was contrary to a previous study. Santos-Parker et al. (2017) reported that moderate-intensity aerobic exercise improved vascular endothelial function significantly in healthy middle-aged and elderly men, which suggested that estrogen had a protective effect on vascular endothelial function (Taddei et al., 1996). Estrogen could improve the damaged vascular endothelial function by increasing the bioavailability of NO, reducing endothelin-1 (ET-1), and generating vasodilatation to promote endothelial healing and increase angiogenesis (Mendelsohn and Karas, 2005; Chakrabarti et al., 2008). Furthermore, previous studies showed that despite the impaired vascular endothelial function, aerobic exercise could still improve the FMD significantly (Black et al., 2009; Green and Smith, 2018). However, at least 65%-80% HR_{max} and longer exercise

duration were required (Moreau et al., 2012), which was also consistent with our results. Therefore, we believed that it was necessary to strengthen exercise stimulation (intensity, duration, or the volume of aerobic exercise) to continuously improve the vascular endothelial function of middle-aged and elderly women, which was consistent with the opinion of Seals et al. (2019).

This study comes with a few limitations that should be taken into consideration. In the process of literature quality evaluation, subjective factors might cause a certain deviation, and there might be some differences in the FMD measurement methods as it was reported that peak expansion measurement cuff release might lead to an underestimation of the real FMD by up to 40% after 60 s (Black et al., 2008), even though this factor seemed to have little impact on the results of the FMD, and these methods were generally well-defined. In addition, only one study on acute exercise was included in the meta-anlysis, it might cause bias to the comparison of exercise duration.

CONCLUSIONS

In summary, this meta-analysis indicated that aerobic exercise, especially 8 weeks or above of vigorous-intensity aerobic exercise, improved the endothelial function in healthy middle-aged and elderly people.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

QY and LY contributed to literature search, figures, study design, data analysis, data interpretation, and writing. GL and HH contributed to data interpretation and writing. YL contributed to study design, figures, data analysis, data interpretation, and writing. All authors contributed to the article and approved the submitted version.

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Differential Effects of Exercise Programs on Neuregulin 4, Body Composition and Cardiometabolic Risk Factors in Men With Obesity

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Background: Neuregulin 4 (Nrg4) is an adipokine that is sensitive to energy expenditure and with a potential role in metabolic homeostasis and obesity. This study examined the effects of 12 weeks of three different exercise training protocols on Nrg4 levels, cardiometabolic risk factors, and body composition parameters in men with obesity.

Methods: Sixty adult men with obesity (Mean ± SD; age: 27.60 ± 8.4 yrs.; height: 168.4 ± 2.6 cm; weight: 96.7 ± 7.2 kg) were randomly allocated into four equal (n = 15) groups: High- Intensity Interval Training (HIIT), Circuit Resistance Training (CRT), Moderate Intensity Continuous Training (MICT) or a control group. The HIIT protocol involved six bouts of 3-min high-intensity exercise (90% VO_{2peak}) followed by 3-min low-intensity exercise (50% VO_{2peak}). The CRT group performed three circuits of resistance training, where each circuit included 11 exercises at 20% of one-repetition maximum (1RM) and 70% of VO_{2peak}, and with a work-to-rest ratio of 2:1 (40-s exercise and 20-s rest) and 60-s recovery between circuits. The MICT group performed 36 min of exercise at 70% of VO_{2peak}. All measurements were taken 72 h before and after the first and last training sessions.

Results: There were significant differences between the groups in fat-free mass (FFM), (effect size (ES): 0.78), fat mass (ES: 0.86), VO_{2peak} (ES: 0.59), high-density lipoprotein cholesterol (HDL-C) (ES: 0.83), low-density lipoprotein (LDL-C) (ES: 0.79), total cholesterol (TC) (ES: 0.90), triglyceride (TG) (ES: 0.52) glucose (ES: 0.39), insulin (ES: 0.61), HOM-IR (ES: 0.91) and Nrg4 (ES: 0.98) ($p < 0.05$). There were no significant changes in very-low-density lipoprotein cholesterol (VLDL-C) (ES: 0.13) levels, or body

weights (ES: 0.51) ($p > 0.05$). Levels of Nrg4 were negatively correlated with LDL-C, TC, TG, VLDL-C, glucose, insulin, HOMA-IR ($p < 0.05$) and positively with HDL-C ($p < 0.05$).

Conclusion: Our results suggest that HIIT and CRT protocols have greater effects than MICT protocol on Nrg4 levels, metabolic and cardiovascular risk factors, and body composition variables in men with obesity.

Keywords: exercise, neuregulin 4 (Nrg4), obesity, HIIT (High Intensity Interval Training), resistance exercise and aerobic exercise

INTRODUCTION

The global epidemic of obesity is associated with comorbidities such as cardiovascular diseases, type 2 diabetes, and dyslipidemia due to hypertrophy of adipose tissue (Weisberg et al., 2003). Adipocytes secrete various bioactive molecules, adipokines, and inflammatory factors such as visfatin, tumor necrosis factor-alpha (TNF-α), and neuregulin-4 (Nrg4; Blüher, 2019; Saeidi et al., 2020a). Some of these adipokines have detrimental effects, while others modulate glucose homeostasis, insulin resistance, lipid metabolism, and obesity-related diseases (Gumà et al., 2020; Saeidi et al., 2020a; Shanaki et al., 2020). Adipose tissue-derived Nrg4 is a signaling molecule enriched in brown adipose tissue (BAT), which targets the liver and is involved in metabolic homeostasis (Blüher, 2019; Tutunchi et al., 2020). Moreover, Nrg4 has an influential role in maintaining energy balance by having anti-lipogenic properties (Wang et al., 2014; Blüher, 2019). Additionally, Nrg4 is an adipokine that is sensitive to energy expenditure and body composition variables (Tutunchi et al., 2020) and the expression of Nrg4 mRNA decreases in obese mice. In overweight humans, the expression of Nrg4 in subcutaneous fat is negatively correlated with body mass index (BMI), body fat, and fatty liver (Wang et al., 2014; Ma et al., 2016; Chen Z. et al., 2017). Furthermore, Nrg4 mRNA expression levels are lower in patients with impaired glucose tolerance or type 2 diabetes than those with normal glucose tolerance, suggesting that Nrg4 may have positive effects on glucose and lipid metabolism (Ma et al., 2016; Tutunchi et al., 2020). The activation of Nrg4 in adipocytes improves metabolic health by increasing adipose tissue angiogenesis (Tutunchi et al., 2020). Thus, it has potential benefits for novel treatments of obesity and associated metabolic complications (Tutunchi et al., 2020).

Different types of exercise training, such as aerobic and resistance training, are associated with benefits such as increased energy expenditure, reduced body fat mass, increased muscle mass, and improvements in insulin resistance and glucose homeostasis in individuals with obesity (Willis et al., 2012; Saeidi et al., 2020b; Zouhal et al., 2020). Frequency, intensity, time, and type of exercise are essential variables in designing an exercise program so as to determine exercise training efficacy and efficiency (Gibson et al., 2018). High-intensity exercise modalities, such as high-intensity interval training (HIIT), are associated with more favorable effects related to increases in post-exercise oxygen consumption (EPOC), body composition variables, and cardiometabolic risk factors in both healthy and individuals with obesity (Batacan et al., 2017; Wewege et al., 2017). Additionally, we recently reported that the intensity of resistance training could determine improvements in body composition, metabolic markers, and inflammatory adipokines in men with obesity (Saeidi et al., 2020b). However, the effects of exercise training and its variables on Nrg4 levels are not well understood. In addition, it is not clear which types of exercise programs are associated with the most efficiency and efficacy in people with obesity.

We speculated that the exercise benefits could be mediated by the activation of Nrg4. To the best of our knowledge, there are no studies on the effects of exercise training on regulating Nrg4 levels in humans. Thus, this study examined two hypotheses: first that exercise training, regardless of modality, positively influences Nrg4 levels, and second, that high-intensity interval training and circuit resistance training increase Nrg4 levels and improve cardiometabolic and body composition markers more than that moderate-intensity continuous training. Therefore, the purpose of this study was to examine the effects of moderate-intensity continuous training, circuit resistance training, and high-intensity interval training on Nrg4 levels, cardiometabolic, and body composition parameters in sedentary males with obesity.

MATERIALS AND METHODS

Ninety participants volunteered for the study, 22 (24%) of whom did not meet the following inclusion criteria: BMI > 30 kg/m^2, being sedentary (no regular physical activity during the last 6 months) and performing only activities of daily living, absent of cardiovascular, metabolic, and endocrine diseases, and not consuming alcohol. Individuals with joint disorders, physical disabilities, and taking prescribed medications and/or supplements that could affect muscle and adipose tissue metabolism were also excluded. Sixty-eight men with obesity (Mean $\pm$ SD; age: 27.6 $\pm$ 8.4 yrs.; height: 168.4 $\pm$ 2.6 cm; weight: 95.7 $\pm$ 3.8 kg; BMI: 32.6 $\pm$ 2.6 kg/m^2) voluntarily participated in this study.

All participants completed a physical examination performed by a physician and clinical exercise physiologist on the first visit. All study procedures were explained during this time, and the participants provided a written consent form and Physical Activity Readiness Questionnaire (PAR-Q; Thomas et al., 1992). The Research and Ethics Committee of the University approved all procedures of this study (Ethics code: IR-IAU1398-25). All

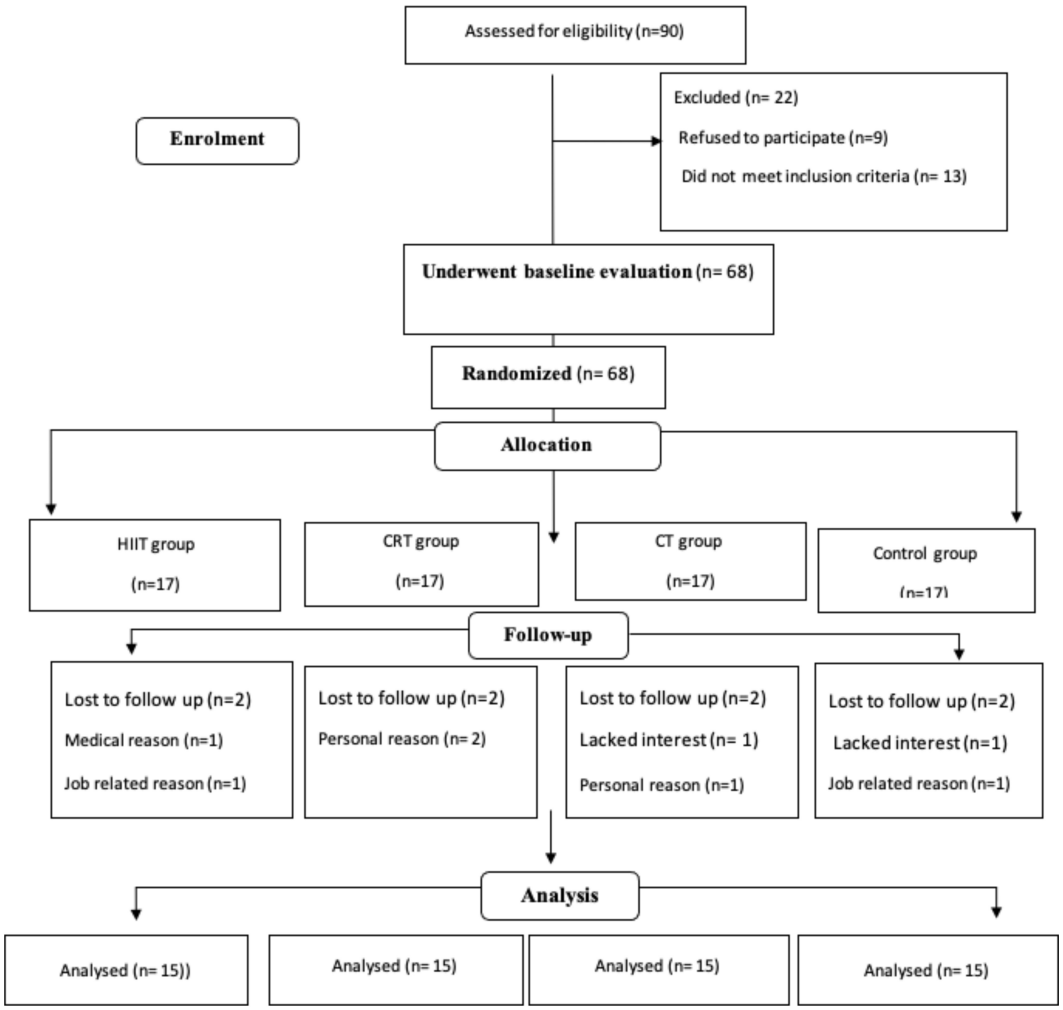

FIGURE 1 | Participant selection flow chart. MICT, Moderate Intensity Continuous Training; CRT, Circuit Resistance Training; and HIIT, High Intensity Interval Training.

procedures were performed according to the latest revision of the Declaration of Helsinki.

Experimental Design

All study procedures were explained 1 week prior to the start of the training programs in a participant familiarization session. Height, weight, and body composition were assessed followed by randomly assignment into one of four groups of equal sizes (n = 17): Moderate-Intensity Continuous Training (MICT), Circuit Resistance Training (CRT), High-intensity interval training (HIIT), and control groups (**Figure 1**). Eight participants withdrew from the study due to a variety of reasons (study duration, medical, occupational, and lack of interest in continuing in the study), resulting in 15 participants per group. Instructions on how to perform the training protocols were provided during the third session when body composition variables and VO_{2peak} were also measured. Twenty-four hours after the VO_{2peak} test, participants in the CRT group performed one maximum repetition test (1-RM) to identify maximum strength based on the Berzisky formula (Brzycki, 1993). Measurements of 1RM and VO_{2peak} tests were repeated every 4 weeks. Following baseline measurements, the three training groups (HIIT, CRT, and MICT) started the 12-week exercise training program (3 sessions per week). Participants in the control group were instructed to maintain their current lifestyles until the end of the study. Study measurements were collected at baseline (72 h before the start of the training protocols) and 72 h after the last session in all the groups at the same time of day (within ∼1 h) and under the same environmental conditions (∼20°C and ∼55% humidity). Participants in the training protocols were asked to follow the same diet for 72 h before baseline and before final measurements were obtained.

Body Composition, Cardio-Respiratory Fitness, and One-Repetition Maximum

Body weights and heights were assessed using a calibrated scale (Seca, Germany) and stadiometer (Seca, Germany), respectively. These data were used to calculate body mass index

(BMI) (kg/m^2). Levels of fat-free mass (FFM), and fat mass (FM) were measured by a bio-impedance analyzer (Medigate Company Inc., Dan-dong Gunpo, South Korea). Evaluation of VO_{2peak} was conducted using a modified Bruce protocol (in a temperature-controlled room, 21–23°C) as reported in previous studies of overweight and obese populations (Hunter et al., 2008; Ghroubi et al., 2009), using an electrically motorized treadmill (H/P/Cosmos, Pulsar med 3p- Sports & Medical, Nussdorf-Traunstein Germany). The physiological criteria used to determine VO_{2peak} [according to American College of Sports Medicine (ACSM) guidelines] included: if subjects reported they were physically exhausted and reached their maximal effort (according to Borg scale), or if the supervisor recognized the subjects had severe dyspnea, dizziness and other limiting symptoms based on guidelines for CPET test of ACSM and American Heart Association (AHA; Thompson et al., 2010; Myers et al., 2014), with a plateau in VO_2 and respiratory exchange ratio (RER) $\geq$ 1.10. Blood pressure was measured with an electronic sphygmomanometer (Kenz BPM AM 300P CE, Japan), and heart rate was monitored with a Polar V800 heart monitor (Finland) throughout the tests. Gas analysis was performed using a gas analyzer system (Metalyzer 3B analyzer, Cortex: biophysik, GMbH, Germany), calibrated before each test. Participants in the CRT protocol lifted their one-repetition maximum (1RM) for 6–8 repetitions for each exercise (see below), in which each 1RM was calculated according to the Berzisky formula (Brzycki, 1993).

Training Protocols

All exercise training sessions included 7-min warm-up and 7-min cool down at 70% of VO_{2peak} and 36 min of a specific exercise training protocol (see below) in the presence of a supervisory exercise physiologist.

The three protocols were matched externally by time (Isotime) and intensity (Isoeffort). All exercise sessions were monitored by heart rate reserve (HRR) which was equalized with the target VO_{2peak}.

The details of the specific exercises are summarized in **Table 1** and described below:

- CRT: 3 sets of 11 circuits (leg extension, knee flexion, sit-ups, chest press, lat pulldown, back extensions, pushups, stationary rowing, biceps curl, military press, and abdominal crunch) with a work-to-rest ratio of 40:20 s and 60 s rest between sets. The load of resistance exercises was 20% of 1RM, and intensity was approximately 70% of VO_{2peak} (Kaikkonen et al., 2000).
- HIIT: 6 sets of 3 min of high-intensity exercise at 90% VO_{2peak} and 3 min of active recovery at 50% VO_{2peak}. The average intensity was 70% of VO_{2peak} (18 min of high-intensity work, 18 min of active recovery).
- MICT: 36 min of jogging or running on the treadmill at 70% of VO_{2peak} (Bartlett et al., 2011).

Heart rate was monitored every minute throughout the exercise sessions in all training groups using a polar heart rate monitor watch (Polar, Made in Finland). Karvonen's formula (Camarda et al., 2008) was used to identify HRR to assess exercise intensity in training groups. The range of HRR used in all exercise sessions was between 65 and 75 % HRR. All VO_{2peak} measurements were retested, and in the CRT group who performed 1-RM testing, the training intensities were readjusted every 4 weeks.

TABLE 1 | Exercise protocols by group (MICT, CRT, and HIIT) and by intensity and duration for each weekly session.

12 Weeks	MICT	CRT	HIIT
Session	**Exercises:** treadmill jogging or running. **Time of each session:** 36 min main training +7 min warm-up and 7 min cooldown. **Intensity:** 70% of VO_{2peak}.	**Exercises:** leg extension, knee flexion, sit ups, chest press, lat pulldown, back extension, pushups, stationary rowing, biceps, military press, and abdominal crunch. **Sets:** 3 Duration of exercise phase: 40 s. **Duration of exercise phase:** 40 s. **Rest interval between exercises:** 20 s. **Rest between each set:** 1 min. **Load:** 20% of 1RM. **Time of each session:** 36 min main training +7 min warm-up and 7 min cooldown. **Intensity:** 70% of VO_{2peak}.	**Exercises:** treadmill jogging or running. **Time of each session:** 36 min main training +7 min warm-up and 7 min. Sets: 6 **Duration of exercise phase:** 3 min **Exercise intensity:** 90% of VO_{2peak}. **Duration of recovery phase:** 3 min. **Recovery intensity:** 50% of VO_{2peak}. **Mean intensity:** 70% of VO_{2peak}.

MICT, Moderate Intensity Continues Training; CRT, Circuit Resistance Training; and HIIT, High Intensity Interval Training.

Nutrient Intake and Dietary Analysis

Changes in dietary intake were evaluated. Three days of food intake (2 weekdays and 1 weekend day) were recorded before and after 12 weeks. The nutrient intakes were computed using the method of McCance and Widdowson (McCance and Widdowson, 2014). Total energy consumption and the amount of energy derived from proteins, fats, and carbohydrates were also determined (**Table 2**).

Blood Markers

All testing was carried out under standard conditions between 8 and 10 am. Fasting blood samples were taken from the right arm 12 h and 72 h before the first exercise session and 72 h after the last session. Blood samples were transferred to EDTA-containing tubes, centrifuged for 10 min at 3,000 rpm, and stored at −70°C. Plasma total cholesterol (TC) and triglycerides (TG) were measured by enzymatic methods (CHOD-PAP) using a Pars tech kit (Tehran, Iran) with a coefficient and sensitivity of 1.1% and 5 mg/dl and 1.6% and 5 mg/dl respectively. High-density cholesterol (HDL-C) and low-density cholesterol (LDL-C) levels were determined using a photometric Pars Testem's Quantitative Detection Kit (Tehran, Iran) with a coefficient and sensitivity of 1.8% and 1 mg/dl and 1.2% and 1 mg/dl, respectively. Very

TABLE 2 | Mean (sd, ±) values of nutritional intake by group.

	Control		MICT		CRT		HIIT	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Total energy (kcal/d)	2, 225 ± 190	2, 306 ± 103	2, 254 ± 216	2, 274 ± 136	2, 203 ± 157	2, 287 ± 98	2, 188 ± 155	2, 283 ± 291
Total protein (g/d)	111 ± 6.30	112 ± 5.73	110 ± 5.47	114 ± 4.53	112 ± 4.13	110 ± 4.43	109 ± 3.50	108 ± 2.63
Protein (g/kg BW/d)	1.05 ± 0.45	1.2 ± 0.4	1.1 ± 0.5	1.2 ± 0.3	1.1 ± 0.4	1.0 ± 0.3	1.0 ± 0.2	1.1 ± 0.3
Total protein (% energy)	18.3 ± 4.1	19.6 ± 3.5	18.1 ± 4.0	19.5 ± 3.0	18.4 ± 4.2	19.7 ± 4.9	18.7 ± 4.4	20.1 ± 5.7
Total CHO (g/d)	295 ± 10.9	304 ± 24.1	305 ± 11.1	316 ± 9.87	285 ± 8.12	289 ± 8.09	241 ± 7.29	244 ± 6.85
Total CHO (% energy)	48.5 ± 6.9	50.8 ± 7.1	50.5 ± 6.3	52.6 ± 7.8	47.4 ± 7.2	50.5 ± 7.0	45.3 ± 9.4	48.0 ± 7.1
Total fat (g/d)	81 ± 5.44	77 ± 7.07	80 ± 5.07	76 ± 5.41	83 ± 5.01	78 ± 4.90	85 ± 6.54	78 ± 5.35
Total fat (% energy)	30.9 ± 7.6	28.8 ± 6.9	31.2 ± 8.8	29.5 ± 7.5	32.5 ± 6.9	29 ± 6.4	34.5 ± 5.9	30.0 ± 6.6

MICT, Moderate Intensity Continues Training; CRT, Circuit Resistance Training; and HIIT, High Intensity Interval Training.

low-density cholesterol (VLDL-C) was calculated using the TG/5 formula (McNamara et al., 1990). Insulin levels were measured with an ELISA kit (Demeditec, Germany) with a sensitivity of 1 ng/ml and the rate of external and internal errors were 5.1 and 8.4%, respectively. Glucose levels were measured with a colorimetric enzymatic kit (Parsazmun, Tehran, Iran) with 5 mg/dl sensitivity. Insulin resistance was assessed using the homeostasis model assessment of insulin resistance (HOMA) according to the formula: HOMA- IR = 22.5 μmol/fasting plasma insulin X fasting plasma glucose (Willis et al., 2012). Nrg4 was measured with an ELISA kit (Kit-ELISA, Germany; Cat number ABIN1571585 with sensitivity of 0.31 ng/ml).

Statistical Analysis

All data were evaluated with SPSS software (version 22), and the normality of the data was assessed by the Shapiro–Wilk test. One-way ANOVA and Tukey's *post-hoc* tests were used for evaluation baseline data of four groups. Interactions between groups were determined by a two-way ANOVA repeated measures test (Groups*time). When a significant difference was detected by ANOVA, mean differences were determined by pairwise comparisons. Correlation between Nrg4 levels and other data was measured with Pearson correlation tests. The sample size was calculated to detect a statistical difference between study variables with a 95% confidence interval (CI) and 80% or greater power value. Additionally, effect sizes (ES) were determined from ANOVA output by partial eta-squared. Moreover, within-group ES were computed using the following equation: ES = (mean post-mean pre)/SD (Field, 2013). According to Hopkins et al. (2009) ES are considered trivial (<0.2), small (0.2–0.6), moderate (0.6–1.2), large (1.2–2.0) and very large (2.0–4.0). Descriptive statistics [means, sd (±)] were used to describe all data. A p-value of <0.05 was used to indicate statistical significance.

RESULTS

Dietary Analysis

No significant differences were observed between the groups in total energy consumption and energy derived from carbohydrate, fat and protein before and after 12 weeks ($p > 0.05$) (**Table 2**).

Body Composition Variables and VO_{2peak}

There were significant interactions between all groups and time for FFM, FM, and VO_{2peak} ($p < 0.05$, ES: 0.78, 0.86, 0.59, respectively). Body fat percent decreased in HIIT (−11%), CRT (−8%), and MICT (−8%) protocols compared with the control group ($p < 0.05$) (**Table 3**). There were increases in FFM following CRT (11%) and HIIT (5%) protocols; however, this increase was significant only in CRT protocol compared with the MICT and control groups ($p < 0.05$) (**Table 3**). There were increases in VO_{2peak} after 12 weeks of HIIT (13%), CRT (10%), and MICT) (7%) compared with the control group ($p < 0.05$) (**Table 3**). There were non-significant decreases in BMI and body weights following the training programs ($p > 0.05$, ES: 0.13, 0.51, respectively) (**Table 3**).

Lipid Profiles

There were interactions between the exercise training groups and time for HDL, LDL, TC, and TG ($p < 0.05$, ES: 0.83, 0.79, 0.90, 0.52, respectively). Plasma levels of HDL increased in the HIIT, CRT, and MICT protocols compared with the control group ($p < 0.05$) (**Table 3**). Nevertheless, this increase was more significant in HIIT and CRT protocols compared to the MICT protocol ($p < 0.05$). Levels of LDL and TC decreased in the HIIT and CRT, and MICT protocols compared with control groups ($p < 0.05$) (**Table 3**). However, LDL levels were reduced in the HIIT and CRT protocols more than in the MICT protocol ($p < 0.05$). Plasma TG levels decreased only in the CRT protocol compared to MICT and control groups ($p < 0.05$). Despite this, VLDL levels were non- significantly decreased in all training protocols ($p > 0.05$, ES: 0.13) (**Table 3**).

Insulin, Glucose, and HOMA-IR and Neuregulin 4

A repeated-measures ANOVA test revealed significant interactions between the exercise groups and time for NRG4, glucose, HOMA-IR, and insulin ($p < 0.05$, ES: 0.98, 0.39, 0.91, 0.61, respectively). Levels of NRG4 were increased in the three training protocols (HIIT, CRT, and MICT) compared with the control group ($p < 0.05$), although this increase was greater in HIIT (160%) and CRT (140%) compared with the MICT protocol (36%) ($p < 0.05$) (**Figure 2**).

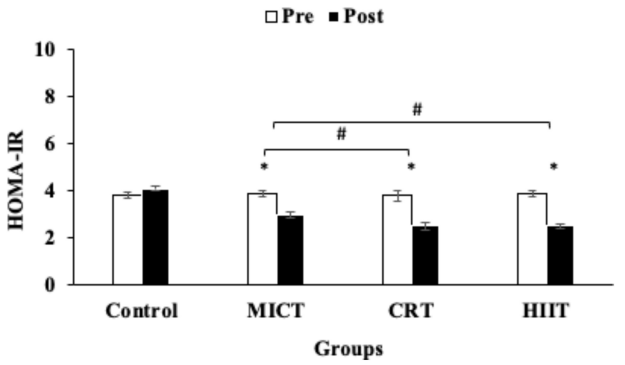

FIGURE 2 | Pre and post training values [mean, sd (±)] for Nrg4 (neuregulin 4) in MICT (Moderate Intensity Continuous Training), CRT (Circuit Resistance Training), and HIIT (High Intensity Interval training) groups. *indicates significant differences from the control group ($P < 0.05$). # indicates significant differences between training groups ($p < 0.05$).

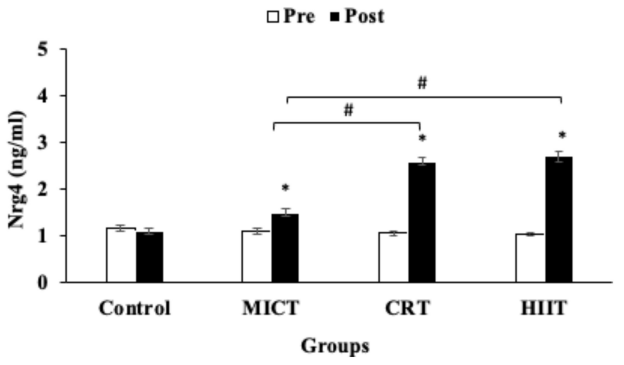

FIGURE 3 | Pre and post training values [mean, sd (±)] for HOMA-IR (Homeostatic Model Assessment-Insulin Resistance), in MICT (Moderate Intensity Continuous Training), CRT (Circuit Resistance Training), and HIIT (High Intensity Interval training) groups. *indicates significant differences from the control group ($P < 0.05$). # indicates significant differences between training groups ($p < 0.05$).

Levels of HOM-IR, and glucose were decreased in the HIIT (−36%, −18%), CRT (−34%, −18%) and MICT (−23%, −15%) protocols compared with the control group ($p < 0.05$) (**Figure 3** and **Table 3**). However, reductions in HOM-IR were greater in CRT and HIIT protocols compared to the MICT protocol ($p < 0.05$) (**Figure 3** and **Table 3**). Plasma levels of insulin were reduced in the decreased following HIIT (−20%), CRT (−20%), and MICT (−9%) protocols, but these reductions were significant only in HIIT and CRT protocols when compared with the control group ($p < 0.05$) (**Table 3**).

There were significant correlations ($p < 0.05$) between Nrg4 with HDL ($r = 0.641$), LDL ($r = -0.568$), TC ($r = -0.459$), glucose ($r = -0.691$), HOMA-IR ($r = -0.624$), body weight ($r = -0.655$), BMI ($r = -0.687$), FFM ($r = 0.554$), FAT ($r = -0.674$) and VO_{2peak} ($r = 0.745$) as determined by Pearson correlations analysis (**Table 4**). Plasma levels of Nrg4 were not correlated ($p > 0.05$) with TG ($r = -0.212$), VLDL ($r = -0.195$) and insulin ($r = -0.225$) levels (**Table 4**).

DISCUSSION

We investigated the effects of three different exercise training protocols on Nrg4 levels and cardiometabolic and body composition variables in sedentary men with obesity. Our current study indicates that levels of Nrg4 were increased by the HIIT, CRT, and MICT protocols, with the increases being greater in HIIT and CRT protocols. In addition, the three training protocols decreased HOMA-IR, TC, and LDL levels, but the decreases for LDL and HOM-IR were greater following HIIT and CRT protocols relative to the MICT protocol. Furthermore,

TABLE 3 | Pre and Post mean (sd, ±) cardiometabolic and body composition variables by group.

	Control		MICT		CRT		HIIT	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Weight (kg)	94.9 ± 4.62	94.9 ± 4.55	95.9 ± 2.60	93.4 ± 2.47	97.2 ± 3.78	94.4 ± 3.56	95.1 ± 4.27	91.2 ± 3.89
BMI (kg/m^2)	31.4 ± 1.68	31.6 ± 1.66	31.5 ± 3.59	30.7 ± 1.53	33.7 ± 2.76	32.2 ± 2.57	33.9 ± 2.31	32.2 ± 2.14
FFM (kg)	29.9 ± 1.53	29.5 ± 1.72	29.6 ± 1.44	29.8 ± 1.37	30.0 ± 2.08	33.2 ± 2.12*#	29.4 ± 1.72	31.0 ± 1.13
FM (kg)	32.8 ± 1.85	33.2 ± 1.70	32.1 ± 1.67	29.6 ± 1.68*	32.6 ± 1.95	30.0 ± 1.62*	32.4 ± 1.18	28.8 ± 1.26*
VO_{2peak}	27.5 ± 2.41	27.2 ± 2.25	29.3 ± 3.26	31.3 ± 2.94*	28.6 ± 2.28	31.6 ± 2.02*	28.4 ± 1.88	32.1 ± 1.76*
HDL (mmol/L)	0.83 ± 0.05	0.83 ± 0.03	0.94 ± 0.03	1.01 ± 0.04*	0.92 ± 0.05	1.23 ± 0.06*#	0.89 ± 0.06	1.17 ± 0.05*#
LDL (mmol/L)	4.08 ± 0.11	4.08 ± 0.10	4.03 ± 0.12	3.75 ± 0.23*	4.06 ± 5.29	3.43 ± 0.13*#	4.13 ± 0.15	3.62 ± 0.11*#
TC (mmol/L)	6.20 ± 0.17	6.33 ± 0.18	6.05 ± 0.15	5.81 ± 0.17*	6.18 ± 0.11	5.63 ± 0.10*	6.20 ± 0.12	5.66 ± 0.11*
TG (mmol/L)	2.69 ± 0.05	2.67 ± 0.05	2.70 ± 0.05	2.66 ± 0.06	2.67 ± 0.06	2.57 ± 0.06*#	2.73 ± 0.06	2.60 ± 0.05
VLDL (mmol/L)	1.22 ± 0.13	1.22 ± 0.11	1.24 ± 0.11	1.22 ± 0.10	1.22 ± 0.13	1.17 ± 0.12	1.25 ± 0.12	1.19 ± 0.11
Glucose (mmol/L)	5.5 ± 0.6	5.7 ± 0.5	5.6 ± 0.5	4.8 ± 0.4*	5.6 ± 0.4	4.6 ± 0.4*	5.7 ± 0.4	4.7 ± 0.4*
Insulin (pmol/L)	110 ± 9.72	113 ± 7.63	110 ± 7.63	100 ± 10.4	109 ± 7.63	87.5 ± 9.7*	108 ± 6.38	86.8 ± 11.1*

*MICT, Moderate Intensity Continues Training; CRT, Circuit Resistance Training; HIIT, High Intensity Interval Training; FFM, fat free mass; FM, fat mass; BMI, body mass index; TG, triglyceride; TC, total cholesterol; HDL, high-density lipoprotein; LDL, low-density lipoprotein; and VLDL, very-low-density lipoprotein. * indicates significant differences compared to the control group ($p < 0.05$). # indicated significant differences between training groups ($p < 0.05$).*

TABLE 4 | Pearson correlation coefficients between Nrg4 and cardiometabolic and body composition variables.

	Cadiometabolic	Pearson *r*-value	*p*-value
Nrg4 (ng/ml)	HDL (mmol/L)	0.841	$p < 0.05$*
	LDL (mmol/L)	−0.768	$p < 0.05$*
	TC (mmol/L)	−0.759	$p < 0.05$*
	TG (mmol/L)	−0.212	$p > 0.05$
	VLDL (mmol/L)	−0.195	$p > 0.05$
	Glucose (mmol/L)	−0.691	$p < 0.05$*
	Insulin (pmol/L)	−0.225	$p > 0.05$
	HOMA-IR	−0.624	$p < 0.05$*
	Weight (kg)	−0.655	$p < 0.05$*
	BMI (kg/m^2)	−0.687	$p < 0.05$*
	FFM (kg)	0.754	$p < 0.05$*
	Fat (kg)	−0.674	$p < 0.05$*
	VO_{2peak}	0.745	$p < 0.05$*

TG, triglyceride; TC, total cholesterol; HDL, high-density lipoprotein; LDL, low-density lipoprotein; VLDL, very-low-density lipoprotein; BMI, body mass index; and FFM, fat free mass.

** indicates significant correlation between levels of the measured variable with Nrg4 (neuregulin 4) levels.*

insulin and HDL levels were improved only by the CRT and HIIT protocols.

Neuregulin 4 is a member of the epidermal growth factor (EGF) family and is expressed in the lungs, heart, and adipose tissue but most commonly in brown fatty tissue (Pfeifer, 2015). Nrg4 consists of an EGF-like domain that acts as an autocrine/paracrine or endocrine factor after proteolytic breakdown (Pfeifer, 2015). Nrg4 reduces obesity in mice and humans who are also exposed to various metabolic diseases (Chen L. L. et al., 2017; Chen Z. et al., 2017). Many studies have examined the tissue effects of Nrg4, especially in the liver and adipose tissues (Chen Z. et al., 2017; Blüher, 2019), where Nrg4 in fat tissue is reduced by body weight (Wang et al., 2014). Our study indicates that improvement in Nrg4 is accompanied by improvements in HOMA-IR by the MICT, CRT, and HIIT exercise protocols, suggesting a positive correlation of Nrg4 with metabolic status (Wang et al., 2014). Moreover, our study demonstrates that increased levels of Nrg4 were associated with reduced plasma glucose and insulin levels and greater fat percent loss; these findings confirmed our hypothesis and support a previous report that high circulating levels of Nrg4 prevents inflammation, improves insulin resistance, and prevents weight gain (Ma et al., 2016). In addition, low plasma levels of Nrg4 in the blood are independently associated with an increased risk of metabolic syndrome in individuals with obesity, and are also negatively correlated with blood glucose levels and body fat mass (Cai et al., 2016). There are no previous studies on the effects of exercise training on Nrg4 levels in humans that we identified. Our study reports that plasma levels of Nrg4 were increased more significantly by HIIT and CRT protocols, which agree with our original hypothesis. In addition, we report greater reductions of LDL, insulin, and HOMA-IR following HIIT and CRT protocols relative to the MICT protocol, further supporting our hypothesis that exercise intensity regulates Nrg4 levels. Additionally, these results confirm our recent study that showed that resistance training intensity is key in modifying adipokines and metabolic markers in men with obesity (Saeidi et al., 2020b). The greater increases in Nrg4 may be related to higher intensity in these protocols, leading to higher metabolic stress and, consequently, increasing catecholamine and hormone (such as growth hormone and glucagon) levels in an intensity-related manner. The effects of exercise on fat tissue lipolysis are greater in the HIIT and CRT protocols compared to the MICT training protocols (Zouhal et al., 2008; Czech, 2017; Dias et al., 2018; Maillard et al., 2018). Thus improvements in Nrg4 are likely related to greater physiological and metabolic stress cocurring during HIIT and CRT protocols, resulting in more physiological adaptations. These adaptations as reported in this study include greater improvements in body composition variables, HDL, LDL, insulin, and HOM-IR following both HIIT and CRT protocols. However, more studies are needed to clarify the mechanisms for regulating Nrg4 activity by exercise training.

Our study demonstrates reductions of LDL levels in the MICT (−7%), CRT (−15%), and HIIT (−13%) training protocols, with reductions in TC levels of 4, 9, and 9% in the three protocols, respectively. This reduction in LDL in CRT and HIIT protocols was significantly greater relative to MICT protocol, and there was a non-significant decrease in TC in the CRT and HIIT protocols. These results support previous studies on the importance of exercise intensity in adjusting lipid profile (Racil et al., 2013; Blüher et al., 2017). Importantly, our results confirm a possible interaction between Nrg4 and obesity (Little et al., 2014; Ma et al., 2016; Saeidi et al., 2020b), and suggest a positive effect of exercise training on obesity and lipid profiles that may be mediated by Nrg4. In addition, we report increases in HDL following HIIT and CRT protocols. Our study (using three different exercise training protocols) and a previous study report (Thompson et al., 2010) indicate that increased levels of Nrg4 are positively correlated with HDL levels. Other studies reported that high-intensity exercise increases glucose metabolism increases, so decreasing/depleting stores of muscle glycogen; consequently, post-exercise muscle glycogen resynthesis occurs at high levels and possibly stimulates lipid metabolism (Jackson et al., 1988; Gillen et al., 2012). Therefore, it is likely that the improvements in the lipid profile we observed may be related to higher HIIT and CRT intensity, leading to increased lipid metabolism in post-exercise times.

STUDY LIMITATIONS

Our study is not without limitations. The lifestyles and dietary intakes of participants were not controlled although they were instructed by a nutritionist and were asked to maintain the same diet throughout the study. We used bioelectrical impedance to measure body composition variables and acknowledge that bioelectrical impedance is not a gold standard method for measuring these markers; however its reliability and validity has been reported previously (Jackson et al., 1988; Ling et al., 2011). The protocols used in our study were not equalized according to calorie expenditure, which might have influenced

the results. Instead, all protocols were matched by time (Isotime) and intensity (Isoeffort).

CONCLUSION

In our study HIIT and CRT caused greater increases in circulating levels of Nrg4 and HDL than those produced by MICT. In addition, both HIIT and CRT also had positive effects on insulin resistance, insulin, and LDL levels. Thus, we conclude that HIIT and CRT may be more useful approaches for reducing metabolic indices associated with cardiovascular disease in sedentary individuals with obesity. However, additional studies are needed to determine the time course response of changes in Nrg4 levels produced by exercise, and analyses are also needed on the effects of HIIT with different rest-to-work ratios and various durations, alone or combined with resistance training.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Research and Ethics Committee of Islamic Azad University (Ethics code: IR-IAU1398-25). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

FT, NK, SM, AS, and HZ contributed to the conception or design of the work. NK, FT, AS, IL, TM, KJ, SRS, SS, and TV contributed to the data acquisition, analysis, or interpretation. MS, TM, KJ, and PD-B drafted the manuscript. All authors critically revised the manuscript, gave final approval, agreed to be accountable for all aspects of work, ensuring integrity and accuracy, and agreed with the order of presentation of the authors.

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Effects of Exercise Training on Bone Health Parameters in Individuals With Obesity: A Systematic Review and Meta-Analysis

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Background: Osteoporosis causes bone fragility, increasing the risk of fractures. Evidence suggests a strong correlation between obesity and fracture risk. Physical training is known to enhance bone resistance and protect from fracture; however, its osteogenic effect in the presence of obesity remains unknown.

Objective: We sought to evaluate the influence of exercise training on bone health indices in individuals with obesity.

Methods: This systematic literature search was conducted using common electronic databases from inception - December 2019. The following key terms (and synonyms searched for by the MeSH database) were included and combined using the operators "AND," "OR," "NOT": [("body mass index" OR obesity OR obese OR overweight OR fat mass) AND ("bone mineral density" OR "bone mineral content" OR "peak bone mass" OR "mechanical loading" OR "Osteoporosis" OR "bone geometry" OR "bone resistance") AND ("exercise training" OR "physical training" OR "strength training," OR "resistance training" OR "aerobic training" OR "combined training")].

Results: After screening, 10 studies (889 initial records) were included in the final analysis (8 different countries, 263 participants). Two studies investigated males, six females, and two, both sexes. The training duration was at least eight weeks with 2–3 sessions/week. Physical training displayed a significant trivial impact on the whole body (WB) BMD (0.13 SMD; 95% CI [0.00, 0.26], $p = 0.046$). Subgroup analyses indicated a significant small increase in the WB BMD (0.27 SMD; 95% CI [0.00, 0.53], $p = 0.048$) in the endurance training group, a non-significant trivial increase in the WB BMD (0.11 SMD; 95% CI [−0.06, 0.29], $p = 0.203$) in the resistance group, and a non-significant trivial increase in the WB BMD (0.03 SMD; 95% CI [−0.26, 0.32], $p = 0.86$) in the combined training group. In addition, a significant small decrease was found in the weight of trained subjects (−0.24 SMD; 95% CI [−0.42, −0.05], $p = 0.011$).

Conclusion: Physical training has little to no effect on the WB BMD in subjects with overweight/obesity. Currently, insufficient evidence to advocate for any specific type of exercise for enhancing bone health exists for overweight/obese individuals. Investigations examining the impact of varying types of physical exercise on WB BMD of obese individuals are needed.

Keywords: bone health, exercise, bone mineral density, bone mineral content, resistance exercise and aerobic exercise, combined training

INTRODUCTION

The worldwide incidence of obesity continues to grow and is largely attributable to an imbalance between energy intake and energy expenditure, which leads to an increase in body fat accumulation, which adversely affects health (Gonnelli et al., 2014). For example, the accumulation of adipose tissue is associated with various non-communicable disorders, including cardiovascular disease, type 2 diabetes, and cancers (Cao, 2011). Moreover, recent data has demonstrated a pathophysiological link between adiposity and osteoporosis (Cortet and Roux, 2016), where it is suggested that there is a decreased bone mineral density (BMD) and an increase in the fracture risk among obese subjects (Shapses and Sukumar, 2012; Gonnelli et al., 2014; Cortet and Roux, 2016).

Osteoporosis is a systemic skeletal disease characterized by a low BMD and microarchitectural deterioration of bone tissue, which leads to an increased risk of developing spontaneous and traumatic bone fractures (Health, 2001), and it represents a major contributor to the global burden of disease (Torres-Costoso et al., 2020). Genetic and hormonal factors, poor diet, excess caloric intake, and/or lower physical activity are known to be involved in the development of obesity and osteoporosis (Shapses and Sukumar, 2012; Weaver et al., 2016).

The relationship between adipose and bone tissue is complex; indeed, a greater body-weight is generally considered to be beneficial to bone health due to the positive effect of mechanical loading on bone formation (Cao, 2011). Moreover, a decreased body mass index (BMI) is a risk factor for lower BMD and predicts greater bone loss in older individuals (Nguyen et al., 1998). In contrast, the potentially positive influence of fat accumulation (i.e., increased weight) on bone health remains controversial; indeed, evidence suggests that excessive adiposity may be detrimental to bone health and increases fracture risk (Shapses and Sukumar, 2012). Furthermore, studies have suggested that the associated increases of BMD and weight are not proportional. Researchers have found in individuals with obesity, that composite indices of femoral neck strength and BMD per unit BMI are significantly lower compared to individuals with normal-weight (Laet et al., 2005; El Khoury et al., 2017). Also, several authors have indicated that fat distribution affects the bone differently; while subcutaneous fat is positively associated with bone mass, visceral and marrow fat are negatively correlated with BMD (Gilsanz et al., 2009; Russell et al., 2010).

Obesity putatively affects bone metabolism through several mechanisms. Both osteoblasts and adipocytes are derived from a common mesenchymal stem cell and agents inhibiting osteoblastogenesis and increasing adipogenesis (Cao, 2011). Obesity is also associated with chronic inflammation, and adipocytes secrete proinflammatory cytokines which can promote osteoclast activity and bone resorption (Cao et al., 2005). In addition, it has been demonstrated that obesity and osteoporosis may be associated with increased production of proinflammatory cytokines and elevated oxidative stress (Wellen and Hotamisligil, 2003; Mundy, 2007). The excessive secretion of leptin and/or decreased production of adiponectin by adipocytes in obesity may also either directly affect the bone formation or indirectly affect bone resorption (Elefteriou et al., 2004; Oshima et al., 2005). Finally, a high-fat diet may alternate intestinal calcium absorption resulting in a decrease in calcium availability for bone formation (Nelson et al., 1998).

Due to the increased prevalence of osteoporosis, non-pharmacological treatment and/or preventive strategies are highly sought-after. Epidemiological and clinical trial research confirms the positive impact of regular physical activity on bone and body composition (Slemenda et al., 1991; Bailey et al., 1999; Specker and Binkley, 2003), and physical exercise is capable of maximizing peak bone mass in younger years and minimizing age-related bone loss in individuals with lean (Babatunde and Forsyth, 2013). These effects are dependent, primarily, on the type of exercise; high impact and resistance exercises are the most recommended strategies to increase BMD and thereby increase bone resistance and decrease fracture risk (Kelley et al., 2001; Weaver et al., 2016). The majority of published studies have investigated the influence of this type of training and showed positive, consistent results (Guadalupe-Grau et al., 2009). Conversely, relatively fewer studies have explored in individuals with obesity the impact of aerobic and combined physical activities (PA) on bone parameters, and incumbent findings remain controversial (El Hage et al., 2009; Guadalupe-Grau et al., 2009). PA modulate bone remodeling through mechanical stimuli, which results in improvements in mineralization and bone geometry. In addition, PA can optimize body composition by increasing lean mass and decreasing fat mass (Guadalupe-Grau et al., 2009).

How obesity influences the effect of PA on bone health is, so far, unclear (Menzel et al., 2015), and the generalizability of previous research conducted in subjects with normal-weight to individuals with overweight/obesity is ambiguous. To date, aerobic, resistance, and other types of exercises have been used to explore the osteogenic response in the presence of obesity, although diverse results have been recorded. Therefore, the first

TABLE 1 | PICOS (participants, interventions, comparisons, outcomes, study design).

PICOS component	Detail
Participants	Individuals with overweight or obesity (BMI $\geq$ 25 kg.m^{-2}; body fat > 25% for men and >30% for women)
Interventions	Exercise training two or more weeks of follow-up (aerobic, resistance and combined training)
Comparisons	Control group/Untrained participants
Outcomes	Physical performances, bone mineral density, bone mineral content, bone geometry, hormone responses.
Study designs	nRCTs, nRnCTs and RCTs

nRCT, non-randomized controlled trial; nRnCT,non-randomized non-controlled trial; RCT, randomized controlled trial.

aim of this systematic review and meta-analysis was to assess the impact of PA on bone health indices in individuals with obesity and the second to investigate whether the type of PA can modulate this effect.

METHODS

Eligibility Criteria

PICOS (Population, Intervention, Comparison, Outcome, and Study design) criteria were used as the inclusion criteria for the current review (see **Table 1**) (Moher et al., 2015). This systematic review included original studies (randomized or non-randomized) for which the full texts were available and that performed interventions with exercise training, included two or more weeks of follow-up, involved individuals with overweight or obesity (BMI $\geq$ 25 kg.m^{-2}), included one or both sexes, and specifically evaluated whole-body BMD, before and after the intervention, in individuals with obesity.

Studies were excluded if they **(1)** did not meet the minimum requirements of an experimental study design (e.g., case reports), **(2)** did not meet the minimum requirements regarding training design (e.g., lack of information on volume, frequency, training methodology), **(3)** were not written in English or French, **(4)** involved individuals were not overweight/obese, **(5)** did not include the measurement of whole body bone mineral density (WB BMD) or **(6)** did not include sufficient information that allows the determination of effect size. Additionally, the following exclusion criteria were adopted to reduce confounding factors: duplicate publications or sub-studies of included studies, studies involving pathologies, and studies associating exercise with a nutritional intervention (e.g., nutrition counseling, balanced or hypocaloric diets, and supplements) or pharmacological drugs. Moreover, review articles were not included in the current systematic review.

Literature Search Strategy

This systematic review and meta-analysis is reported in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement and the Cochrane Handbook for Systematic Reviews of Interventions (Higgins and Deeks, 2011).

We searched the following electronic databases (up to December 2019) without a period limit: Cochrane Library, PubMed, Science Direct, Scopus, SPORTDiscus, and Web of Science. Additionally, a manual search for published studies in Google Scholar was conducted for the gray literature analysis. The following key terms (and synonyms searched for by the MeSH database) were included and combined using the operators "AND," "OR," "NOT": [("body mass index" OR obesity OR obese OR overweight OR fat mass) AND ("bone mineral density" OR "bone mineral content" OR "peak bone mass" OR "mechanical loading" OR "Osteoporosis" OR "bone geometry" OR "bone resistance") AND ("exercise training" OR "physical training" OR OR "strength training," OR "resistance training" OR "aerobic training" OR "combined training")]. Only eligible full texts in English or French were considered for analysis.

Three investigators among the authors (AB, KS, and REH) independently performed searches in the electronic databases, and disagreements were solved by consensus.

Study Selection

Inclusion or exclusion of articles was decided with the application of the PICOS criteria (see **Table 1**) to the title, abstract, and/or full text of articles. The data-collection process is presented in **Figure 1** (Liberati et al., 2009). The titles and abstracts of the selected articles were independently assessed by two researchers (AB and AJ). The reviewers were not blinded to the authors, institutions, or journals associated with the manuscripts. Abstracts that provided insufficient information about the inclusion and exclusion criteria were retrieved for full-text analysis. Furthermore, the researchers independently analyzed the full text and determined the eligibility of the studies, and disagreements were resolved by consensus. To avoid double-counting participants or to clarify questions about the methods, the corresponding authors were contacted if needed. Furthermore, when necessary, the corresponding author was also contacted *via* e-mail to provide data not included in the published research. Two researchers (AB and KS) independently performed the data extraction, and disagreements were resolved by consensus.

Quality Assessment

Study quality was assessed using the Physiotherapy Evidence Database (PEDro) scale (http://www.pedro.fhs.usyd.edu.au), which has been shown to have good reliability and validity (Maher et al., 2003). The PEDro scale has 11 possible points that examine external validity (criterion 1) and internal validity (criteria 2–9) of controlled trials and whether there is sufficient statistical information for interpreting results (criteria 10–11). The items of the scale are: (i) eligibility criteria were specified; (ii) subjects were randomly allocated to groups; (iii) allocation was concealed; (iv) groups were similar at baseline; (v) subjects were blinded; (vi) therapists who administered the treatment were blinded; (vii) assessors were blinded; (viii) measures of key outcomes were obtained from more than 85 % of subjects; (ix) data were analyzed by intention to treat; (x) statistical

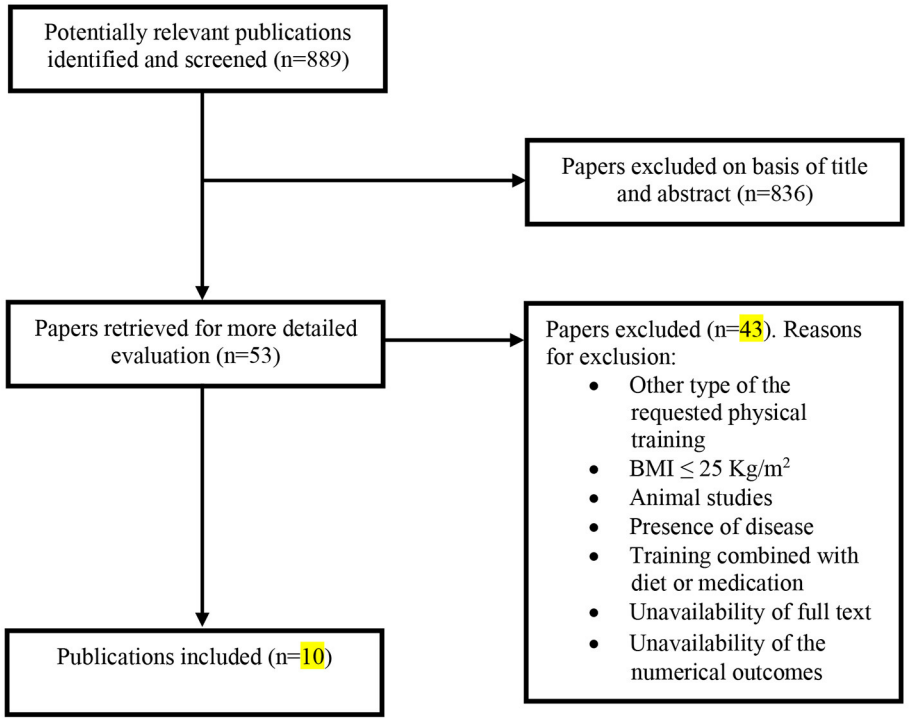

FIGURE 1 | Flow diagram for the selection of studies. Selection process for research articles (n = 10) included in this systematic review. Adapted version of the recommendations in the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) statement (Moher et al., 2010).

comparisons between groups were conducted, and (xi) point measures and measures of variability were provided. The first criterion is not included in the final score. Moreover, because of the nature of physical activity interventions, patient and therapy blinding and allocation are difficult; therefore, the total score a trial could receive was 8 points. A cut-off of 6 on the PEDro scale was used to indicate high-quality studies, as this has been reported to be sufficient to determine high quality vs. low quality in previous studies (Maher et al., 2003). The studies were evaluated by two experienced investigators (AB and AJ) and in the event of disagreement, a third reviewer (HZ) was invited to review.

Statistics

Effect sizes (ES) were computed to discern the standardized effect of acute and long-term training on the outcome variables (WB BMD (g/cm^2) and weight (kg)). Mean group differences in WB BMD and weight between pre-and post-intervention periods were divided by a pooled SD, based on the assumption of a large correlation ($r = 0.7$) for each group. The standardized mean differences (SMD) were calculated to determine Cohen's d for each study and Hedge's g was used to account for potential bias in small sample sizes.

Given that BMD and weight values are continuous data, the weighted mean difference method was used for combining study effect size estimates. Weighting assigned to each study group comparison in the analysis was in the inverse proportion to the variance.

When data could be pooled, these effect sizes and 95% confidence intervals (95% CI) were calculated using random-effects models (DerSimonian and Laird approach), that account for true variation in effects between studies, as well as random error within a single study. Values for ES were defined as trivial (<0.2), small (0.2–0.6), moderate (0.6–1.2), large (1.2–2.0), and very large >2 (Hopkins et al., 2009). A negative effect size value indicated that the WB BMD or the weight decreased after the intervention, while a positive effect size indicated there was an increase. Cochrane's Q and the I^2 index were used to assess heterogeneity. The alpha value for statistical significance for Q was set at $p < 0.10$, because it tends to suffer from low differential power (Higgins et al., 2003). For I^2, values of 25, 50, and 75% were used to indicate low, moderate, and high heterogeneity, respectively (Higgins et al., 2003). Furthermore, Z test analysis was used to examine if the overall ES's were significantly different from zero.

We contacted authors to obtain any missing numerical data needed for analysis. For interventions with 2 or more arms including the same type of training (El Hage et al., 2009), in the meta-analysis, we combined the 2 arms using standard procedures, as outlined in Section 9.3.9 of the Cochrane Handbook (Higgins and Deeks, 2011). Subgroup analysis was used to investigate differences in the magnitude of the exercise-induced osteogenic effect across studies dependent to the type of training (endurance, resistance, and combined training). Potential publication bias was assessed through visual inspection of funnel plots. All statistical analyses were conducted using

TABLE 2 | Characteristics of the studies that examined the effect of exercise training on bone health indices in individual with obesity.

Study	PEDro scale	Population/sex	Sample size	Country	Age, years (mean ± SD or range)	Characteristics of exercise training	Duration (weeks)
Endurance training							
El Hage et al. (2009)	8	Women	N: 21 Ex1: normal weight: 7 Ex2: overweight: 8 Ex3: obese: 6	Lebanon	16.2 ± 1.8	Endurance training: running and collective game	12
Kim et al. (2015)	7	Men	N: 39 Ex: 29 CG: 10	Korea	25.3 ± 2.8	Aerobic exercise: treadmill running	8
Berro et al. (2020)	7	Women	N: 28 Ex: 14 CG: 14	Leba-non	18–35	Endurance training: treadmill running	48
Resistance or Strength training							
Huang et al. (2017)	9	Women	N: 35 Ex: 18 CG: 17	Taiwan	68.9 ± 4.9	Elastic band resistance training	12
Cunha et al. (2018)	10	Women	N: 62 Ex1S: 21 Ex3S: 20 CG: 21	Brazil	68.0 ± 4.3	Free weight and machines	12
Warren and Schmitz (2009)	9	Women	N: 148 Ex: 72 CG: 76	USA	36.4 ± 5.5	Strength training	96
Cornish and Chilibeck (2009)	8	Women and men	N: 51 ALA: 25 ALA men: 14 ALA women: 11 Pla: 26 Pla men: 14 Pla women: 12	Canada	65.4 ± 0.8	Resistance training	12
Romero-Arenas et al. (2013)	7	Women and men	N: 37 HRC: 16 ST: 14 CG: 7	Spain	61.6 ± 5.3	High-resistance circuit (HRC) training vs. traditional strength training (ST)	12
Bocalini et al. (2009)	7	Women	N: 25 Ex: 15 CG: 10	Brazil	57–75	Strength training	24
Combined training							
Bolam et al. (2016)	10	Men	N: 42 HI: 13 Mod: 15 CG: 14	Sweden	50–74	Upper body RE + high-dose impact loading or moderate dose impact loading	36
Choquette et al. (2011)	9	Women	N: 100 -Pla: 26 -ISO: 26 -Ex + Pla: 25 -Ex + ISO: 23	Canada	50–70	Resistance and aerobic exercise	24

PEDro scale, physiotherapy evidence database scale; SD, standard deviation; N, number of subjects; G, group; C, control; Ex, exercise; RE, resistance exercise; HI, high-dose impact loading; MOD, moderate dose impact loading; Alpha-linolenic acid, ALA; ISO, isoflavones; Pla, Placebo.

R (version 4.0.3; The R Foundation for Statistical Computing, Vienna, Austria) (The R Development Core Team; R Core Team, 2020) and the "metafor" package (Viechtbauer, 2010). Unless otherwise stated, $p < 0.05$ was used to demarcate statistical significance.

RESULTS

Study Selection

Overall, our search yielded 889 records (**Figure 1**). After the screening of titles, abstracts, and full texts, 10 studies were included in our final analysis, and the characteristics of these studies are shown in **Table 2**. These studies were performed in 8 different countries (Lebanon, Canada, Brazil, USA, Taiwan, Korea, Spain, and Sweden), where 2 studies investigated only male subjects, 6 studies investigated only females, and 2 studies investigated both sexes. These papers included 15 training groups (9 for females, 5 for men, and 1 for men and females) and involved 263 trained participants in total. One study (Bocalini et al., 2009) included the measurement of body weight only. The training duration was at least 8 weeks and ranged, for the majority of studies, between 12 and 48 weeks. One study reported a training duration of 96 weeks (Warren and Chua, 2008). The training frequency was at least 2–3 sessions per week, and six studies reported compliance to the training, which ranged between 71 and 94%. The included long-term studies (24 > weeks) were classified as 'high-quality' studies (mean 8.2 in the PEDro scale score) (**Table 3**).

Table 4 summarizes the studies that examined the effects of exercise training (endurance training, strength and resistance training, and combined training) on bone health indices in individuals with obesity. Nine studies, consisting of 14 trained groups (8 for females, 5 for men, and 1 for men and females) and 240 participants, were included in the meta-analysis investigating the effect of exercise on the WB BMD, as shown in the forest plot (**Figure 2**). Our analysis indicated a significant trivial increase in the WB BMD (0.13 SMD; 95% CI [0.00, 0.26], $p = 0.046$). The I^2 (0.0%) parameter indicated the homogeneity of the results, whilst the visual inspection of the funnel plot (**Figure 3**) did not reveal a substantial asymmetry.

The subgroup of endurance training, as shown in the forest plot (**Figure 2**), included 3 studies (3 groups: 2 females and 1 man) and 57 participants. A significant small increase in the WB BMD (0.27 SMD; 95% CI [0.00, 0.53], $p = 0.048$) was detected among these studies, and the I^2 (0.0%) parameter indicated homogeneity of the results.

The subgroup of resistance training (**Figure 2**) included 5 studies (8 groups: 6 females, 1 males, and 1 man and females) and 153 participants. The result showed a non-significant trivial increase in the WB BMD (0.11 SMD; 95% CI [−0.06, 0.29], $p = 0.203$) was detected among these studies. The I^2 (0.0%) parameter indicated homogeneity of the results.

The subgroup of combined training (**Figure 2**) included 2 studies (3 groups: 1 females, 2 males) and involved 53 participants; it assessed the impact of the combined training

TABLE 3 | Physiotherapy evidence database (PEDro) score of the included longitudinal studies.

Study	Eligibility criteria	Randomized allocation	Blinded allocation	Group homogeneity	Blinded subjects	Blinded therapists	Blinded assessor	Drop out /15 %	Intention to treat analysis	Between-group comparison	Point estimates and variability	PEDro score
El Hage et al. (2009)	•	•	○	•	○	○	•	•	•	•	•	8
Kim et al. (2015)	•	•	○	•	○	○	•	•	○	•	•	7
Huang et al. (2017)	•	•	•	•	•	○	•	•	○	•	•	9
Cunha et al. (2018)	•	•	•	•	•	•	•	•	○	•	•	10
Cornish and Chilibeck (2009)	•	•	•	•	○	○	•	•	○	•	•	8
Romero-Arenas et al. (2013)	•	•	○	•	○	○	•	•	○	•	•	7
Bocalini et al. (2009)	•	•	○	•	○	○	•	•	○	•	•	7
Bolam et al. (2016)	•	•	•	•	•	○	•	•	•	•	•	10
Choquette et al. (2011)	•	•	•	•	•	○	•	•	○	•	•	9
Berro et al. (2020)	•	•	○	•	○	○	•	•	○	•	•	7

TABLE 4 | Studies examined the effects of exercise training on bone health indices in individual with obesity.

	Reference	Gender, Number of participants (N), and age (yrs)	Intervention	Outcomes 1 physical performance, weight, body composition	Outcomes 2 DMO, CMO, bone geometry, hormones	Effect size SMD [95% CI]
Endurance training	El Hage et al., 2009	Women; N: 21 Ex1 normal Weight: 7 Ex2 overweight: 8 Ex3 obese: 6 Age: 16.2	Ex1, Ex2, and Ex3: 3d*w×90–60 min per session, running at 70% MAV, strengthening and proprioceptive exercises, stretching, and collective games.	No modifications on weight and body composition.	Ex2 and Ex3: ↑ Legs BMC Ex3: ↑ WB BMC. Ex1, Ex2 and Ex3: ↑ total and sub-total BMD for the 3 groups.	WB BMD; 0.57 [0.01, 1.14]
	Kim et al., 2015	Men; N: 39 Ex: 29 CG: 10 Age: 25.3	CG: no exercise Ex: 4d*w, 65–75% VO_2 max to burn ∼600 Kcal per session.	Ex: ↓ weight, ↓ BMI, ↓ WC, ↓ trunk fat %, ↓ total fat %. CG: ↑ WC. Higher – weight changes, BMI, WC, trunk fat %, total fat % in Ex compared to CG.	Ex: no changes in BMD, ↓ FPI, ↓ HOMA-IR, ↑ HDL-C, ↓ LDL-C, total adiponectin ↓, ↓ leptin, ↑ HMW/TAdip, ↓ $1.25(OH)_2D$, ↑ OC, ↑ $_{uc}$OC, ↑ $_{uc}$OC/OC. Higher – changes in FPI, HOMA-IR, LDL-C, total adiponectin, and leptin compared to CG. Higher + changes in HDL-C, HMW/TAdip, OC, and $_{uc}$OC in Ex compared to CG.	WB BMD; 0.17 [−0.20, 0.53] Weight; −0.48 [−0.87, −0.10]
	Berro et al., 2020	Women; N: 28 Ex: 14 CG: 14 Age: 18–35	Ex: 3d*w, 45 min, 60% VO_2 max, treadmill running CG: no exercise.	Ex: ↓ weight, ↓ BMI, ↓ FM, ↓ FM%, ↓ WC, ↓ HC, ↓ trunk FM%, ↑ maximal str, ↑ MAV. CG: ↑ weight, ↑ BMI, ↑ HC. Higher + changes in CSI, BSI, ISI, VJ, RMHS, MAV, and VO_2 max (ml/mn/kg et l/mn) in the ETG compared to CG. Higher - weight changes, BMI, FM, FM%, WC, and HC in the Ex compared to CG.	Ex: ↑ WB BMC, ↑ L1-L4 BMD CG: ↓ CSI, ↓ BSI, ↓ ISI. Higher + changes in CSI, BSI, and ISI in the ETG compared to CG.	WBBMD; 0.21 [−0.32, 0.74] Weight; −0.32 [−0.85, 0.22]
Resistance or Strength training	Huang et al., 2017	Women; N: 35, Ex: 18 CG: 17 age >60 yrs	Ex: Elastic band resistance training (12 w, 3d*w), 55 min, 10 min of warm-up 40. min of elastic band RE and 5 min of cooling down. CG: a 40-min course about home exercise.	Ex: Fat in the right upper extremity, left upper extremity, total fat, and fat % had decreased. Higher – changes in the right upper extremity fat, total fat, and fat % in Ex compared to Con.	Ex: WB BMD ↑, Z-score and T-score ↑.	WB BMD; 0.53 [0.04, 1.03] Weight; −0.03 [−0.49, 0.43]
	Cunha et al., 2018	Women; N: 62 Ex1: 21 Ex2: 20 NCG: 21 Age: 68 yrs	3d*w, 12 w for Ex1 and Ex2. Ex1: 1 set of 10 to 15 reps per exercise. Ex2: 3 sets of 10 to 15 reps per exercise. CG: no exercise.	Ex1: ↑ Tstr, ↑ LM. Ex2: ↑ Tstr, ↑ LM, ↓ TBF%. Higher + change of Tstr in Ex2 compared to Ex1. Higher – changes of TBF% in Ex2 compared to Ex1.	No effect on bone density. Higher + changes of Z score in Ex2 compared to Ex1.	Ex1/WB BMD; 0.00 [−0.43, 0.43] Ex2/WB BMD; 0.12 [−0.31, 0.55]

(Continued)

TABLE 4 | Continued

	Reference	Gender, Number of participants (N), and age (yrs)	Intervention	Outcomes 1 physical performance, weight, body composition	Outcomes 2 DMO, CMO, bone geometry, hormones	Effect size SMD [95% CI]
	Cornish and Chilibeck, 2009	Women and men; N: 51 ALA: 25 ALA men: 14 ALA women: 11 Pla: 26 Pla men: 14 Pla women: 12 Age: 65.4	ALA G: Flaxseed oil (14g of ALA per day) + RT. Pla: 30ml of corn oil per day + RE. RE for both G: 3d*w progressive RE for major muscle groups.	ALA and Pla: ↑ leg press, ↑ chest press, ↑ LM, ↑ muscle thickness elbow flex and ext, ↑ knee ext, ↓ FM% and weight. Pla: women ↑ muscle knee flexor thickness. Higher + changes in muscle knee flexors thickness in ALA men. ALA men: ↓ IL-6 concentration.	ALA and Pla: ↑ hip BMC, ↑ hip BMD, ↑ WB BMC. Pla: WB BMD ↑.	Men/WBBMD; 0.11 [−0.41, 0.64] Women/WBBMD; 0.12 [−0.45, 0.69]
	Romero-Arenas et al., 2013	Women and men; N: 37 HRC: 16 ST: 14 CG: 7 Age: 61.6	ST: 2d*w, 2 sets of 3 exercises, 12 reps at 50% of 6RM, 10 reps at 75% of 6RM, 1 min rest between exercises. HRC: the same exercise as ST, exercises executed consequently in 2 circuits separated with 5 min. CG: no exercise.	HRC and ST: ↑ isokinetic str, ↑ LM Higher + changes in isokinetic str in HRC and ST comp to CG. HRC: ↓ FM%, ↓ FM. Higher - changes in FM and FM% in HRC comp to CG. Higher + changes in LM in HRC comp to CG. HRC: ↑ VO_{2LM} (ml/kg*min) for 1, 2 and 3 min. HRC: ↓ energy expenditure for 1, 2, and 3 min.	HRC and ST: ↑ WB BMD.	HRC/WB BMD; 0.13 [−0.36, 0.62] ST/WB BMD; 0.09 [−0.43, 0.62]
	Berro et al., 2020	Women; N: 29 Ex: 15 CG: 14 Age: 18–35	Ex: 3d*w, 45 min, 75% RM, 8 to12 reps, 4 to 5 exercises per muscle group. CG: no exercise.	Ex: ↓ weight, ↓ BMI, ↓ FM, ↓ FM%, ↓ WC, ↓ HC, ↑ LM, ↑ maximal str, ↑ MAV, TBS cor + %Δ VJ, TBS cor + %Δ RMHS. Higher + changes in the VJ, RMHS, MAV, and the VO_2 max (ml/min/kg) in the Ex. Higher - changes in the weight, BMI, FM, FM%, WC, and HC in the Ex. CG: ↑ weight, ↑ BMI, ↑ HC, ↓ CSI, ↓ BSI, ↓ ISI.	Ex: ↑ WB BMC, ↑ L1-L4 BMD, ↑ TBS, ↑ SI, ↑ CSI, ↑ BSI, ↑ ISI. CG: ↑ WB ↑ BMI, ↓ CSI, ↓ BSI, ↓ ISI. Higher + changes in the L1-L4 BMD, CSI, BSI, ISI, in the Ex compared to CG.	WBBMD; −0.20 [−0.71, 0.31] Weight; −0.28 [−0.80, 0.23]
	Bocalini et al., 2009	Women; N: 35 Ex: 23 CG: 12 Age: 57–75	Ex: 3d*w, 10 min warm up, progressive RM (50% - 85%), 3 sets*10 reps for upper and lower muscles. CG: no exercise.	Ex: ↑ muscle str for the lower and upper body, ↓ weight. Higher – changes in the weight in the Ex compared to CG.	Ex: no changes in bone parameters. CG: ↓ LS BMD, ↓ FN BMD.	Weight; −0.19 [−0.61, 0.22]
Combined training	Bolam et al., 2016	Men; N: 42 HI: 13 Mod: 15 CG: 14 Age: 50–74 yrs	4 d*w, for 36 w. Upper body RE and either high-dose impact loading (HI; 80 jumps per session) or moderate-dose impact loading (MOD; 40 jumps per session).	Higher + changes in Arm LM in HI compared to CG. Higher + changes in 6-meter fast walk-in Mod compared to HI and CG.	Higher - changes in Hip BMD in the Mod G compared to HI and Con. Higher – changes in Troc BMD in Mod G compared to HI. No effects on Testosterone, SHBG, Estradiol.	HI/WB BMD; 0.00 [−0.54, 0.54] MOD/WB BMD; 0.08 [−0.43, 0.59]

(Continued)

TABLE 4 | Continued

Reference	Gender, Number of participants (N), and age (yrs)	Intervention	Outcomes 1 physical performance, weight, body composition	Outcomes 2 DMO, CMO, bone geometry, hormones	Effect size SMD [95% CI]
Choquette et al., 2011	Women; N: 100 -Pla, n: 26 -ISO, n: 26 -Ex + Pla, n: 25 -Ex + ISO, n: 23 Age: 50–70 yrs	Pla: Cellulose ISO: 70 mg daily dose of isoflavones. Ex + Pla: 1h*3d*w, 30 min of RT, and 30 min of ET. RE: free weights and selective plate machines, 1 set/12–14 reps/60% in M1 to 4sets/4–6 reps/85% in M6. ET: cycle ergometer and a treadmill, started at 40–50% of HR and increased up to 70–85%. -Ex + ISO.	Pla: leg FM ↓. ISO: leg FM ↓. Ex + Pla: ↓ HC, ↓ WC, ↓ TFM, ↓ Arms FM, ↓ Legs FM, ↑ TLM, ↑ Arms LM, ↑ Appendicular LM, ↓ %TFM, ↓ % Trunk FM, ↓ % Arms FM, ↓ % Legs FM. Ex + ISO: ↓ HC, ↓ WC, ↓ TFM, ↓ Arm FM, ↓ Legs FM, ↓ Trunk FM, ↑ TLM, ↑ Arms LM, ↑ Legs LM, ↑ Appendicular LM, ↓ %TFM, ↓ % Trunk FM, ↓ % Arms FM, ↓ % Legs FM.	ISO: ↓ TH BMD. Ex + Pla: ↑ LDL-C, ↑ Glucose. Pla: ↑ LDL-C. ISO: ↓ Insulin, ↓ Homa.	WB BMD; 0.00 [−0.46, 0.46] Weight; −0.06 [−0.52, 0.40]

N, number of subject; G, group; C, control; Ex, exercise; d, day; W, week; reps, repetitions; RM, maximal resistance; VO_2 max, maximal oxygen consumption; MAV, maximal aerobic velocity; HR, heart rate; ET, endurance training; RE, resistance exercise; HI, high-dose impact loading; MOD, moderate dose impact loading; BMC, bone mineral content; BMD, bone mineral density; WB, whole body; LS, lumbar spine; TH, total hip; FN, femoral neck; TBS, trabecular bone score; WC, waist circumference; HC, hip circumference; troc, trochanter; str, strength; Mstr, muscle strength; Tstr, total strength; FM, fat mass; TBF, total body fat; TFM, total fat mass; LM, lean mass; TLM, total lean mass; VJ, vertical jump; RMHS, maximal resistance half squat; CSI, compression strength index; BSI, binding strength index; ISI, impact strength index; SI, strength index; Alpha linolenic acid, ALA; ISO, isoflavones; Placebo, Pla; HOMA, homeostasis assessment model; %Δ, percentage of variation; FPI, fasting plasma insulin; HOMA-IR, homeostatic model assessment for insulin resistance; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; HMW/TAdip, ratio of high molecular weight adiponectin to total adiponectin; 1.25$(OH)_2$D, 1,25-dihydroxy-Vitamin D; OC, osteocalcin; $_{uc}$OC, undercarboxylated osteocalcin; IL-6, interleukin 6; ↑, increase; ↓, decrease.

on the WB BMD. A non-significant trivial increase in the WB BMD (0.03 SMD; 95% CI [−0.26, 0.32], $p = 0.86$) was detected among these studies. The I^2 (0.0%) parameter indicates the homogeneity of the results. Concerning the impact of the type of physical training on the WB BMD, the comparison between the subgroups (**Figure 2**) did not reveal any significant differences ($p = 0.46$).

Five studies (6 groups: 5 females, 1 male), involving 124 participants, investigated the impact of physical activity on weight (**Figure 4**). The results showed a small significant decrease in the weight of subjects (−0.24 SMD; 95% CI [−0.42, −0.05], $p = 0.011$). The I^2 (0.0%) parameter indicated homogeneity of the results.

DISCUSSION

To the best of our knowledge, this is the first meta-analysis to have investigated the influence of the type of physical activity intervention on bone mass in individuals with overweight/obesity. The results show that physical activity is likely to increase the WB BMD in individuals with obesity.

The findings of the present study are supportive of two recent meta-analyses (Ashe et al., 2021; Clemente et al., 2021). Ashe and colleagues (Ashe et al., 2021) suggested that physical activity has little effect on the total hip BMD, and little to no effect on femoral neck BMD, lumbar spine BMD, and WB BMD in males. The other meta-analysis assessed the effects of small-sided games-based training programs on BMD in untrained adults (Clemente et al., 2021) and reported a trivial effect and small effect for this type of training on the WB BMD and lower limbs BMD, respectively when compared to the control groups. The modest osteogenic expression found in the WB BMD, compared to other bone sites, could reflect a site-specific effect for physical exercise. In addition, while the benefits of weight-bearing exercises on bone are well established in the literature (Weaver et al., 2016; Beck et al., 2017), such benefits are generally related to baseline BMD. Accordingly, BMD is, therefore, not expected to largely increase in subjects with overweight, since they already have high BMD values (Qiao et al., 2020).

To further understand the influence of physical exercise on bone, it is important to better comprehend how varying weight statuses may be related to bone health. Accordingly, our results demonstrated a small, but significant, reduction in body weight. Indeed, this outcome extends previous meta-analytic results showing that physical activity promotes weight loss in adolescents with obesity (Stoner et al., 2019). Weight loss is generally associated with bone loss and increased fracture risk in both males and females (Cummings and Nevitt, 1994; Meyer et al., 1995, 1998; Langlois et al., 1996; Lee et al., 2010). Hence, this meta-analysis suggests that the osteogenic effect of the exercise could be tempered by the associated weight loss and that physical activity has the potential to overcome its detrimental effect on BMD among individuals with overweight/obesity. Indeed, these results support the beneficial use of a physical activity to promote weight loss and preserve bone mass in subjects with overweight/obesity.

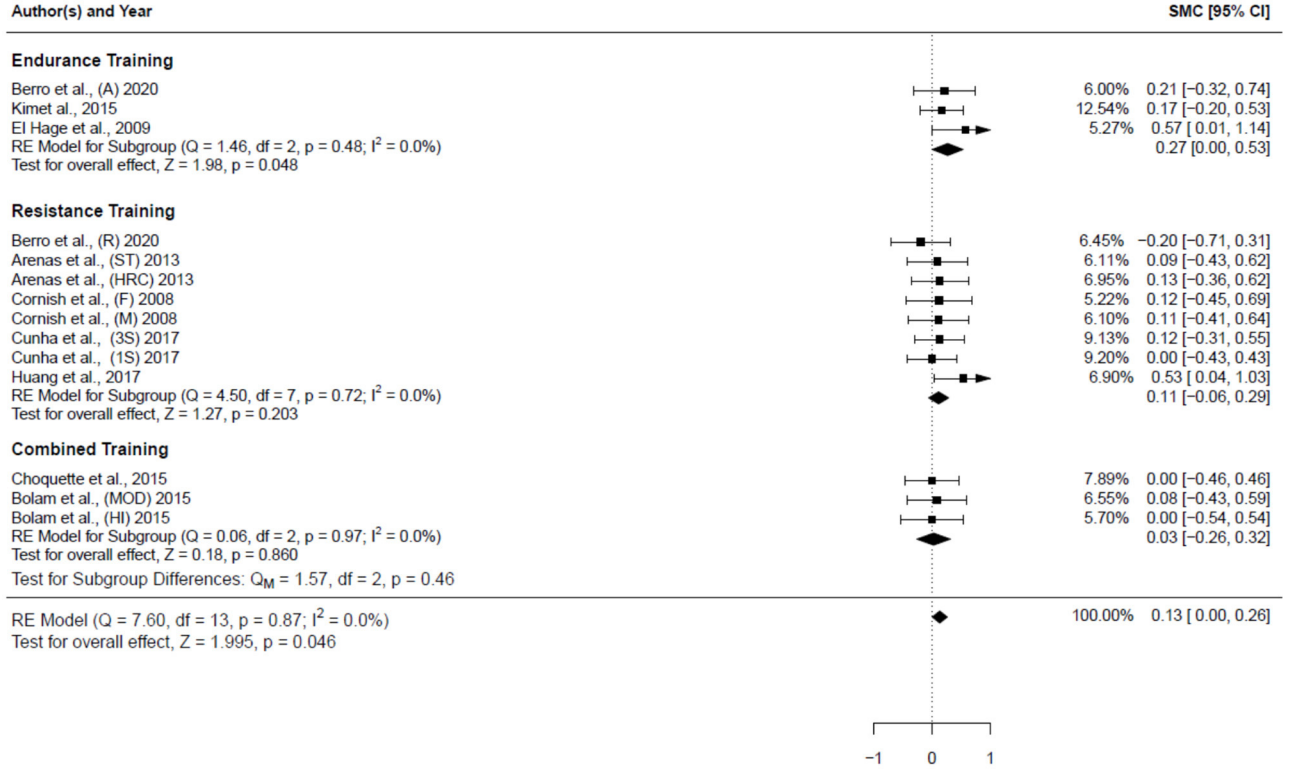

FIGURE 2 | Forest plot for the estimated standardized effect size changes in WB BMD. Three subgroups were included endurance training, resistance training, and combined training. The black squares represent the standardized mean difference, while the left and right extremes of the squares represent the corresponding 95% confidence intervals. The middle of the black diamond represents the overall standardized mean difference, while the left and right extremes of the diamond represent the corresponding 95% confidence intervals. SMC, standardized mean change; A, aerobic; R, resistance; ST, strength training; HRC, Hight resistance circuit; F, females; M, males; 3S, 3 sets; 1S, 1 set; MOD, moderate dose impact loading; HI, high-dose impact loading.

Effects of Exercise Training on Bone Health Indices in Individuals With Obesity

Effects of Endurance Training

Our analysis showed that endurance training slightly increases the bone density in individuals with obesity. The three studies included (El Hage et al., 2009; Kim et al., 2015; Berro et al., 2020) were of high quality, included both sexes, and the population was restricted to adolescents (El Hage et al., 2009) and adults (Kim et al., 2015; Berro et al., 2020). Two studies used treadmill running as an intervention (Kim et al., 2015; Berro et al., 2020), and the third one used team sports (El Hage et al., 2009). Albeit in the short duration period, the highest increase in the WB BMD was noted among adolescents with obesity who used collective aerobic exercise (El Hage et al., 2009) compared to the treadmill running adult populations (Kim et al., 2015; Berro et al., 2020). It is relatively well established in the literature that the potential of the osteogenic response during adolescence is higher compared to adulthood (Bonjour et al., 2009; Golden and Abrams, 2014). Interestingly, the osteogenic effect of the collective aerobic exercise appeared to be higher than the classic treadmill running. Aerobic exercise is a low-cost and easy to perform physical activity; indeed, these two characteristics are important when compliance is needed to obtain the desired effect. The studies included in this review indicated that weight-bearing aerobic exercise can have a positive impact on bone health among adolescents and adults with obesity.

Effects of Resistance or Strength Training

Our analysis demonstrated that resistance training has little to no effect on the WB BMD. The training programs ranged between 12 to 96 weeks and involved free weights, elastic bands, machines, and circuit training as the interventions for both genders. A previous meta-analysis (Kelley et al., 2001) examined the impact of the resistance exercise on BMD in females (consisting of 29 studies) and found that resistance exercise had a positive effect on BMD at the lumbar spine of all females, and the femur and radius sites for postmenopausal females. However, we did not include these parameters in our analytical model, which precludes any direct comparison.

In our analysis, seven papers had an average participant age of more than 55 years (Bocalini et al., 2009; Cornish and Chilibeck, 2009; Romero-Arenas et al., 2013; Huang et al., 2017; Cunha et al., 2018), and postmenopausal females represented the majority of the subjects. Age is an important factor which influences the responsiveness of bone to exercise. It was previously reported (Berger et al., 2010) that the peak bone mass (PBM) for the

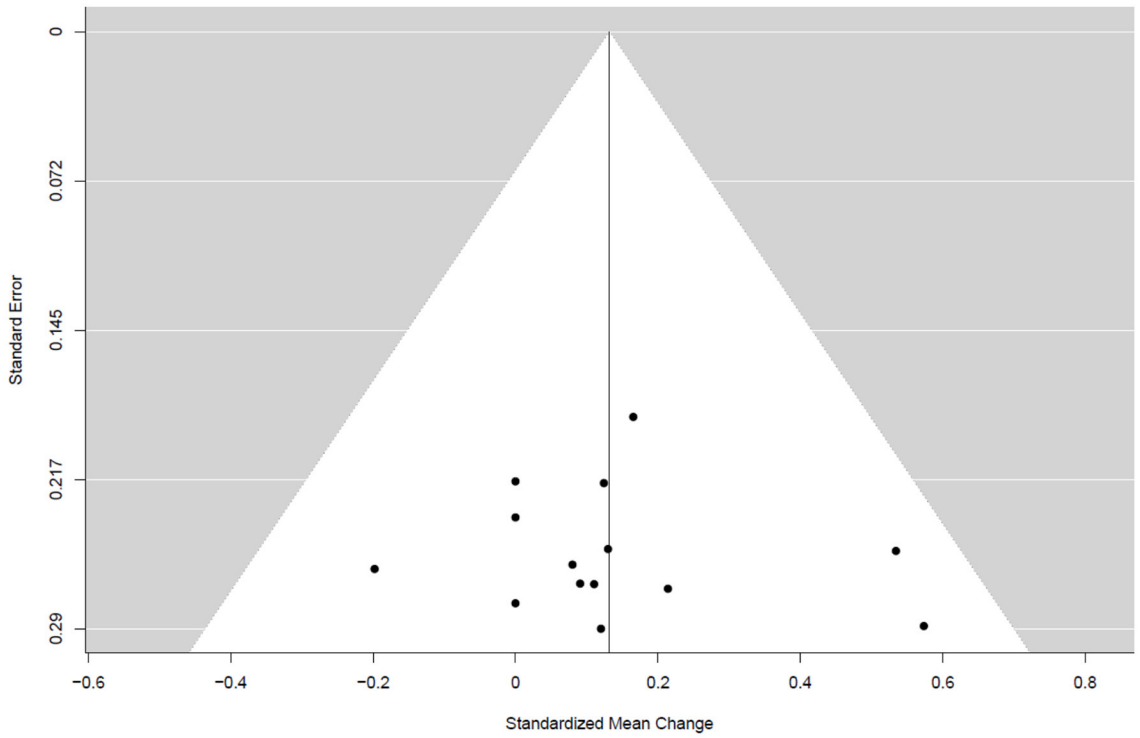

FIGURE 3 | Funnel plots for WB BMD in the whole group.

trabecular and cortical bone occurs before 40 and 33 years for females and males, respectively. Bone loss begins after PBM and accelerates in females with the onset of menopause (Weaver et al., 2016). Accordingly, physical activity must overcome bone loss before inducing any gain. In addition, as mentioned earlier, obesity is associated with higher BMD (Qiao et al., 2020); thus, the osteogenic response would be expected to be low among subjects with overweight/obesity.

The greatest increase in WB BMD was reported in the study of (Huang et al., 2017) despite the short duration of their training program. One possible explanation for this result is the trivial weight loss observed in this investigation. In fact, this study reported the smallest effect concerning weight loss among all studies. Also, these authors did not control for dietary factors, which can influence the impact of exercise, weight variation, and, consequently, the bone mass (Huang et al., 2017). Resistance training is recognized as an efficacious, non-pharmacological intervention to enhance bone mass (Weaver et al., 2016; Beck et al., 2017). Despite the absence of a significant effect, considering the population age and weight status, this result might suggest that the resistance exercise can combat the bone loss associated with aging in individuals with overweight/obesity.

Effects of Combined Training

This meta-analysis showed that the combined exercise training had little to no effect on the WB BMD. Two studies were included in the analysis; Choquette et al. (2011) and Bolam et al. (2016) investigated the influence of the combined exercise training on WB BMD in males and females with overweight/obesity, with an age range between 50 and 74 years. A previous meta-analysis in postmenopausal females showed that the risk of fractures in the combined exercise groups was significantly lower than in controls. The percentage change of BMD of the spine, trochanter, femoral neck, but not the total hip, was in favor of the intervention group. Our observed effect size may be attributable to the age and the weight status of participants, which can potentially diminish the osteogenic response of the physical exercise. Nevertheless, the present results suggest that the combined training may have the potential to combat bone loss in older adults.

Notably, the subgroups comparison did not reveal any significant differences among the types of training. The highest effect was observed in the endurance training, in which the age of the samples was lower compared to the other subgroups.

Although we have presented a novel addition to the literature, this meta-analysis has some limitations that must be mentioned. The number of studies that investigated the impact of endurance and combined training on bone health in subjects with overweight/obesity was relatively small. Furthermore, the training program's duration was highly variable, ranging from eight (Kim et al., 2015) to ninety-six (Warren and Schmitz, 2009) weeks. To this point, the resorption activity in a basic multicellular unit in adult human bone takes ∼3 weeks and

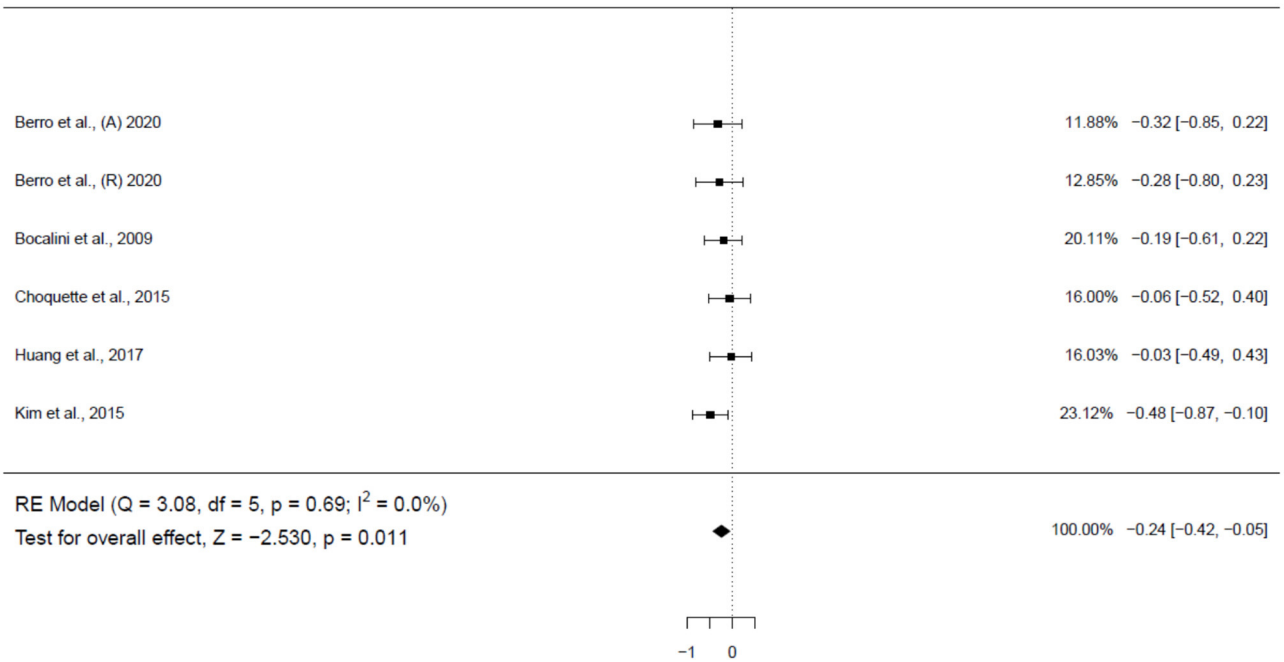

FIGURE 4 | Forest plot for the estimated standardized effect size changes in weight. The black squares represent the standardized mean difference, while the left and right extremes of the squares represent the corresponding 95% confidence intervals. The middle of the black diamond represents the overall standardized mean difference, while the left and right extremes of the diamond represent the corresponding 95% confidence intervals. SMC, standardized mean change A, aerobic; R, resistance.

the formation response 3 to 4 months (Sims and Martin, 2014), which means that a longer program could induce a higher osteogenic effect and as such be more impactful. Also, age and gender are two well-known moderators of the relation between fat and bone (Dolan et al., 2017). For example, younger individuals tend to exhibit a higher osteogenic effect with physical activity compared to older subjects (Bonjour et al., 2009; Golden and Abrams, 2014). The studies incorporated in this analysis included both sexes, adolescents, adults, and the elderly. Unfortunately, due to the few number of studies we identified in our analysis, sex and age could not be considered as moderators, in addition to the type of physical exercise.

CONCLUSION

Our systematic review and meta-analysis suggests that physical training have little to no effect on the WB BMD in subjects with overweight obesity. This conclusion, however, is based upon a limited number of available studies. Furthermore, there is also insufficient evidence at this time to advocate a specific type of exercise for enhancing bone health. Additional well-designed randomized controlled trials, investigating the impact of different types of physical exercise on bone health, in both sexes, and among individuals with overweight/obesity, especially during adolescence and adulthood, are needed before any firm recommendations can be made.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary files, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

HZ, AJB, and RE contributed to the conception or designof the work. AJB, SK, AJ, and RE independently performed searches in the electronic databases, evaluated articles, and extracted data for the review. All authors confirm responsibility for the following: review conception and design, interpretation reviewed the results, and approved the final version of results, manuscript preparation, writing, editing, the manuscript.

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Impact of 12-Week Moderate-Intensity Aerobic Training on Inflammasome Complex Activation in Elderly Women

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Aging often associates with a chronic low-grade inflammatory status that can be consequent to the activation of Toll-like receptors (TLRs) and the downstream NLR family pyrin domain containing 3 (NLRP3) inflammasome and causes a chronic secretion of pro-inflammatory cytokines. Since exercise has known anti-inflammatory effects, we investigated the effect of Nordic walking training on inflammasome activation and downstream effectors in elderly women. A population of elderly women was divided into EXP (n = 29) that completed 12 weeks of the moderate-intensity aerobic training program and CTRL (n = 29), performing no activity. Blood samples were taken before and after the first (T1-pre and T1-post, respectively) and last (T2-pre and T2-post, respectively) exercise unit. Inflammasome activation status was assessed by whole blood NLRP3 and TLR4 expression by RT-qPCR. Serum levels of IL-1β, IL-6, TNFα, and IL-18 cytokines were assayed by multiplex fluorescent beads-based immunoassays or ELISA. NLRP3 and TLR4 levels were reduced 2 folds between T1-pre and T2-pre and induced at T2-post, compared to T2-pre, by 2.6- and 2.9-fold, respectively. A single exercise bout elicited a 1. 38-, 1. 5-, and 1.36-fold rise of IL-1β, TNFα, and IL-6 concentration, respectively, although not significant, at the beginning of the training (T1-pre vs. T1-post), a 1.4-fold decrease for IL-1β and TNFα at the end of the training (T1-pre vs. T2-pre), and a 2-, 1.8- and 1.26-fold increase after the last exercise session (T2-pre vs. T2-post) for the three cytokines. When stratifying the population based on BMI in normal weight (NW) and overweight (OW), NLRP3 and TLR4 expression was affected only in NW. As for inflammatory cytokines, IL-1β was modulated in NW at the beginning of the training, whereas in OW at the end of the training; for TNFα, this time-dependent modulation was significant only in OW. Applied aerobic training affected the resting expression of inflammasome constituents (NLRP3 and TLR4) and levels of

downstream effectors (IL-1β, TNFα, and IL-6). However, at the end of the program, participants acquire an acute inflammatory response to exercise that was absent at baseline. Future studies would have to define the molecular mechanisms associated with, and how to potentiate, the exercise-associated inflammatory response.

Keywords: aging, NLRP3 inflammasome, pro-inflammatory cytokines, physical activity, aerobic exercise

INTRODUCTION

Aging is associated with several biological changes that profoundly affect cell and tissue functions and hesitates in the increased risk of developing diseases, frailty, injuries, disability, hospitalization, and, consequently, mortality. Among the plethora of altered functions, aging associates with a persistent state of chronic low-grade inflammation (LGI), also referred to as inflammaging (Franceschi et al., 2000). LGI is, in turn, associated with onset and development of most diseases including metabolic dysfunctions (e.g., obesity, impaired glucose tolerance, metabolic syndrome, and type 2 diabetes), muscle-skeletal failure (e.g., osteopenia, osteoporosis, and sarcopenia), cancers, neurodegenerative diseases, and aging itself (Franceschi et al., 2007).

Inflammation is a complex homeostatic response to harmful stimuli that protects the organism and promotes tissue repair and regeneration after injury, by orchestrating the innate immune response (Franceschi et al., 2007). The innate immune response is primed by the activation of pattern-recognition receptors (PRRs), as the Toll-like receptors (TLRs), C-type lectin receptors (CLRs), retinoic acid-inducible gene (RIG)-I-like receptors (RLRs), and NOD-like receptors (NLRs), by pathogen-associated molecular patterns (PAMPs). Injured cells can also activate the innate inflammatory response through the release of endogenous damage-associated molecular patterns (DAMPs), in the absence of pathogens (Gomarasca et al., 2020). During aging, alterations in T cell function, immune-senescence, extracellular matrix alterations, unfavorable changes in body composition (increased fat mass), and foci of chronic infections feed the presence of DAMPs and PAMPs into the circulation and keep the inflammatory response chronically activated (Franceschi et al., 2007; Mejias et al., 2018). This condition, known exactly as the LGI, is characterized by slightly and chronically increased plasma levels of pro-inflammatory cytokines, such as interleukin (IL)-6, IL-1β, tumor necrosis factor (TNF)α, and C-reactive protein (CRP). This pro-inflammatory state is caused by the activation of TLRs, upon PAMPs and DAMPs recognition, and the downstream signaling that culminates in the activation of NF-κB and MAPK pathways that promotes the expression of pro-inflammatory cytokines (Kawasaki and Kawai, 2014).

A key modulator of age-related systemic LGI is the NLR family pyrin domain containing 3 (NLRP3) inflammasome. Inflammasomes are multimeric protein complexes that assemble in the cytosol after sensing both PAMPs and DAMPs. Inflammasomes are involved in the activation of caspase-1 that in turn processes the pro-inflammatory cytokines IL-1β and IL-18 into their bioactive mature forms. The NLRP3 inflammasome needs to be primed by the activation of the TLR4 that, throughout NF-κB signaling, leads to the increased expression of the NLRP3 protein (Guo et al., 2015). Among others, aging is associated with elevation of intracellular and extracellular levels of uric acid, reactive oxygen species (ROS), free fatty acids (fFAs), ceramides, free cholesterol, oxidized low-density lipoproteins (LDL), advanced glycation end products, as well as with the alteration of the microbial community and the consequent increase in the level of microbial-derived constituents in blood. All these age-associated danger signals, contribute to the activation of the NLRP3 inflammasome and the subsequent triggering of the so-called age-related inflammation (or inflammaging) (Gritsenko et al., 2020). Thus, the NLRP3 inflammasome is a major sensor of age-related accumulation of DAMPs, in absence of apparent infection (Youm et al., 2013).

Powerful and effective regulators of chronic LGI are physical exercise and training. Both aerobic and resistance chronic exercises exert beneficial effects on the modulation of the inflammatory response (Gerosa-Neto et al., 2020; Padilha et al., 2021), reduction of chronic LGI in the elderly population (Dalle et al., 2017; Duggal et al., 2019), as well as, on the improvement of the metabolic profile (Lira et al., 2017; Da Silva et al., 2020). Exercise, which is defined as a planned, structured, and repeated physical activity (PA), can reduce not only the baseline inflammatory status of chronic LGI but also the acute inflammatory response against harmful stimuli (Beyer et al., 2012). However, only a few studies investigated the effects of PA on inflammasome activation and none has focused on Nordic walking. A resistance training (RT) program in healthy elderly reduced the protein expression of TLR4 and its downstream signaling effectors, leading to an overall improvement of the inflammatory status (Rodriguez-Miguelez et al., 2014). Similarly, another study related to RT intervention in elderly demonstrated the downregulation of NLRP3 protein and a decreased caspase-1-to-pro-caspase-1 ratio in peripheral blood mononuclear cells (PBMCs), highlighting the possible beneficial effect of RT in limiting the inflammatory reactivity (Mejias-Pena et al., 2017). A recent study has demonstrated that also moderate-intensity chronic aerobic exercise may reduce TLR4 and NLRP3 mRNA expression in PBMCs and circulating levels of IL-1β and IL-18 in young males (Khakroo Abkenar et al., 2019). Moreover, it was revealed that 12 weeks of moderate-intensity aerobic training combined with RT brought cardiometabolic benefits in adults with metabolic syndrome (Da Silva et al., 2020). Previously published papers indicated that Nordic walking training induces a reduction in circulating levels of the autophagy protein high mobility group box 1 (HMGB1) in elderly women and an increase the myokine

irisin (Gmiat et al., 2017). Thereby, moderate-intensity aerobic training, as Nordic walking, seems to positively affect the inflammatory status and it happens mainly throughout the modulation of the innate immunity function (Padilha et al., 2021), whose activation mainly relay on TLR4 signaling and the consequent inflammasome activation, especially in the elderly subjects that experience an age-associated deregulation of the TLR4-associated inflammatory pathways (Kawasaki and Kawai, 2014; Mejias et al., 2018). Nordic walking, also known as "Scandinavian walking with poles," is a popular outdoor activity based on specially designed poles for the purpose of activating the upper body during walking. It combines and stimulates skiing, sport walking, and trekking skills: by activating the upper body muscles, the use of poles may increase the length of each step, finally resulting in a faster gait and improved metabolism. Elderly are the most enthusiastic performers maybe because of its open-air, nature friendly, and social behaviors but also because perceived exertion and joint overload are limited thanks to the use of poles, despite the higher heart rate and oxygen consumption compared to a standard walk (Skorkowska-Telichowska et al., 2016). Interestingly, although the moderate intensity, Nordic walking is emerging as a powerful and effective strategy to counteract frailty and, particularly, metabolic- (Muollo et al., 2019) and mobility-associated aspects of frailty, as increased risk of fracture and skeletal muscle wasting (Ossowski et al., 2016; Xu et al., 2016). Within this context, this study aims to unravel the effect of a 12-week Nordic walking moderate aerobic training program on the expression of the main components of inflammasome complex (TLR4 and NLRP3) in whole blood and the downstream cytokine effectors (IL-1β, IL-18, TNFα, and IL-6) in elderly women.

MATERIALS AND METHODS

Study Design

In this intervention case-control study, 70 elderly women (age = 68 ± 8 years old), with a sedentary behavior [according to the American College of Sports Medicine guidelines (ACSM, 2010)] were recruited among church communities, senior citizens' clubs, and universities of the third age. Participants were randomly assigned to the experimental (EXP, n = 35) group, engaged in a 12-week Nordic walking training program, and control (CTRL, n = 35) group, not involved in any activity. Recruitment and testing of EXP and CTRL subjects took place at the same period. At enrolment, all subjects underwent a medical examination and were asked to provide information regarding prescribed medications. Exclusion criteria were: uncontrolled hypertension (diastolic blood pressure > 100 mmHg), history of cardiac arrhythmia, cardio-respiratory disorders, and orthopedic problems. Body composition and 2,000 m walking test were determined 1 week prior to the start of the experiment and after 12 weeks of training. Participants belonging to the EXP group were familiarized with the right technique for walking with Nordic walking poles. All subjects were characterized for weight, BMI, and hematological and biochemical markers (as reported in the subsection "Blood Collection and Sample Preparation").

From the original cohorts, six subjects who either did not attend one blood sampling or did not meet the compliance criteria to training (i.e., <90% participation), were excluded. By the end, 29 subjects were included in the EXP group and 29 in the CTRL group. In order to verify the existence of a BMI-dependent response to the training program, participants were sub-grouped in normal weight (NW; EXP, n = 14; CTRL, n = 12), with BMI < 25 kg/m^{-2}, and overweight/obese (OW; EXP, n = 15; CTRL, n = 17), with BMI ≥ 25 kg/m^{-2}.

The population was not stratified according to Vitamin D intake, since many participants used to take this supplement as a general recommendation from the Ministry of Health for seniors in Poland. The levels of Vitamin D were measured to be in the range of 32–55 ng/mL for the whole population.

The study received the official approval of the Bioethical Committee of the Regional Medical Society in Gdansk (KB-34/18) and was registered as clinical trial with the ID: NCT03417700, in accordance with the Declaration of Helsinki. All participants were given detailed information about experiment, procedures, risks, and benefits of the study and gave their written consent to participation.

Training Protocol

Participants from EXP group met three times a week (Monday, Wednesday, and Friday), 1 h after eating a light breakfast. In order to avoid the impact of different diets on training response, all the participants were given the same breakfast on the day the tests were collected and were asked not to change their eating habits during the training period. CTRL subjects were also asked to maintain unaltered their lifestyle habits and to keep their PA level below 150 min/week (ACSM, 2010). Both groups were instructed not to perform any additional physical activity during the study period. Each training session lasted 1 h, and consisted of 10-min warm-up, 40-min specific Nordic walking training, and 10-min cool-down. Subjects were equipped with standard Nordic walking poles. The same group of research assistants and coaches checked attendance of participants, supervised all training sessions, and performed the tests. Nordic walking training was performed with 60–70% intensity of the maximal heart rate (HR) obtained during the supervised 2000 m walking test. This test was performed on a flat floor, according to the previously described procedure (Mieszkowski et al., 2018). This test, as well as each training unit, was monitored using Garmin Forerunner 405 with a built-in GPS in order to record distance. The model of Garmin Forerunner 405 was equipped with additional HR sensor. The participants were encouraged to maintain the highest possible pace during the 2,000 m walking test to achieve the highest intensity, but they were not allowed to run. Time was measured using photoelectric cells (Racetime 2 SF, Microgate, Bolzano, Italy) with an accuracy of 0.001 s. The start of the movement was signaled by the instructor. The information about 60–70% HR max intensity achieved during every training session was monitored for each participant individually by coach. To evaluate the maximal oxygen capacity a mathematic formula

was applied: VO_2 max = 116.2-2.98 Time-0.11HR-0.14Age-0.39BMI (Laukkanen et al., 2000; Kortas et al., 2015). The EXP group completed 12 weeks of Nordic walking training, which included 36 training units. During the entire training program, participants in the EXP group covered a total distance of almost 120 km. Only subjects, who attended at least 90% of the total amount of training units, were considered as completing the protocol.

Blood Collection and Sample Preparation

Blood samples were taken from the antecubital vein by two professional nurses. For the EXP group, blood samples were collected at baseline, before and after the first session of Nordic walking (T1-pre and T1-post, respectively), immediately before the last exercise session, after 12 weeks of training (T2-pre), and immediately after the last training session (T2-post). Post-exercise blood drawings were performed within 15 min from the end of the exercise session. For the CTRL group, blood was sampled only at T1 and T2, corresponding to the T1-pre and T2-pre, respectively, of the EXP cohort. The blood was collected at rest, under fasting condition, between 7:00 and 8:00 a.m. Ethylendiaminotetraacetate dipotassium salt (K2EDTA)-anticoagulated blood (K2EDTA Vacutainer®, Becton Dickinson, and Co., Franklin Lakes, NJ, United States) was used for hematological characterizations and RNA extraction. The hematological assessment was performed only at T1-pre and T2-pre for the EXP group and at both T1 and T2 for the CTRL group, and included: hemoglobin [Hb], hematocrit (Ht%), red blood cells (RBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin content (MCHC), red cells distribution width – coefficient of variation (RDW-CV), platelets count (Plt), mean platelet volume (MPV), total white blood cell count (WBC), absolute and relative counts of neutrophils (Neu), lymphocytes (Ly), monocytes (Mo), eosinophils (Eo), and basophils (Ba). Serum was obtained from blood collected into SST II Advance™ tubes with clot activator (Becton Dickinson, and Co.) and was used for the characterization of the metabolic [total cholesterol (TChol), high-density lipoproteins (HDL), low-density lipoproteins (LDL), triglycerides (TG), iron, and ferritin] and inflammatory (as described below) profiles, measured only at T1-pre and T2-pre for the EXP group and at both T1 and T2 for the CTRL group. Samples were centrifuged at 2,000 × *g*, for 10 min, at 4°C and stored at −80°C until later analysis. Guidelines for the correct management of the pre-analytical phase were strikingly followed (Banfi et al., 2010; Dugue et al., 2018; Faraldi et al., 2020). Since [Hb], Ht%, RBC, MCV, MCH, MCHC, RDW-CV, Plt, and MPV remained stable during the observation and did not differ among any of the groups, and are, however, mostly irrelevant to the aim of the current study, they were not further discussed.

Further, 25-hydroxy vitamin D [25-(OH)D], the most reliable marker of vitamin D status (Ferrari et al., 2017), was measured by high-performance liquid chromatography (HPLC) coupled with mass spectrometry (MS) on a Shimadzu LCMS 8050 HPLC system Nexera X2 column with Agilent Eclipse Plus C18 1.8 μm 2.1 × 100 mm columns, according to Gmiat et al. (2017). The measurement was performed at baseline in order to verify that all participants were in a state of sufficiency. Importantly, according to Polish guidelines for seniors, all participants used to be supplemented with vitamin D.

RNA Extraction

Total RNA was extracted from whole blood using the Direct-Zol miniprep Kits (Zymo Research Co., Orange, CA, United States), following manufacturer instructions. Briefly, three volumes of TRI Reagent® were added to 250 μl of whole blood. After mixing thoroughly and centrifuging at 12,000 × *g* for 30 s at RT, the supernatant was transferred into RNase-free tubes for the subsequent RNA purification. After adding an equal volume of ethanol 95% and mixing, the sample was transferred into the Zymo-Spin™ IC Column and centrifuged at 12,000 × *g*, for 30 s, at RT. Thereafter, the sample was digested with DNase I (6 U/μl) for 15 min at RT. The columns were further washed and the RNA was eluted in 15 μl of DNase/RNase-Free Water by centrifugation at 12,000 × *g* for 30 sat RT. RNA concentration was quantified using a NanoDrop spectrophotometer (Thermo Fisher Scientific, Waltham, MA, United States). RNA purity and integrity were assessed by considering the 260/280 nm and 260/230 nm absorbance ratios, visualized at NanoDrop spectrophotometer and through 1% agarose gel electrophoresis.

Gene Expression Analysis in Whole-Blood

Total RNA was reverse transcribed using the iScript cDNA Synthesis Kit (Bio-Rad Laboratories, Hercules, CA, United States). RT-qPCR was carried out on a StepOne Plus instrument (Applied Biosystems, Foster City, CA, United States), using TaqMan™ Gene Expression Master Mix and premade 6-Carboxyfluorescein (FAM)-labeled TaqMan assay for *TLR4* (Hs00152939_m1), *NLRP3* (Hs00918082_m1), *PPIB* (Hs00168719_m1), *PGK1* (Hs99999906_m1), *ACTB* (Hs99999903_m1) (Thermo Fisher Scientific). The thermal protocol was as follows: 50°C for 2 min, 95°C for 10 min, followed by 40 amplification cycles at 95°C for 15 s and 60°C for 60 s. Results, reported as quantification cycle (Cq) values, were analyzed by the GenEx software ver. 6 (Exiqon A/S, Vedbaek, Denmark). The relative expression of each gene was calculated by the $2^{-\Delta\Delta Cq}$ method, using PPIB and PGK1 as reference genes. Analysis of target genes was performed on the overall included subjects and on subjects stratified for BMI. Results on gene expression are reported as median (minimum value to maximum value).

Selection of Reference Genes

PPIB, PGK1, and ACTB were assayed as reference genes. Expression level analysis was performed comparing normalized expression levels calculated as follows:

$$\Delta Cq = Cq\ rg - geomean\ rg \quad (1)$$

Cq rg: quantification cycle of a reference gene in a sample.

geomean rg: geometrical mean of the Cq of PPIB, PGK1, and ACTB of all samples.

The heatmap analysis was performed using the tool provided by GenEx software (Exiqon). An in-depth analysis of the normalization strategies was performed as previously described (Faraldi et al., 2019). Expression stability of PPIB, PGK1, and ACTB, was analyzed using the NormFinder (Andersen et al., 2004) and GeNorm (Vandesompele et al., 2002) algorithms provided by the GenEx software.

Cytokines Analysis

The pro-inflammatory cytokines IL-1β, IL-6, and TNFα were quantified in serum with a multiplex customized Human High Sensitivity Cytokine B Premixed Mag Luminex Performance Assay (R&D Systems, Minneapolis, MN, United States). Samples were analyzed in duplicates and read on a MAGPIX® Multiplex System (Luminex® Co., Austin, TX, United States). The assay sensitivities were 0.146 pg/mL for IL-1β, 0.135 pg/mL for IL-6, and 0.250 pg/mL for TNFα. Values under the last point of the standard curve, but above the blank and the minimum detectable dose (0.03 pg/mL for IL-1β, 0.08 pg/mL for IL-6, and 0.13 pg/mL for TNFα) were derived by the Bio-Plex Manager Software. The intra-assay (CV_i) and inter-assay (CV_b) coefficients of variation of each analyte were 1.7 and 11.1% for IL-1β, 2.0 and 11.4% for IL-6, and 1.7 and 11.6% for TNFα, respectively.

IL-18 concentrations were measured in serum by Human Total IL-18 ELISA (R&D Systems), following manufacturer instructions. The assay-specific sensitivity was 5.15 pg/mL. Maximum intra-assay (CV_i) and inter-assay (CV_b) coefficients of variation were 3.1% and 8.7%, respectively. Readings were performed at λ = 450 nm subtracted of the corresponding readings at λ = 570 nm on a Victor X3 (PerkinElmer, Waltham, MA, United States). Body mass, body composition, and body mass index (BMI) were determined using a multi-frequency impedance analyzer (In $Body_{720}$, Biospace, South Korea). The measurements were performed twice, 1 week before and after the entire intervention, according to McLester et al. (2020).

Statistical Analysis

The minimum sample size was determined with G*Power (v3.1.9.7) based on IL-6 serum concentrations as the primary endpoint index, being this cytokine largely described as the prototypic mediator of innate immune response and exercise-dependent metabolic regulation (Chowdhury et al., 2020). For sample size calculation was assumed a two-tailed type I α error of 0.05, a power (1-β error probability) > 0.95, a pre-to-post-intervention difference of 18%, and a standard deviation of ±18.54 pg/mL, according to Gmiat et al. (2018). The total estimated sample size was 22.

Statistical analysis was performed with Prism® v6.01 (GraphPad Software Inc., La Jolla, CA, United States). The D'Agostino-Pearson's normality test (omnibus K2 test) defined non-parametric distributions for most of the parameters analyzed. Thereby, in the descriptive analysis, data are reported as the median and range (minimum to maximum), while the statistical analysis was conducted with non-parametric tests.

In EXP and CTRL groups, age, height, and Vitamin D were analyzed by non-parametric Mann–Whitney test.

In the EXP group, time-dependent changes were analyzed by non-parametric repeated measures Friedman's test with Dunn's multiple comparisons (T1-pre vs. T1-post vs. T2-pre vs. T2-post). Comparison of time-dependent changes between EXP and CTRL group and comparison between NW and OW within and between EXP and CTRL groups were performed by two-way ANOVA with Sidak's multiple comparison test. Differences were considered statistically significant if p-values < 0.05, and only significant data were discussed in the text.

The effect size has been calculated by Kendal W for Friedman's tests, Cohen's eta-squared for two-way ANOVA, and Cohen's d for the *post hoc* tests.

RESULTS

Characterization of the Study Cohort

The characterization of EXP subjects before the beginning (T1-pre) and before the last exercise session of the 12-week Nordic walking training (T2-pre), and CTRL subjects over the same period (T1 and T2, respectively) is detailed in **Tables 1**, **2** and **Supplementary Table 1**. The characterization of the cohorts at these two time-points allows avoiding the alteration of some hematological markers due to the effect of acute exercise, meaning after a session of training, rather than highlighting the differences due to the chronic exercise.

The two cohorts, compared before and after the intervention (T2-pre for EXP), resulted homogenous for age and most of the measured parameters. Within each cohort, NW and OW subjects differed for weight and BMI at T1 and T2 in both EXP and CTRL. Basophils were increased in OW subjects at both T1 and T2 of the CTRL cohort, while they were reduced between T1 and T2 in the EXP-NW cohort. No time-dependent change was observed in CTRL, although lymphocyte absolute count increased from T1 to T2 in the entire group and in the OW cohort. The results of the ANOVA tests and the related effect size are reported in **Supplementary Table 2**.

Considering the metabolic markers, HDL was lower in EXP-OW compared to EXP-NW at both time-points, and decreased in NW subject at the end of the training (**Supplementary Table 1**). In the EXP group, a time-dependent, although clinically irrelevant, reduction of serum iron was recorded in OW subjects, and at T2 compared to CTRL considering the whole population or the OW subgroup. Importantly, baseline 25-(OH)D concentrations did not differ among any of the cohorts.

Expression and Stability of PPIB, PGK1, and ACTB Genes

According to previously published studies (Dheda et al., 2004; Falkenberg et al., 2011), we selected PPIB, PGK1, and ACTB as possible candidates reference genes to normalize RT-qPCR data in whole blood samples. Following a cluster analysis to exclude co-regulation, ΔCq analysis revealed a more scattered expression of ACTB compared to PPIB and PGK1 (**Figures 1A–C**) and a more constant of PPIB and PGK1 in all samples at all

TABLE 1 | Anthropometrical characteristic of the study cohort.

	CTRL (n = 29)					EXP (n = 29)						
	T1		T2		p-value T1 vs. T2	T1-pre		T2-pre		p-value T1-pre vs. T2-pre	p-value T1 CTRL vs. T1-pre EXP	p-value T2 CTRL vs. T2-pre EXP
	Median (min-to-max)	p-value NW vs. OW	Median (min-to-max)	p-value NW vs. OW		Median (min-to-max)	p-value NW vs. OW	Median (min-to-max)	p-value NW vs. OW			
Age (years)	68.0 (60.00–78.0)		/		/	69.0 (60.0–78.0)		/		/	0.568	/
NW	67.5 (62.0–76.0)	0.763	/	/	/	70.0 (60.0–78.0)	0.502	/	/	/	0.446	/
OW	71.0 (60.0–78.0)		/		/	67.0 (60.0–76.0)		/		/	0.836	/
Height (m)	1.63 (1.50–1.74)		/			1.63 (1.53–1.78)		/		/	0.243	/
NW	1.64 (1.53–1.74)	0.360	/	/	/	1.65 (1.53–1.78)	0.883	/	/	/	0.962	/
OW	1.61 (1.50–1.70)		/		/	1.63 (1.53–1.75)		/		/	0.398	/
Weight (kg)	67.80 (54.40–93.00)		67.70 (54.10–93.80)		0.928	67.35 (54.40–94.20)		65.80 (54.80–95.60)		0.460	0.731	0.664
NW	63.90 (54.40–73.30)	**0.004**	63.25 (54.10–73.80)	**0.004**	1.000	58.70 (54.40–73.80)	**<0.001**	57.60 (54.80–73.80)	**<0.001**	0.996	0.966	0.971
OW	71.80 (58.50–93.00)		72.20 (57.70–93.80)		0.986	68.50 (65.80–94.20)		68.70 (65.10–95.60)		0.258	1.000	1.000
BMI (kg/m^2)	26.00 (20.70–34.60)		25.90 (20.60–34.90)		0.909	25.30 (19.80–33.00)		24.80 (19.60–33.10)		0.995	0.261	0.280
NW	23.75 (20.70–24.90)	**<0.001**	23.43 (20.60–25.30)	**<0.001**	1.000	22.60 (19.80–24.70)	**<0.001**	22.20 (19.60–24.70)	**<0.001**	0.941	0.681	0.816
OW	27.90 (25.30–34.60)		27.10 (24.90–34.90)		0.992	26.90 (25.20–33.00		26.50 (24.50–33.10)		0.915	0.813	0.771

Description of weight, BMI, and hematologic markers in the entire study cohort and for the sub-cohorts stratified based on BMI (BMI < 25 kg/m^2: NW; BMI > 25 kg/m^2: OW, as determined at recruitment). Data are expressed as median (range) since the non-parametric distribution, as assayed by D'Agostino-Pearson's test. Comparison of age and height between EXP and CTRL were assessed using Mann–Whitney test. Within-group (EXP and CTRL) time-dependent changes in NW and OW subjects were performed by the means of two-way ANOVA with Sidak's multiple comparison post hoc test. Statistically significant (p-values < 0.05) differences are indicated in bold. NW, normal weight subjects; OW, overweight subjects; BMI, body mass index.

TABLE 2 | White blood cell counts.

	CTRL (n = 29)					EXP (n = 29)						
	T1		T2		p-value T1 vs. T2	T1-pre		T2-pre		p-value T1-pre vs. T2-pre	p-value T1 CTRL vs. T1-pre EXP	p-value T2 CTRL vs. T2-pre EXP
	Median (min-to-max)	p-value NW vs. OW	Median (min-to-max)	p-value NW vs. OW		Median (min-to-max)	p-value NW vs. OW	Median (min-to-max)	p-value NW vs. OW			
WBC ($\times 10^9$/L)	5.58 (3.93–7.90)		5.74 (4.02–9.21)		0.475	5.73 (3.53–8.89)		5.52 (3.71–10.18)		1.000	0.997	0.821
NW	5.83 (5.06–7.90)	0.819	5.69 (4.47–8.80)	1.000	0.999	5.30 (3.95–7.56)	0.641	4.79 (3.79–6.86)	0.232	0.968	0.716	0.577
OW	5.53 (3.93–7.41)		5.74 (4.02–9.21)		0.375	6.00 (3.53–8.89)		6.25 (3.71–10.18)		0.977	0.751	0.985
Neu (%)	50.80 (38.40–70.10)		50.80 (31.90–66.00)		0.635	52.60 (31.70–77.40)		50.70 (38.10–69.30)		0.323	0.905	0.999
NW	49.30 (38.40–65.40)	0.995	50.60 (31.90–66.00)	0.974	0.932	51.40 (31.70–77.40)	0.998	48.50 (41.00–67.20)	0.986	0.747	1.000	1.000
OW	53.60 (39.90–70.10)		51.40 (42.20–65.90)		0.977	53.50 (35.90–67.60)		52.00 (38.10–69.30)		0.874	1.000	1.000
Ly (%)	35.40 (19.10–48.20)		37.10 (21.80–54.90)		0.296	34.85 (13.20–54.90)		37.95 (20.50–49.20)		0.280	0.989	0.995
NW	38.70 (23.10–47.20)	0.964	37.05 (23.80–54.90)	0.957	0.834	37.20 (13.20–54.90)	0.999	39.00 (20.50–49.20)	0.976	0.662	1.000	1.000
OW	33.80 (19.10–48.20)		37.10 (21.80–46.30)		0.758	34.10 (25.80–49.10)		36.70 (21.50–47.60)		0.874	1.000	1.000
Mo (%)	8.20 (6.10–11.00)		8.20 (5.60–11.00)		0.431	8.60 (5.50–13.30)		8.90 (5.60–13.40)		0.889	0.462	0.277
NW	8.05 (6.90–10.70)	1.000	7.70 (5.60–10.90)	0.999	0.711	8.50 (6.20–12.00)	1.000	8.70 (5.70–13.40)	1.000	1.000	0.972	0.709
OW	8.50 (6.10–11.00)		8.30 (5.90–11.00)		0.966	8.80 (5.50–13.30)		9.30 (5.60–11.90)		0.938	0.966	0.978
Eo (%)	2.90 (0.90–15.90)		2.70 (0.80–6.30)		0.256	2.70 (0.60–5.10)		2.45 (0.70–5.00)		0.987	0.154	0.583
NW	3.00 (1.20–8.00)	0.998	2.95 (1.40–5.70)	0.988	1.000	2.60 (1.10–5.10)	1.000	2.30 (0.80–5.00)	1.000	1.000	0.865	0.854
OW	2.50 (0.90–15.90)		2.70 (0.80–6.30)		0.196	2.70 (0.60–5.00)		2.50 (0.70–4.50)		1.000	0.712	1.000
Ba (%)	0.70 (0.30–1.60)		0.70 (0.20–1.50)		0.346	0.70 (0.30–1.30)		0.60 (0.20–1.10)		0.113	0.621	0.381
NW	0.55 (0.30–1.30)	**0.050**	0.45 (0.20–1.10)	**0.028**	0.756	0.70 (0.40–1.30)	0.986	0.60 (0.20–0.80)	0.812	**0.021**	0.793	1.000
OW	0.80 (0.40–1.60)		0.80 (0.40–1.50)		0.853	0.70 (0.30–1.10)		0.60 (0.30–1.10)		1.000	0.166	0.454

(Continued)

TABLE 2 | (Continued)

	CTRL (n = 29)					EXP (n = 29)						
	T1		T2		p-value T1 vs. T2	T1-pre		T2-pre		p-value T1-pre vs. T2-pre	p-value T1 CTRL vs. T1-pre EXP	p-value T2 CTRL vs. T2-pre EXP
	Median (min-to-max)	p-value NW vs. OW	Median (min-to-max)	p-value NW vs. OW		Median (min-to-max)	p-value NW vs. OW	Median (min-to-max)	p-value NW vs. OW			
Neu (×10^9/L)	2.92 (1.58–4.55)		3.04 (1.74–5.29)		0.975	2.84 (1.80–5.01)		2.86 (1.69–7.06)		0.839	0.936	0.981
NW	3.02 (2.24–4.16)	0.998	3.14 (1.74–4.64)	1.000	0.988	2.37 (1.89–5.01)	0.820	2.45 (1.75–4.13)	0.341	0.857	0.989	0.931
OW	2.92 (1.58–4.55)		2.88 (2.12–5.29)		0.953	3.39 (1.80–4.89)		3.07 (1.69–7.06)		1.000	0.889	0.984
Ly (×10^9/L)	1.93 (0.98–3.05)		2.22 (1.24–3.28)		**0.025**	1.82 (0.83–3.32)		2.06 (1.11–3.17)		0.367	0.986	0.654
NW	2.34 (1.17–3.05)	0.412	2.17 (1.24–3.28)	0.968	0.933	1.78 (0.83–3.32)	0.937	2.07 (1.11–2.91)	0.878	0.921	0.572	0.576
OW	1.90 (0.98–2.92)		2.24 (1.25–3.13)		**0.030**	2.05 (1.16–2.95)		2.19 (1.27–3.17)		0.745	0.852	1.000
Mo (×10^9/L)	0.48 (0.34–0.67)		0.48 (0.27–0.76)		0.903	0.48 (0.32–0.92)		0.45 (0.30–0.84)		0.910	0.733	0.937
NW	0.51 (0.40–0.61)	0.929	0.47 (0.30–0.76)	1.000	0.897	0.47 (0.33–0.69)	0.835	0.44 (0.31–0.84)	0.770	0.993	0.990	0.999
OW	0.44 (0.34–0.67		0.50 (0.27–0.76)		0.665	0.51 (0.32–0.92)		0.49 (0.30–0.78)		1.000	0.588	0.952
Eo (×10^9/L)	0.15 (0.07–1.06)		0.16 (0.06–0.33)		0.341	0.13 (0.04–0.30)		0.13 (0.03–0.34)		0.994	0.114	0.503
NW	0.19 (0.07–0.48)	1.000	0.21 (0.08–0.27)	0.989	0.998	0.13 (0.06–0.27)	0.986	0.11 (0.03–0.23)	0.971	1.000	0.582	0.642
OW	0.14 (0.07–1.06)		0.15 (0.06–0.33		0.448	0.15 (0.04–0.30)		0.15 (0.05–0.34)		1.000	0.828	1.000
Ba (×10^9/L)	0.04 (0.02–0.10)		0.04 (0.01–0.09)		0.740	0.04 (0.02–0.07)		0.04 (0.01–0.07)		0.374	0.507	0.306

White blood cell counts in the entire study cohort and for the sub-cohorts stratified based on BMI (BMI < 25 kg/m^2: NW; BMI > 25 kg/m^2: OW, as determined at recruitment). Data are expressed as median (range) since the non-parametric distribution, as assayed by D'Agostino-Pearson's test. Within-group (EXP and CTRL) time-dependent changes in NW and OW subjects were performed by the means of two-way ANOVA with Sidak's multiple comparison post hoc test. Statistically significant (p-values < 0.05) differences are indicated in bold. NW, normal weight subjects; OW, overweight subjects; WBC, white blood cell count; Neu, neutrophils count; Mo, monocytes count; Ly, lymphocytes count; Eo, eosinophils count; Ba, basophils count.

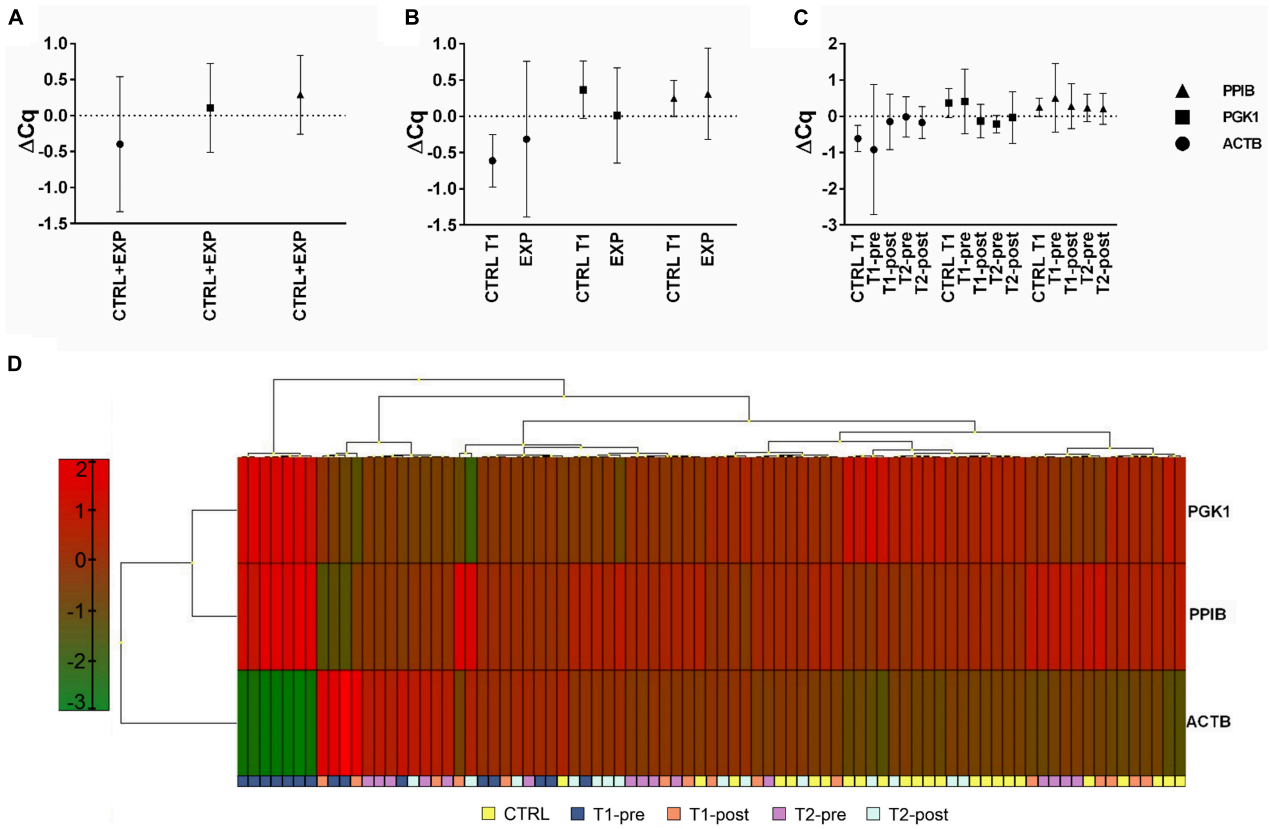

FIGURE 1 | Expression profile of potential housekeeping genes, ACTB, PGK1, and PPIB, in whole blood. Expression profile of the three selected potential reference genes (ACTB, PGK1, and PPIB). Expression level of each potential reference gene was analyzed considering **(A)** samples from all groups (CTRL+EXP), **(B)** samples from the EXP (T1-pre, T1-post, T2-pre, and T2-post), and CTRL groups separated, and **(C)** samples from each time-point separated. **(D)** Heat-map of the expression profiles of ACTB, PGK1, and PPIB, from each group (CTRL, T1-pre, T1-post, T2-pre, and T2-post). Different colors represent differential gene expression.

time-points (**Figure 1D**). Descriptive characteristics of the potential reference genes and the relative expression stability analysis are summarized in **Supplementary Tables 3**, **4**.

The expression stability of candidate genes was evaluated by NormFinder and GeNorm algorithms: PPIB and PGK1 were the best ranked genes, by NormFinder, while the combination PPIB-PGK1 was identified by GeNorm. Therefore, PPIB and PGK1 were used as reference genes.

Effect of Training on NLRP3 and TLR4 Expression in Whole Blood

NLRP3 and TLR4 mRNA expression levels were determined in whole blood. Both NLRP3 and TLR4 showed similar expression profiles: while a single bout of aerobic exercise at the beginning of the training protocol (T1-pre vs. T1-post) did not induce any significant changes in the expression of both genes, they were induced at T2-post, compared to T2-pre [NLRP3 T2-pre: 0.090 (0.049–0.459) vs. NLRP3 T2-post: 0.237 (0.064–0.594); TLR4 T2-pre: 0.087 (0.046–0.765) vs. TLR4 T2-post: 0.256 (0.047–0.660)]. Additionally, both NLRP3 and TLR4 expression levels were significantly reduced between T1-pre and T2-pre [NLRP3: 0.172 (0.072–1.680) vs. 0.090 (0.049–0.459); TLR4: 0.184 (0.068–1.296) vs. 0.087 (0.046–0.765)] (**Figure 2A**).

However, when comparing EXP and CTRL, the two groups differed at the beginning of the training (T1-pre vs. T1) for both genes. While in the CTRL group, no time effect was recorded, the EXP group showed a significant reduction of both genes at T2-pre compared to T1-pre (**Figure 2B**).

When analyzing the trained cohort sub-grouped in NW and OW, no differences were found at any time-point between the two sub-groups (**Figure 2C**).

The NW sub-population was affected by the acute intervention since it showed a significant increase in the NLRP3 gene expression [T1-pre vs. T1-post) (0.180 (0.072–1.679) vs. 0.218 (0.069–0.528), respectively] and this acute effect was seen also at the end of the training, when comparing T2-pre vs. T2-post, even though not significantly (**Figure 2C**). The chronic exercise, on the other hand, induced a significant decrease in both NLRP3 and TLR4 expression at the rest time-points (T1-pre vs. T2-pre) [NLRP3: 0.180 (0.072–1.679) vs. 0.088 (0.062–0.237); TLR4 0.216 (0.068–1.300) vs. 0.081 (0.046–0.292)] in the NW cohort (**Figures 2C,D**).

When comparing the NW and OW cohorts between the EXP and CTRL groups, besides the lack of differences in NLRP3, the

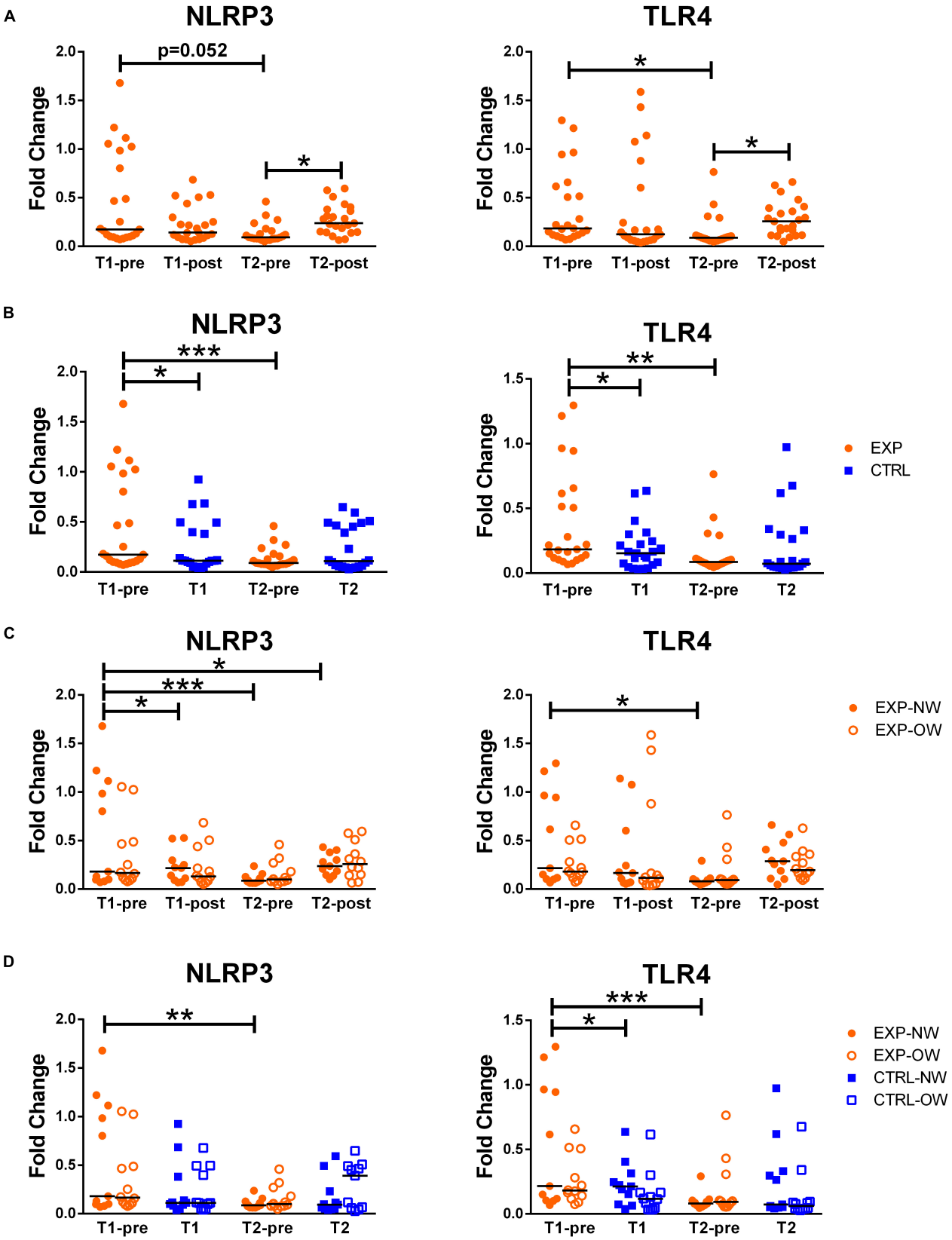

FIGURE 2 | Continued

FIGURE 2 | Expression of inflammasome-related genes in whole blood samples. Fold change of NLRP3 and TLR4 gene expression, normalized on PPIB and PGK1, in whole blood from elderly women underwent a 12-week aerobic Nordic walking training program (EXP, $n = 29$) and untrained controls (CTRL, $n = 35$). **(A)** Expression of NLRP3 and TLR4 in the whole EXP group before and after the first (T1-pre and T1-post) and the last (T2-pre and T2-post) sessions of Nordic walking. **(B)** Expression of NLRP3 and TLR4 in the whole EXP group (orange dots), before the first and the last (T1-pre and T2-pre) Nordic walking session, and in the CTRL (blue squares) over the same observation period (T1, T2). **(C)** Expression of NLRP3 and TLR4 in the whole EXP cohort, grouped based on BMI in normal weight (NW, full dots) and overweight (OW, empty dots) subjects. **(D)** Expression of NLRP3 and TLR4 in the EXP (orange dots) and CTRL (blue squares) cohorts, grouped based on BMI in normal weight (NW, full symbols) and overweight (OW, empty symbols) subjects. Asterisks indicated significant differences according to the different statistic tests applied: $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

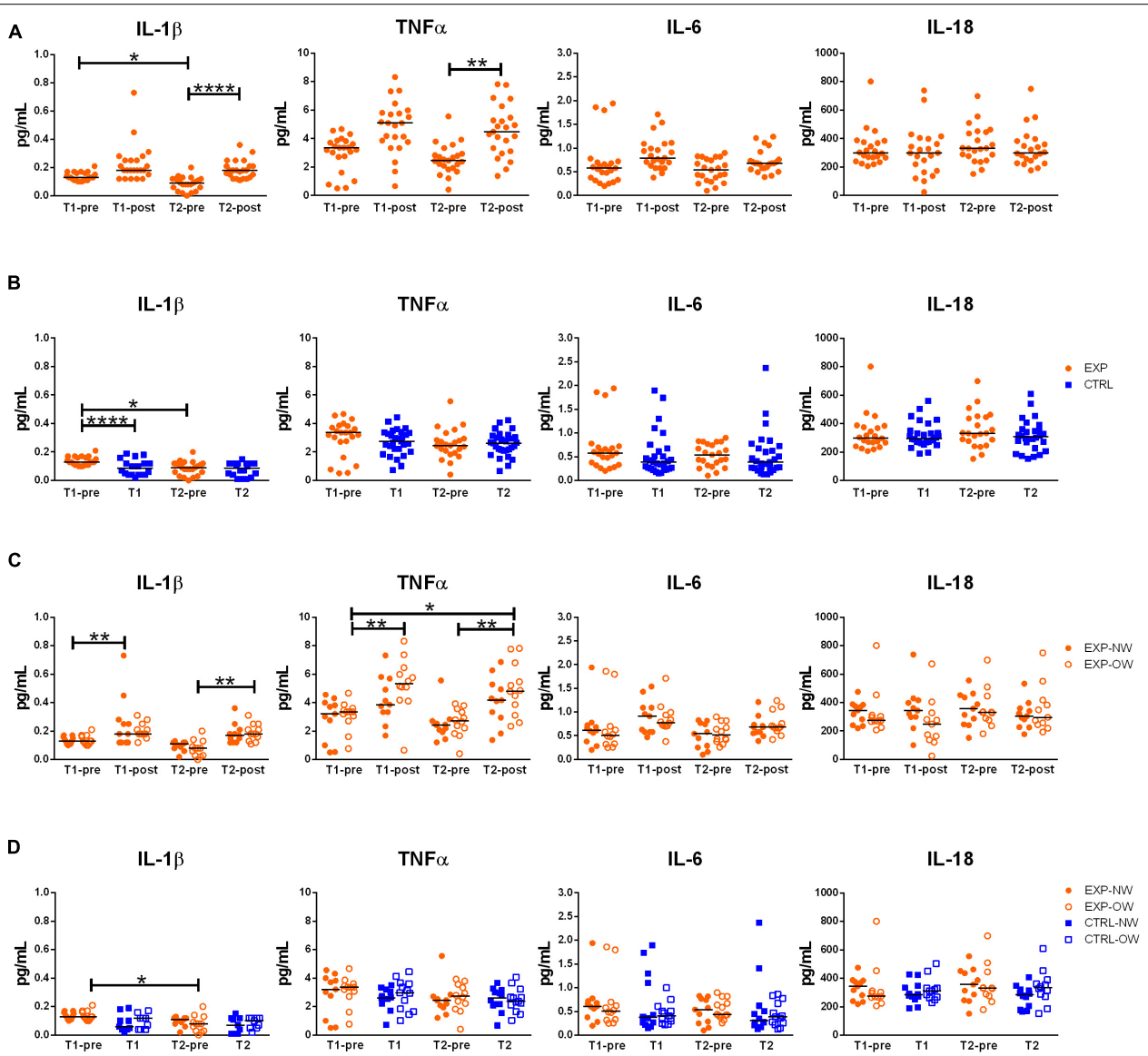

FIGURE 3 | Circulating levels of relevant cytokines associated to inflammasome pathway activation. Concentrations of IL-1β, TNFα, IL-6 and IL-18 in sera from elderly women underwent to a 12-week aerobic Nordic walking training program (EXP, $n = 29$) and untrained controls (CTRL, $n = 35$). **(A)** Circulating levels of the assayed cytokines in the whole EXP group before and after the first (T1-pre and T1-post) and the last (T2-pre and T2-post) sessions of Nordic walking. **(B)** Circulating levels of the assayed cytokines in the whole EXP group (orange dots), before the first and the last (T1-pre and T2-pre) Nordic walking session, and in the CTRL (blue squares) over the same observation period (T1, T2). **(C)** Circulating levels of the assayed cytokines in the EXP cohort, grouped based on BMI in normal weight (NW, full dots) and overweight (OW, empty dots) subjects. **(D)** Circulating levels of the assayed cytokines in the EXP (orange dots) and CTRL (blue squares) cohorts, grouped based on BMI in normal weight (NW, full symbols) and overweight (OW, empty symbols) subjects. Asterisks indicated significant differences according to the different statistic tests applied: $^{*}p < 0.05$; $^{**}p < 0.01$; $^{****}p < 0.0001$.

expression level of TLR4 resulted slightly higher, but significant, in EXP-NW than CTRL-NW at the beginning of the training (**Figures 2C,D**).

The results of the ANOVA tests and the related effect size are reported in **Supplementary Table 5**.

Effect of Training on the Release of Inflammasome- and Metabolic Inflammation-Related Cytokines

In order to determine the effect of the aerobic training program on inflammasome activation and inflammatory status, the circulating concentrations of related cytokines (IL-1β and IL-18, as markers of inflammasome activation, and TNFα and IL-6, as markers of metabolic inflammation) were determined. IL-18 levels neither were affected by the intervention in the EXP group, nor were different between EXP and CTRL groups, or between NW and OW cohorts (**Figure 3**). A single exercise bout elicited a rise in blood concentrations of the other cytokines, although not significant at the beginning of the training, but highly significant for IL-1β and TNFα at the end of the training (T2-pre vs. T2-post) [IL-1β: 0.090 pg/mL (0.000–0.200) pg/mL vs. 0.180 pg/mL (0.110–0.360) pg/mL; TNFα: 2.450 pg/mL (0.410–5.560) pg/mL vs. 4.490 pg/mL (1.380–7.820) pg/mL], while for IL-6, this increase is not significant (**Figure 3A**). No changes were detected between the CTRL and the EXP groups at the two time-points except for IL-1β that decreased at the beginning of the training (T1-pre vs. T1, **Figure 3B**).

When considering the EXP group divided between NW and OW sub-cohorts (**Figure 3C**), a single bout of exercise induced a general increase of IL-1β in both sub-cohorts, however, significant at the beginning of the training (T1-pre vs. T1-post) for NW [0.130 pg/mL (0.100–0.170) pg/mL vs. 0.180 pg/mL (0.120–0.730) pg/mL] and at the end of the training (T2-pre vs. T2-post) for OW [0.080 pg/mL (0.000–0.200) pg/mL vs. 0.180 pg/mL (0.110–0.310) pg/mL]. On the other hand, as effect of the chronic intervention (T1-pre vs. T2-pre), the cytokine decreased, though not significantly, in both sub-cohorts [NW: 0.130 pg/mL (0.100–0.170) pg/mL vs. 0.110 pg/mL (0.020–0.130) pg/mL; OW: 0.130 pg/mL (0.100–0.210) pg/mL vs. (0.080 pg/mL (0.000–0.200) pg/mL]. For TNFα, only the OW subjects were affected by a modulation of the cytokine, characterized by a strong increase after the first bout compared to baseline (T1-pre vs. T1-post) [3.360 pg/mL (0.770–4.670) pg/mL vs. 5.340 pg/mL (0.660–8.330) pg/mL], a decrease after the 12-week training (T1-pre vs. T2-pre) [2.740 pg/mL (0.410–3.930) pg/mL], and an increase after the last bout of exercise (T2-pre vs. T2-post) [4.830 pg/mL (2.350–7.820) pg/mL]. Additionally, an overall increase of TNFα was observed between the beginning (T1-pre) and the end of the training (T2-post). Even though IL-6 followed the same pattern of the two other cytokines, no differences were significant. Curiously, no differences were observed between the NW and OW cohorts (**Figure 3C**).

Finally, no differences were observed between EXP and CTRL groups within the stratification in NW and OW (**Figure 3D**).

The results of the ANOVA tests and the related effect size are reported in **Supplementary Table 5**.

DISCUSSION

Our study aimed at analyzing the behavior of inflammasome activation in post-menopausal women performing 12-week moderate-intensity aerobic Nordic walking training. Obtained findings reveal that Nordic walking training program is associated with a reduced expression of inflammasome components at rest and, importantly, the acquisition of a post-exercise pro-inflammatory response at the end of the training period, as indicated by the modulation of NLRP3 and TLR4 mRNA, and IL-1β and TNFα levels. Interestingly, when participants are grouped based on BMI it emerges that NLRP3 response is more pronounced in NW subjects than in OW. The downstream markers of inflammasome activation status, IL-1β, and of the inflammatory status, TNFα, showed a similar course: their level increases after the first bout of exercise, decreases after the 12-week training, and newly increases in response to the last exercise session. Noteworthy, in case of IL-1β, this modulation is significant for NW at the beginning of the training, whereas for OW at the end of the training; in case of TNFα, this time-dependent modulation was significant only in OW. This suggests a beneficial effect of Nordic walking in both NW and OW due to the reduction of inflammasome marker IL-1β. Importantly, vitamin D status, which is known to potentially affect the innate immunity response (Arababadi et al., 2018), was comparable among the EXP and CTRL groups and the relative sub-cohorts.

The importance of studying inflammasome resides in the fact that its activation may be the cause of (or may contribute to) the onset and development of several diseases (Guo et al., 2015) and particularly, since the involvement of NLRP3 inflammasome in age-associated chronic LGI, linked to the onset of several age-related diseases (Mejias-Pena et al., 2017). Aging may represent a key determinant of the responsivity to aerobic exercise. In a recent study, an 8-week Nordic walking training reduced the TLR4 and NLRP3 mRNA expression and circulating levels of IL-1β and IL-18 in young males (Khakroo Abkenar et al., 2019) while, in the present study, the most relevant result, is represented by the (re)acquisition of a post-acute exercise inflammatory response. In order to investigate whether the activation status of the inflammasome machinery, in response to aerobic activity in elderly women, was reflected into the circulation, mRNA expression level of NLRP3 and TLR4 was analyzed in whole blood. The choice of whole blood, as the assay matrix, was driven by the fact that inflammasome pathways can be activated in virtually all blood cells, but red blood cells (Tran et al., 2019), other than in other tissues. Therefore, whole blood expression of inflammasome markers may picture the integrated innate response potential against danger signals which may drive, in turn, LGI (Gomarasca et al., 2020). Gene expression analysis is completed by a normalization study to determine the best suitable reference genes in RT-qPCR analysis, in order to obtain the most reliable results (Mahoney et al., 2004; Faraldi et al., 2018).

Exercise training-dependent alteration of immune function is associated with the activation of several local and systemic responses. For instance, exercise activates purinergic signaling (ATP, ADP, adenosine, related receptors, and enzymes) (Moritz

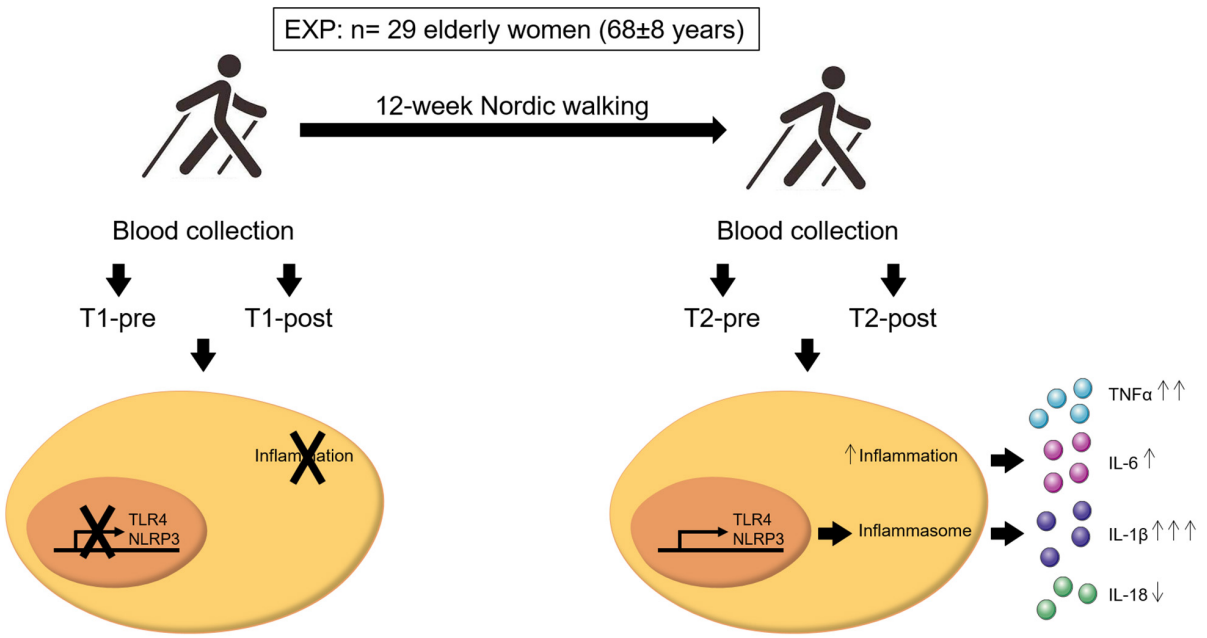

FIGURE 4 | Schematic representation of the important findings of this study. A population of 29 elderly women (age 68 ± 8 years old) completed 12 weeks of Nordic walking moderate-intensity aerobic training program (EXP). Blood samples were taken before and after the first (T1-pre and T1-post, respectively) and last (T2-pre and T2-post, respectively) exercise unit, and inflammasome- and inflammation-related markers were assessed. Aerobic training mildly affected the inflammasome-related inflammatory response. Participants acquired an acute inflammatory response to exercise, that was absent at baseline, that resulted in the activation of NLRP3 and TLR4 gene expression and the consequent release in the blood stream of the inflammasome- and inflammation-related cytokines IL-1β, IL-6, TNFα, and IL-18. In particular, a single bout of exercise at the end of the training induced a significant upregulation of IL-1β (↑↑↑) and TNFα (↑↑), a non-significant increase of IL-6 (↑), and a non-significant down-modulation of IL-18 (↓). The arrows next to the cytokines refer to the entity of the effect of the upregulation or downregulation of these cytokines.

et al., 2021). The purinergic system has relevance in inflammatory response and, particularly, in the shift from the pro-inflammatory response to acute, intense exercise to the anti-inflammatory response associated with chronic exercise (Cardoso et al., 2021). Exercise improves immune functions via the stimulation of the neuroendocrine secretion of catecholamines and the activation of their signaling (Simpson et al., 2021). Similarly, PA, together with diet, affects the composition of gut microbiota with profound effects on immune function, but also on muscle strength and dynamics (Strasser et al., 2021).

Many pieces of evidence have demonstrated that PA has inhibitory effects on inflammasome activation. This control may take place in different ways, indirectly by targeting pro-inflammatory compounds (fFA, ceramides) that are increased in LGI and aging (Ringseis et al., 2015) or, as it happens consequently to neuronal stimulation of myofibers, via the perturbation of plasma membrane integrity and potassium gradient across the membrane (Gaidt and Hornung, 2018), and directly by triggering TLRs expression and their downstream signaling. For instance, IL-18, the designated marker of NLRP3 inflammasome activation status was decreased by 43% in men and women with metabolic syndrome in response to a 12-week aerobic interval training program (three times a week) (Stensvold et al., 2012). Similarly, IL-18 was decreased in patients with metabolic syndrome undergone 12-week combined (endurance and strength) training program (three times a week) (Troseid et al., 2009), in T2DM subjects following a 6-month aerobic moderate-intensity exercise training program (four times/week) (Kadoglou et al., 2007a,b), and 8-week high-intensity exercise training on a rowing ergometer (three times/week), in obese subjects (Leick et al., 2007). However, similarly to our results, other authors failed in evidencing any putative improvement of the inflammatory status marked by IL-18. For instance, Christiansen et al. (2010) did not observe any decrease in circulating IL-18 concentrations in obese men and women following a 12-week aerobic exercise training program performed three times a week, possibly because of the relatively moderate intensity of the exercise. Also, RT has been shown to target innate immunity and inflammasome activation: in healthy elderly 8-week RT decreased the protein expression of TLR2 and TLR4 as well as the expression of several TLRs signaling-associated molecules (e.g., MyD88, TRIF, NF-κB, and MAPK) and plasma levels of the CRP (Rodriguez-Miguelez et al., 2014). On the contrary, Mejias-Pena et al. (2016) did not record any change in the expression of TLR2, TLR4, MyD88, and TRIF, in peripheral blood mononuclear cells (PBMCs) from older subjects after 8 weeks of aerobic exercise training, suggesting the possibility that the type of exercise might be a determinant of the TLRs-mediated anti-inflammatory effect of exercise. The lack of control for confounding variables, in the available studies, prevents a definitive elaboration on potential benefits of this interventional practice, according to a recently

published systematic review (Sanchez-Lastra et al., 2020). However, Nordic walking is a potentially beneficial exercise strategy for overweight and obese people. Based on the twelve good-to-fair quality selected studies, the authors of this study evidenced that subjects performing Nordic walking experienced significant improvement in parameters such as fasting plasma glucose, abdominal adiposity, and body fat compared with the values recorded at baseline, but no significant improvements were found when compared with control groups (Sanchez-Lastra et al., 2020). Notably, adiposity in the elderly may be associated with a deregulated glucose metabolism, and hence to an increased risk of T2DM, via a deregulation of immune cells and, specifically of CD8+ cytotoxic subsets (Bosslau et al., 2021).

In regards to the inflammasome activation status, our results revealed that the last bout of exercise determined an increase of both NLRP3 and TLR4 mRNA, indicating that, possibly, the ability to activate inflammasome is acquired during the training program, since such a response was absent after only one bout of exercise at the beginning of the training. This effect may be linked to the restoration of whole blood NLRP3/TLR4 mRNA expression at the end of the program, compared to the first sampling. As a consequence, these results demonstrated that although the circulating inflammatory profile may be fairly affected by the moderate-intensity aerobic training, the system alertness to endogenous and exogenous danger signals (TLR4 and NLRP3 mRNA) may be restored by the activity. Still, few limitations must be mentioned. The CTRL group, in our study, was not involved in any training program and, hence, the comparison was made between Nordic walking and sedentary lifestyle. Therefore, future investigations should aim at comparing the effect of different kinds of physical activities. This study lacks an objective and standardized method of assessment of the participants' effort, as well as of their baseline physical activity level [e.g., via the international physical activity questionnaire (IPAQ)] and thereby, it is not possible to define if the effects on inflammasome activation and inflammatory response may be related to the effort spent. Only females have been considered and for future research gender-dependent differences should also be considered. Other limitations are related to the eventual lack of comparison between subjects with different inflammatory statuses at the beginning of the training. This additional comparison would highlight the effect of Nordic Walking training on inflammation. Further, gene expression analysis of cytokines would have given additional information related to the inflammatory status of PBMCs while their circulating levels represent the net result of their systemic expression. Finally, body composition analysis would have a greater significance than anthropometrical measures in describing the cohorts and, eventually, their intervention-related changes. An important strength of this study is represented by the robust study of normalization, based on validated algorithms, applied to gene expression analysis: indeed, different normalization strategies may lead to different results and, for each experimental set, it is recommended to select the most appropriate method.

CONCLUSION

Despite a fair effect on the resting whole blood expression of inflammasome constituents (NLRP3 and TLR4) and circulating levels of the downstream inflammasome related cytokine IL-1β, a 12-week moderate-intensity aerobic training program (Nordic walking) allows the partial acquisition of the acute exercise-induced inflammatory response at the end of the training compared to the total absence of response observed at the beginning of the program (**Figure 4**). Specifically, a post-training acute exercise-induced response was recorded for NLRP3 and TLR4 expression, while IL-1β and TNFα changes were driven by the overweight participants.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://zenodo.org/, doi: 10.5281/zenodo.5789001.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Bioethical Committee of the Regional Medical Society in Gdansk. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

EZ and GL: conception. MG, KM, EZ, and GL: study design. KM and MFl: data acquisition. MG, KM, MFa, MFl, and SP: data analysis. MG, KM, MFa, EZ, and GL: data interpretation. MG, KM, MFa, and GL: manuscript drafting. MFl, SP, GB, and EZ: manuscript revision. MG, KM, MFa, MFl, SP, GB, EZ, and GL: final approval. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2022.792859/full#supplementary-material

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REVIEW
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Multimodal Benefits of Exercise in Patients With Multiple Sclerosis and COVID-19

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Multiple sclerosis (MS) is a demyelinating disease characterized by plaque formation and neuroinflammation. The plaques can present in various locations, causing a variety of clinical symptoms in patients with MS. Coronavirus disease-2019 (COVID-19) is also associated with systemic inflammation and a cytokine storm which can cause plaque formation in several areas of the brain. These concurring events could exacerbate the disease burden of MS. We review the neuro-invasive properties of SARS-CoV-2 and the possible pathways for the entry of the virus into the central nervous system (CNS). Complications due to this viral infection are similar to those occurring in patients with MS. Conditions related to MS which make patients more susceptible to viral infection include inflammatory status, blood-brain barrier (BBB) permeability, function of CNS cells, and plaque formation. There are also psychoneurological and mood disorders associated with both MS and COVID-19 infections. Finally, we discuss the effects of exercise on peripheral and central inflammation, BBB integrity, glia and neural cells, and remyelination. We conclude that moderate exercise training prior or after infection with SARS-CoV-2 can produce health benefits in patients with MS patients, including reduced mortality and improved physical and mental health of patients with MS.

Keywords: COVID-19, multiple sclerosis, blood-brain barrier, glia, physical exercise, myelin

Abbreviations: 4-HNE, 4-hydroxynonenan; ACE2, Angiotensin-converting enzyme 2; BBB, Blood-brain barrier; BDNF, Brain-derived neurotrophic factor; CB1, Cannabinoid type 1 receptor; CNS, Central nervous system; COVID-19, Coronavirus disease-19; CREB, cAMP response element-binding protein; CRP, C-reactive protein; GFAP, Glial fibrillary acidic protein; HCoVs, Human coronaviruses; HPA, Hypothalamic-pituitary-adrenal; IFN-ϒ, Interferon-gamma; IL-1ra, Interleukin-1 receptor antagonist; MERS-CoV, Middle east respiratory syndrome-related coronavirus; MMPs, Matrix metalloproteinases; MS, Multiple sclerosis; NMDA, N-methyl-D-aspartate receptor; Pgc-1α, Peroxisome proliferator-activated receptor gamma (PPAR-γ) coactivator 1-alpha; ROS, Reactive oxygen species; SIRT1, Silent mating type information training; TNF-α, Tumor necrosis factor-alpha; Tregs, T regulatory cells; VEGF, Vascular endothelial growth factor; WHO, World health organization.

INTRODUCTION

The coronavirus disease 2019 (COVID-19) pandemic that started in 2020 has caused death and disease throughout the world (Morley, 2020), leading to emergency measures such as extended locking-downs of communities, public places, stay at home requirements (Faulkner et al., 2020), and a disruption of daily life, leading to social insulation, and loneness (Berg-Weger and Morley, 2020). The SARS-CoV-2 virus can dissipate to the central nervous system (CNS) and culminates in delirium, depression and other mental, and psychological disorders due to the increased levels of inflammatory cytokines in the brain (Perez et al., 2020). SARS-CoV-2 is a neuroinvasive virus that not only triggers circulatory inflammation, but also affects various components of the CNS (Mao et al., 2020b; LiY-C and Hashikawa, 2020). Infection with COVID-19 leads to a disruption of the blood-brain barrier (BBB) disruption, increased reactivity of astrocytes (astrogliosis), microgliosis, myelin damage and demyelination, and neuronal loss and the formation of plaques (Gowrisankar and Clark, 2016; Ahmadirad and Ghasemi, 2020; Li L. et al., 2020; Calcagno et al., 2020; Desforges et al., 2020; Nordvig et al., 2021; Tavčar et al., 2021). The burden of infection with COVID-19 varies in different individuals, such that some individuals experience no signs of infection while others who suffer autoimmunity and inflammatory diseases such as multiple sclerosis (MS) may experience more intense symptoms of infection (Zindler and Zipp, 2010; Wilson et al., 2020).

Many features of MS and COVID-19 disease share similarities, including immune and BBB dysfunction, increased reactivity of brain residence cells (astrogliosis, microgliosis), demyelination and plaque formation, and finally neuronal loss (Thompson, 2000; Sofroniew, 2009; Brosnan, 2013; Rempe et al., 2016; Troletti et al., 2016). Inflammation in both diseases is associated with plaque formation (Minagar et al., 2002; Minagar and Alexander, 2003; Nordvig et al., 2021). The formation of plaques/lesions leads to neurological symptoms that result from neuronal loss and oxidative stress (Gilgun-Sherki et al., 2004; Kostic et al., 2013). Many patients with MS disease become less active and this inactivity leads to an accumulation of adipose tissue. Accrued adipose tissue triggers inflammatory conditions and plaque formation (Elenkov et al., 2005; Maes et al., 2009). Concurrent exposure of MS patients with the hyper-inflammation associated with coronavirus infection exposes individuals with MS at a higher risk of psychological issues and a more extensive neuropathology (Miller et al., 2009; Iwata et al., 2013; Rethorst et al., 2013; Gasmi et al., 2020).

The high transmissibility and dissemination of the coronavirus has led to a race to develop treatments for COVID-19 using inactivated/killed whole virus and convalescent plasma to improve immune responses (See et al., 2008; Dai and Gao, 2021). These vaccines can cause liver pathology and a robust response of the immune system especially in T cells and antibody titers in individuals with COVID-19 (Weingartl et al., 2004; Zhao et al., 2005; Shi et al., 2006; Ip et al., 2014; Al-Amri et al., 2017). Despite these complications, many patients with MS and who are infected with COVID-19 will receive vaccinations against COVID-19. It is not clear that how long the benefits of the COVID-19 vaccines will provide protection. Lifestyle changes, as proposed by the World Health Organization (WHO) (World Health Organization, 2004), involving increased daily physical activity is an alternate non-pharmacological procedure in the management of COVID-19 where engaging in physical activity or physical therapy could inhibit COVID-19 transmission (Sang et al., 2020) and also improve psychological, neurological, and physical health (Chekroud et al., 2018; Mücke et al., 2018; Schuch and Stubbs, 2019).

There is no molecular-based evidence regarding the effects of COVID-19 in individuals with MS. The goal of this review is to describe the effects of the coronavirus pandemic on patients with MS and the neuroprotective roles of exercise.

LITERATURE SEARCH STRATEGY

A comprehensive revision was performed using electronic databases including Medline, ISI Web of Knowledge, PubMed, Google Scholar, and Scopus on studies related to human and experimental subjects, from inception until February 2022. We included studies involved MS, COVID-19, and exercise and investigations on mechanisms, using the following key terms: "coronavirus or COVID-19", "MS disease or patients", "coronavirus and nervous system", "coronavirus pathways infecting central nervous system", "coronavirus and cytokine storm", "coronavirus and BBB disruption", "coronavirus and microglia activation", "coronavirus and astrocyte activation", "microglia and astrocytes in health and pathology", "coronavirus/MS and demyelination", "coronavirus and plaque/lesion formation", "coronavirus and neural loss", "MS and cytokine storm", "MS and inflammation", "MS and BBB permeability", "MS and plaque/lesion formation", "COVID-19/MS and mental or psychological or mood or anxiety or depression problems", "COVID-19 and loneliness", "COVID-19 and stress", "loneliness and stress", "COVID-19 and socio-psychological stress", "COVID-19/MS physical inactivity", "physical inactivity and mental/psychological/mood problems", "physical inactivity and inflammation", "inflammation and depression", "exercise adaptions in mental/mood/psychological/metabolic/central nervous system", "exercise and the changes in myokines", "exercise and immune system or inflammation", "exercise and monoamines and neurotransmitters", "exercise and BBB", "exercise and microglial/astrocytes changes", "exercise and neurotrophic/growth factors", "neurotrophic/growth factors and depression", "exercise and endocrine adaptations", "exercise and neurological/mental/mood disorders", "exercise and stress reduction", "exercise and endocannabinoids", "endocannabinoids and mood", "monoamines and psychological problems", "exercise and oxidative stress", "exercise and MS", "exercise during COVID-19", "exercise and opioids", "opioids and mood", "exercise and stress".

CORONAVIRUS AS A NEURO-INVASIVE VIRUS

The worldwide pandemic caused by COVID-19 led to a novel disease related to severe acute respiratory syndrome coronavirus-

2 infection (SARS-CoV-2) (Zhu N. et al., 2020; Zhou et al., 2020). SARS-CoV-2 is beta-coronavirus that is closely associated with the SARS-CoV virus dissipated in 2002–2004 (Zhou et al., 2020). Human coronaviruses (HCoVs) were initially categorized into seven strains 1) SARS-CoV, 2) Middle East Respiratory Syndrome-related Coronavirus (MERS-CoV), 3) HCoV-229E, 4) HCoV-OC43, 5) HCoV-NL63, 6) HCoV-HKU1, and 7) the novel SARS-CoV-2 (Bohmwald et al., 2018). The genome of HCoVs encodes proteins and glycoproteins such as the spike glycoprotein, membrane glycoprotein, envelope glycoprotein, nucleocapsid protein, RNA polymerase and some genes for accessory proteins (Cheng Q. et al., 2020). Three (MERS-CoV, SARS-CoV, and SARS-CoV-2) out of the seven HCoVs cause severe respiratory illness with high incidence and mortality rates (Cheng Q. et al., 2020). SARS-CoV-2 shares 79 and 50% genomic similarities to SARS-CoV and MERS-CoV, respectively (Lu et al., 2020). The respiratory tract is the main target tissue for HCoVs (Abdelaziz and Waffa, 2020), though 5% of patients that need ventilatory support (Day, 2020) have a 40–50% mortality rate (Zhu J. et al., 2020; Weiss and Murdoch, 2020). Mortality rates due to COVID-19 are high in the elderly, likely due to the presence of comorbidities such as cardiovascular diseases, smoking, lung disease, obesity and diabetes (Zhu N. et al., 2020; Richardson et al., 2020), although fatal outcomes can also occur in otherwise healthy younger patients but with high viral loads (Chen et al., 2020). Common clinical features associated with infection with COVID-19 include fever, sore throat, dry cough, shortness of breath, and sometimes reproductive system dysfunction (Zhu J. et al., 2020; Richardson et al., 2020; Maleki and Tartibian, 2021).

SARS-CoV-2 infects neuronal cells of the CNS and peripheral nervous system (Mao et al., 2020b; Li et al., 2020c). Animal and post-mortem analysis indicates the thalamus, brainstem, cerebrum, hypothalamus, and cortex are the most infected areas (Gu et al., 2005; McCray et al., 2007; Netland et al., 2008). Neurological presentations such as headache, impaired consciousness, cognitive deficits, dizziness, acute ischemic stroke, intracerebral hemorrhage, nausea, and vomiting, anosmia, hypogeusia are frequent signs of neurovirulent infections with SARS-CoV-2 (Li et al., 2020b; Mao et al., 2020b). Brain edema and partial neurodegeneration have been identified in autopsies of COVID-19 patients (Ahmadirad and Ghasemi, 2020). These neurological signs occur in 88% patients with severe infections of COVID-19 (Mao et al., 2020b), while other reports suggest that one third of patients have neurological symptoms 2–3 weeks after infection with the virus (Mao et al., 2020a; Mao et al., 2020b). The brain is affected by SARS-CoV-2 infection after changes are observed in the respiratory tract, although this is not always the case (Filatov et al., 2020).

SARS-CoV-2 binds to angiotensin-converting enzyme 2 (ACE2) receptors that are primarily expressed on airway epithelial cells, lung parenchyma, vascular endothelial cells, renal cells, and cells in the small intestine (Kuhn et al., 2004; Lu et al., 2013; Qian et al., 2013; Zhu Y. et al., 2020), although this receptor is not expressed in brain tissue (Wei et al., 2020). The presence of ACE2 receptors is not sufficient to predispose host cells to CoV-induced infection (Gandhi et al., 2020). There are several mechanisms by which SARS-CoV-2 virus can enter the CNS and consequently damage neurons, including the trans-neuronal route which infects sensory and motor nerve terminals, especially the olfactory nerves in the nasal cavity, and subsequent entry to the CNS through retrograde transportation via motor proteins (Swanson and Mcgavern, 2015). The virus can also access respiratory and cardiovascular centers in the brainstem during the early stages of the disease and lead to respiratory failure, inflammation and demyelination reactions through plaque formation (Netland et al., 2008; Mori, 2015; Desforges et al., 2020; Steardo et al., 2020). The neuroinvasive nature of infection with COVID-19 was shown in intranasal swabs from mice infected with SARS-CoV where the virus reached the thalamus and brainstem areas via olfactory nerves (Kumari et al., 2021). The anosmia frequently reported by patients infected with COVID-19 provides additional evidence for an important role for viral entry into the CNS via olfactory nerves (Gandhi et al., 2020).

A second pathway for the entry of the virus is the hematogenous pathway which allows for viral entry of most HCoV strains by infecting peripheral monocytes, T lymphocytes, and macrophages (Wan et al., 2021). The infected immune cells can cross the BBB of the ventricular choroid plexus through transcytosis (Gu et al., 2005; Chan et al., 2013; Desforges et al., 2014; Calcagno et al., 2020). A third pathway for viral entry into the CNS involves the microvascular endothelial cells of the BBB (Ahmadirad and Ghasemi, 2020). Endothelial cells of the BBB express receptors for SARS-CoV such as ACE2 and CD209L (Li et al., 2007). Glial cells and neurons in the brainstem, sub-fornical organ, paraventricular nucleus, nucleus tractus solitarius, rostral ventrolateral medulla potentially also express ACE2 receptor for SARS-CoV-2 (Xia and Lazartigues, 2010; Gowrisankar and Clark, 2016). Viral spike glycoproteins interact with ACE2 receptors on the surface of capillary endothelial cells to disrupt the integrity of the BBB (Baig et al., 2020). Infection with the SARS-CoV-2 increases the permeability of the BBB as a result of a cytokine storm due to the direct cytopathic effects of the virus (Calcagno et al., 2020; Nordvig et al., 2021), suggesting that ACE inhibitors could be a treatment option in some infected patients with hypertension and/or diabetes (Yan et al., 2020). The interaction of SARS-CoV-2 with ACE2 receptors on vascular endothelial cells can increase blood pressure, disrupt the BBB or lead to intracerebral hemorrhage (Calcagno et al., 2020). Stimulation of the immune system by SARS-CoV-2 can compromise BBB integrity through two main mechanisms: downregulation of tight-junction proteins (Desforges et al., 2020) and activation of resident glial cells, particularly astrocytes, in the CNS (Arbour et al., 2000).

Peripheral T lymphocytes, neutrophils, natural killer cells, and monocyte/macrophages secrete matrix metalloproteinases (MMPs) to increase BBB permeability by downregulating tight junction proteins such as occludin and cluadin-5 (Nordvig et al., 2021). Hyper-inflammation induced by SARS-CoV-2 stimulates astrocytes (Calcagno et al., 2020), a highly heterogeneous group of cells with plasticity in form and function. Astrocytes regulate homeostasis in the CNS by transporting ions and protons,

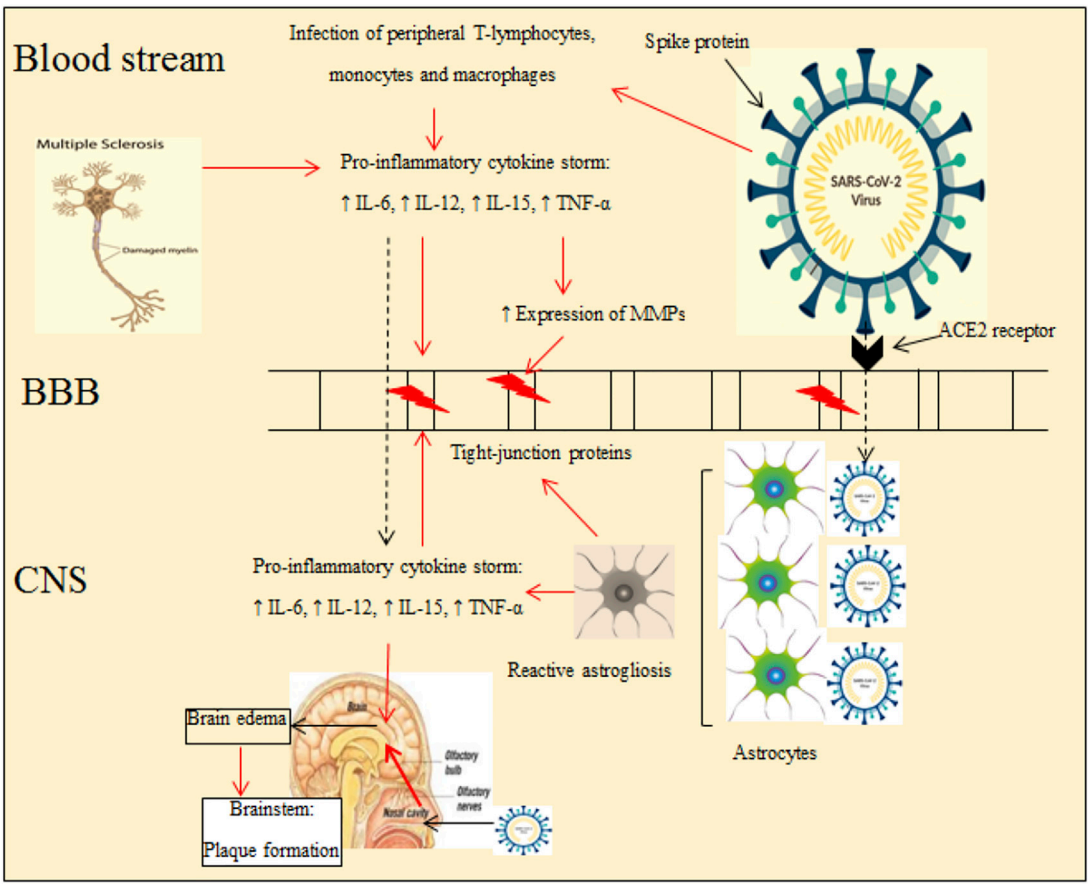

FIGURE 1 | Neuro-invasive mechanisms of SARS-CoV-2 and common effects of COVID-19 and MS diseases on brain and its components. Hyper-inflammation induced by MS and coronavirus increases BBB permeability and plaque formation in the brainstem. Reactivation of astrocytes induced by pro-inflammatory cytokines also impacts the BBB. The red arrows indicate the detrimental effects of inflammation produced by both MS and SARS-CoV- 2 (ACE2, angiotensin-converting enzyme 2; MMPs, matrix metalloproteinases; MS, multiple sclerosis; CNS, central nervous system; BBB, blood-brain barrier).

eliminating and catabolizing neurotransmitters, scavenging reactive oxygen species (ROS) and providing neurons with energy substrates. Astrocytes maintain the cytoarchitecture by connecting their end-feet to the vasculature to regulate BBB permeability (Verkhratsky and Nedergaard, 2018; Verkhratsky and Zorec, 2019). Astrocytes are targeted by CoVs through binding to ACE2 and Toll-like receptors expressed on astroglial cells (Calcagno et al., 2020; Li et al., 2020a). The interaction of astrocytes and microglia with pathogens such as SARS-CoV-2 leads to reactive astrogliosis and microgliosis (**Figures 1**, **2**) (Chen et al., 2021; Tavčar et al., 2021). Reactive astrogliosis is defined by changes morphology (hypertrophy) and increased gene expression, such as glial fibrillary acidic protein (GFAP) (Ben Haim et al., 2015), which modulates the stability of astrocytes and the severity of brain injury (Trautz et al., 2019). Morphological alterations of these glial cells are congruent with the loss of physical connections between reactive astrocyte and endothelial cells and are associated with increased BBB permeability (Chupel et al., 2018). Additionally, astrogliosis increases the production of pro-inflammatory cytokines, chemokines and other inflammatory signals that disrupt the BBB and consequently promotes neuronal inflammation (Wang et al., 2020). Patients infected with COVID-19 experience damage to both white and gray matter and have demyelinating lesions (plaques) throughout the brain, including the cerebellum, brainstem, and spinal cord (Arbour et al., 2000; Yeh et al., 2004; Algahtani et al., 2016; Li et al., 2016; Morfopoulou et al., 2016) (**Figure 1**).

Collectively, SARS-CoV-2 is a neuroinvasive virus that enters the CNS and leads to inflammation, changes in glial cells BBB permeability, plaque formation and demyelination (Yachou et al., 2020).

SUSCEPTIBILITY OF PATIENTS WITH MS TO CORONAVIRUS INFECTION

Disruption of the BBB leads to inflammation in both patients with MS and in experimental autoimmune encephalomyelitis (EAE), an animal model of MS (Brosnan, 2013; Troletti et al., 2016; Razi et al., 2022a). Several agents modulate BBB permeability in MS/EAE, including adhesion molecules, ROS,

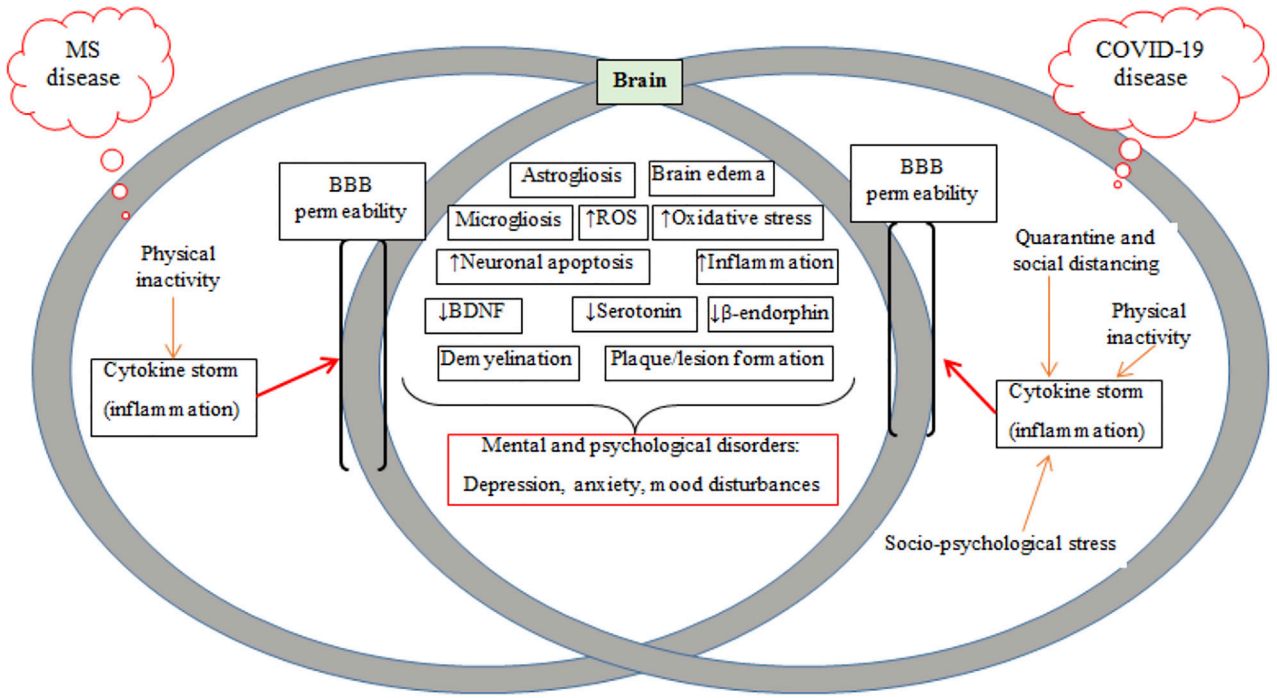

FIGURE 2 | Schematic of mechanisms common in MS and COVID-19 diseases, including BBB breakdown/permeability and mental and psychological disorders. MS, multiple sclerosis; BBB, blood-brain barrier; ROS, reactive oxygen species; BDNF, brain-derived neurotrophic factor.

pro-inflammatory cytokines such as IL-1β, interferon-gamma (IFN-ϒ), and tumor necrosis factor-alpha (TNF-α) (Abbott et al., 2006; Alvarez et al., 2011; Baeten and Akassoglou, 2011). Partial dysregulation of tight junction proteins are mediated by inflammatory immune mechanisms and the activation of MMPs by resident activated glial cells and pro-inflammatory immune cells (Rempe et al., 2016). Additionally, astrocytes are the first glial cells to respond to immune insults (Sofroniew, 2009).

Plaque/lesion progression is a hallmark of MS pathology. Oligodendrocytes, as glial cells forming myelin sheaths around axons involved in conducting neuronal impulses, are targeted by the immune system in acute lesions in patients with MS (Thompson, 2000). Although the origin of the disease is unknown, it is assumed that there is an interaction between receptors on T cells with myelin antigens present on major histocompatibility complex (MHC) class II molecules, which are expressed on macrophages/microglia and astrocytes (Minagar and Alexander, 2003). Activated lymphocytes and macrophages cross the BBB and interact with resident cells in the CNS such as microglia and astrocytes (Smith et al., 1998) (**Figure 1**). Pro-inflammatory cytokines activate microglia and increase quinolinic acid levels in the CNS and also stimulate apoptosis (**Figure 2**) (Myint et al., 2007; McNally et al., 2008). Astroglial loss in MS patients has several detrimental consequences on brain health, including decreased re-uptake of glutamate from the synaptic cleft to induce neuronal excitotoxicity, decreased metabolic support via lactate production, and increased oxidative stress through the loss of astrocyte produced antioxidants (Phillips and Fahimi, 2018). Additionally, quinolinic acid acts on ionotropic N-methyl-D-aspartate receptor (NMDA) and glutamate receptors to inhibit the upregulation of neurotrophic factor like brain-derived neurotrophic factor (BDNF) mediated by cAMP response element-binding protein (CREB) (Hardingham et al., 2002; Santana-Martínez et al., 2019). Inflammatory mediators such as pro-inflammatory cytokines (TNF-α, IFN-ϒ) and chemokines are released by proliferating lymphocytes (Fujiwara and Kobayashi, 2005). The release of these mediators leads to an imbalance between pro- and anti-inflammatory (IL-4 and IL-10) cytokines in favor of inflammatory mechanisms (Minagar and Alexander, 2003; Raivich and Banati, 2004). Thus, MS is likely also associated with cytokine storms (Link, 1998). Myelin is injured by toxic substances such as oxygen and nitrogen free radicals produced by an auto-activated immune system and myelin then is phagocytized by macrophages and microglia (Smith et al., 1998; Rus et al., 2006). Injured myelin disrupts the axonal conduction and gradually leads to axonal loss and lesion or plaque formation (Van Asseldonk et al., 2003; Waxman, 2006). Patients in the early phases of MS experience functional disorders derived from produced hyper-inflammation (**Figure 2**) (Minagar et al., 2002; Minagar and Alexander, 2003), but the clinical symptoms observed later derive from degenerative changes or lesions (Chang et al., 2002).

The formation of lesions or plaques is a hallmark in the pathology of MS, with imaging of lesions confirmed by MRI technology used to diagnose the severity of MS (Ge, 2006). With

respect to the diagnosis of relapsing-remitting (RR) or progressive MS, the lesions in white and gray matter have been categorized into two general groups: inflammatory and degenerative lesions (although they are sometimes also classified as acute and chronic lesions) that are heterogeneous in size and shape (Barkhof et al., 1992). Collectively, the lesions are distributed in most regions of the brain such as periventricular and sub-cortical regions, corpus callosum, brainstem, and optic nerves (Paolillo et al., 1997). These underlying inflammatory and demyelinating conditions can predispose patients with MS to more severe neurological changes and challenges following COVID-19 infection, as this virus causes symptoms that are also present in MS (Naucler et al., 1996; Ahlqvist et al., 2005; Donati et al., 2005). Inhibiting the migration of oligodendrocyte precursor cells to demyelinated sites by SARS-CoV-2 may be another mechanism for exacerbating neuronal damage in MS following infection with COVID-19 (Kong et al., 2003; Campbell et al., 2017). Some currently used vaccines could be harmful in some patients with autoimmunity such as MS, since the most of these vaccines contain the S1 subunit of spike protein of the SARS-CoV-2 in an inactivated form (Dai and Gao, 2020; Ong et al., 2020; Biström et al., 2021), which can activate systemic and central inflammation (Rhea et al., 2021). Importantly, SARS-CoV-2 can cause MS plaques in the brainstem to extend to other regions controlling the respiratory system (Berger et al., 2020; Li et al., 2020b).

THE CONCURRENT EFFECTS OF COVID-19 AND MS ON MENTAL AND PSYCHOLOGICAL FACTORS

Common strategies to combat viral infections include quarantine and social isolation, which can cause mental and psychological disorders including acute and chronic stress, anxiety and depression (Garcovich et al., 2020; Hwang et al., 2020; Pan et al., 2020; Wilkialis et al., 2021). These countermeasures are associated with sedentary behaviors that decrease physical exercise (Booth et al., 2000; Lightfoot, 2011; Gasmi et al., 2020). Neurological diseases such as MS also decrease physical activity (Lee et al., 2003; Barnes and Yaffe, 2011). Physical inactivity and socio-psychological stress lead to inflammatory conditions with oxidative damage to lipids, proteins and DNA in the brain (Liu et al., 1996; Radak et al., 2001). Chronic stress associated with the coronavirus pandemic produces physiological changes such as increased release of cytokines, cortisol and catecholamines, and can lead to depression (Raison and Miller, 2003; Iwata et al., 2013; Mariotti, 2015; Dos Santos, 2020; Ramezani et al., 2020; Mohammadkhanizadeh and Nikbakht, 2021).

As many as 60% of patients with MS suffer with depression and anxiety (Bakshi et al., 2000; Grytten et al., 2016). There are several mechanisms that can culminate in depression in patients with MS and SARS-CoV-2 infection (Machado and Gutierrez, 2020). The stress response is largely regulated by the hypothalamic-pituitary-adrenal (HPA) axis (which releases cortisol) and the sympathetic nervous system (which releases epinephrine and norepinephrine) (Silverman and Deuster, 2014). The initial response to stress is characterized with increases in catecholamine secretion and is associated with the trafficking leukocytes from the spleen to the circulation (Silverman and Deuster, 2014). Increased cortisol levels induced by chronic stress reduces immune function (McEwen et al., 1997; Sapolsky et al., 2000; Dhabhar, 2009), increasing the susceptibility of patients with MS to infection (Chaudhry et al., 2021; Zabalza et al., 2021). High levels of cortisol and inflammatory cytokines in both MS and COVID-19 downregulate the protective effects of BDNF against neuronal cell apoptosis (Zhao et al., 2007; Kudielka et al., 2009; Tollenaar et al., 2009). Peripheral and central BDNF levels are reduced in MS (Connor et al., 1997; Baker et al., 2010; Laske et al., 2010; Pereira et al., 2013), and are associated with signs of depression and anxiety due to reduced release of synaptic proteins and neurotransmitters (monoamines and opioids) (Egan et al., 2003; Hariri et al., 2003; Vaynman et al., 2006). Increased neuronal apoptosis in the brain causes neuronal loss and reduces the number of synapses (Glantz et al., 2006; Zhou et al., 2019). Neuronal excitotoxicity produced by high glutamate concentrations in patients with MS could be another mechanism to downregulate BDNF by inhibiting CREB binding to DNA (Zou and Crews, 2006; Kostic et al., 2013). Oxidative stress is important in the pathogenesis of neuro-inflammatory diseases such as MS (LeVine, 1992; Sayre et al., 2008). Although multiple factors contribute in oxidative stress, including glutamate induced activation of ionotropic receptors (Gilgun-Sherki et al., 2004). Oxidative stress causes damage to DNA that further increases apoptosis and neurodegeneration (Bohr et al., 1998; Schmitz et al., 1999).

Inflammation and depression is common in patients with MS and those infected with COVID-19, (**Figure 2**) (Möller et al., 2013; Phillips and Fahimi, 2018). Increased pro-inflammatory markers [IL-6, IL-1β, TNF-α, and C-reactive protein (CRP)] occur in individuals with depression (Laske et al., 2008; Steiner et al., 2012), supported by reports of anti-depressant effects of TNF-α antagonists (Soczynska et al., 2009; Fond et al., 2014; Abbott et al., 2015). Pro-inflammatory cytokines activate the kynurenine pathway to form quinolinic acid or kynorenic acid; quinolinic acid activates NMDA receptors and inhibits the upregulation of *Bdnf* gene by blocking CREB function (Hardingham et al., 2002). Downregulation of BDNF expression reduces neurogenesis and neurotransmission (Galic et al., 2012; Calabrese et al., 2014).

Pro-inflammatory cytokines activate microglial cells in the CNS (McCusker and Kelley, 2013), which then intensifies inflammatory activation by secreting other pro-inflammatory cytokines such as IL-1β, TNF-α, IL-6, and IFN-γ (Smith et al., 2012). This excessive production of pro-inflammatory cytokines increases the firing rates of adrenergic neurons that inhibit beta-endorphin neurons (Rivier, 1995; Boyadjieva et al., 1997; Borsody and Weiss, 2002). This can lead to mood changes in MS patients with COVID-19 infection as mood is regulated by endorphin levels (**Figure 2**) (Fichna et al., 2007). Two other mechanisms whereby patients with MS and infected with COVID-19 can experience alterations in mood, depression and anxiety are increased activation of inhibitory GABAergic neurons that

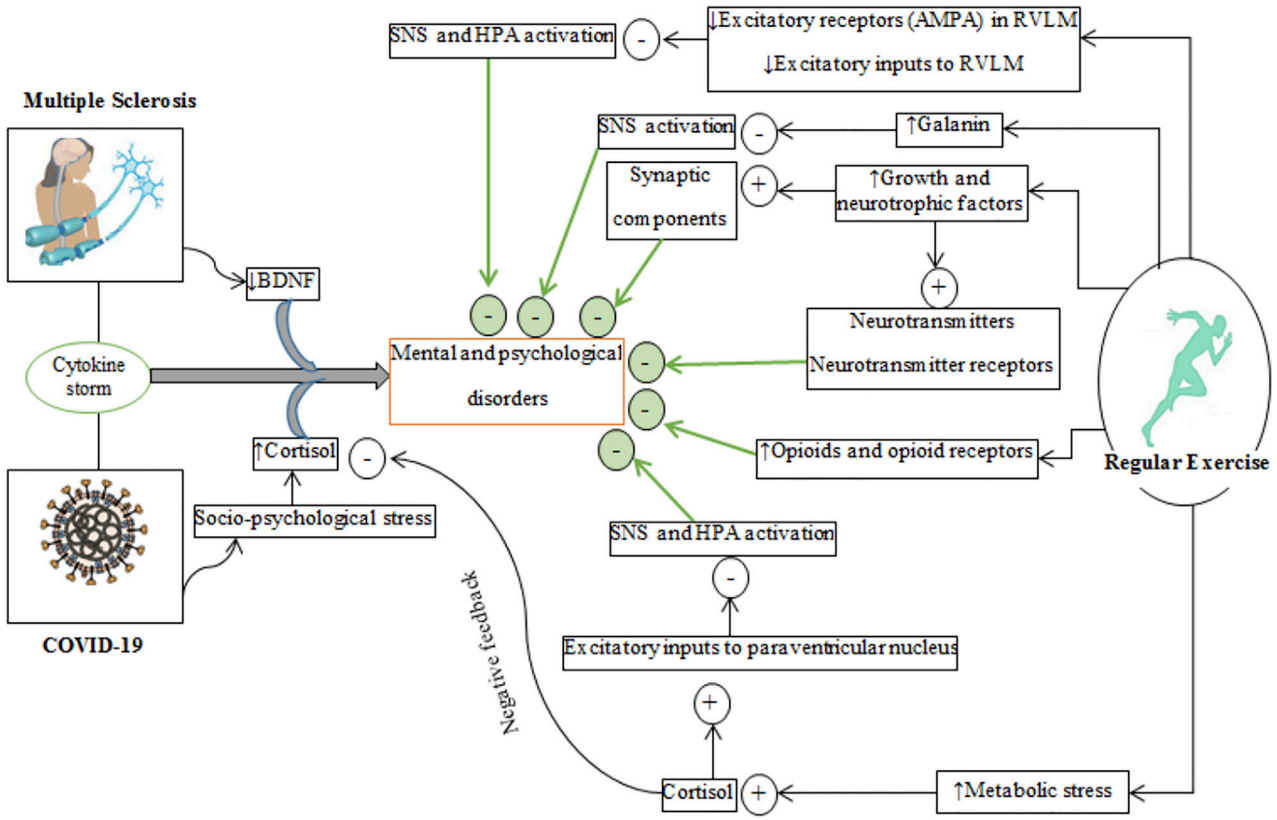

FIGURE 3 | Schematic representation of converged pathways associated with mental and psychological disorders at (left side) and regular exercise that inhibit these disorders (right side) in multiple sclerosis and COVID-19. Plus (+) marks represent the incremental effects of exercise while minus (–) marks represent decreased effects of exercise. The effects of regular exercise are shown by thick green arrows. HPA, hypothalamus-pituitary-adrenal axis; SNS, sympathetic nervous system; BDNF, brain-derived neurotrophic factor; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; RVLM, rostral ventrolateral medulla.

reduces firing rate of serotonergic neurons (Manfridi et al., 2003; Brambilla et al., 2007), and reduced activation of serotonin transporters that lower serotonin levels in nerve terminals (Haase and Brown, 2015). Importantly, central inflammation can cause neural apoptosis in some brain areas regulating these psychological states is also related to mitochondrial dysfunction (Cotman and Berchtold, 2002; Butterfield et al., 2014; Kiliaan et al., 2014).

In summary, increased levels of pro-inflammatory cytokines change neuroendocrine function, neurotransmitter metabolism and neuroplasticity to cause detrimental effects on psychological states (Elenkov et al., 2005; Maes et al., 2009; Miller et al., 2009; Rethorst et al., 2013). Individuals with clinical depression and anxiety disorders experience changes in mood, energy loss, and reduced exercise levels (Dinas et al., 2011).

EXERCISE TRAINING IN MS AND COVID INFECTION

Regular physical exercise improves cardiovascular/aerobic capacity and brain health (Colcombe and Kramer, 2003; Etnier et al., 2006). Exercise causes adaptations to organs such as the liver, skeletal muscles, adipose tissue, and brain at molecular and cellular levels (Gomez Pinilla, 2006; Rasmussen et al., 2009; Di Liegro et al., 2019). Exercise exerts its beneficial effects on the brain by producing neurotrophic, growth and myokine factors and also by altering neurotransmission, improving BBB integrity, increasing remyelination and improving the immune system (refer to **Figures 3–6**) (Lin and Kuo, 2013; Phillips et al., 2014).

EXERCISE TRAINING AND MENTAL AND PSYCHOLOGICAL HEALTH

Exercise is often recommended in the management of chronic diseases such as neurological, endocrine, psychological, and mental diseases (Weyerer and Kupfer, 1994; Brandt and Pedersen, 2010; Carek et al., 2011; Pedersen and Saltin, 2015; Baek, 2016; Spielman et al., 2016; Smith and Merwin, 2021). The beneficial effects of exercise on mental health markers involve reduced stress, anxiety and depression (**Figure 3**), increased neurotrophic and growth factors, increased neurotransmitters and synaptogenesis, elevated endogenous opioid levels markers, and changes in the expression and affinity of some central monoamine receptors (**Figure 3**) (Lin and Kuo, 2013; Phillips and Fahimi, 2018).

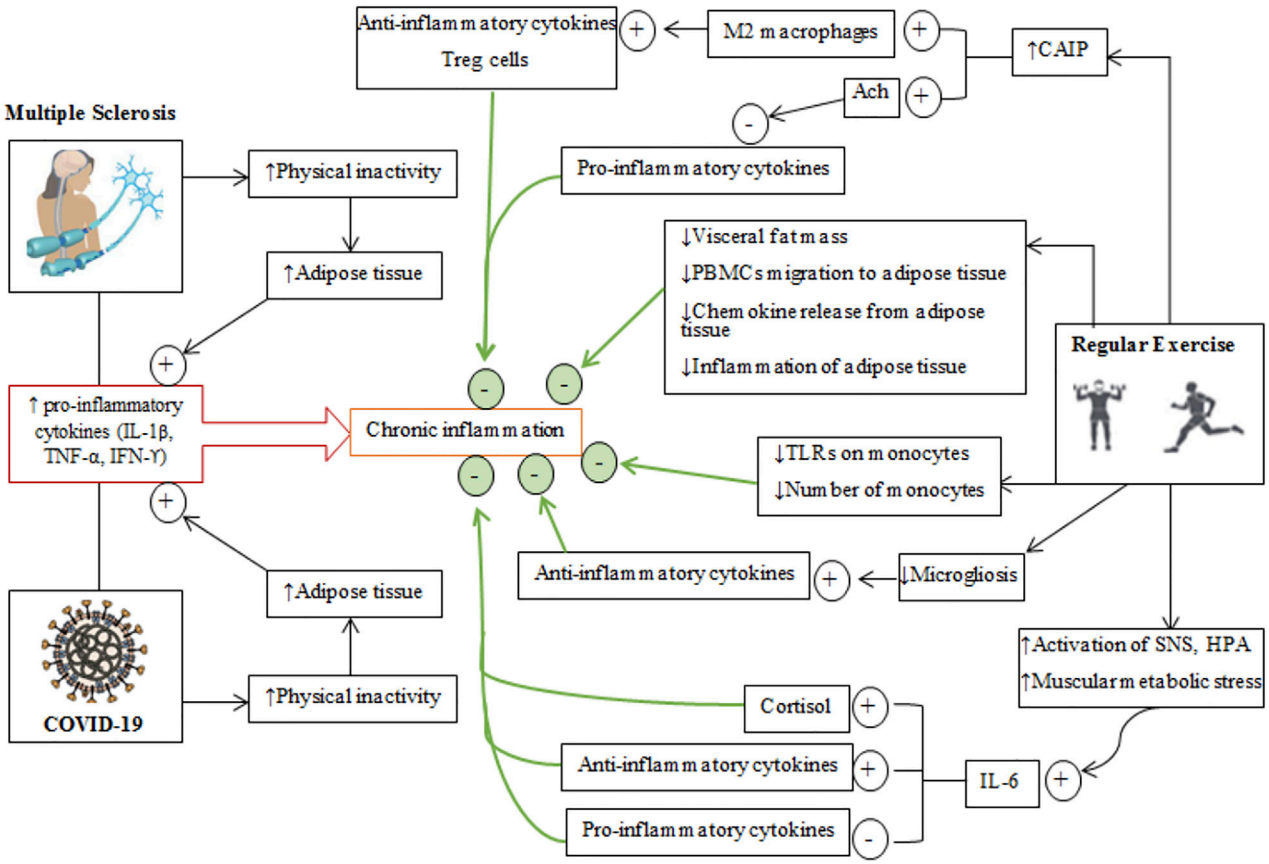

FIGURE 4 | Schematic depiction of the effects of regular exercise on inflammatory conditions in multiple sclerosis and COVID-19 diseases. The generally common and different pathways through which MS and COVID-19 cause an inflammatory condition are shown on the left while isolated effects of regular exercise that regulate/modulate inflammation are shown on the right. Plus (+) signs represent the incremental effects of exercise, while minus (–) signs represent decreased effects of exercise. The cumulative effects of regular exercise are shown by the thick green arrows and green circles. Treg cells, T regulatory cells; PBMCs, peripheral blood mononuclear cell; CAIP, cholinergic anti-inflammatory pathway; SNS, sympathetic nervous system; ACh, acetyl choline; TLRs, toll-like receptors.

Trained males and females have lower responses of cortisol and heart rate related to socio-psychological stress (Rimmele et al., 2009), and as a consequence have lower anxiety and improved mood when encountering stressful conditions such as the COVID-19 pandemic (Silverman and Deuster, 2014). Physical exercise in sedentary individuals generally reduces HPA and sympathetic nervous system activity (Zschucke et al., 2015), which can improve lower stress levels such as during the COVID-19 pandemic (**Figure 3**) (Luger et al., 1988; Deuster et al., 1989), primarily by: 1) secreting cortisol due to metabolic exercise stress (Webb et al., 2013) added to socio-psychological stress (e.g., induced by COVID-19) for igniting negative feedback which negatively regulates hypothalamus and pituitary glands (Basso and Suzuki, 2017), 2) reduced excitatory input from the cardiovascular system to the rostral ventrolateral medulla (which regulates sympathetic activity) inhibits the sympathetic nervous system and lowers peripheral levels of cortisol (Mischel et al., 2015). Exercise training downregulates the number of AMPA (GluR3 subunit) receptors in the rostral ventrolateral medulla area (Subramanian et al., 2014), 3) increased cortisol levels induced by exercise influences central levels of endocannabinoids, which can reduce/inhibit excitatory inputs to the paraventricular nucleus and as a consequence inactivates the HPA axis and sympathetic activation (Ziegler et al., 2010), and 4) upregulation of β-endorphin (Yermal et al., 2010). The nuclei of neurons producing β-endorphin are located in the arcuate nuclei of hypothalamus, and their terminals are distributed throughout the CNS including the hypothalamic paraventricular nucleus which inhibits corticotropin-releasing hormone secretion (Sarkar and Zhang, 2013), and 5) increased galanin expression in the locus coeruleus, which suppresses the release of norepinephrine in the locus coeruleus (Voss et al., 2011; Lin and Kuo, 2013; Phillips and Fahimi, 2018). Experiments in animals (Murray et al., 2010) and humans (Legakis et al., 2000) demonstrate that exercise upregulates galanin expression in the locus coeruleus. Thus, exercise buffers the effects of stress from socio-psychological events such as the coronavirus pandemic and other behaviors causing anxiety (Brailovskaia et al., 2021).

Growth and neurotrophic factors have functional interactions, such that increases in one influence the release of the other in the brain (Deyama et al., 2019). Neurotrophic and growth factors mediate the beneficial effects of exercise on neurons,

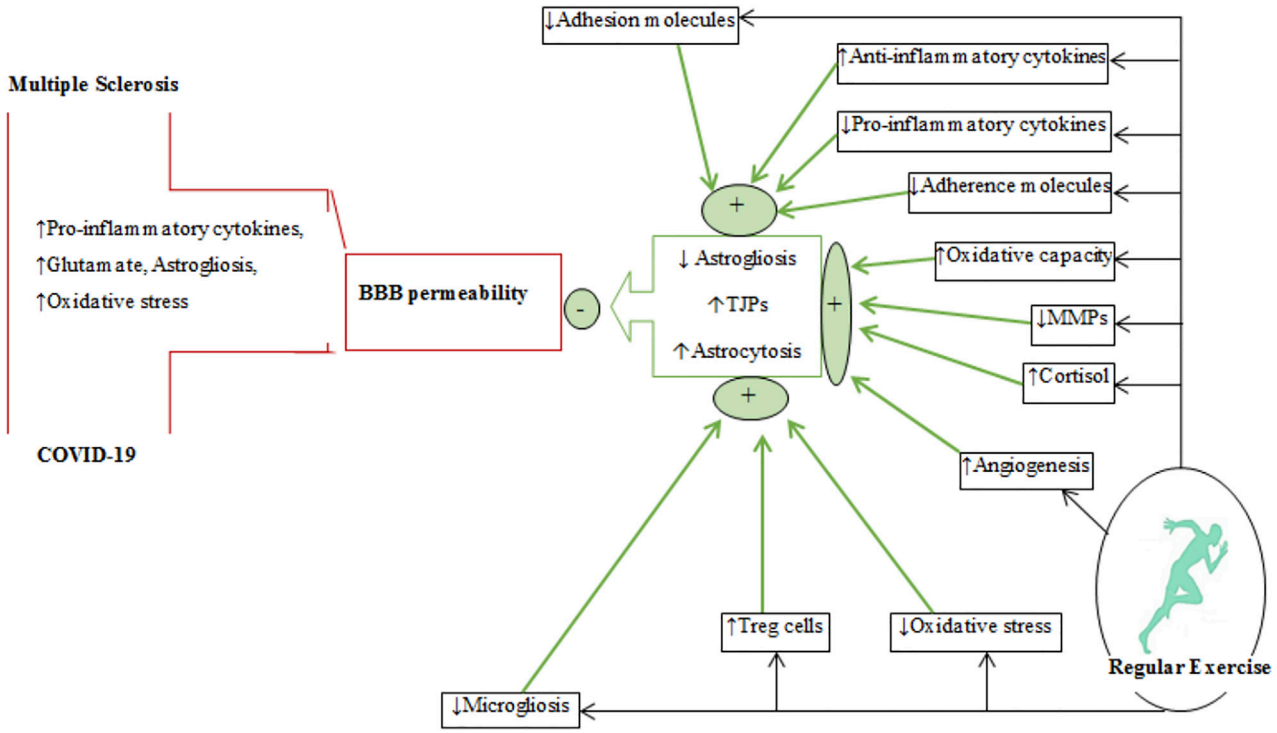

FIGURE 5 | A schematic diagram depicting the effects regular exercise on BBB permeability in multiple sclerosis and COVID-19. The plus (+) and minus (–) signs represent increases and decreases, respectively. The effects of regular exercise are shown by thick green arrows. MMPs, matrix metalloproteinases; Treg, T regulatory cells; TJPs, tight-junction proteins; BBB, blood-brain barrier.

vascularization and neurotransmission (Basso and Suzuki, 2017; Delezie and Handschin, 2018). Thus, exercise training improves psychological, mood and mental health partly through the release of neurotrophic and growth factors (Cotman and Berchtold, 2002; Kandola et al., 2019). Central and peripheral levels of BDNF expression are lower in patients with MS and depression and this may be exacerbated during infection with COVID-19, as supported by findings that anti-depressant treatments and exercise training raise BDNF levels (White and Castellano, 2008; Silverman and Deuster, 2014). Exercise alters the levels of some monoamines (e.g., norepinephrine, serotonin, and tryptophan) to impact BDNF expression (Ivy et al., 2003; Phillips and Fahimi, 2018). In addition, endocannabinoids released from exercising muscles and neural terminals bind to their receptors (CB1 and CB2) to increase BDNF levels (Tantimonaco et al., 2014). BDNF increases the expression of tryptophan hydroxylase, which is involved in serotonin biosynthesis in neurons of the raphe nucleus (Siuciak et al., 1998; van Praag, 2009). BDNF increases synaptophysin and synaptobrevin levels which mediate neurotransmitter release (Kondo and Shimada, 2015); levels of synaptophysin and synaptobrevin are altered in cognitive disorders (Cotman and Engesser-Cesar, 2002). Exercise improves synaptic function by upregulating genes related to membrane trafficking, neurotransmitter vesicle recycling, synaptic plasticity (Bolton et al., 2000; Schinder and Poo, 2000). Thus, regular physical exercise reduces stress induced loss of BDNF levels and can alleviate depression, anxiety and improve mood (Marais et al., 2009; Szuhany and Otto, 2020). It is likely that neuroendocrine changes can dampen socio-psychological disorders associated with the coronavirus pandemic in patients with MS (Meeusen et al., 2001; Lin and Kuo, 2013; Pedersen and Saltin, 2015; Basso and Suzuki, 2017) (**Figure 3**).

Exercise promotes the release of endocannabinoids (Carek et al., 2011), which have roles in mood, analgesia, memory and reward systems (Basso and Suzuki, 2017). Plasma endocannabinoids are lipophilic and readily passes the BBB to interact with cannabinoid receptors in the brain. Eight days of voluntary exercise increased anandamide levels and the density and activity of hippocampal cannabinoid CB1 receptors (Hill et al., 2010), which are associated with neuronal plasticity in the brain (Tantimonaco et al., 2014). Binding to central or peripheral endocannabinoids receptors produces different responses (Raichlen et al., 2012), and can produce analgesia (Agarwal et al., 2007), stimulate reward centers in the brain to reduce anxiety and enhance euphoria (Dietrich and McDaniel, 2004), improve mood and lower depression by increasing the expression of BDNF (Tantimonaco et al., 2014), and finally modulate the release of dopamine (increased) and GABA (decreased) (Raichlen et al., 2012).

Exercise increases the levels of serotonin, dopamine and norepinephrine levels in the brain and spinal cord (Meeusen et al., 2001), and also increases the affinity of dopamine receptors (Lin and Kuo, 2013), decreases dopamine breakdown (Petzinger

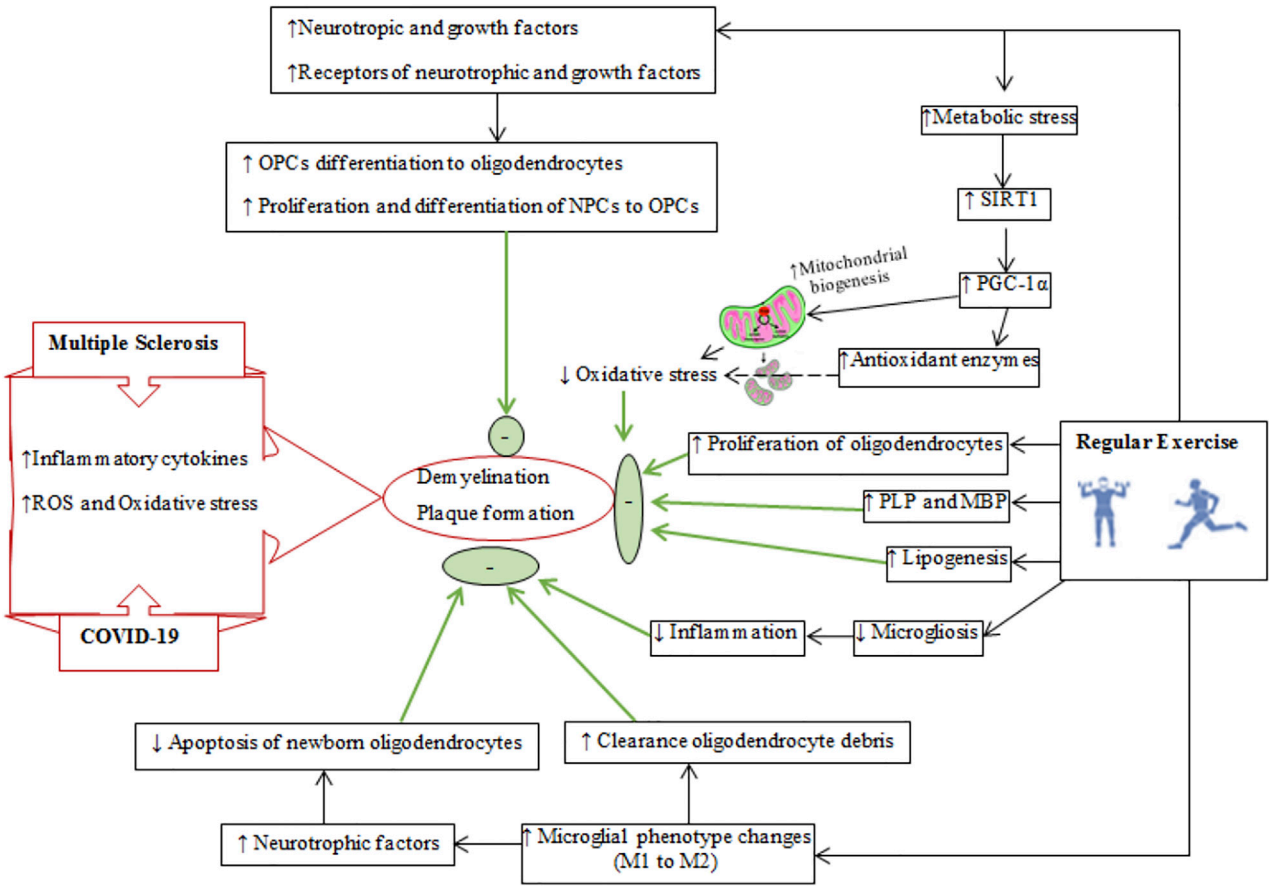

FIGURE 6 | Schematic diagram of demyelination and plaque formation in multiple sclerosis and COVID-19. The reparative effects of regular exercise on reducing demyelination and improving remyelination are shown on right side. The final effects of regular exercise are shown by thick green arrows and green circles and oval shapes. SIRT1, silent mating type information training; PGC-1α, peroxisome proliferator-activated receptor-gamma coactivator-1 alpha; PLP, myelin proteolipid protein; MBP, myelin basic protein; NPCs, neuronal precursor cells; OPCs, oligodendrocyte precursor cells.

et al., 2007), promotes mRNA expression levels of dopamine D2 receptors; collectively, these changes reduce motor dysfunction (Vučković et al., 2010), reduces loss of dopaminergic neurons induced by inflammation (Reiss et al., 2009), and increases dopamine synthesis (Lin and Kuo, 2013). The increased content of cerebral norepinephrine and dopamine induced by exercise are mediated by tyrosine hydroxylase, biosynthesizing enzyme common to the production of both neurotransmitters (Sutoo and Akiyama, 2003; Ma, 2008; Lin and Kuo, 2013). Norepinephrine regulates synaptic plasticity, increases neuronal survival and regeneration, and enhances mood (Ma, 2008; Basso and Suzuki, 2017).

Physical exercise increases the availability of tryptophan to stimulate the synthesis of serotonin in the brain (Chaouloff, 1989), while also modulating serotonin receptors (5-HT1A, 5-HT1B, and 5-HT2A) in anxiety and depression (Chennaoui et al., 2000; Guszkowska, 2004). Decreases in cerebral 5-HT1A and 5-HT2A receptors occur in patients with cognitive impairment such as MS (Meltzer et al., 1998). Stimulation of post-synaptic 5-HT1A and 5-HT3 receptors produces anti-depressant effects by upregulating hippocampal BDNF levels (Kondo et al., 2015; Kondo and Shimada, 2015).

Endogenous opioids such as β-endorphin, enkephalins, endorphins, and dynorphins are released from the anterior pituitary gland in response to exercise (Basso and Suzuki, 2017). In addition to increasing opioid peptides, exercise also modulates the binding affinity of endogenous opioids to mu (μ), kappa (κ) and delta (δ) receptors (Boecker et al., 2008). Exercise modulation of the sensitivity and number of opiate receptors (especially μ receptors) in the brain is associated with positive alterations in mood, depression, anxiety, analgesia, euphoria, and stress (Boecker et al., 2008; Dinas et al., 2011; Tantimonaco et al., 2014; Arida et al., 2015).

Collectively, regular physical exercise reduces the mental and psychological challenges associated with COVID-19 pandemic in patients with autoimmune diseases such as MS patients who are highly susceptible to mental and psychological issues. The positive changes induced by exercise training are mediated by alterations of neurotransmitters, neurotrophic factors, opioids, and their receptors.

EXERCISE TRAINING AND IMMUNITY

Chronic inflammation, which is associated with increased cytokine levels, is a pathologic hallmark of both MS and COVID-19 (Pedersen, 2009; Pedersen, 2017). Pro-inflammatory cytokines stimulate all aspects of acute phase responses, including the production of many acute phase proteins such as C-reactive protein (CRP) and IL-6 (Pedersen et al., 2001) and also upregulation of matrix metalloproteinases (MMPs) (Kurzepa et al., 2005).

Exercise produces anti-inflammatory effects through a variety of mechanisms that mitigate chronic inflammation in some diseases related to autoimmunity and hyper-inflammation (**Figure 4**) (Brandt and Pedersen, 2010; Gleeson et al., 2011). Plasma levels of pro-inflammatory cytokines (TNF-α, IL-1β, and IL-6) are increased during and after physical exercise (Pedersen et al., 2001), with increases in IL-6 greater than production other cytokines (Aral et al., 2014). Increases in IL-6 during exercise are due to activation of the SNS and activation of the HPA axis (Pedersen et al., 2001). Adipocytes, macrophages in adipose tissue, monocytes, brain, liver and exercising muscles are the primary sources of IL-6 during exercise (Gleeson et al., 2011; Coelho Junior et al., 2016). Muscle-derived IL-6 attenuates the production of some pro-inflammatory by cytokines (IL-1β, TNF-α) released by inflammatory cells and adipose tissue (Pedersen et al., 2001; Gleeson et al., 2011). Furthermore, IL-6 upregulates circulatory anti-inflammatory cytokines such as interleukin-1 receptor antagonist (IL-1ra), IL-10, and IL-4 (Pedersen et al., 2001; Suzuki et al., 2002; Steensberg et al., 2003; Pervaiz and Hoffman-Goetz, 2011). IL-1ra is mainly secreted by monocytes and macrophages and inhibits the pro-inflammatory functions of IL-1β (Freeman and Buchman, 2001), while IL-10 is mostly produced by Treg, Th1, Th2, and Th17 cells and also monocytes, macrophages, dendritic cells, B, and $CD8^+$ T cells (Maynard and Weaver, 2008). IL-10 can downregulate the levels of MHC, intercellular adhesion molecule 1 (ICAM1), and costimulatory molecules (CD80 and CD86) on antigen-presenting cells (APCs) (Maynard and Weaver, 2008). Inhibition of some pro-inflammatory cytokines and adaptive immune components is another role of IL-10 in mitigating the capacity of effector T cells to maintain inflammatory responses (Moore et al., 2001; Maynard and Weaver, 2008). IL-6 secreted from exercising muscles augments cortisol release (which is an anti-inflammatory agent) (Steensberg et al., 2003).

Increased vagal tone occurs during regular physical exercise and after some adaptations to exercise (Rodrigues et al., 2014). The parasympathetic nervous system activates the cholinergic anti-inflammatory pathway, consisting of vagal afferents, motor and efferent projections (Bonaz et al., 2016). Afferent projections deliver the information on peripheral immune conditions to the CNS (Pavlov and Tracey, 2012). Increased cholinergic anti-inflammatory pathway activation promotes macrophage transformation from M1 to M2 subtypes; the M2 phenotype produces anti-inflammatory cytokines and T regulatory cells (Tregs) which nave roles in immune suppression (Rocha et al., 2016). On the other hand, acetylcholine released from efferent outflows activates nicotinic receptor α7 on immune cells to prevent further release of pro-inflammatory cytokines (Pavlov et al., 2006).

Patients with MS and COVID-19 are often forced to adopt sedentary lifestyle, which leads to an accumulation of visceral fat, which is strongly associated with the infiltration of pro-inflammatory cytokines, macrophages, T cells and the appearance of chronic systemic low-grade inflammation (Ouchi et al., 2011) as well as the migration of peripheral blood mononuclear cells to adipose tissue (Gautier et al., 2009; Zeyda et al., 2011). Increases in the size of adipocytes and the number of infiltrated immune cells stimulate the recruitment of macrophages to adipocytes that is mediated by chemokine ligand 2 and 3 (CCL2, CCL3) (also known as MIP1α) (Bruun et al., 2005). Immune cells in adipocytes release chemokines that mediate upregulation of complimentary chemokine receptors on peripheral blood mononuclear cells (Gleeson et al., 2011). Exercise inhibits the migration of peripheral blood mononuclear cells to inflamed adipose tissue (Kawanishi et al., 2013) through the secretion of chemokines from other sources and thereby causes an internalization of chemokine receptors on peripheral blood mononuclear cells (Maffei et al., 2009). Further, exercise reduces the release of chemokines from adipose tissue, which attenuates the infiltration of macrophages (Kanda et al., 2006). Thus, exercise reduces the migration of peripheral blood mononuclear cells to adipose tissues and reduces inflammation by expediting the phenotypic conversion of macrophages from M1 (pro-inflammatory type) to M2 (anti-inflammatory) (Kawanishi et al., 2010).

Exercise reduces the expression of tissue ICAM1, which regulates the docking of inflammatory cells on the endothelium, extracellular matrix, epithelium, and mediates the interaction between T cells and target cells; hence, regular exercise inhibits macrophage infiltration into adipose tissues (Zoppini et al., 2006; Kawanishi et al., 2010). Exercise-induced reductions in circulatory inflammation also occur by modulating monocytes (Gleeson et al., 2006). Toll-like receptors are transmembrane proteins that are highly expressed on monocytes, where they have important roles in the diagnosis of microbial pathogens and tissue damage (Kaisho and Akira, 2006). Activation of Toll-like receptors produces pro-inflammatory cytokines (Takeda et al., 2003). Trained individuals have reduced expression levels of Toll-like receptors (TLR1, 2, and 4) in their monocytes and lower release of inflammatory cytokines (Stewart et al., 2005; Flynn and McFarlin, 2006; Gleeson et al., 2006; Oliveira and Gleeson, 2010). In addition, regular physical exercise attenuates the number of circulating inflammatory monocytes ($CD14^{low}$ $CD16^+$), which are rich in Toll-like receptors (TLR4) on their surfaces (Skinner et al., 2005; Timmerman et al., 2008; Simpson et al., 2009). Exercise reduces visceral fat mass and lowers the production of pro-inflammatory adipokines (TNF-α, leptin, retinol binding protein 4), lipocalin 2, IL-18, chemokine ligand 2 (CCL2 or MCP-1), CXC-chemokine ligand 5, angiopoietin-like 2 to create an anti-inflammatory environment (Petersen and Pedersen, 2005; Lim et al., 2008; Mathur and Pedersen, 2008; Mujumdar et al., 2011).

Reduced levels of Treg cells leads to autoimmunity and stimulates immune responses to exogenous antigens (Fernandez et al., 2008; Paust et al., 2011). These cells can suppress immune responses by producing forkhead box P3 proteins (Sakaguchi, 2005). Thus, increasing the numbers of circulatory Treg cells, for example by exercise, can limit inflammation in diseases such as MS (Yeh et al., 2006; Duffy et al., 2019; Goverman, 2021). In response to antigen stimulation, Treg cells causes the release of anti-inflammatory cytokines (IL-10, TGF-β) and the change of T helper 1 (Th1) cells (pro-inflammatory phenotype) to anti-inflammatory Th2 cells by increasing forkhead box P3 proteins (Yeh et al., 2009).

Peripheral and central levels of pro-inflammatory cytokines including TNF-α, IFN-ϒ, IL-6, and IL-1β are increased in neurological diseases (Mee-Inta et al., 2019). Activation of microglia by pro-inflammatory cytokines results in microgliosis which is then followed by the production of large amounts of IL-1β (Mee-Inta et al., 2019). Increase in IL-1β mediated by microglia exacerbates inflammation in the CNS by reactivating infiltrated lymphocytes and also by incurring astrogliosis (Pekny and Pekna, 2014). Exercise suppresses microgliosis and reduces inflammation by increasing the levels of anti-inflammatory cytokines (Mee-Inta et al., 2019).

EXERCISE TRAINING AND BBB PERMEABILITY

Regular exercise alters BBB permeability by causing changes in tight-junction proteins (occludin, claudins, and zonula occludens (ZOs)) and in supporting astroglial cells (**Figure 5**). There is insufficient evidence that exercise alters the function of these proteins in MS, although a recent report indicates that endurance exercise after EAE induction (animal model of MS) increases claudin-4 and occluding levels (Souza et al., 2017). Some clinical evidence suggests that exercise affects BBB permeability by increasing oxidative capacity and reducing inflammation (Souza et al., 2017; Chupel et al., 2018). Activation of matrix metalloproteinases (MMPs) produced by reactivated astrocytes and invasive T lymphocytes can disrupt cerebrovascular base-membrane and endothelial tight junction proteins in inflammatory diseases such as MS (Rempe et al., 2016). Exercise training modulates the concentrations of permeable BBB markers such as MMPs and S100β in MS patients (Zimmer et al., 2018; Negaresh et al., 2019). The effects of exercise on BBB changes in nonclinical conditions were monitored using peripheral markers such as serum levels of S100β (*S100 calcium-binding protein B, a protein expressed by mature astrocytes*) were used to monitor changes in BBB permeability (Koh and Lee, 2014). Exercise-induced changes in BBB permeability is related to hyperthermia, increases in circulatory concentrations of ammonia, adrenaline, noradrenaline, inflammatory mediators, central neurotransmitters (serotonin and glutamate), ROS production, and growth factors (Watson et al., 2006; Nierwińska et al., 2008; Małkiewicz et al., 2019; Suzuki et al., 2020). Increased lactate produced during exercise triggers the expression of hypoxia-inducible factor 1-alpha (HIF1-α) followed by the activation of vascular endothelial growth factor (VEGF)-A expression in astrocytes (Zimmer et al., 2019) and disruption of the tight-junction proteins (claudin-5 and occluding) to alter BBB permeability (Brambilla et al., 2005). Thus, exercise in nonclinical conditions increases BBB permeability to meet the increased neural demands caused by physical exercise.

Astrocytes interact with tight-junction proteins to maintain BBB integrity (Razi et al., 2022a). Astrocytes are glial cells having extensive connections with adjacent cells such as endothelial cells. Inflammatory conditions cause these glial cells to undergo astrogliosis that is characterized by morphological and functional changes with upregulation of GFAP (Abbott et al., 2006; Liddelow and Barres, 2017). Glutamate excitotoxicity, oxidative stress and pro-inflammatory cytokines (IL-6, IL-1β, and TNF-α) trigger astrogliosis and increases in BBB permeability in diseases such as MS and COVID-19 (Burda and Sofroniew, 2014). Thus, reducing reactive astrogliosis by downregulation of GFAP can be a therapeutic target for diseases involving disorders of BBB permeability (**Figure 5**) (Alvarez et al., 2013), although there is limited evidence to support this, especially in diseases involving inflammatory conditions (Razi et al., 2022a). Six-weeks of exercise training in an animal model of MS reduced both GFAP expression and astrogliosis (Mandolesi et al., 2019). Some potential mechanisms for exercise-induced downregulation of GFAP include: 1) reduced levels of cytokines released by activated microglia and astrocytes, 2) upregulation of Tregs in the CNS and phenotype alterations of Th1 to Tregs, 3) suppression of ROS production and oxidative stress, and 4) inhibition of microglial activation (Santin et al., 2011; Bernardi et al., 2013; Radak et al., 2016; Gentile et al., 2019; Quan et al., 2020) by increased secretion/expression of anti-inflammatory cytokines (IL-1ra, IL-10, and IL-4) and upregulation of the CD200 and CD200R glycoproteins on neurons and microglial cells, respectively (Mee-Inta et al., 2019). Inhibition of microglial activation in autoimmune diseases reduces the release of microglia-mediated pro-inflammatory cytokines such as IL-1β and consequent reduction of astrocyte activation (Mee-Inta et al., 2019). Exercise-induced cortisol release downregulates the expression of GFAP by astrocytes (Bernardi et al., 2013). Exercise-induced angiogenesis, reportedly, promotes astrocyte proliferation to strengthen the neurovascular unit (NVU) and preserve the integrity of the BBB (Li et al., 2005).

In summary, regular exercise improves BBB integrity in some neurological conditions by mitigating inflammatory states, increasing tight-junction proteins, promoting angiogenesis, and favouring astrogliocytosis (astrocyte proliferation) over astrogliosis.

EXERCISE TRAINING AND PLAQUE REDUCTION THROUGH REMYELINATION

Myelin is required for the conduction of neural impulses, and demyelination of neurons and white matter atrophy is a characteristic of MS (Bando, 2020). Oligodendrocytes and oligodendrocyte precursor cells are susceptible to damage by

inflammatory mediators, ROS, and oxidative stress in MS (Yoon et al., 2016; Feter et al., 2018). Damaged oligodendrocytes and demyelination lead to the neurological deficits (Cheng J. et al., 2020). Oligodendrocyte precursor cells are quiescent cells that can migrate to demyelinated areas to restore myelin (Jiang et al., 2017; Jensen et al., 2018). Physical exercise can affect the remyelination process by influencing the neuronal microenvironment in the CNS (**Figure 6**) and by a variety of related mechanisms including increased upregulation of neurotrophic and growth factors such as BDNF, insulin-like growth factor-1 (IGF-1), neurotrophin-3 (NT-3), and their receptors [tyrosine kinase receptor B (TrkB) and IGF-1R] to influence neural precursor cells and oligodendrocyte precursor cells to proliferate and differentiate to oligodendrocyte precursor cells and oligodendrocytes, respectively (Gallo and Armstrong, 2008; Ahn et al., 2016; Jensen and Yong, 2016; Tomlinson et al., 2016; Feter et al., 2018). These neurotrophic and growth factors influence myelin production by stimulating the phosphoinositide 3-kinases (PI3K)-Akt-mTOR pathway (Carson et al., 1993; Cao et al., 2003; Lin et al., 2005).

Exercise increases silent mating type information training (SIRT1) levels (Sarga et al., 2013), which upregulates peroxisome proliferator-activated receptor gamma (PPAR-γ) coactivator 1-alpha (PGC-1α), a transcriptional factor for mitochondrial biogenesis (Cheng J. et al., 2020). Increases in mitochondria reduces ROS-derived oxidative stress and protects myelin from oxidative damage in MS (Rafalski et al., 2013; Ng et al., 2015). Physical exercise lowers levels of 4-hydroxynonenan (4-HNE, a marker of lipid peroxidation) in the brain (Yoon et al., 2016). Importantly, improved mitochondrial function due to exercise training increases the activity of acetyl-CoA carboxylase 1 and 2, enzymes that provide malonyl-CoA for synthetizing long-chain fatty acids required for remyelination (Sedel et al., 2015; Sedel et al., 2016). Furthermore, increased exercise-induced PGC-1α reduces demyelination by increasing antioxidant enzyme levels to offer greater protection from oxidative stress (St-Pierre et al., 2006), while also promoting remyelination by modulating lipid production, oligodendrocyte differentiation and myelin proteins such as myelin basic protein and proteolipid protein (Camacho et al., 2013; De Nuccio et al., 2015).

Regular physical exercise inactivates microglia and also modulates their phenotype conversion from M1 (inflammatory) to M2 (neuroprotective) (Kohman et al., 2012; Franco and Fernandez-Suarez, 2015). M1 microglia cause oligodendrocyte apoptosis and suppress remyelination by increasing antigen presentation and producing toxic cytokines, while M2 microglia have neurotrophic effects by mitigating local inflammation, clearing oligodendrocyte debris and releasing neurotrophic factors (Miron et al., 2013). Exercise leads to an upregulation of the fractalkine receptor proteins (*CX3CL1/CX3CR1; mediators of chemotaxis and adhesion of immune cells*) to polarize microglia to a neuroprotective phenotype (IGF1/Iba1 positive microglia), and increase their phagocytic activity to expedite the clearance of myelin debris (Vukovic et al., 2012; Ransohoff and El Khoury, 2016). M2 microglia trigger the differentiation of oligodendrocyte precursor cells and attenuate apoptosis of newborn oligodendrocytes as important components of remyelination (Miron et al., 2013). Additional mechanisms for exercise-induced remyelination include increases in the density of remyelinated axons, and a restored g-ratio (*g-ratio measures myelin thickness and is calculated by dividing the inner axon dimeter by the outer myelin diameter*) (Feter et al., 2018; Jensen et al., 2018).

PRACTICAL CONSIDERATIONS

Survivors of acute viral respiratory diseases such as COVID-19 endure neuropsychological deficits and a poor quality of life (QOL) that can last for 1 year or more (Yeo, 2020; Gentil et al., 2021). Patients can experience muscle weakness and atrophy, tendon, and neuromuscular impairments in intensive and long-term health care (Gentil et al., 2021). Engaging in regular physical exercise prior to infection (and even early after infection) can reduce these complications (Calverley et al., 2020; Hekmatikar et al., 2021) and also limit mental and physical stress (Silverman and Deuster, 2014). The beneficial adaptions to exercise training can occur within 4 days to 26 weeks (Batouli and Saba, 2017). The World Health Organization (WHO) recommends individuals undertake at least 150 min exercise with moderate-intensity or 75 min with high-intensity exercise per week (Norum, 2005).

Viral infection in MS patients is often associated with an increased risk of relapsing (De Keyser et al., 1998). Patients with MS are sensitive to increases in body temperature during exercise sessions (Razi et al., 2022b), and a supervised muscle strengthening training program should be modified according to the stage of the disease (Smith et al., 2006; Pedersen and Saltin, 2015). Physical exercise is not recommended during any systemic viral disease, since inflammatory reactions in muscle cells and coronary artery walls increase the risk of cardiac sudden death during infection (Inciardi et al., 2020). The average time from initiation to clinical recovery from COVID-19 infection is 2 weeks (Barker-Davies et al., 2020) and this period can last from 3 to 6 weeks for patients with severe clinical disease (Woods et al., 2020).

Inclusion of the resistance training in daily activities for at least two sessions per week expedites recovery from infection (Norum, 2005). The resistance training program recommended amid the COVID-19 pandemic involves a low number of repetition (≤6 repetition) and a long periods of rest between sets (≥3 min) (Gentil et al., 2021). This training protocol is suitable for patients with MS who are also infected with the SARS-CoV-2 virus, since patients with MS are sensitive to hyperthermia and an additional respiratory infection can further limit participation in aerobic exercise (Albesa-Albiol et al., 2019; Garnacho-Castaño et al., 2021). Resistance training can improve mood, and limit states of depression and anxiety (Gordon et al., 2017; Gordon et al., 2018), while progressive strength training for 12 weeks in MS patients is the best strategy to promote muscle strength and improve depression, fatigue, and QOL (Dalgas et al., 2009; 2010).

Exercise also improves immunological responses in MS and COVID-19 (Gentile et al., 2019; da Silveira et al., 2021). Moderate

level of exercise improves the ability of the immune system to limit viral infections (Nieman et al., 2011). Vigorous exercise improves the anti-inflammatory actions of IL-10 (Wang et al., 2012). High-intensity exercise improves neuronal conduction velocity (van Meeteren et al., 1997), while moderate and resistance aerobic exercise increases remyelination of axons (Bobinski et al., 2011). The beneficial effects of exercise on body tissues, especially the brain, are mediated through neurotrophic factors (Tari et al., 2019). Moderate level of exercise increases serum levels of neurotrophic factors in patients with MS and also in healthy individuals (Gold et al., 2003). Thus, physical exercise with moderate intensity can improve brain health of patients with MS during and after the COVID-19 pandemic.

CONCLUSION

Regular physical exercise mitigates mental and psychological disorders associated with COVID-19 infections in patients with MS by causing changes in neurotransmitters, neuromodulators, opioids, and neurotrophic and growth factors. Regular exercise leads to positive changes in central and peripheral immune systems and induces an anti-inflammatory milieu to limit the effects of the cytokine storm associated with MS and COVID-19. Thus, regular exercise training has pronounced central and peripheral effects that can be used as prophylactic and reparative interventions to improve brain health.

AUTHOR CONTRIBUTIONS

OR, BT, and NZ conceptualized and wrote the first draft. IL, HZ, OR, and SR-R developed the study concept. KG, KS, and HZ reviewed and edited the final version of manuscript. All authors contributed to the article and approved the submitted version.

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BAIBA Involves in Hypoxic Training Induced Browning of White Adipose Tissue in Obese Rats

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In recent years, obesity has become an important risk factor for human health; how to effectively prevent and reduce the occurrence of obesity is a hot research topic in recent years. Hypoxic training effectively improves abnormalities of lipid metabolism caused by obesity. The current study explored the effects of hypoxic training on BAIBA secretion and white fat browning in inguinal fat in obese rats. Analyses were performed by HPLC/MS/MS—MS/MS, RT-q PCR and western blot methods. The findings showed that 4 weeks of hypoxic training reduced body weight, Lee's index, and regulated blood lipid profile in obese rats. Hypoxic training up-regulated BAIBA concentration in gastrocnemius muscle and circulation in obese rats. Hypoxic training significantly upregulated expression of PPARα and UCP-1 in inguinal fat of obese rats and increased white fat browning. The findings showed that BAIBA may involve in improveing blood lipid profile and white fat browning by modulating PPARα and UCP-1 expression.

Keywords: hypoxic training, obesity, BAIBA, lipid metabolism, browning

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1 INTRODUCTION

Obesity is a major public health concern. The global obesity prevalence has increased by approximately 1.5-fold for adults and 2-fold for children in the past 20 years (WHO, 2020). China CDC reported that the national overweight rate for adults aged 18 and above was 30.1% in 2015 and the prevalence of obesity rate was 11.9% (Wang et al., 2017), and is currently on the rise. Studies are currently, exploring effective methods for preventing and reducing occurrence of obesity.

Mammals have two main types of adipose tissue including the white adipose tissue (WAT) and brown adipose tissue (BAT) (Mueller, 2016; Jeremic et al., 2017). White adipose tissue originates from Myf5-progenitor cells, which are characterized by accumulation of single-compartment lipid droplets and few mitochondria. The main function of white adipose tissue is energy storage. Brown adipocytes originate from Myf5+ progenitor cells and are characterized by multicompartmental lipid droplets containing abundant mitochondria. The main function of brown adipocytes is to maintain body temperature though non-shivering thermogenesis (Wu et al., 2012). The inner mitochondrial membrane of brown adipocytes is rich in uncoupling protein 1 (UCP-1), which causes uncoupling of electron transfer and ATP production processes of mitochondrial oxidative respiration. Uncoupling of these two processes results in emission of large amounts of energy in form of heat and energy consumption, however, this thermogenic effect is highly regulated in the body (Rodriguez et al., 2020).

There is a third type of adipocytes similar BAT called beige/brite adipocyte occurs in WAT. Beige adipocytes are differentiated from WAT under induced conditions. Beige cells have similar

thermogenic capacity as brown adipocytes under fully activated conditions. Moreover, beige adipocytes have a similar origin as WAT but have unique marker genes such as Transmembrane protein 26 (TMEM-26), CD137 and T-box 1 (Tbx1) (Dempersmier and Sul, 2015; Mueller, 2016).

Cold exposure and fasting induces production of beige fat in WAT through a process known as white fat browning (Poher et al., 2015). An important hallmark of white fat browning is increased expression of UCP-1 and increased thermogenesis. The classical pathway of white fat browning is association of PR domain-containing 16 (PRDM16) with CCAAT-enhancer binding proteins-β (C/EBP-β) through its zinc finger domain. This association forms a transcriptional complex that induces peroxisome proliferator-activated receptor-α/γ (PPARα/γ) and peroxisome proliferator-activated receptor-γ coactivator 1-α (PGC1-α) expression. Peroxisome proliferator activated receptor-γ coactivator1-α (PRDM16 binds to PPAR-γ and PGC-1α to form a complex which induces UCP-1 expression and browning (Kim et al., 2016; Jeremic et al., 2017).

Exercise is a good method for weight loss. Regular exercise increases energy expenditure of the body, reduces body fat, and improves body composition (Wang et al., 2019). Expression levels of beige adipocyte markers (such UCP-1 and PRDM16) in white fat, mainly in subcutaneous fat are upregulated by different exercise modalities (such as free spinning wheel, running platform and swimming) and different intervention durations (one-time or long-term), indicating that regular exercise promotes rodents white fat browning (Gorgens et al., 2015; Rocha-Rodrigues et al., 2016).

The mechanism of exercise-induced white fat browning may be regulated by other pathways besides activation of the sympathetic nervous system. In addition to consumption of a large amount of energy during exercise, skeletal muscle secretes several myokines to regulate metabolic disorders caused by obesity (Gorgens et al., 2015; Hoffmann and Weigert, 2017). A previous study reported that exercising muscles secrete interleukin-6 (IL-6) which is released into the blood (Steensberg et al., 2000). Studies are currently exploring the muscle as an endocrine organ, and cytokines secreted and released by muscles are called "myokines." Some of the myokines released by muscles includeIL-6 (Steensberg et al., 2000), fibroblast growth factor 2 (FGF21) (Fisher et al., 2012), brain-derived neurotrophic factor (BDNF), irisin (BDNF), and myokine (Irisin) (Bostrom et al., 2012). These muscle factors can act on the liver and adipose tissue to regulate glucolipid metabolism (Hoffmann and Weigert, 2017; Castillo-Armengol et al., 2019; Kirk et al., 2020).

β-Aminoisobutyric acid (BAIBA) has been reported recently as a newly discovered muscle factor (Kammoun and Febbraio, 2014; Roberts et al., 2014). BAIBA is a natural metabolite of thymine and valine secreted by myocytes during exercise. BAIBA exists as two main enantiomeric isomers in organisms, D-BAIBA and L-BAIBA. D- BAIBA is produced by the cytosolic thymine metabolic pathway, which mainly involves dihydropyrimidine dehydrogenase (DPYD), dihydropyrimidinase (DPYS) and β-ureidopropionase (β-ureidopropionase enzymes). L-BAIBA is produced through catabolism of the branched-chain amino acid, L-valine through transamination of L-methyl-malonyl semialdehyde (L-MMS), a downstream product of L-valine, and L-glutamic acid by the action of mitochondrial 4-aminobutyric acid transaminase enzyme (Tanianskii et al., 2019). BAIBA reduces body fat and its activity is not dependent on changes in energy intake or physical activity levels, but rather on changes in basal oxygen consumption. BAIBA can promote white adipose tissue thermogenesis through adrenaline-dependent and-independent pathways andwhite fat browning (Kitase et al., 2018). In addition, exercise increases plasma BAIBA levels and plasma BAIBA levels are negatively correlated with the risk of metabolic diseases (Kammoun and Febbraio, 2014; Roberts et al., 2014).

Previous studies conducted by our group reported that Hypoxic Training is effective in regulating lipid metabolism in obese rats. The findings showed that the main mechanisms involve skeletal muscle fatty acid metabolism and liver lipid metabolism (Lu et al., 2014; Lu et al., 2016; Gao et al., 2020). However, effect of Hypoxic exercise on BAIBA secretion in obese rats has not been fully elucidated. Moreover, studies have not fully explored whether BAIBA is involved in regulation of lipid metabolism and white fat browning induced by Hypoxic exercise. In the study, a hypoxic exercise model was established in obese rats. In addition, mechanisms of the effects of hypoxic exercise on lipid metabolism and white fat browning in obese rats were explored by determining the levels of BAIBA.

MATERIALS AND METHODS

Animals and Experimental Design

A total of 110 3-week-old male SD rats were used to establish an obesity model in the current study. Animals were housed in separate cages, five rats per cage, under a light/dark cycle of 12 h/12 h, a temperature of 22 ± 1°C and 40%–60% humidity. Animals were fed with normal chow feeding for a week, then randomly assigned to two groups. The first group comprised 20 rats fed with normal chow and 90 rats fed with high-fat chow diet *ad libitum*. Through our previous research, we found that the success rate of obesity model modeling was about 60%, so 90 animals were used to establish theobesity model. The feed composition was as follows: normal feed diet comprising experimental rat growth maintenance pellet feed (3.40 kcal/g, 65% of energy from carbohydrate, 12% of energy from fat); high-fat feed comprising high-fat feed from Research Diets, United States (item no. D12451, 4.73 kcal/g, 35% of energy from carbohydrate, 45% of energy from fat). Animals were fed for 12 weeks then 32 obese rats were selected from the high-fat diet group based on their body weight and Lee's index.

The calculation formula of Lee's index:

$$\text{Lee's index} = \frac{\sqrt[3]{body\ weight\ (g)}}{body\ length\ (cm)} * 10^3$$

During the exercise and hypoxia intervention, each group was guaranteed to have no less than 10 rats. However, due to factors such as injury during the training process, the number of rats in

each group included in this study for statistical analysis was eight in each group. The standard group (NC group) and the standard exercise group (NE group) were selected from the normal diet group, with eight rats in each group. Obese rats were randomly assigned to obese normoxic control group (ONC group), obese normoxic exercise group (ONE group), obese hypoxic control group (OHC group), and obese hypoxic exercise group (OHE group), with eight rats in each group. Rats in each group had similar body weight and training condition. Exercise intervention was performed in the NE, ONE, and OHE groups; hypoxic intervention was performed in the OHC and OHE groups. The duration of intervention was 4 weeks in all groups. The experiments were reviewed and approved by the ethical committee on treatment and handling of experimental animals.

Exercise Intervention Programs

All groups underwent 2 weeks of running table adaptation training. The training speed was increased from 16 m/min to 25 m/min and the exercise time was increased from 20 min/d to 60 min/d during the 2 weeks. Endurance training was conducted using a horizontal animal running platform with a running speed of 20 m/min in the OHE group and 25 m/min in the ONE group for 1 h/days, 5 days/weeks and the training lasted for 4 weeks. The previous experiments showed that the blood lactate concentration of the rats in the OHE group and the ONE group was basically the same during exercise, that is, the exercise intensity of the HE group and the NE group was the same (He et al., 2012).

Hypoxia Intervention Program

Hypoxia generator (GA15FF-13 twin-screw air compressor and CA-200AT nitrogen generator) purchased from Tianjin Senro Technology Co., Ltd. was used to create an atmospheric pressure hypoxic experimental environment. OHC and OHE groups were subjected to a hypoxic intervention by simulating living and/or training in an altitude of 3,500 m (oxygen concentration of 13.6%) for a period of 4 weeks.

Sample Collection

Sampling was performed after 48 h after the end of the last exercise to minimize effect of acute exercise on the relevant indexes. Rats were weighed and the body length determined, then 10% trichloroacetaldehyde hydrate for intraperitoneal anesthesia was administered intraperitoneally to induce anesthesia at a dose of 0.3 ml/100 g body weight. After induction of anesthesia, blood was drawn from the abdominal aorta and centrifuged for subsequent analysis. Inguinal fat and gastrocnemius muscle were sectioned on ice, divided in two portions and immediately wrapped with numbered tin foil. Samples were placed in liquid nitrogen, and later transferred to −80° ultra-low temperature refrigerator for further use. Blood lipid indexes were analyzed using a Hitachi 7,600 automatic biochemical analyzer following the manufacturer's instructions.

Real Time qPCR

Relative expression levels of PPAR α and UCP-1 mRNA in inguinal fat were determined by RT-qPCR.

TABLE 1 | Real-time PCR Primer sequences.

Primer Name	Primer Sequence	Product Length/Bp
UCP-1-F	TCCCTCAGGATTGGCCTCTAC	101
UCP-1-R	GTCATCAAGCCAGCCGAGAT	
PPAR-α-F	TCCACGAAGCCTACCTGAAGAACT	187
PPAR -α-R	AATCGGACCTCTGCCTCCTTGTT	
β-Actin-F	GAAGTGTGACGTTGACATCCG	282
β-Actin-R	GCCTAGAAGCATTTGCGGTG	

Total inguinal fat RNA was extracted using TriZol reagent (Invitrogen, United States) according to the manufacturer's instructions. Purity of total RNA was determined by agarose gel electrophoresis and images were obtained with EUV-LDUV gel imaging system (KoreaBiotech, Korea).

cDNA synthesis and RT-qPCR assays were performed using M-MLV reverse transcriptase and Premix Taq™ (Ex Taq™ Version 2.0), which were purchased from Bao Biological Engineering (Dalian) Co. Primers were synthesized by Beijing Tianyi Huiyuan Biotechnology Co., Ltd. β-Actin was used as the housekeeping gene. The primer sequences used are presented in **Table 1**. ddCT method was used to determine the relative expression of target genes.

Western Blot Analysis

Protein expression levels of PGC-1 α, PPAR α, and UCP-1 in inguinal fat samples were determined by western blot.

Total inguinal fat protein was extracted using tissue protein lysate. Protein concentration was determined using BCA protein quantification kit according to the manufacturer's instructions. The required sampling volume for each sample was determined based on the protein concentration and the samples were mixed with 2 * loading buffer to obtain 50 μg of total protein for each sample.

10% separation gel was prepared and gel electrophoresis of the samples was performed. The electrophoresis voltage was 120 V and electrophoresis was terminated when bromophenol blue electrophoresis reached the bottom of the gel. After electrophoresis, samples were transferred to PVDF membrane at a constant current of 300 mA for 1 h. Membranes were blocked with 5% skimmed milk powder prepared with TBST. The membrane was then immersed and slowly shaken on a shaker and at room temperature for 60 min. Membranes were incubated with primary antibody (see **Table 2** for the primary antibody dilution ratio) overnight at 4°C. Samples were washed thrice with western wash solution under slow shaking on a side-swinging shaker for 5–10 min. Horseradish peroxidase (HRP)-labeled secondary antibody was diluted with western secondary antibody diluent at a ratio of 1: 10,000. The membrane was incubated with the diluted secondary antibody for 1 h at room temperature on a side-swinging shaker with slow shaking. Membranes were washed using western wash solution on a shaker under slow shaking thrice for 5–10 min. Liquid A: liquid B of the ECL luminescence reagent was prepared based on the size of the membrane in a 1:1 ratio by volume. The ECL was evenly placed on the PVDF film, then the X-ray film was obtained film strips of the same size were obtained. The X-ray film was then placed directly on top of the film. When the strips were clear enough, they were rinsed in water,

TABLE 2 | Dilution ratio of primary antibodies.

Primary antibody	Antibody Manufacturers	Catalog No.	Dilution ratio
PPAR-α	Abcam	ab24509	1:3,000
PGC-1 α	Abcam	ab54481	1:1,000
UCP-1	Abcam	ab23841	1:1,000
β-Actin	CST	4,967	1:1,000

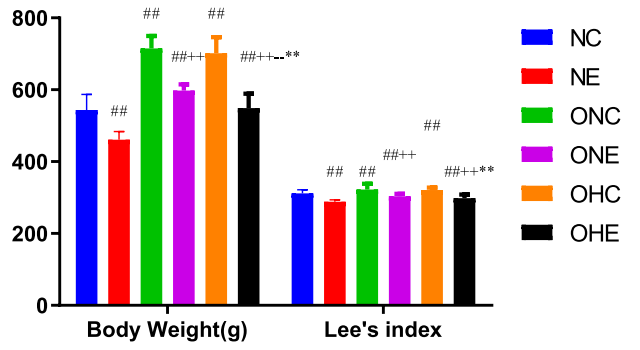

FIGURE 1 | Changes in Body Weight and Lee's Index of Rats after Normoxic and Hypoxic Exercise. $n = 8$, $^{\#}p < 0.05$ vs. NC group; $\#\#p < 0.01$ vs. NC group; $+p < 0.05$ vs. ONC group; $++p < 0.01$ vs. ONC group; $-p<0.05$ vs. ONE group; $--p<0.01$ vs. ONE group; $^{*}p < 0.05$ vs. OHC group; $^{**}p < 0.01$ vs. OHC group; following tables same.

and the X-ray film was placed into the fixing solution. The developed negative was scanned and the image was analyzed in grayscale using IPP6 software. Statistical analysis was performed on the obtained grayscale values. β-Actin was used as an internal control and the results were expressed as protein/internal reference protein expression levels.

HPLC/MS/MS Analysis

Samples were weighed and homogenized with distilled water. The homogenate was centrifuged at 13,200 rpm for 1 min, and the supernatant was obtained for subsequent analysis. 400 μl of protein precipitant (including internal standard) was added to 100 μl of the supernatant and the mixture was vortexed for 1 min. The sample was the let to stand for 5 min, then centrifuged at 13,200 rpm for 4 min, and the supernatant was obtained for subsequent analysis.

Blood was drawn from the abdominal aorta, and serum was obtained by centrifugation at 3,000 rpm for 15 min. The supernatant was further centrifuged at 13,200 rpm for 4 min.

Concentration of BAIBA in the samples was determined by HPLC-MS/MS method. Thee LC liquid phase was an Ultimate 3,000 high performance liquid chromatograph (DIONEX, United States) and the MS mass spectrometer was an API 3200 Q TRAP liquid mass spectrometer (AB, United States). The standards, methanol and nitrile were of analytical purity (Fisher, United States).

Statistical Analysis

Statistical analysis was conducted using SPSS (United States). Data were presented as mean ± SD and differences among groups were compared by ANOVA. Correlation analysis was performed using bivariate correlation analysis, with $R \geq 0.8$ being highly correlated, $0.8 > R > 0.3$ being moderately correlated, and $R \leq 0.3$ being lowly correlated. A $p < 0.05$ was considered statistically significant.

RESULTS

Effects of Normoxia Exercise and Hypoxia Exercise on Body Weight and LEE's Index in Rats

The findings showed that the body weight of rats in the ONC and OHC groups was significantly higher compared with that of rats in the NC group (**Figure 1**). Notably, the body weight of rats in the ONE and OHE groups was significantly lower compared with that of rats in the ONC group ($p < 0.01$). Analysis showed that the body weight of rats in the OHC group was not significantly different from that of rats in the ONC group ($p > 0.05$). However, the body weight of rats in the OHE group was significantly lower compared with that of rats in the ONE group ($p < 0.01$). These findings indicate that normoxic and hypoxic exercise reduces body weight of obese rats. Lee's index of rats in ONE and OHE groups was significantly lower compared with that of rats in the ONC group ($p < 0.01$). Lee's index of rats in OHC group was not significantly different from that of rats in the ONC group ($p > 0.05$). These findings indicate that normoxic and hypoxic exercise decreases the Lee's index in obese rats.

Effects of Normoxia Exercise and Hypoxia Exercise on Levels of Blood Lipids in Rats

The findings showed that level of TG of rats in the ONC group was significantly higher compared with that in the NC group ($p < 0.01$, **Table 3**). In addition, LDL level was significantly lower compared with that in the NC group ($p < 0.01$, **Table 3**). Levels of TC, TG, and LDL of rats in the ONE group were significantly lower compared with the levels in the ONC group ($p < 0.01$). However, analysis showed no significant difference in HDL levels between the ONE group and ONC group ($p > 0.05$). Levels of TC, TG, HDL, and LDL of rats in the OHC group were not significantly different from the levels in the ONC group ($p > 0.05$). The finding showed that levels of TC, TG and LDL of rats in the OHE group were significantly lower compared with the levels in the HC and NC groups ($p < 0.01$, $p < 0.01$). Notably, HDL level of rats in the OHE group was significantly higher compared with the level of HDL in the OHC and ONC groups ($p < 0.01$, $p < 0.01$). Analysis showed that the level of LDL of rats in the OHE group was significantly lower compared with level in rats in the ONE group ($p < 0.01$).

TABLE 3 | Changes in Blood Lipid levels in Rats after Normoxic and Hypoxic Exercise (n = 8).

	NC	NE	ONC	ONE	OHC	OHE
TC (mmol/L)	1.23 ± 0.25	0.88 ± 0.21$^{\#\#}$	1.41 ± 0.17	0.97 ± 0.12$^{++\#\#}$	1.30 ± 0.10	0.86 ± 0.23$^{++**\#\#}$
TG (mmol/L)	0.27 ± 0.04	0.17 ± 0.03$^{\#}$	0.47 ± 0.17$^{\#\#}$	0.31 ± 0.03^{++}	0.41 ± 0.11$^{\#\#}$	0.25 ± 0.08^{++**}
HDL (mmol/L)	0.38 ± 0.04	0.41 ± 0.10	0.33 ± 0.05	0.38 ± 0.06	0.35 ± 0.05	0.46 ± 0.13^{++**}
LDL (mmol/L)	0.37 ± 0.10	0.30 ± 0.06$^{\#}$	0.31 ± 0.02$^{\#}$	0.27 ± 0.02^{++}	0.28 ± 0.05	0.23 ± 0.03$^{\#\#++--**}$

n = 8, $^{\#}p < 0.05$ vs. NC group; $^{\#\#}p < 0.01$ vs. NC group; $+p < 0.05$ vs. ONC group; $++p < 0.01$ vs. ONC group; $-p < 0.05$ vs. ONE group; $--p < 0.01$ vs. ONE group; $*p < 0.05$ vs. OHC group; $**p < 0.01$ vs. OHC group; following tables same.

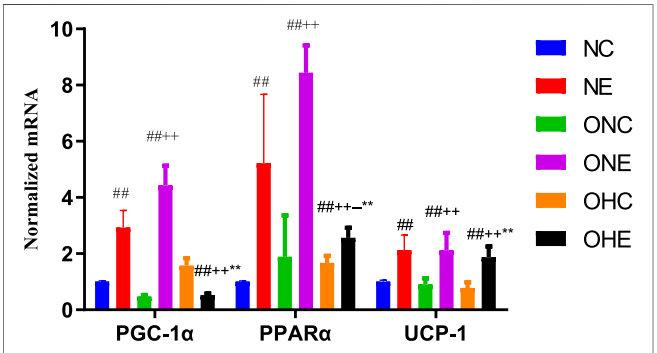

FIGURE 2 | mRNA expression levels of PGC-1α, PPAR α, and UCP-1 in inguinal fat of rats. mRNA expression levels of PPARα and UCP-1 in inguinal adipose of rats of NC, NE, ONC, ONE, OHC, and OHE groups were determined using β-Actin as a reference gene. n = 8, $^{\#}p < 0.05$ vs. NC group; $^{\#\#}p < 0.01$ vs. NC group; $+p < 0.05$ vs. ONC group; $++p < 0.01$ vs. ONC group; $-p < 0.05$ vs. ONE group; $--p < 0.01$ vs. ONE group; $*p < 0.05$ vs. OHC group; $**p < 0.01$ vs. OHC group; following tables same.

Effects of Normoxia Exercise and Hypoxia Exercise on mRNA Expression Levels of PGC-1α, PPAR α and UCP-1 in Inguinal Fat of Rats

The findings showed that the mRNA expression levels of *PGC-1α*, *PPAR α*, and *UCP-1* in the inguinal fat of rats in the NE group were significantly higher compared with the levels in rats in the NC group ($p < 0.01$, **Figure 2**). In addition, mRNA expression levels of *PGC-1α*, *PPAR α*, and *UCP-1* in the inguinal fat of rats in the ONE group were significantly higher compared with the levels in rats in the ONC group ($p < 0.01$). Analysis showed that the mRNA expression levels of *PPAR α* and *UCP-1* in the inguinal fat of rats in the OHE group were significantly higher compared with the levels in rats in the ONC and OHC groups ($p < 0.01$, $p < 0.01$, $p < 0.01$).

Effects of Normoxia Exercise and Hypoxia Exercise on Protein Expression Levels of PGC-1α, PPAR α and UCP-1 in the Inguinal Fat of Rats

The findings showed that the protein expression levels of PGC-1α and UCP-1 in the inguinal fat of rats in the NE group were significantly higher compared with protein levels in rats in the NC group ($p < 0.01$, **Figure 3**). Protein expression levels of PGC-1α, PPAR α, and UCP-1 in the inguinal fat of rats in the ONE group were significantly higher compared with the protein levels in rats in the ONC group ($p < 0.01$). Protein expression levels of PGC-1α, PPAR α, and UCP-1 in the inguinal fat of rats in the OHC group were significantly higher compared with the protein levels in rats in the ONC group ($p < 0.01$). Protein expression levels of PPAR α and UCP-1 in the inguinal fat of rats in the OHE group were significantly higher compared with the protein levels in rats in the ONC OHC groups ($p < 0.01$, $p < 0.01$, $p < 0.01$). Moreover, the protein expression level of PGC-1α in the OHE group was significantly higher compared with the protein level in rats in the ONC group ($p < 0.01$).

Effects of Normoxic Exercise and Hypoxic Exercise on Expression Level of BAIBA in Gastrocnemius Muscle and Blood of Rats

The findings showed that the levels of BAIBA in the gastrocnemius muscle and blood of rats in the NE group were significantly higher compared with the levels in the NC group ($p < 0.01$, **Table 4**). In addition, level of BAIBA in gastrocnemius muscle of rats in the ONE group was significantly higher compared with the level in rats in the ONC group ($p < 0.01$). However, analysis showed no significant difference between BAIBA level in the blood of rats in the OHC group and that in the ONE group ($p > 0.05$). Level of BAIBA in gastrocnemius muscle of rats in the OHE group was significantly higher compared with the level in rats in the ONC and OHC groups ($p < 0.01$, $p < 0.01$). Moreover, level of BAIBA in the blood of rats in the OHE group was significantly higher compared with the level in rats in the OHC group ($p < 0.01$). However, analysis showed no significant difference in the level of BAIBA in rats in the ONC and ONE groups ($p > 0.05$, $p > 0.05$).

Relationship Between BAIBA Level, and Blood Lipid Level and Browning Index in Rats

The findings showed that BAIBA level in rat gastrocnemius muscle was negatively correlated with rat TC level and positively correlated with PPARα and UCP-1 mRNA expression levels in rat inguinal fat (**Table 5**). Moreover, blood BAIBA level was negatively correlated with rat TC level and positively correlated with PPARα and UCP-1 mRNA expression level in rat inguinal fat ($p < 0.05$, $p < 0.01$).

DISCUSSION

The findings of the current study showed that hypoxic exercise and normoxic exercise significantly reduced body weight and

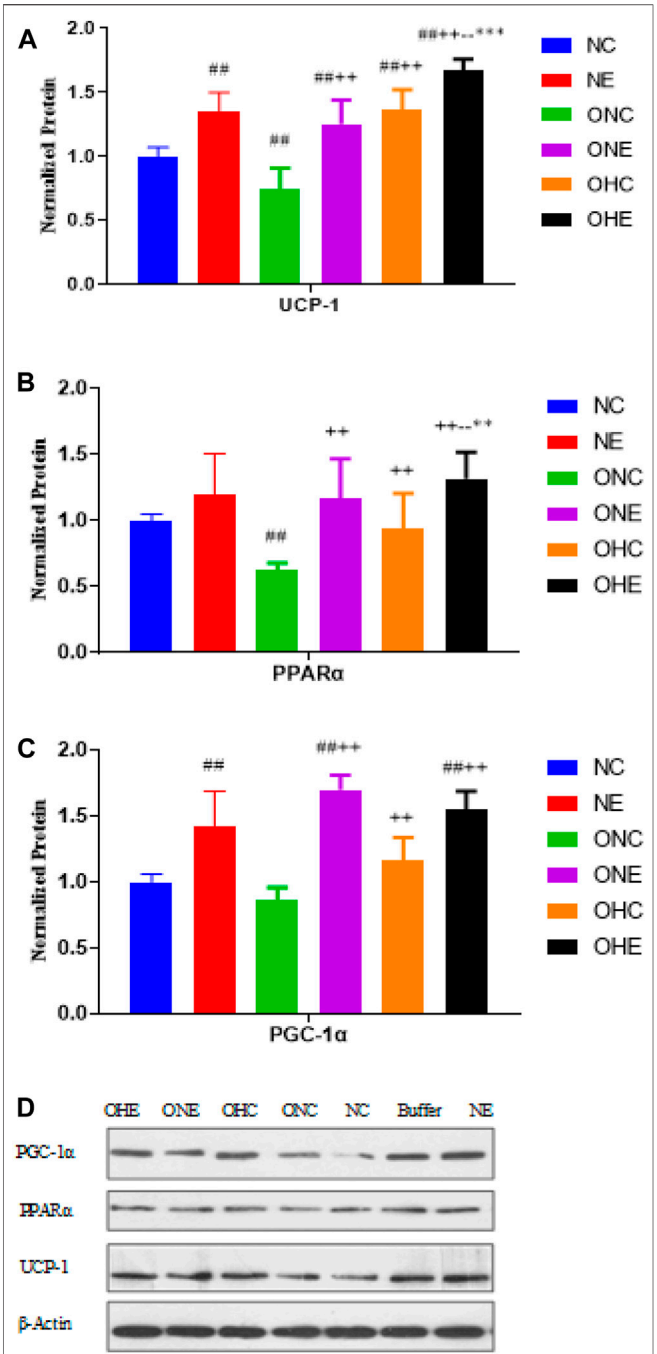

FIGURE 3 | Protein expression levels of UCP-1 **(A)**, PPAR α **(B)** and PGC-1α **(C)** in inguinal adipose of rats after normoxic and hypoxic exercise. Protein expression levels of PPARα and UCP-1 in inguinal adipose of rats in NC, NE, ONC, ONE, OHC, and OHE groups were expressed as relative levels based on the expression level of β-Actin **(D)**. $n = 8$, $^{\#}p < 0.05$ vs. NC group; $^{\#\#}p < 0.01$ vs. NC group; $+p < 0.05$ vs. ONC group; $++p < 0.01$ vs. ONC group; $-p < 0.05$ vs. ONE group; $--p < 0.01$ vs. ONE group; $^{*}p < 0.05$ vs. OHC group; $^{**}p < 0.01$ vs. OHC group; following tables same.

Lee's index in obese rats ($p < 0.01$). In addition, hypoxic exercise had a significantly higher effect in reduction of body weight in obese rats was more effective in improving Lee's index compared with the effect of exercise under normoxia. These findings indicate that hypoxic exercise intervention in is more effective in weight reduction in obese rats compared with exercise under normal conditions.

Moreover, hypoxic exercise significantly improved lipid metabolism. Obese people present with significantly higher TC, TG and LDL levels and significantly lower HDL levels compared with healthy individuals. Abnormal lipid metabolism leads to changes in lipid parameters, which is an important risk factor for obesity-induced cardiovascular diseases. However, in this study, the HDL and LDL of the ONC group even tended to be lower than those of the NC group, and further research is needed. Exercise can improve lipid metabolism in the body, as indicated by decrease in TC, TG, and LDL levels and increase in HDL levels in rats subjected to exercise. Notably, high-intensity interval training induced significantly higher effects on levels of lipid parameters compared with the effect of moderate-intensity aerobic training. Acute hypoxic exposure decreases the levels of triglycerides with 50 and 48 carbons, whereas TGs containing 48-50 carbons are mainly associated with adipogenesis (Kennedy et al., 2001). Studies report that acute hypoxic exposure leads to increase in free fatty acid levels in the blood resulting in increased fatty acid metabolism. Long-term hypoxic exposure (6 weeks or 30 days) causes significant decrease in TG, however, the trends in TC, HDL, and LDL levels are not correlated with the duration of hypoxic exposure and/or degree of hypoxia (Kennedy et al., 2001; Siques et al., 2014; Song et al., 2020). The findings of the current study indicated that normoxic and hypoxic exercise reduced TC, TG, and LDL levels in obese rats. However, the effect of hypoxic exercise in improving HDL and LDL was significantly higher compared with the effect of normoxic exercise. Du et al. (2020) reported consistent findings that increase in altitude was correlated with decrease in TG levels and the decrease was highly correlated hypoxic exposure, however, the effects on TC, LDL, and HDL levels were not correlated with degree of hypoxic exposure.

The preliminary research of our research group shows that 4 weeks of hypoxic training can be achieved by increasing Sterol Regulatory Element Binding protein-1C (SrebP-1C), acetyl-Coa Carboxylase 1 (ACC1) and Fatty Acid in the liver The expression level of Synthetase (FASN) and the expression level of Carnitine Palmitoyl Transferase 1A (CPT1A) in liver can be down-regulated to improve liver lipid metabolism by inhibiting fatty acid synthesis in liver, increasing fatty acid transfer and oxidation in liver, and thus improving body lipid metabolism. (Jing Wen, 2018).

Oxygen concentration affects adipocyte function. Exposure of white adipocytes to hypoxic conditions in culture leads to changes in the expression of over 1,000 genes. The secretion of several adipokines associated with inflammation is upregulated by hypoxia and shifts from oxidative metabolism to anaerobic glycolysis. Glucose utilization is increased in hypoxic adipocytes with a corresponding increase in lactate production. Importantly, hypoxia induces insulin resistance in adipocytes and leads to the development of adipose tissue fibrosis (Trayhurn, 2013; Gozal et al., 2017).

Exercise also induces functional changes in adipose tissue. In particular, after Bostrom et al. (2012) showed in 2012 that irisin as

TABLE 4 | Levels of BAIBA in Gastrocnemius Muscle and Blood of Rats after normoxic and hypoxic exercise (n = 8).

	NC	NE	ONC	ONE	OHC	OHE
Muscle BAIBA(ng/mg)	1.12 ± 0.04	6.47 ± 1.24##	1.68 ± 1.02	7.07 ± 2.50 ++##	2.89 ± 1.13	3.72 ± 1.12++--
Blood BAIBA(ng/mL)	23.28 ± 7.50	37.94 ± 8.49##	35.56 ± 12.17	44.28 ± 22.17##	14.09 ± 2.72++	39.15 ± 16.89**

*n = 8, #p < 0.05 vs. NC group; ##p < 0.01 vs. NC group; +p < 0.05 vs. ONC group; ++p < 0.01 vs. ONC group; -p < 0.05 vs. ONE group; --p < 0.01 vs. ONE group; *p < 0.05 vs. OHC group; **p < 0.01 vs. OHC group; following tables same.*

TABLE 5 | Relationship between BAIBA and blood lipid, and browning index in Rats (n = 8).

		Muscle BAIBA	Blood BAIBA
		Correlation Value (p Value)	Correlation Value (p Value)
TC	Pearson correlation	–0.478 (0.006)%%	–0.422 (0.016) %
TG		–0.313 (0.082)	–0.277 (0.124)
HDL		0.167 (0.360)	0.139 (0.448)
LDL		–0.338 (0.059)	–0.194 (0.288)
PPARα mRNA		0.791 (0.000) %%	0.355 (0.046) %
UCP-1 mRNA		0.568 (0.001) %%	0.550 (0.001) %%
PGC-1α protein		0.372 (0.187)	0.164 (0.273)
PPARα protein		0.425 (0.015) %	0.339 (0.058)
UCP-1 protein		0.246 (0.175)	0.279 (0.122)

%significant difference p < 0.05, %%significant difference p < 0.01.

a myokine can promote white fat browning, more and more studies in recent years have demonstrated that exercise can promote white fat browning, especially the promotion effect of aerobic exercise is more obvious (Rocha-Rodrigues et al., 2016; Rodriguez et al., 2017).

Hypoxic training can promote the expression of UCP-1 through various mechanisms, including the activation of AMPK signaling pathway *in vivo* by hypoxic exercise, which on the one hand can inhibit AMPK levels in hypothalamus, resulting in decreased appetite and downregulation of body fat, and on the other hand, AMPK can stimulate increased expression of PGC-1α mRNA and protein, thus inducing the conversion of white fat to brown fat. However, on the other hand, it has also been shown that hypoxia downregulates neuropeptide Y (NPY) expression levels and that NPY gradually decreases with increasing altitude and duration of hypoxia. In contrast, NPY may inhibit UCP-1 expression by specifically expressing Y5R on the surface of BAT(Kotz et al., 2000). It indicates that hypoxia can affect UCP-1 expression by affecting NPY. The effect of hypoxia on UCP-1 is still unclear and needs to be explored in further studies.

The current study explored the effects of hypoxic exercise on the expression levels of PPARα and UCP-1 in white fat of obese rats and the correlation od these levels with BAIBA level. The findings showed that both normoxic and hypoxic exercise upregulated expression of *PPAR α* mRNA in inguinal fat of rats. Notably, upregulation of *PPAR α* mRNA expression was higher under normoxic exercise compared with that under hypoxic exercise. In addition, hypoxic and normoxic exercise upregulated *UCP-1* mRNA expression in rat inguinal fat, and the finding showed no significant difference between the two forms of exercise on upregulation of *UCP-1* mRNA expression. Normoxic and hypoxic exercise upregulated PPAR α and UCP-1 protein expression in inguinal fat of obese rats, with a higher effect observed under hypoxic exercise. Moreover, hypoxic environment upregulated PPAR α protein expression in inguinal fat of obese rats. In this study, the mRNA expression trends of PGC-1α and other indicators are not completely consistent with the protein expression trends, which may be due to the fact that PGC-1α has a co-activation effect with PPAR-γ and PRDM-16 during browning, mainly at the mRNA level. In this study, we found that hypoxic exercise had an advantage in promoting UCP-1 protein expression, but hypoxic training did not seem to have an advantage over normoxic training in promoting the expression of PPARα and PGC-1α. This may involve appetite suppression and enhanced hepatic lipid metabolism induced by hypoxia and hypoxic training. On the other hand, this study selected the most favorable oxygen concentration for improving lipid metabolism, which may not be the optimal oxygen concentration for promoting browning, and the exact mechanism needs to be further investigated.

PPARα regulates gene transcription by acting on the promoter of target genes, regulates lipid metabolism, suppresses inflammatory responses, and promotes white fat browning. Hypoxic exposure, hypoxic exercise, cold exposure and starvation can affect PPARα expression. Studies in mice exposed to hypoxia found elevated ACC expression in the liver, increased lipid synthesis in the liver, and significantly lower PPARα mRNA expression and reduced fatty acid oxidation. Subsequently, it was found that hypoxic exercise decreased CPT1 protein expression, inhibited the transport of long-chain fatty acids into mitochondria, and increased hepatic fatty acid transport, thereby reducing body weight and body fat in rats (Tong, 2005; Piguet et al., 2010; Urdampilleta et al., 2012). In

this study, we found that both hypoxic and low aerobic exercise upregulated PPARα mRNA expression in adipose tissue, and exercise had a more significant effect on promoting PPARα mRNA.

BAIBA is a non-protein amino acid (Tanianskii et al., 2019) and exercise significantly affects BAIBA secretion. Notably, BAIBA is mainly produced and secreted during muscle contraction. Stautemas et al. (2019) reported that the concentration of D-BAIBA in blood under resting state was approximately 67-fold higher compared with levels of L-BAIBA (1734 ± 821 nM vs. 29.3 ± 7.8 nM). In addition, D-BAIBA increased by approximately 13% and L-BAIBA increased by approximately 20% after 1 h of cycling at maximum output power intensity. The two conformations of BAIBA play roles as muscle factors (Stautemas et al., 2019). A study by Roberts et al. reported that BAIBA concentrations in gastrocnemius muscle of wild-type mice increased by approximately 5.2-fold ($p < 0.01$) after 3 weeks of free spinning exercise, and blood BAIBA levels increased by approximately 19% ($p < 0.01$) (Roberts et al., 2014).

In this study, we found Hypoxic exercise also led to an increase in BAIBA secretion in the gastrocnemius muscle, but the increase was lower than that of normoxic exercise; hypoxic exercise could ameliorate the decrease in blood BAIBA concentration caused by the hypoxic environment and restore it to a level exceeding normoxic rest. Previous studies have shown that staying at high altitude results in increased metabolism of BCAA, BCAA catabolism in skeletal muscle begins with the transamination of α-KG to glutamate by branched-chain aminotransferase, yielding branched-chain ketoacids that are ultimately oxidized as succinyl -CoA in the citric acid cycle (CAC). BAIBA is a metabolite of valine in BCAA (Wagenmakers, 1992; Makowski et al., 2005; Chicco et al., 2018). It is not clear whether BCAA metabolism caused by hypoxic environment causes valine deficiency, which may be one of the reasons affecting the concentration of BAIBA in hypoxic environment. The results of this study also suggest that future research needs to further explore the influencing factors of hypoxic environment down-regulating BAIBA secretion in blood, and whether supplementation of BAIBA can further enhance the fat-reducing effect of hypoxic exercise requires further research.

In the current study, correlation analysis showed that BAIBA concentration in skeletal muscle was significantly and positively correlated with inguinal fat *PPAR α* mRNA expression level, UCP-1 mRNA expression level, and PPAR α protein expression level. Moreover, the findings showed that BAIBA concentration in blood was significantly correlated with inguinal fat *PPAR α* mRNA expression level and *UCP-1* mRNA expression level. These findings indicate that BAIBA modulates the transcriptional process of inguinal fat PPAR α and UCP-1 by regulating skeletal muscle secretion, thus regulating occurrence of white fat browning, and ultimately regulating blood lipid profile, weight loss and fat loss. Browning of white fat in obese rats occurs in visceral fat and white fat with high tissue specificity. In addition, browning of visceral fat is mainly modulated by sympathetic nerve activity and adrenaline secretion. Browning of subcutaneous fat is mainly induced by inter-tissue signaling crosstalk (Cross-talk) of cytokines, mainly muscle factors (Rodriguez et al., 2017).

CONCLUSION

The finding showed that 4-weeks hypoxic exercise induced reduction in body weight and Lee's index and improved blood lipid profile in obese rats. In addition, 4-weeks hypoxic exercise upregulated BAIBA concentration in gastrocnemius muscle and circulation, upregulated PPARα and UCP-1 expression in inguinal fat and increased white fat browning in obese rats. The findings showed that BAIBA may involves in improveing blood lipid profile lipid metabolism and white fat browning by modulating PPARα and UCP-1 expression.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusion of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The animal study was reviewed and approved by Institutional Ethics Committee of China Institute of Sport Science (protocol code cissla-2014032, 2014-02-26).

AUTHOR CONTRIBUTIONS

JF, XW, CY, and XW developed protocols and conducted experiments. JF wrote the manuscript. YL and LF conceived the study, finalized the study design, provided oversight for its conduct, and revised the manuscript. All authors contributed to the article and approved the submitted version.

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Effects of Yoga on Blood Glucose and Lipid Profile of Type 2 Diabetes Patients Without Complications: A Systematic Review and Meta-Analysis

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Background: Type II diabetes mellitus (T2DM) has become a worldwide public health problem. Although it has been empirically established that physical activity is a promising therapeutical approach to the prevention and management of T2DM, the effectiveness of yoga on T2DM has not yet reached an agreement across studies and also needs an updated synthetic examination.

Purpose: The purpose of this study was to examine the effect of yoga training on diabetes-related indicators compared with usual care.

Methods: The review protocol of this study has been registered in the PROSPERO with a registration number CRD42021267868. A systematic literature search through electronic databases was conducted to identify yoga-based intervention (i.e., randomized controlled trial [RCT]; e.g., yogic postures, movements, breathing, and meditation) studies reporting outcomes on glycosylated hemoglobin (HbA1c), fasting blood glucose (FBG), postprandial blood glucose (PPBG), total cholesterol (TC), triglycerides (TG), and body mass index (BMI). A number of two researchers manually reviewed and assessed each article using the Cochrane Risk of Bias Tool 2.0. The literature search identified 296 eligible entries, of which 13 were finalized after screening using predefined inclusion and exclusion criteria. The extracted data (group mean and standard deviation at posttest) were synthesized using random-effects meta-analyses. Finally, potential moderators were explored using subgroup analysis and sensitivity analysis.

Results: The standardized mean difference for the effects of yoga was significant on HbA1c (MD = −0.47; 95%CI: −0.77, −0.16; $Z = 3.02$, $p = 0.003$), FBG (SMD = −0.92; 95%CI: −1.55, −0.29; $Z = 2.87$, $p = 0.004$), PPBG (SMD = −0.53; 95%CI: −0.86, −0.21; $Z = 3.20$, $p = 0.001$), and TG (SMD = −0.32; 95%CI: −0.54, −0.10; $Z = 2.86$, $p = 0.004$). However, yoga effect was not observed on TC (SMD = −0.84; 95%CI: −1.71, 0.04; $Z = 1.87$, $p = 0.06$) and BMI (MD = −0.63; 95%CI: −1.42, 0.16; $Z = 1.57$, $p = 0.12$).

Conclusion: The findings suggest that yoga can improve the biochemical indices of blood glucose and the lipid profile of patients with T2DM. Therefore, yoga can be prescribed as an effective and active complementary treatment for T2DM. However, this

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study only tested yoga as a short-term treatment. In the future, rigorous RCTs with a larger sample size may be carried out to examine the long-term effect of yoga on T2DM.

Systematic Review Registration: https://www.crd.york.ac.uk/PROSPERO/display_record.php?RecordID=267868, identifier: CRD42021267868.

Keywords: meta-analysis, type 2 diabetes, yoga, blood glucose, lipid profile

INTRODUCTION

Type 2 diabetes mellitus (T2DM) has become a serious public health problem across the globe, which is typically characterized by impaired insulin secretion and insulin resistance, and seriously affects the quality of life of patients. Individuals with T2DM were subjected to many life-threatening health problems, resulting in higher medical care costs, faded quality of life, and a higher risk of mortality (Baena-Díez et al., 2016). According to the International Diabetes Federation, the prevalence of diabetes worldwide is now estimated to be over 10%, and the cases of diabetes were dominated by T2DM. By 2045, the absolute number of patients with T2DM will increase by 46% (Sun et al., 2022). Obviously, T2DM has become a global epidemic. Apart from uncontrolled factors, physical inactivity, unhealthy diet intake, overweight, and obesity were thought to be the main contributors to diabetes (Hu, 2011). Regardless of the type of diabetes, patients were required to control their blood glucose through receiving medication, exercise prescriptions, and special dietary plans. However, therapies incorporating exercise were believed to be one of the safe and healthy approaches for T2DM treatment. Currently, there are burgeoning unconventional auxiliary treatment options available for patients with diabetes for better control of their blood glucose levels such as yoga, massage therapy, and acupuncture (Pandey et al., 2011). Ascertaining the effectiveness of yoga on T2DM through empirical examination is therefore warranted. To date, yoga as adjuvant therapy for diabetes has not yet been thoroughly investigated for agreement across studies, especially for patients with T2DM without severe metabolic syndrome (Dutta et al., 2021).

According to the American Diabetes Association, moderate-intensity aerobic exercise plays a pivotal role in managing diabetes-induced metabolic disorders (American Diabetes Association, 2019). In fact, insufficient physical activity leads to an increased risk of obesity (Kim et al., 2017), which is a possible reason for the occurrence of T2DM and also a considerable predictor of mortality and complications in patients with T2DM (American College of Sports Medicine, 2013). Therefore, one of the key points of T2DM care is to provide a tailored physical activity recommendation while taking into account patients' complex health conditions (Lin et al., 2012). As a mind-and-body integrated exercise, yoga capitalizes on the capability of meditation to enhance physical health, which appears particularly suitable for the fitness condition of patients with diabetes. Yoga is underpinned by one of the six philosophical systems in ancient India advocating the ideal way of lifestyle. Meanwhile, yoga can be of moderate exercise intensity under specific types of training (Larson-Meyer, 2016). In recent years, yoga has been much more fashionable across the globe, and the use of yoga as a therapy is developing rapidly (Jeter et al., 2015). Practically, yoga has played a significant role in the treatment and prevention of diabetes. Several experimental studies examining the effectiveness of yoga on T2DM have shown favorable results in blood glucose, lipid profile, oxidative stress, blood pressure, anthropometric indicators, and quality of life (Gordon et al., 2008; Hegde et al., 2011, 2020; Shantakumari and Sequeira, 2013; Datey et al., 2017; Balaji et al., 2020; Sharma et al., 2020; Nair et al., 2021; Ranga et al., 2021; Sivapuram et al., 2021). Additionally, favorable results were also observed in mental health, functional capacity, wellbeing, sleep quality, and body composition among patients with diabetes, as a result of receiving yoga practice (Akhtar et al., 2013; Innes and Selfe, 2016; Miles et al., 2016; Rshikesan et al., 2017).

Prior intervention studies examining the response of T2DM to yoga training showed typical characteristics across philosophical underpinning, exercise type, dosage, and population. The Hindu religious perspectives underlie the physical practices of yoga which also frames the ideology for yogic meditation (De Michelis, 2005). Most yogic practices adopted in the interventions focused primarily on postures (asanas), usually with the additions of breath control (pranayama), and/or sometimes also incorporating elements of concentration or meditation; however, this varies with different styles or schools of yoga (e.g., Hatha vs. integrated yoga) that are practiced in different areas of the world and/or for different purposes (De Michelis, 2005; Sengupta, 2012). The reported dosage of promising yoga interventions showed variations in the duration of each yoga session (45–90 min), frequency (2–3 times/week to every day), and total length of intervention (40 days−6 months). The reported age of subjects ranged from 30 to 83.7 years. It is worth noting that most of the study subjects are adults from India, which may be due to the origin of yoga in India (Jeter et al., 2015).

Type 2 diabetes mellitus is typified by hyperglycemia in the presence of insulin resistance (American Diabetes Association, 2014). Therefore, keeping glucose levels within a healthy range is the most important recommendation for diabetes management (Inzucchi et al., 2012). Other key-related hemodynamic and metabolic abnormalities characterizing T2DM include elevated blood pressure, dyslipidemia, and increased oxidative stress (Wellen and Hotamisligil, 2005; Innes and Vincent, 2007; Rana et al., 2007). It is known that people who are overweight or obese have a higher risk of developing T2DM. Body mass index (BMI) is a measurement of body weight status. A meta-analysis with prospective cohort studies reported that overweight (BMI: 25–30 kg/m^2) or obese (BMI: >30 kg/m^2) individuals were more likely to have T2DM (Mi et al., 2020). It can be seen that BMI plays an

important role in diabetes management. Considering the above reasons, blood glucose, lipid profile, and BMI were considered the variables of interest in this study. Furthermore, the risk for T2DM increases with age, and even individuals as early as childhood are also subjected to such disease (Yoon et al., 2006). Among those under 60 years old, 41.1% of deaths are diabetes-related (IDF Diabetes Atlas, 2015). Given that, T2DM as a life-threatening risk factor can occur at any time point across the entire lifespan, which deserves more epidemiological and medical investigation for a healthier way of prevention and/or treatment.

Despite the studies of systematic review and meta-analysis having been published previously (Ramamoorthi et al., 2019; Wibowo et al., 2021), the role of yoga in diabetes treatment, especially whether yoga can significantly improve blood glucose and lipid profile, was still under debate. Besides, most of the prior meta-analyses had included prediabetes or T2DM patients with other symptoms such as severe metabolic syndrome rather than merely patients with T2DM alone and rarely confirmed whether the patients have complications, making it difficult to generalize the results to other settings. In addition, the inclusion criteria of prior studies were problematic in that the inclusion of low-dose (i.e., intervening length $\leq$4 weeks) studies would jeopardize the credibility of the results. Given the above factors, this study was an update as follows. First, we strictly restricted our analyses to subjects with T2DM. This study only evaluated the effect of yoga on patients with uncomplicated T2DM, not taking into account patients with complications, prediabetes, gestational diabetes, etc., and further explored the effect of yoga on patients of different ages. Second, we rigorously developed inclusion criteria based on the Cochrane manual and included only randomized controlled trials, excluding case–control or quasi-experimental studies, and low-dose studies. Finally, in addition to observing the effects of yoga on blood glucose and lipid profiles, we also assessed the effect of yoga on BMI, which is necessary because most patients with T2DM are with abnormal BMI. Not only that, we expanded the search scope and refreshed the search years. Therefore, the purpose of this study was to evaluate available evidence from existing randomized controlled trials concerning the effect of yoga-based intervention (e.g., yogic postures, movements, breathing, and meditation) on the biochemical indicators of blood glucose, lipid profile, and BMI in patients with T2DM and expected to provide an evidence-based reference for the treatment of diabetes with yoga practice.

METHODS

The review protocol of this study has been registered in the PROSPERO and assigned the registration number CRD42021267868. All review procedures followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) recommendation (Moher et al., 2009). The PRISMA checklist can be found in the **Supplemental Materials**.

Identification of Studies

The Cochrane handbook provided the guideline for conducting and reporting this systematic review and meta-analysis study (Higgins, 2011). A systematic search was conducted to investigate the yoga effect on T2DM. After identifying relevant Medical Subject Headings (MeSH), six electronic databases were searched by the first author including Cochrane Library, Medline, EMBASE, PubMed, Web of Science, and FMRS from their inception through July 2021. The keywords used (in possible combinations and variations) in the search were built as follows: (yoga [MeSH] OR exercise [MeSH] OR physical activity [MeSH]) AND (type 2 diabetes mellitus [MeSH]) AND (fasting blood glucose [MeSH] OR fasting blood sugar [MeSH] OR fasting plasma glucose [MeSH]) AND (postprandial blood sugar [MeSH] OR postprandial blood glucose [MeSH]) AND (total cholesterol [MeSH]) AND (Triglycerides [MeSH]) AND (glycated hemoglobin [MeSH] OR glycosylated hemoglobin [MeSH] OR HbA1c [tiab]) AND (body mass index OR BMI [tiab]) AND (randomized controlled trial [MeSH] OR RCT [tiab]). Titles and abstracts of the citations were scanned to identify potential articles. Potentially eligible articles were retrieved for more detailed review. Eligible trials were limited to adult human subjects, and only trials published with the full text and written in English were included in this study.

Criteria for Inclusion and Exclusion

The following eligibility criteria were applied for screening: (1) setting and population: adult patients with T2DM (as diagnosed by a clinician, or using recognized diagnostic criteria) with confirmed disease regardless of gender; (2) study design: randomized controlled trials; (3) intervention: yoga-based intervention (e.g., yogic postures, movements, breathing, and meditation) / program; (4) comparison: the control group only receives usual care or alternate program; (5) geographic origin: countries across the globe; (6) language: English; (7) age: $<$70 years old; (8) study duration: 10–24 weeks. Studies violating one of the following criteria were excluded: (1) suspected patients with T2DM without further confirmatory testing; (2) literature review; (3) with no control group; (4) without sufficient data/statistics for referential analysis and/or without full text; and (5) patients with gestational diabetes mellitus. The electronic database search was complemented with a manual search to identify any missing studies from the reference lists of all included articles. A number of two research assistants (C and D) independently screened each article and any discrepancies in eligibility were discussed before a decision was made for inclusion or exclusion.

Risk of Bias Assessment

The risk of bias was evaluated using the Cochrane Risk of Bias Tool (Higgins, 2011) by two research assistants (C and D) independently, which rated each article against six criteria: (1) selection bias: whether details of random sequence generation and allocation concealment were sufficiently described; (2) performance bias: whether blinding of participants and personnel as well as blinding efficacy was sufficiently described; (3) detection bias: whether blinding of outcome assessment was sufficiently described; (4) attrition bias, whether details and reasons of attrition and exclusion were sufficiently described; (5) reporting bias: whether concerns for possible selective outcomes reporting were clearly stated; (6) other bias: whether any other

important concerns that were not addressed in the above five criteria were described. The assessment to each criterion has three levels: low risk of bias, unclear risk of bias, and high risk of bias. A third research assistant (L) would jump in to solve the problem whether there were discrepancies between the two research assistants.

Data Extraction

The information and data extraction for the included articles were conducted by two research assistants (C and D) independently using a predefined data extraction form, including the first author name, year of publication, sample size, intervention duration, research design, demographic details (i.e., gender and age), and group mean and standard deviation (SD) at posttest. The extracted data and information from the included studies were entered and saved in an Excel spreadsheet using a purpose-built template. For RCTs with three-arms or more, only data from the yoga group and control group were used for comparison. Any disagreement between the two reviewers was discussed until a consensus was reached. But if disagreement was not resolved, a third research assistant (L) would sort out the conflict.

Data Synthesis and Meta-Analysis

The literature review and meta-analysis were conducted using EndNote X8 and Review Manager (Revman) version 5.4.1, respectively, for bias processing, assessment, heterogeneity test, pooled data evaluation, bias graph, forest plot, and funnel plot. The meta-analysis only examined continuous outcome variables, and each mean difference was weighted according to the inverse variance method (weighted mean difference [MD]) (Higgins, 2011). When the same outcome was measured by different scales, the mean difference was standardized by dividing it by the within-group SD; the results were then weighted and the average is taken (standardized mean difference [SMD]). The MD or SMD in each study was pooled with a random-effects model (Higgins and Green, 2008). The p-value was set at 0.05 to be statistically significant. Within-group heterogeneity was evaluated using the I^2 statistic (negligible: $p > 0.10$, heterogeneous: $p \leq 0.10$), with low, moderate, and high heterogeneity levels set to be I^2 values of 25, 50, and 75%, respectively (Higgins et al., 2003). For subgroup analyses, the heterogeneity between groups was also calculated using the I^2 statistic.

Subgroup and Sensitivity Analyses

Substantial differences were observed in session length, publication year, sample size, and age, across included studies, and thus, subgroup analysis was used. Subgroup analysis is one of the important methods to analyze the heterogeneous results or to answer questions about specific patients, intervention types, or research types. The subgroup moderator analysis was used to explain higher heterogeneity in this meta-analysis and examine whether the effects of yoga differed according to (1) session length, (2) age, and (3) sample size. To further investigate, sensitivity analysis was conducted for the meta-analyses by bringing out each study one by one from the meta-analysis and

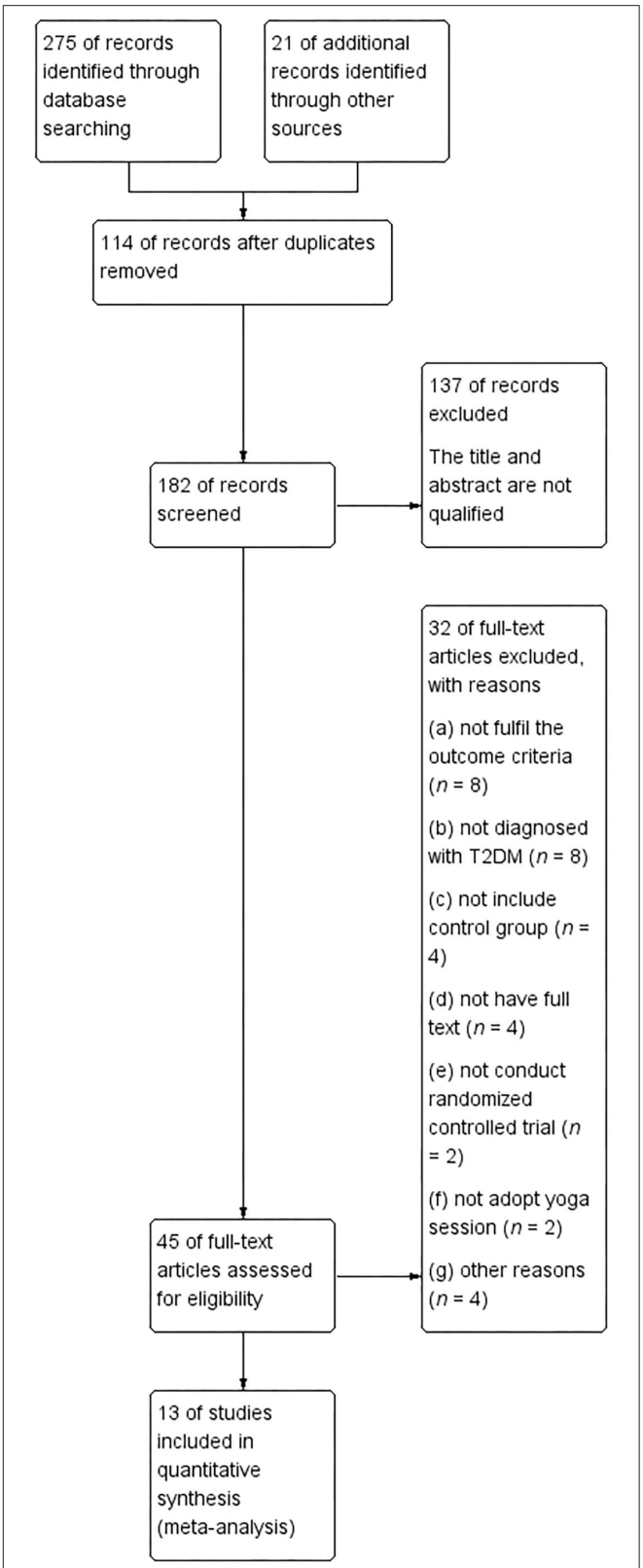

FIGURE 1 | Flow chart for study inclusion and exclusion process.

recomputing the effect size and I^2 to evaluate the influence of each study on the newly emerged effect size.

Publication Bias

A funnel plot was used for assessing the publication bias. A higher risk of publication bias depicts a much asymmetric distribution in the plot. The funnel plot is a commonly used method of identifying publication bias in meta-analysis. In the absence of bias, the dots in the plot should be clustered into an inverted funnel. If there is severe publication bias, the funnel plot looks asymmetrical and there is a blank in the bottom corner of the plot. In this case, the synthesized effect sizes calculated by meta-analyses may overestimate the overall efficacy of the included interventions (Higgins, 2011).

RESULT

Study Selection

Based on the participant, interventions, comparisons, and outcomes (PICO) principle (Higgins, 2011), the initial search retrieved 296 entries from six databases. After removing 114 duplicates, there were 182 studies left. We further excluded 137 articles with irrelevant titles and abstracts. After the first round of screening, 251 articles were excluded and 45 studies with full text received a further evaluation. In the second round of screening, 32 studies were excluded because of the following reasons: (a) not fulfill the outcome criteria ($n = 8$); (b) not diagnosed with T2DM ($n = 8$); (c) not include control group ($n = 4$); (d) not have full text ($n = 4$); (e) not conduct randomized controlled trial ($n = 2$); (f) not adopt yoga session ($n = 2$); and (g) other reasons ($n = 4$). Finally, only 13 RCT studies were included in the meta-analyses (**Figure 1**).

Study Characteristics

Key characteristics of eligible studies are shown in **Table 1**. Overall, the total sample size was 1,335 (intervention = 672, control = 663). The sample sizes across all eligible studies ranged from 40 to 300. All participants were adults with T2DM (M_{age} = 53.2; M_{age} range: 41.3–63.8 years). A number of two studies did not specify patients' average age but were also included as an exception because the age range was provided meeting the inclusion criteria. The length of yoga intervention varied from 10 to 24 weeks with the majority of the studies receiving 12 weeks (62%) of intervention (Hegde et al., 2011, 2020; Shantakumari and Sequeira, 2013; Datey et al., 2017; Sreedevi et al., 2017; Ranga et al., 2021; Sivapuram et al., 2021; Viswanathan et al., 2021) and few studies using 24 weeks (two studies: 15%) (Gordon et al., 2008; Sharma et al., 2020), 16 weeks (two studies: 15%) (Balaji et al., 2020; Gupta et al., 2020), and 10 weeks (one study: 8%) (Nair et al., 2021). Each yoga session lasted for 45–120 min per day, and the frequency was 1–7 days per week. The majority of the studies did not explicitly state the yoga style used during the intervention.

Quality and Risk of Bias Assessment

Quality and risk of bias assessment were conducted based on the Cochrane Risk of Bias Tool (Higgins, 2011). The summaries of the risk of bias across the 13 eligible studies are displayed in **Figures 2**, **3**. The majority of studies (78%) were at low risk of bias for selective reporting. For random sequence generation, seven studies (54%) showed low risk, three studies (23%) were an unclear risk, and three studies (23%) have high risk. For allocation concealment, six studies (46%) adequately concealed allocation; three studies (23%) clearly stated that subjects and researchers were likely to predict distribution results, and four studies (31%) did not mention anything concerning allocation concealment. For binding of participants and personnel, all research was high risk and unclear risks. The rest of the risk biases was low risk and unclear risk (refer to **Figures 2**, **3** for details).

Primary Outcomes

Effect of Yoga on HbA1c

Data involving 927 subjects with T2DM from nine eligible studies were analyzed to examine the effect of yoga on glycated hemoglobin (HbA1c). A number of four studies showed significant HbA1c decreases in the yoga group following the intervention compared to the control group (**Figure 4**). Results from the meta-analysis showed a significant overall mean difference favoring yoga group (MD = −0.47; 95%CI: −0.77, −0.16; $Z = 3.02$, $p = 0.003$). Heterogeneity was clearly significant for the pooled result of HbA1c ($df = 8$, $p < 0.0001$, $I^2 = 82\%$). We carried out subgroup analyses to investigate the potential sources of heterogeneity.

Effect of Yoga on FBG

Data involving 1,130 subjects with T2DM from 11 eligible studies were analyzed to ascertain the effect of yoga on fasting blood glucose (FBG). A total of seven studies showed significant FBG decreases in the yoga group following the intervention compared to the control group (**Figure 5**). Results from the meta-analysis showed a significant standardized mean difference favoring yoga group (SMD = −0.92; 95%CI: −1.55, −0.29; $Z = 2.87$, $p = 0.004$). Heterogeneity was clearly significant for the pooled result of FBG ($df = 10$, $p < 0.0001$, $I^2 = 96\%$). We carried out subgroup analyses to investigate the potential sources of heterogeneity.

Effect of Yoga on PPBG

Data involving 726 subjects with T2DM from six eligible studies were analyzed to find out the effect of yoga on postprandial blood glucose (PPBG). The majority of the studies showed significant PPBG decreases in the yoga group following the intervention compared to the control group (**Figure 6**). Results from the meta-analysis showed a significant standardized mean difference favoring yoga group (SMD = −0.53; 95%CI: −0.86, −0.21; $Z = 3.20$, $p = 0.001$). Heterogeneity was clearly significant for the pooled result of PPBG ($df = 5$, $p < 0.0001$, $I^2 = 76\%$). We thus carried out sensitivity analyses to investigate the potential sources of heterogeneity.

Secondary Outcomes

Effect of Yoga on TC

Data involving 924 subjects with T2DM from eight eligible studies were analyzed to find out the effect of yoga on total cholesterol (TC). Only four studies showed significant TC

TABLE 1 | Characteristics of the included intervention studies.

Study	N (female%)	Age mean (SD)	Group	Duration (min/d)	Frequency (d/wk)	Study length (wk)	Outcomes
Balaji et al. (2020)	69 (32%)	49.6 (5.9)	YG, CT	60	3	16	Outcome measures included fasting blood sugar (FBS), postprandial blood sugar (PPBS), HbA1c, blood urea, and serum creatin.
Datey et al. (2017)	74 (0%)	41.3 (11.4)	AHJ+YG, YG, CT	60	7	12	Outcome measures included fasting blood sugar (FBS) postprandial blood sugar (PPBS) and the hemoglobin A1c (HbA1c).
Gordon et al. (2008)	154 (80.5%)	63.8	PT, YG, CT	120	1	24	Outcome measures included lipid profile, fasting blood glucose, concentration of MDA, PLA2 activity, and POX.
Gupta et al. (2020)	78 (44%)	50.6 (8.5)	YG, CT	45	3	16	Outcome measures included Hba1c, weight, body mass index, waist circumference, systolic blood pressure, diastolic blood pressure, FPG, total cholesterol, triglycerides, LDL cholesterol, and HDL cholesterol.
Hegde et al. (2011)	123	58.6 (9.4)	YG, CT	–	3	12	Outcome measures included BMI, waist circumference, waist-to-hip ratio, blood pressure, fasting plasma glucose (FPG), postprandial plasma glucose (PPPG), and Hba1c.
Hegde et al. (2020)	40 (50%)	57.3 (5.8)	YG, SYG	75–90	6	12	Outcome measures included MDA, GSH, vitamin C, SOD, fasting plasma glucose, glycosylated hemoglobin (Hba1c), waist circumference, body mass index (BMI), and blood pressure.
Nair et al. (2021)	45 (47%)	50.3 (4.3)	YG, CT	60	4	10	Outcome measures included fasting blood sugar (FBS), tail moment (TM), olive tail moment (OTM), total antioxidant capacity (TAC), low-density lipoprotein (LDL), high-density lipoprotein (HDL), triglycerides (TG), total cholesterol (TC), blood pressure, waist-to-hip ratio (WHR), and body mass index (BMI).
Ranga et al. (2021)	100	–	YG, CT	–	5	12	Outcome measures included blood pressure (BP), fasting blood glucose (FBG), postprandial blood glucose, and Hba1c.
Shantakumari and Sequeira (2013)	100 (49%)	45.0 (9.5)	YG, CT	60	7	12	Outcome measures included triglycerides (TG), total cholesterol (TC), HDL cholesterol, LDL cholesterol, and high-density lipoprotein e cholesterol (HDLeC).
Sharma et al. (2020)	104 (45%)	–	YG, CT	40	5	24	Outcome measures included glucose (fasting and postprandial) and lipid profile, body mass index (BMI), waist-to-hip ratio (WHR), TG, TC, HDL-c (enzymatic direct HDL method), low-density lipoprotein (LDL), very low-density lipoprotein (VLDL). AIP, The logarithm of molar ratio of TG to high-density lipoprotein cholesterol (TG/HDL cholesterol), was calculated.
Sivapuram et al. (2021)	81	56.7 (18.2)	YG, CT	60	7	12	Outcome measures included fasting blood sugar (FBS), postprandial blood sugar (PPBS), Hba1c, lipid profile, and BMI.
Sreedevi et al. (2017)	67 (100%)	51.9 (7.0)	PYG, YG, CT	60	2	12	Outcome measures included fasting plasma glucose, Hba1c, quality of life, Pharmacological adherence, BMI, waist-to-hip ratio (WHR), blood pressure, and total cholesterol.
Viswanathan et al. (2021)	300 (35%)	51.8 (7.7)	YG, CT	50	5	12	Outcome measures included anthropometric measurements, BMI, blood pressure, Pittsburgh sleep quality index (PSQI), glycated hemoglobin (HbA1c), TG, TC, HDL-C (enzymatic direct HDL method), low-density lipoprotein (LDL), very low-density lipoprotein (VLDL).

Age was the average of all subjects in each study.

YG, yoga group; CT, control group; AHJ, Ayurveda herbal juices; PT, conventional physical training; SYG, sham yoga; PYG, peer sports yoga.

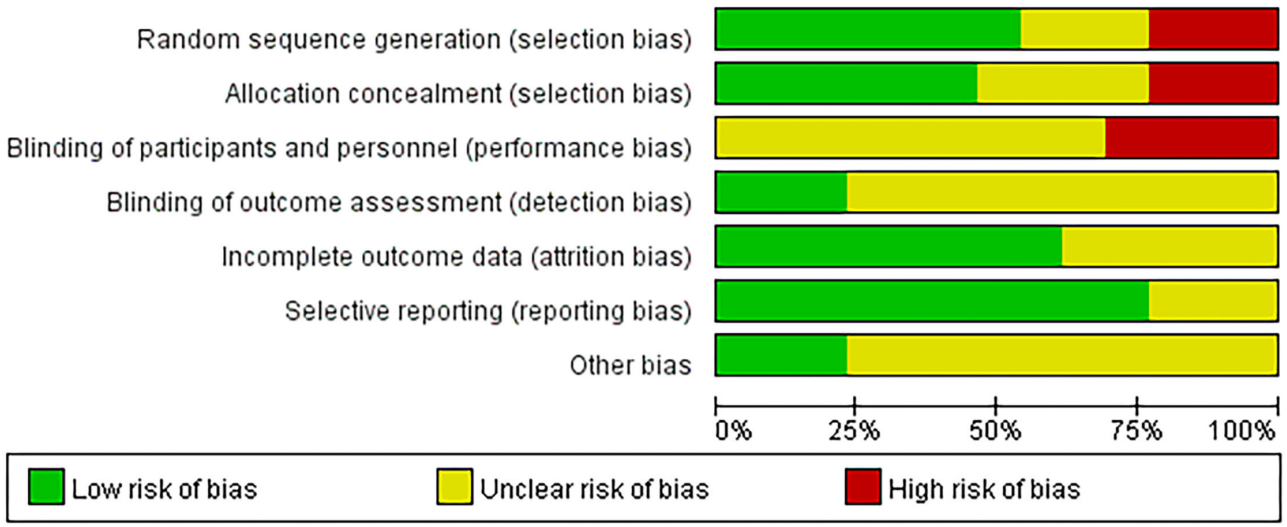

FIGURE 2 | Risk of bias graph: judgment on each risk of bias item presented as a percentage across all included studies.

changes in the yoga groups following the intervention compared to the control groups (**Figure 7**). Results from the meta-analysis showed no significant overall standardized mean difference (SMD = −0.84; 95%CI: −1.71, 0.04; $Z = 1.87$, $p = 0.06$). Heterogeneity was clearly significant for the pooled result of TC ($df = 7$, $p < 0.001$, $I^2 = 97\%$).

Effect of Yoga on TG

Data involving 862 subjects with T2DM from seven eligible studies were analyzed to determine the effect of yoga on triglycerides (TG). A total of four studies showed significant TG decreases in the yoga group following the intervention compared to the control group (**Figure 8**). Results from the meta-analysis showed a significant standardized mean difference favoring yoga group (SMD = −0.32; 95%CI: −0.54, −0.10; $Z = 2.86$, $p = 0.004$). Heterogeneity was clearly significant for the pooled result of TG ($df = 6$, $p = 0.03$, $I^2 = 58\%$). We carried out subgroup analyses to investigate the potential sources of heterogeneity.

Effect of Yoga on BMI

Data involving 857 subjects with T2DM from eight eligible studies were analyzed to examine the effect of yoga on body mass index (BMI). The majority of the studies showed no significant BMI changes in the yoga groups following the intervention compared to the control groups (**Figure 9**). Results from the meta-analysis showed no significant overall mean difference between groups (MD = −0.63; 95%CI: −1.42, 0.16; $Z = 1.57$, $p = 0.12$). Heterogeneity was clearly significant for the pooled result of BMI ($df = 7$, $p < 0.001$, $I^2 = 76\%$).

Heterogeneous Interpretation

HbA1c

With subgroup moderator analysis, we observed that the effect of yoga differs according to the yoga session length. Subgroup analysis showed that (**Figure 10**) the heterogeneity was 15% when the session length was more or <60 min (MD = −0.32; 95%CI: −0.49, −0.15; $Z = 3.63$, $p < 0.001$); the heterogeneity was 91% when each session was equal to 60 min (MD = −0.80; 95%CI: −1.56, −0.04; $Z = 2.07$, $p = 0.04$). The heterogeneity increased after pooling the data, indicating that the difference in each session length was likely to be the source of heterogeneity.

FBG

With subgroup moderator analysis, we observed that the effect of yoga on FBG differs according to the individual's age. Subgroup analysis result showed (**Figure 11**) that patients ≤50 years old were with 31% heterogeneity (SMD = −0.77; 95%CI: −1.09, −0.46; $Z = 4.80$, $p < 0.001$); patients older than 50 years old were with 97% heterogeneity (SMD = −0.98; 95%CI: −1.85, −0.11; $Z = 2.21$, $p = 0.03$). The heterogeneity increased after pooling the data, indicating that difference in each individual's age was likely to be the source of heterogeneity.

PPBG

With subgroup moderator analysis, we did not observe that the effect of yoga differs according to how they were grouped. Further, a sensitivity analysis identified that a more restricted analysis of the data did not affect the merging effect size. After excluding the study with high heterogeneity (Balaji et al., 2020), the pooled data of the rest studies changed insignificantly, indicating that the sensitivity was low, and the results were relatively robust.

TG

With subgroup moderator analysis, we observed that the effect of yoga differs according to the sample size of the study. Subgroup analysis showed that (**Figure 12**) the heterogeneity was 0% when the sample size outweighed 100 subjects (SMD = −0.30; 95%CI: −0.46, −0.13; $Z = 3.49$, $p < 0.001$); the heterogeneity was 76% when sample size was ≤100 subjects (SMD = −0.35; 95%CI:

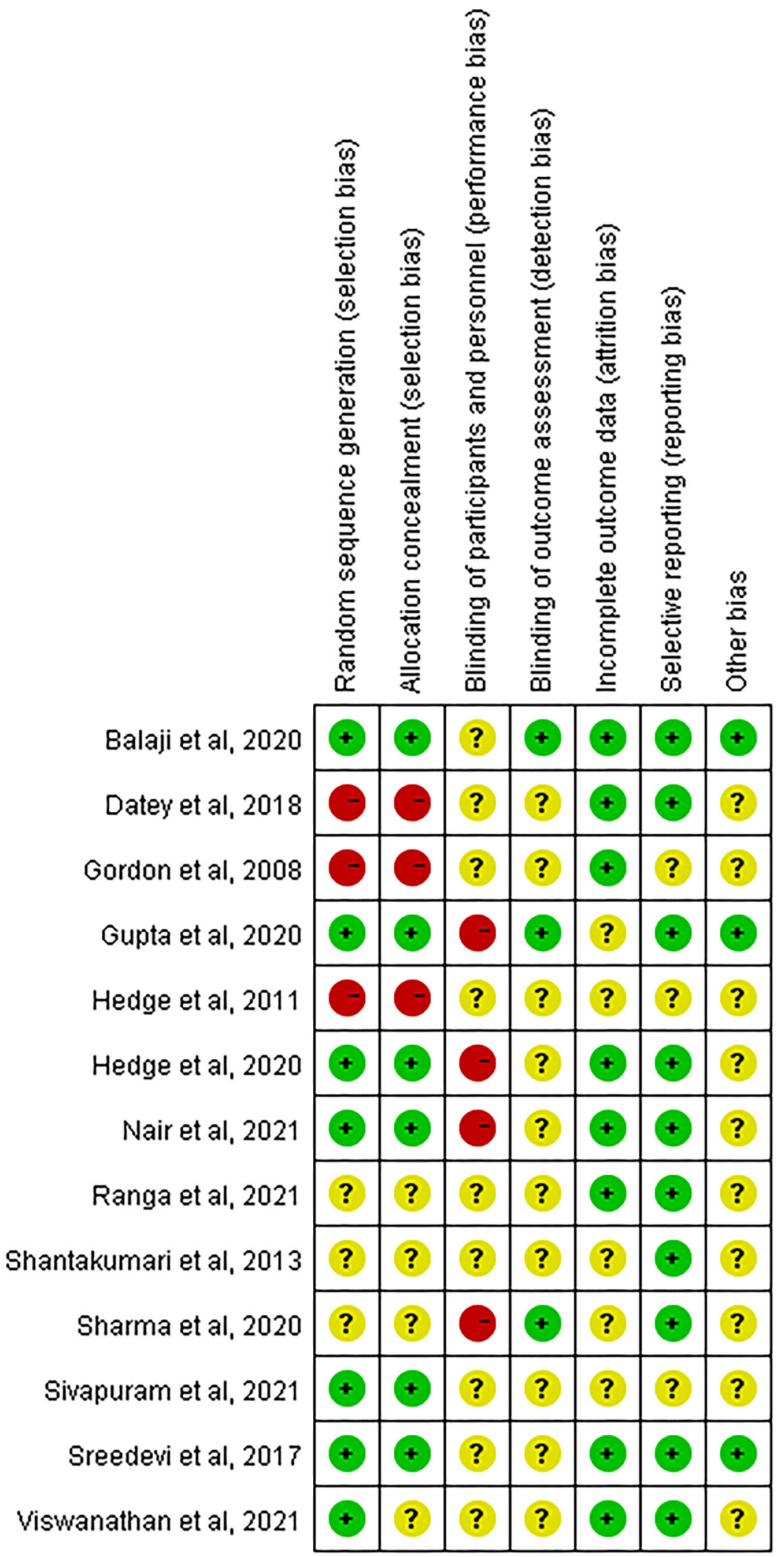

FIGURE 3 | Risk of bias summary: judgment on each risk of bias item for each included study.

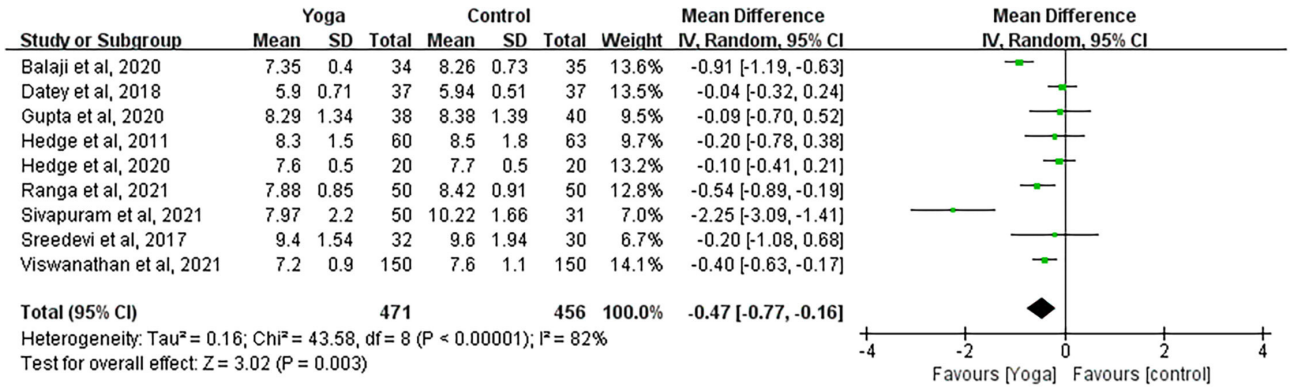

FIGURE 4 | Forest plots for the effect of yoga on HbA1c.

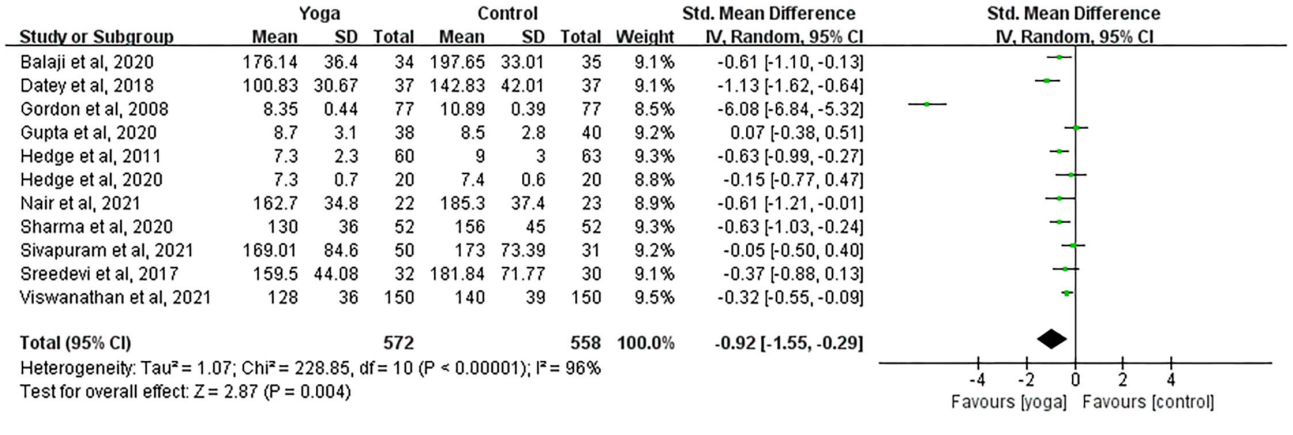

FIGURE 5 | Forest plots of the effect of yoga on FBG.

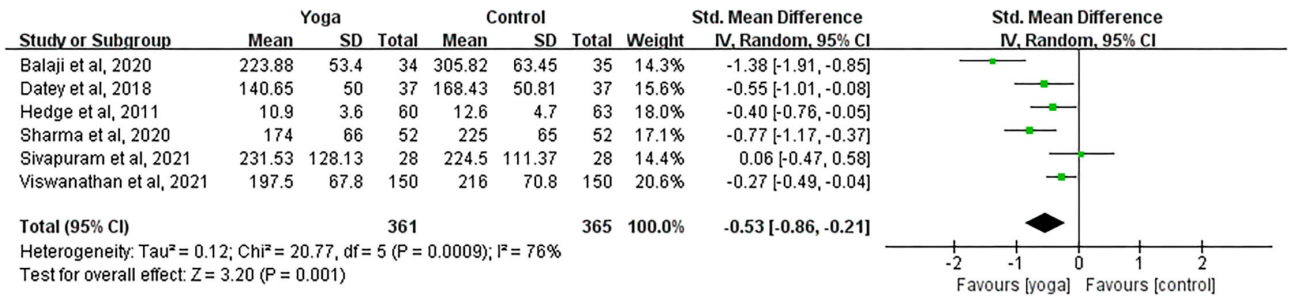

FIGURE 6 | Forest plots of the effect of yoga on PPBG.

−0.82, 0.13; $Z = 1.44$, $p = 0.15$). The heterogeneity increased after pooling the data, indicating that difference in each sample size was likely to be the source of heterogeneity.

Publication Bias

Only when the number of studies involving in the meta-analysis was more than ten, a funnel plot was eligible for assessing publication bias. The funnel plots for outcomes of FBG looked approximately asymmetrical as assessed by visual examination, indicating a high possibility of bias (**Figure 13**).

DISCUSSION

The purpose of the study was to evaluate available evidence from existing RCTs concerning the effect of yoga-based intervention on the biochemical indicators of blood glucose and lipid

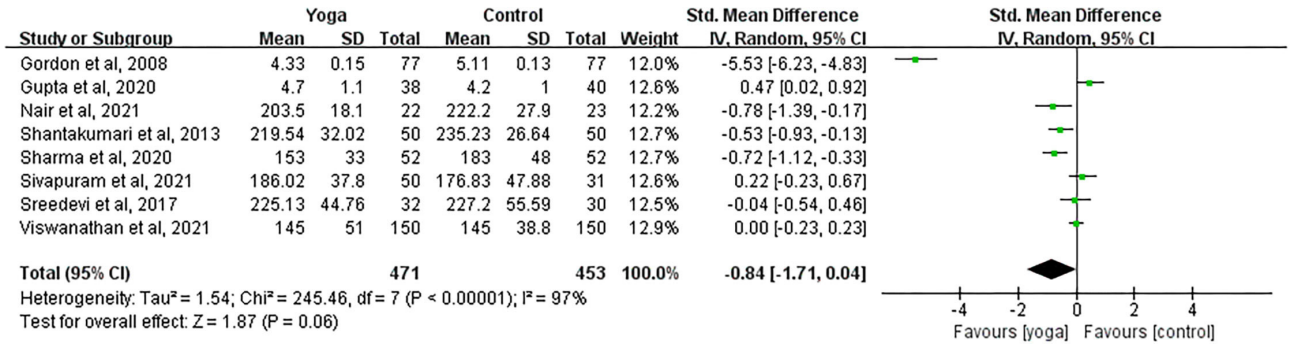

FIGURE 7 | Forest plots of the effect of yoga on TC.

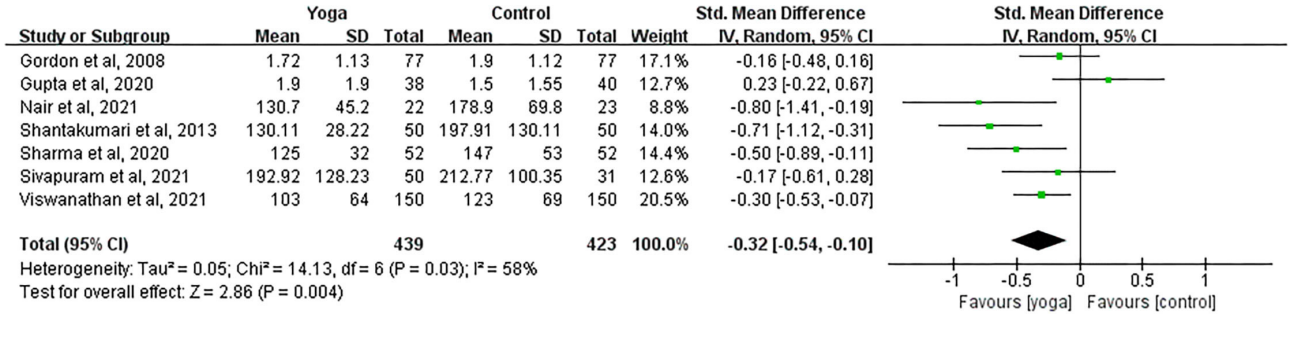

FIGURE 8 | Forest plots of the effect of yoga on TG.

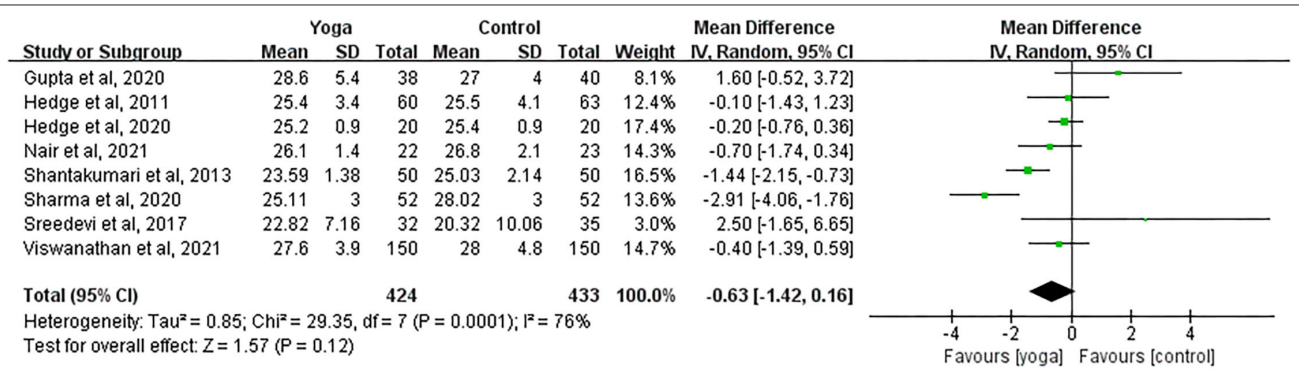

FIGURE 9 | Forest plots of the effect of yoga on BMI.

profile in patients with T2DM. Chronic hyperglycemia was the major characteristic of T2DM, which was commonly diagnosed depending on HbA1c, FBG, and PPBG levels (American Diabetes Association, 2016). The results of the meta-analyses incorporating evidence from the 13 RCTs with a total of 1,335 patients with T2DM suggested that yoga can significantly improve HbA1c, FBG, PPBG, and TG levels. The study supported that yoga training improves blood glucose control and prevents T2DM biomarkers from worsening. The synthesized evidence approved yoga as an effective complementary treatment for patients with T2DM. These findings were in line with a recently published meta-analysis yielding favorable effects of yoga on specific metabolic syndrome (Chu et al., 2016).

Although not a primary focus of this study, an insignificant effect of yoga practice on BMI was observed, which is consistent with prior work (Boulé et al., 2001). A possible reason behind this was that yoga training decreases muscle insulin resistance and increases glucose disposal through a number of mechanisms that would not necessarily be relevant to losing weight. However, results must be interpreted with caution because of the limited number of selected studies in the analysis. Therefore, additional investigation

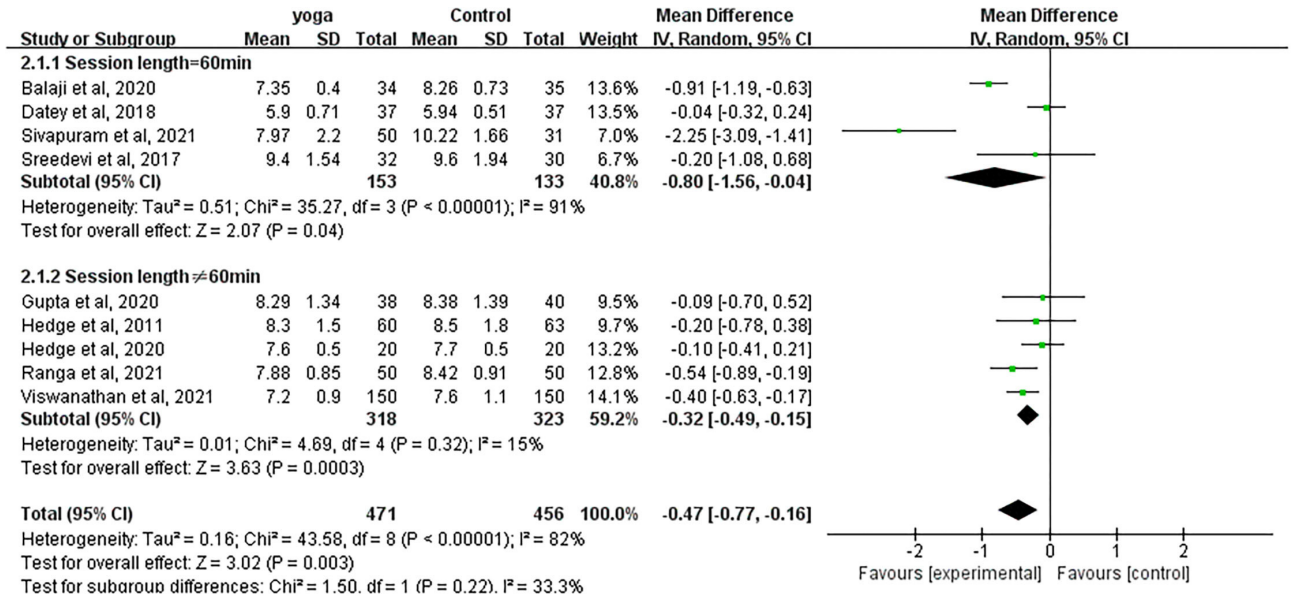

FIGURE 10 | Forest plots of the subgroup analysis on Hba1c.

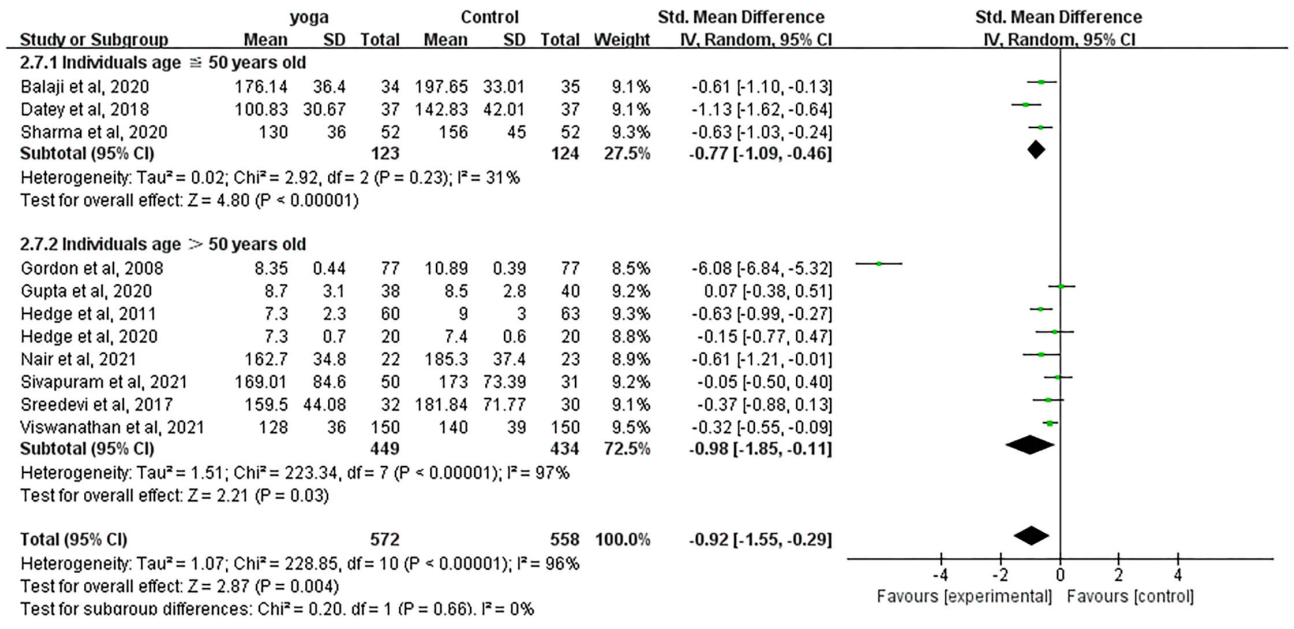

FIGURE 11 | Forest plots of the subgroup analysis on FBG.

is necessary to discover the impact of yoga practice on body mass.

Furthermore, there was significant heterogeneity among primary and secondary outcome variables across different studies, which remained even after adjusting the impacts of different types of intervention and characteristics of participants (potential moderators). This indicated that there were still important variations between the included studies that made them considerably different from each other. We explained all results except for PPBG through subgroup analysis. For the pooled result of PPBG, after excluding the study with high heterogeneity (Balaji et al., 2020), the pooled data from the rest studies showed no substantial change, indicating that the sensitivity was low, and the results were relatively reliable.

Another aspect that needs to be taken into account while explaining these results was that in cases where diabetes

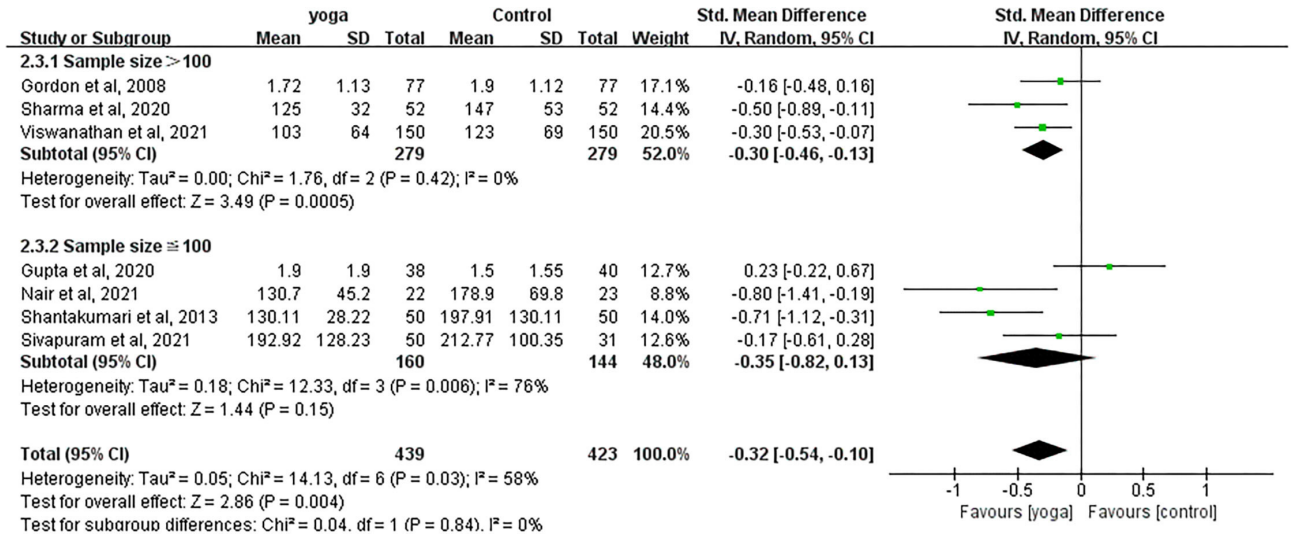

FIGURE 12 | Forest plots of the subgroup analysis on TG.

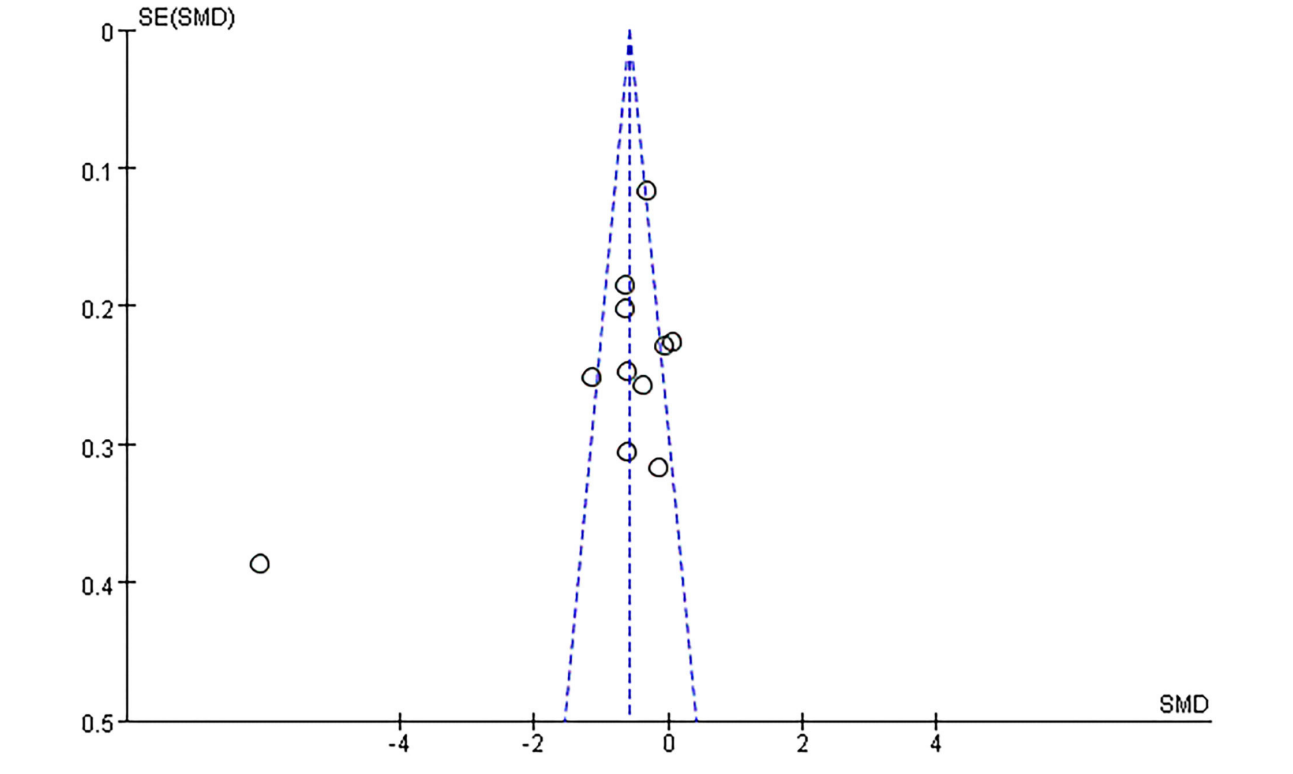

FIGURE 13 | Funnel plot on FBG of patients with T2DM.

treatment adopting both pharmaceutical and yoga approaches yield sizable effect, yoga as an ingredient to such treatment was hard to ascertain its sole effectiveness. All studies included in the present meta-analyses administered yoga to the participants together with other pharmaceutical treatments. Given such a condition, caution should be taken when concluding that the favorable effect on T2DM was solely due to the adjunct yoga treatment. Because both treatment and control groups incorporated pharmacological intervention, these favorable effects may also be caused either by positive yoga–medicine

interaction or yoga alone. To observe or repeat these add-on effects, yoga is better recommended as an adjuvant treatment to pharmaceutical prescriptions for patients with diabetes.

Even though RCT is widely believed to provide the most reliable evidence of causality for clinical tests, the RCTs selected for these meta-analyses had extra problems through the lens of risk of bias. The overall risk of bias for each included trial was either unclear or high. Typically, only statistically significant results suggesting a favorable effect are more likely to be published. Moreover, although yoga has been reported to be a generally safe exercise (McCall et al., 2013), in the context of T2DM, none of the studies reported any adverse events following a yoga program. It is not known whether in other studies, participants experienced any adverse events, or whether authors failed to report these adverse events. Finally, it is a strong recommendation from this study that more RCT-based examinations should be carried out revealing how yoga as an adjuvant therapy plays its role in T2DM treatment. Additionally, it may need a longer time for yoga to take its effect on diabetes, and thus, future research should incorporate interventions with a longer intervening session to monitor the changes in blood glucose, blood lipids, and body composition.

This study depicted the influence of yoga as a complementary treatment on biochemical indicators of blood glucose and lipid profile in patients with T2DM. The main limitation of this review is information insufficiency from the included studies, such as some studies did not report age and session length, which could have an impact on the results. Therefore, caution should be seriously taken concluding the reductive effect of yoga based on the extracted information. It might be possible that the overall effect sizes change substantially if the data reports were more complete. Publication bias and small sample sizes also did not make us convincingly state that the short-term effects of yoga interventions can be generalized to the long-term management of T2DM. Whereas, we strictly controlled the quality of the included studies, the fact is that the pooled results of the meta-analyses still showed large heterogeneity despite our attempts to identify possible sources of heterogeneity through subgroup analysis. Additionally, due to our limited access to online databases, our search may miss some databases such as Scopus. Finally, as indicated in the results, the majority of RCTs published to date exploring T2DM's response to yoga originated in India, suggesting the need for conducting strict trials with patients with T2DM of other races and/or in other developed and developing countries.

CONCLUSIONS

The findings of this study suggested that yoga treatment can improve the indices of blood glucose and lipid profile in patients with T2DM (simultaneously receiving pharmacological treatment). Therefore, yoga can be regarded as an effective complementary treatment to T2DM for the short term (i.e., 10–24 weeks). Future research is needed to highlight high-quality trials with standardized yoga plans to verify the long-term reductive effect of yoga on T2DM-related indicators. However, given the aforementioned limitations and potential bias in the present study, more large-scale and rigorous RCTs must be carried out to reaffirm our current findings.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

SC participated in the search strategy, data analysis, heterogeneous interpretation, and manuscript writing. SC, SD, and YL participated in the data extraction. SD and YL reviewed the search results and critical review of the manuscript. TY participated in the preliminary study design. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fspor.2022.900815/full#supplementary-material

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REVIEW
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Roles and Molecular Mechanisms of Physical Exercise in Sepsis Treatment

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Physical exercise is a planned, purposeful action to keep a healthy lifestyle and improve physical fitness. Physical exercise has been widely used as a non-pharmacological approach to preventing and improving a wide range of diseases, including cardiovascular disease, cancer, metabolic disease, and neurodegenerative disease. However, the effects of physical exercise on sepsis have not been summarized until now. In this review, we discuss the effects of physical exercise on multiple organ functions and the short- and long-time outcomes of sepsis. Furthermore, the molecular mechanisms underlying the protective effects of physical exercise on sepsis are discussed. In conclusion, we consider that physical exercise may be a beneficial and non-pharmacological alternative for the treatment of sepsis.

Keywords: physical exercice, sepsis, organ failure, outcome, molecular mechanism

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1 INTRODUCTION

Sepsis is defined as a life-threatening organ failure caused by a dysregulated host response to infection and affects approximately 19.4 million individuals each year (Prescott and Angus, 2018). In recent years, there have been several interventions utilized to improve the survival of patients with sepsis. As a result, the mortality of in-hospital sepsis patients has declined, from 35% to 18%, making for many sepsis survivors (Kaukonen et al., 2014; Prescott and Angus, 2018). However, emerging data suggest that one-third of the survivors die within a year, and one-sixth have clinical sequelae including cognitive dysfunction, physical incapacity, exacerbation of chronic medical conditions, and mental problems (Iwashyna et al., 2010; Yende et al., 2014; Prescott and Angus, 2018; Venet and Monneret, 2018). The reasons for poor long-term outcomes after sepsis are complex and include residual organ damage. During sepsis, multiple organ systems, including the respiratory, renal, cardiovascular, neurological, hepatic, and hematological systems, are typically impaired simultaneously, resulting in poor clinical outcomes (Lelubre and Vincent, 2018). Multiple organ failure may remain despite successful treatment for sepsis. Therefore, effective interventions that target multiple organ systems are critical for improving the short- and long-time outcomes of sepsis.

Physical exercise is a planned, purposeful action to maintain a healthy lifestyle and improve physical fitness (WHO, 2010). Physical exercise has been widely used as a non-pharmacological approach to preventing and improving a wide range of diseases, including cardiovascular disease, cancer, metabolic disease, and neurodegenerative disease (Gleeson et al., 2011; Kim et al., 2014). For example, the obesity-associated metabolic disease was improved by moderate- or high-intensity exercise (Wang et al., 2017). In addition, physical exercise was able to inhibit cancer metastasis, ameliorate the side effects of cancer treatment, and prevent cancer-related death. Furthermore, there is emerging evidence that physical exercise acts on multiple organ systems under various conditions (Sabaratnam et al., 2022). However, the effects of physical exercise on sepsis have not been summarized until now. This review outlines the effects of physical exercise on multiple organ

functions and the short- and long-term outcomes of sepsis. To clarify the role of physical exercise in sepsis, it is crucial to understand the molecular mechanisms mediating the protective impacts of physical exercise. Therefore, the molecular mechanisms underlying the protective effects of physical exercise on sepsis are also discussed.

2 EFFECTS OF PHYSICAL EXERCISE ON MULTIPLE ORGAN FUNCTION AND THE OUTCOMES OF SEPSIS

2.1 Effects of Physical Exercise on Cardiovascular Function

The cardiovascular system is frequently impaired in sepsis. Cardiovascular dysfunction is characterized by a total decrease in left ventricular diastolic and systolic functions, which leads to arterial hypotension (Rong et al., 2021). Sepsis patients with cardiovascular dysfunction have a higher mortality rate than those with normal cardiovascular function during hospitalizations (Merx and Weber, 2007). After hospitalizations, sepsis survivors have a 13-fold increased risk of cardiovascular events compared with survivors of other diseases (Yende et al., 2014). Therefore, cardiovascular dysfunction is the leading problem in sepsis patients during and after hospitalizations.

Several studies have demonstrated that physical exercise promotes metabolic flexibility, myocardial remodeling, and angiogenesis, which have been considered to prevent and treat cardiovascular dysfunction in various diseases (Wu et al., 2019a). Mehanna et al. (2007) demonstrated that exercise preconditioning attenuated the alterations in arterial pressure and heart rate of Wistar rats at 5 h following lipopolysaccharide (LPS) injection, suggesting that exercise training alleviated cardiovascular abnormalities during sepsis. Similarly, Chen et al. (2007) showed that exercise-trained rats had lower basal levels of heart rate and arterial pressure, as well as less severe cardiac injury at 72 h following LPS treatment. This study also found that exercise training before sepsis reduced plasma levels of pro-inflammatory cytokines and nitrate, which are potential mechanisms of the positive effects of physical exercise on cardiovascular function in sepsis (Chen et al., 2007). Furthermore, cardiovascular function measured by ejection fraction after sepsis was alleviated by exercise preconditioning (Sun et al., 2020; Khoshkhouy et al., 2021). Overall, these animal studies suggest that cardiovascular dysfunction may be ameliorated by physical exercise preconditioning in sepsis.

2.2 Effects of Physical Exercise on Renal Function

Septic patients often develop uropenia with increased serum creatinine and urea. Those who meet consensus criteria for acute kidney injury (AKI) are deemed to have sepsis-associated AKI. A survey suggested that over 60% of patients with sepsis have AKI (Poston and Koyner, 2019). Sepsis patients with AKI have a higher mortality rate than patients without AKI. Therefore, AKI has been long-regarded as an independent risk factor of mortality in sepsis during hospitalization (Poston and Koyner, 2019). Furthermore, a study involving 2,617 sepsis survivors revealed that they have a 2.7-fold increased risk of readmission for AKI compared with survivors for other diseases (Prescott and Angus, 2018). Here, we investigate whether physical exercise acts on AKI in sepsis.

In an ischemic-reperfusion model, physical exercise can prevent and attenuate renal dysfunction in healthy individuals (de Lima et al., 2019). In gentamicin-associated acute kidney injury, physical exercise promotes the recovery of renal structure and function by restoring redox balance (Oliveira et al., 2017). Interestingly, several studies have shown that exhaustive exercise is associated with kidney injury (Wu et al., 2012; Hosoyamada et al., 2016; Gundlapalli et al., 2021). In mice with sepsis, the impairment of kidney tubules is less severe with physical exercise (Sossdorf et al., 2013). In contrast, Húngaro et al. (2020) found that physical exercise increased the renal tubulointerstitial space and expression levels of NGAL, a gene related to kidney injury, and TLR4, suggesting that physical exercise enhances renal dysfunction after LPS treatment. Therefore, the effects of physical exercise on renal function are unclear and may depend on the intensity and duration of physical exercise.

2.3 Effects of Physical Exercise on Neurological Function

Sepsis-associated encephalopathy is one of the most common complications in sepsis. Approximately 70% of septic patients suffer consciousness, delirium, concentration deficiency, anxiety, depression, and cognitive dysfunction during hospitalization (Molnár et al., 2018). About 50% of sepsis survivors acquire long-time cognitive dysfunction, including deficiency in memory, attention, executive function, verbal skills, and mental problems after hospitalization (Davydow et al., 2012; Molnár et al., 2018). Moreover, sepsis-associated encephalopathy is responsible for poor sepsis outcomes resulting in high hospitalization costs. Therefore, it is essential to prevent and treat neurological dysfunction during sepsis.

There is ample evidence that physical exercise alleviates structural brain abnormalities and cognitive dysfunction in a wide range of brain diseases, including Alzheimer's disease, Huntington's disease, and Parkinson's disease (Gubert and Hannan, 2021). Physical exercise enhances neuroplasticity, neurogenesis, angiogenesis, and synaptic activity to improve brain structure and function in various brain disorders (Sujkowski et al., 2022). In relation to traumatic brain injury, Morris et al. (2016) reported that physical exercise improved cognitive dysfunction. In sepsis, the endocannabinoid system and cyclooxygenase enzyme play central roles in cognitive dysfunction by regulating neuroinflammation. Moosavi Sohroforouzani et al. (2020) found that the escape distance and latency to reach the platform in the LPS treatment group were longer than those in the LPS+ treadmill aerobic exercise group, and exercise preconditioning reduced cannabinoid receptor 2 receptor levels as well as cyclooxygenase-2 levels, suggesting that treadmill aerobic exercise had a beneficial

effect on cognitive function by regulating the endocannabinoid system and cyclooxygenase in sepsis. In *Trypanosome cruzi* infection, exercise preconditioning decreases the parasite peak and contributes to the survival of neurons and neuronal hypertrophy (Moreira et al., 2014). These results show that exercise preconditioning ameliorates neurological dysfunction in sepsis.

2.4 Effects of Physical Exercise on Other Organ Functions

As discussed above, physical exercise preconditioning has protective effects on cardiovascular and neurological functions in sepsis. Here, we discuss whether physical exercise improves other organ functions in sepsis. de Araújo et al. (2012) firstly found that physical activity reduced the static elastance of the lung, alveolar collapse, lung collagen and fiber content, and neutrophil levels in bronchoalveolar lavage fluid. Subsequent studies verified that pulmonary surfactant function was impaired; neutrophil influx in the liver and lung, capillary plugging, and expression levels of lung interleukin 6 (IL-6) were increased in sepsis, but voluntary running reversed these septic responses (Tyml et al., 2017). Similarly, preconditioning exercise prevented aggravations of lung injury by mediating purinergic system and oxidative stress under septic condition (Miron et al., 2019). These animal studies suggest that lung and liver functions can be improved by exercise preconditioning during sepsis. In addition, Al-Nassan and Fujino (2018) demonstrated that a mild exercise preconditioning could preserve muscle mass and prevent atrophy during sepsis. Furthermore, exercise preconditioning increased survival, ameliorated multiple organ damage, and recovered pro- and anti-inflammatory balance by modifying gut microbiota composition (Kim and Kang, 2019).

Overall, the above findings indicate that exercise preconditioning protects against multiple organ failure during sepsis in experimental models. Clinical research demonstrates that early physical rehabilitation in septic patients might improve physical function and reduce the inflammatory response at 6–12 months post-hospital discharge (Kayambu et al., 2011; Kayambu et al., 2015; Ahn et al., 2018). Therefore, physical exercise may be a non-pharmacological method to improve multiple organ dysfunction in sepsis.

2.5 Effects of Physical Exercise on the Outcomes of Sepsis

Here, we discuss whether physical exercise affects the outcomes of sepsis. Based on experimental models, several studies have suggested that regular exercise alters the morbidity of sepsis and increases the survival rate (Sossdorf et al., 2013; Kim and Kang, 2019; Wang et al., 2021). In a clinical study, Wang et al. (2014) indicated an association between physical exercise preconditioning and susceptibility to sepsis. They concluded that individuals with low rates of physical exercise and high rates of watching television presented with higher morbidity and mortality of sepsis (Wang et al., 2014). However, sepsis survivors have a significant reduction in exercise capacity and physical activity that may continue even 3 months after hospitalization (Borges et al., 2015), and little information is available regarding the effects of post-hospital exercise on the long-term outcomes of sepsis.

Although the effects of physical exercise in improving organ function of sepsis are different in different organ systems, several studies show that exercise preconditioning can ameliorate sepsis-mediated multiple organ failure and reduce morbidity and mortality of sepsis (summarized in **Table 1**). In conclusion, we consider that physical exercise preconditioning may be a beneficial and non-pharmacological alternative for preventing and treating sepsis and is suitable for any individual.

3 MOLECULAR MECHANISMS OF PHYSICAL EXERCISE IN SEPSIS TREATMENT

3.1 Mitochondrial Quality Control

3.1.1 Mitochondrial Biogenesis

Mitochondrial quality is controlled by various processes such as mitochondrial biogenesis, mitochondrial fusion/fission, and mitophagy. Mitochondrial biogenesis contributes to the production of new mitochondria and mitochondrial content. These processes are controlled by biogenesis signals, such as PGC-1α, NRF-1, NRF-2, AMPK, SIRT1, and TFAM. PGC-1α plays a central role in mitochondrial biogenesis and is activated by the SIRT1-AMPK pathway, which then interacts with NRF-1 and NRF-2 in both the mitochondria and nucleus (Song et al., 2021). In the mitochondria, PGC-1α binds to NRF-1 and NRF-2, coactivating TFAM, which in turn mediates mitochondrial DNA translation, transcription, and replication (Song et al., 2021). In the nucleus, PGC-1α binding to NRF-1 and NRF-2 induces nuclear translocation of mitochondrial proteins, which are then imported into the mitochondria (Song et al., 2021). During sepsis, the expression levels of PGC-1α, TFAM, NRF-1, and NRF-2 are increased in multiple organ tissues, including the liver, heart, brain, and lungs, in the initial stage and decreased in the late stage (Rayamajhi et al., 2013; Vanasco et al., 2014; Wu et al., 2019b). Haden et al. (2007) first demonstrated that mitochondrial biogenesis induction could restore basal metabolism in *Staphylococcus* aureus sepsis. Thereafter, MacGarvey et al. (2012) showed that targeted induction of mitochondrial biogenesis could attenuate multiple organ dysfunction in sepsis. In addition, several studies have repeatedly verified that PGC-1α overexpression attenuates multiple organ dysfunction in sepsis (Tran et al., 2011; Yi et al., 2020; Li et al., 2021). Various proteins of mitochondrial biogenesis have been found to be increased after exercise. A systematic review showed that physical exercise increased the expression levels of PGC-1α, NRF-1, NRF-2, and TFAM and promoted mitochondrial biogenesis in Parkinson's disease (Nhu et al., 2021). In addition, Zhang and Gao (2021) found that physical exercise protects against cardiovascular disease by promoting mitochondrial biogenesis. Therefore, physical exercise could enhance

TABLE 1 | Effects of physical exercise on multiple organ function and outcomes of sepsis.

Organ	Effects	Molecular mechanisms	Reference
Heart	Attenuate the alterations in arterial pressure and heart rate	—	Mehanna et al. (2007)
	Attenuate basal levels of heart rate, arterial pressure and cardiac injury	Reduce levels of pro-inflammatory cytokines and nitrate	Chen et al. (2007)
	Ameliorate cardiac injury	Reduce levels of pro-inflammation, oxidative stress and apoptosis	Khoshkhouy et al. (2021)
	Ameliorate cardiovascular dysfunction reflected by ejection fraction	Inhibit GCN2-eIF2α/ATF4 pathway	Sun et al. (2020)
Kidney	Ameliorate kidney tubular damage	Increase lysophosphatidylcholines and decrease inflammatory cytokines	Sossdorf et al. (2013)
	Expand the renal tubulointerstitial space	Increase levels of NGAL and TLR 4	Húngaro et al. (2020)
Brain	Reduce escape distance and latency to arrive the platform	Inhibit endocannabinoid system and COX	Moosavi Sohroforouzani et al. (2020)
	Contribute to survival of neuron and neuronal hypertrophy	Increased levels of TGF-β and TNF-α	Moreira et al. (2014)
Lung	Enhance pulmonary surfactant function	Reduce levels of pro-inflammation and neutrophil influx in lung	Tyml et al. (2017)
	Ameliorate lung injury	Reduce density of purinergic enzymes and receptors, and oxidative stress	Miron et al. (2019)
	Ameliorate pulmonary edema	Decrease levels of pro-inflammation and restore redox balance	Wang et al. (2021)
	Reduce static elastance of lung and alveolar collapse	Decrease content of lung collagen and fiber, levels of neutrophils in BALF	de Araújo et al. (2012)
Liver	—	Reduce neutrophil influx in liver	Tyml et al. (2017)
	Make no effect on liver damage	—	Sossdorf et al. (2013)
Skeletal muscle	—	Reduce capillary plugging and increase eNOS	Tyml et al. (2017)
	Preserve muscle mass and prevent atrophy	—	Al-Nassan and Fujino (2018)
Outcomes	Alter the morbidity of sepsis and increase the survival rate of sepsis	Modify gut microbiota	Sossdorf et al. (2013), Kim and Kang (2019), Kayambu et al. (2011), Wang et al. (2014), Ahn et al. (2018), Wang et al. (2021)

multiple organ functions through the induction of mitochondrial biogenesis.

3.1.2 Mitochondrial Dynamics

Mitochondrial fusion and fission regulate mitochondrial number and size. These processes are mediated by the fission proteins, Drp1 and Fis1, and the fusion proteins, Mfn2, Mfn1, and OPA1. In mitochondrial fusion, homo- and hetero-oligomeric structures are formed by Mfn1 and Mfn2 to link two neighboring mitochondria for outer membrane fusion, and OPA1 directly promotes inner membrane fusion (Chan, 2012). During mitochondrial fission, Drp1 translocates from the cytosol to the mitochondria and forms Drp1 complexes to constrict the mitochondrial tubule. The parent mitochondria are then segregated into two daughter mitochondria (Losón et al., 2013). In sepsis, the fusion proteins Mfn2 and OPA1 are decreased, and the fission protein Drp1 is increased in the liver, heart, and immune cells (Gonzalez et al., 2014; Shen et al., 2018). Inhibition of Drp1 and overexpression of Mfn2 improve organ dysfunction and poor outcomes in sepsis (Gonzalez et al., 2014; Deng et al., 2018; Wu et al., 2019b). Jang et al. (2018) found that physical exercise enhanced the expression of Mfn2, OPA1, and p-Drp1 Ser637 and balanced mitochondrial fusion and fission. In addition, treadmill exercise enhances learning skills and memory in Alzheimer's disease by balancing mitochondrial fusion and fission (Yan et al., 2019).

3.1.3 Mitophagy

Mitophagy is the selective elimination of aged and damaged mitochondria, which can help maintain mitochondrial homeostasis. The import of PINK1 to the inner mitochondrial membrane is blocked when a damaged mitochondrion is detected, resulting in the accumulation of PINK1 on the outer mitochondrial membrane. PINK1, which is activated through auto-phosphorylation, can phosphorylate ubiquitin, a substrate of PINK1, which then induces the recruitment of Parkin to damaged mitochondria. After that, PARK2 is activated by phosphorylation, which binds to the outer mitochondrial membrane and autophagy adaptor proteins, including OPTN and NDP52, ultimately resulting in autophagosomes (Lazarou et al., 2015). Finally, autophagosomes fuse with a lysosome, degrading damaged mitochondria. In sepsis, mitophagy is induced in the initial stage, but lysosomal degradation is impaired in the late stage, leading to multiple organ dysfunction (Chien et al., 2011; Hsieh et al., 2011). Knockdown of PINK1 or PARK2 exacerbates multiple organ dysfunction during sepsis (Kang et al., 2016). These suggest that complete induction of mitophagy presents as a therapeutic target

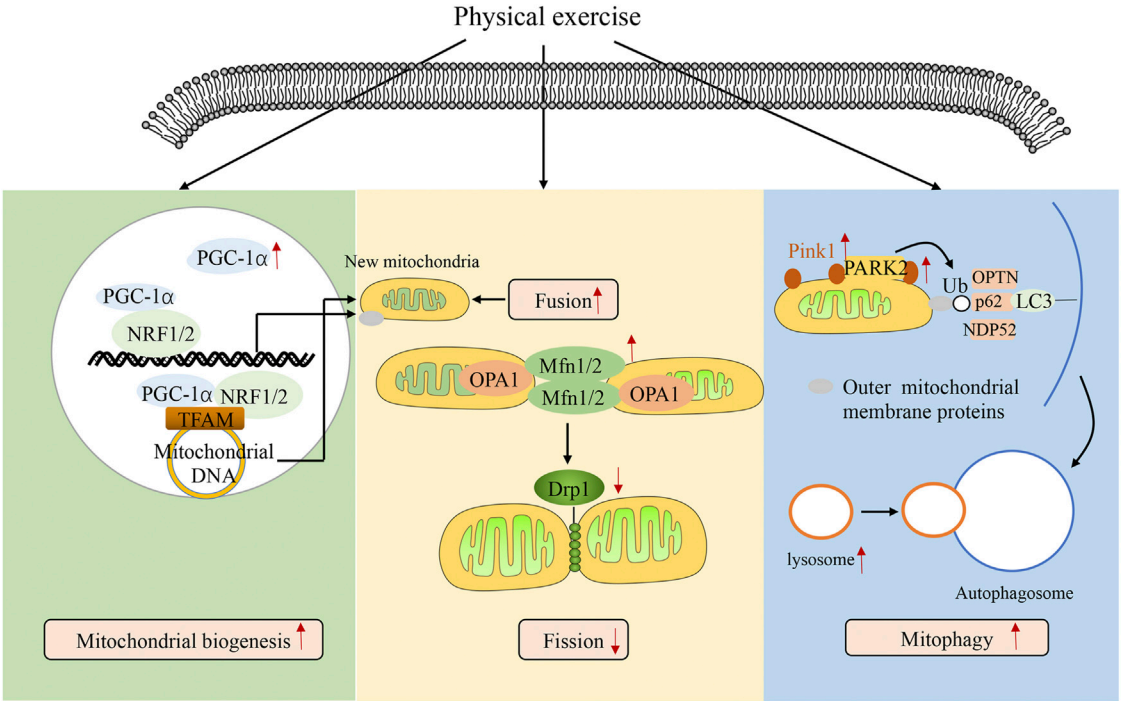

FIGURE 1 | Physical exercise regulating mitochondrial quality control. The figure shows how physical exercise mediates mitochondrial quality control. Mitochondrial quality is controlled by various processes, including mitochondrial biogenesis, mitochondrial fusion/fission, and mitophagy. Processes of mitochondrial biogenesis are controlled by biogenesis signals such as PGC-1α, NRF-1, NRF-2, AMPK, SIRT1, and TFAM. PGC-1α plays a central role in mitochondrial biogenesis, interacting with NRF-1 and NRF-2 in both the mitochondria and nucleus. In the mitochondria, PGC-1α binds with NRF-1 and NRF-2, coactivating TFAM, which in turn mediates mitochondrial DNA translation, transcription, and replication. In the nucleus, PGC-1α binds with NRF-1 and NRF-2, inducing the nuclear translation of mitochondrial proteins, which are imported into the mitochondria. Mitochondrial fusion and fission are mediated by fission proteins such as Drp1 and fusion proteins such as Mfn2, Mfn1, and OPA1. PINK1 import to the inner mitochondrial membrane is inhibited when it detects a damaged mitochondrion, resulting in the accumulation of PINK1 on the outer mitochondrial membrane. PINK1 phosphorylates ubiquitin, a substrate of PINK1, which then induces the recruitment of Parkin to the damaged mitochondria. Then, PARK2 is phosphorylated and binds to outer mitochondrial membrane proteins and autophagy adaptor proteins, ultimately resulting in mitophagy. Physical exercise promotes mitochondrial quality control.

during sepsis. There is evidence that physical exercise enhances the recruitment of PARK2 to the outer mitochondrial membrane to stimulate mitophagy in cardiovascular disease (Wu et al., 2019a; Memme et al., 2021). Furthermore, Hwang et al. (2018) demonstrated that physical exercise reduced the expression levels of P62 and enhanced the expression of LAMP2 and cathepsin L, suggesting that physical exercise promotes lysosomal degradation. Therefore, physical exercise could reverse sepsis-induced disruption of the lysosomal degradation and promote complete induction of mitophagy. Collectively, previous results have suggested that physical exercise improves organ dysfunction by regulating mitochondrial quality control (**Figure 1**).

3.2 Systemic Inflammation

Sepsis is characterized by hyperinflammatory responses and immunosuppression in the initial and late stages of the disease, respectively. Hyperinflammatory responses are the leading cause of organ dysfunction. During sepsis, innate immune cells recognize pathogen-associated molecular patterns *via* pattern recognition receptors, activating numerous signaling pathways in the cell (Cecconi et al., 2018). Activation of these pathways results in the downstream activation of MAP3K7, which then activates the JNK-p38-ERK pathways, IRFs, and NF-κB (Lawrence, 2009). Finally, inflammatory cytokines, including IL-6, IL-12, TNF-α, and IL-1β, are released, inducing endothelial dysfunction and cell damage in multiple organ tissues. Damage-associated molecular patterns produced by tissue injury have the same function as pathogen-associated molecular patterns and amplify immune responses (Timmermans et al., 2016). These factors induce multiple organ dysfunction in sepsis.

Numerous studies have shown that physical exercise improves organ dysfunction by reducing systemic inflammation in sepsis patients. Wang et al. (2021) found that aerobic exercise decreased lung neutrophil content and the mRNA expression levels of IL-6, TNF-α, Glu1, CXCL-1, and HMGB1 in the lung to improve respiratory dysfunction. Shimojo et al. (2019) showed that swimming decreased serum inflammatory cytokines and increased anti-inflammatory cytokines by decreasing dopamine. Miron et al. (2019) demonstrated that physical exercise decreases serum IL-6 and IL-1β expression following LPS treatment. Tyml et al. (2017) showed that voluntary running protects against respiratory dysfunction, hepatic dysfunction, and neutrophil influx by reducing inflammation in sepsis. Collectively, these studies conclude that physical exercise

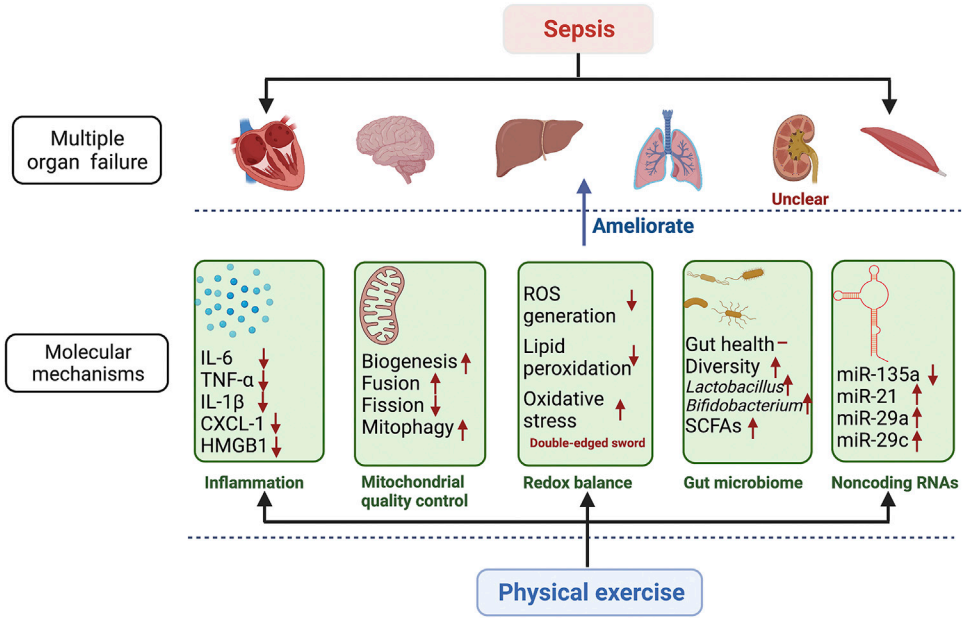

FIGURE 2 | Molecular mechanisms involved in the beneficial effects of physical exercise on multiple organ failure in sepsis. The figure shows how physical exercise regulates multiple organ failure through these potential molecular mechanisms. The potential molecular mechanisms include inflammation, mitochondrial quality control, redox balance, gut microbiome, and noncoding RNAs. These potential molecular mechanisms regulated by physical exercise ameliorate sepsis-induced multiple organ failure, including respiratory, cardiovascular, neurological, hepatic, hematological, and muscle systems. The effects of physical exercise on renal dysfunction are unclear in sepsis. IL-6 interferon 6; TNF-α tumor necrosis factor α; IL-1β interferon 1β; CXCL-1 chemokine (C-X-C motif) ligand 1; HMGB1 high mobility group 1; ROS reactive oxygen species; SCFAs short-chain fatty acids; miR-135a microRNA 135a; miR-21 microRNA 21; miR-29a microRNA 29a; miR-29c microRNA 29c.

improves organ dysfunction by reducing systemic inflammation in sepsis.

3.3 Redox Balance

Oxidants and antioxidants are involved in various diseases. The oxidative burst promotes the production of reactive oxygen species (ROS) and reactive nitrogen species. To maintain cellular homeostasis, antioxidant enzymes, including glutathione peroxidase, superoxide dismutase, and catalase, act as oxidant scavengers and decrease the cellular level of oxidants (Mantzarlis et al., 2017). In the past decades, several studies have suggested that ROS are induced during sepsis and involved in the development of sepsis-induced multiple organ dysfunction (Jung et al., 2000; Pleiner et al., 2003; Ritter et al., 2003). A clinical study showed that the antioxidant potential was increased to a greater extent in sepsis survivors than in non-survivors (Cowley et al., 1996). Further research verified that the balance between oxidants and antioxidants was disrupted in sepsis, resulting in oxidative stress, cell death, and organ injury (Miliaraki et al., 2022).

Converging studies have suggested that ROS are involved in mediating the effects of physical exercise. Adams et al. suggested that physical exercise decreased ROS generation, resulting in improving acetylcholine-mediated vasodilatation and reducing Ang II-mediated vasoconstriction (Adams et al., 2005). In addition, Miron et al. (2019) found that physical exercise reduces lung lipid peroxidation and reactive species. Furthermore, Wu et al. (2020) demonstrated that physical exercise alleviated the increased ROS levels and apoptosis in kidney tissues. However, Mendonça et al. (2019) found that pre-infection exercise aggravates acute infections by aggravating oxidative stress. A review summarized that prolonged endurance exercise promoted oxidative stress, whereas moderate physical exercise reduced oxidative stress (Gomez-Cabrera et al., 2021). Therefore, physical exercise is considered a double-edged sword for redox balance, depending on the intensity and duration of physical exercise.

3.4 Gut Microbiome

There are trillions of microbiota in the human gastrointestinal tract that play diverse roles in health and disease. Recent breakthroughs in technology, such as metagenome and 16S ribosomal RNA sequencing, have enabled progress in understanding the gut microbiome. This has led to an enormous increase in research elucidating the association between the gut microbiome and diseases. In sepsis, a study revealed that the levels of beneficial *Lactobacillus* and *Bifidobacterium* were decreased, and the abundance of pathogenic *Pseudomonas* and *Staphylococcus* was increased (Shimizu et al., 2006). Disruption of the gut microbiome at both the functional and compositional levels promoted multiple organ dysfunction in patients with sepsis (Liu et al., 2019). Moreover, disruption of the gut microbiome increased the susceptibility of rats to sepsis (Haak and Wiersinga, 2017). It also reported that intervention with three microbiota-derived short-chain fatty acids could improve multiple organ dysfunction in sepsis (Haak and Wiersinga, 2017). These new insights suggest

that the gut microbiome plays an essential role in mediating sepsis-induced multiple organ dysfunction.

There is evidence that exercise may affect the gut microbiome, which can then modulate multiple organ dysfunction in sepsis. For example, physical exercise changes the composition of the gut microbiome, including an increase in the abundance of beneficial *Lactobacillus* and *Bifidobacterium* (Queipo-Ortuño et al., 2013). Modifying the composition of the gut microbiome by exercise preconditioning can increase survival, ameliorate multiple organ damage, and restore pro- and anti-inflammatory balance in sepsis (Kim and Kang, 2019). Physical exercise also increases short-chain fatty acid levels in both humans and rodents, which is beneficial for multiple organ dysfunction in sepsis (Allen et al., 2018). Physical exercise enhances SCFA levels by increasing SCFA-producing bacteria, including the propionate producer *Propionibacterium freudenreichii* and the butyrate producers *Faecalibacterium prausnitzii* (Húngaro et al., 2020; Ramos et al., 2022). Furthermore, physical exercise increases the diversity of the gut microbiome and decreases gut transit time. Therefore, the gut microbiome may be a bridge between physical exercise and sepsis.

3.5 Noncoding RNAs

Non-coding RNA (ncRNA) is a class of RNA molecules that cannot encode proteins or peptides, mainly including microRNA (miRNA), long non-coding RNA (lncRNA), circular RNA (circRNA), and small interfering RNA (siRNA) (Matsui and Corey, 2017). ncRNA binds to many molecular targets to form a regulatory network, initiating specific cellular biological responses. In addition, ncRNA can regulate gene expression, influence intracellular signaling, and participate in epigenetic modifications, thus playing a crucial role in various disease (Matsui and Corey, 2017). Many studies have demonstrated that multiple miRNAs, such as mi-R210, miR-23b, and miR-29a, can suppress NF-κB and IL-6 expression in sepsis by regulating the function of the immune cells (Qi et al., 2012; Benz et al., 2016). In addition, a study showed that lncRNA HOTAIR regulates cardiomyocyte TNF-α synthesis in a murine sepsis model (Wu et al., 2016). Furthermore, recent research suggested that mcircRasGEF1B protected cells from infection by regulating the stability of mature ICAM-1 mRNAs (Ng et al., 2016). In conclusion, there is growing evidence that ncRNA is involved in regulating pathophysiological processes in sepsis.

Physical exercise has been reported to regulate various ncRNA, including circulating miRNAs (Baggish et al., 2011). For example, exercise training increased cell proliferation *via* downregulating the levels of miR-135a (Improta-Caria et al., 2020). In the traumatic brain injury model, physical exercise could attenuate cognitive dysfunction *via* upregulating the levels of miR-21 (Hu et al., 2015). Interestingly, physical exercise can improve cardiovascular dysfunction *via* upregulating the levels of miR-29a and miR-29c, which are associated with inflammatory cytokines released in sepsis (Soci et al., 2011).

4 CONCLUSION

Studies have shown that exercise preconditioning can improve cardiovascular, neurological, respiratory, and hepatic dysfunction in sepsis, and increase the survival of sepsis patients. Nevertheless, doubts remain about the effectiveness of this therapy in sepsis. Thus, there is a need for more clinical research to evaluate whether physical exercise can attenuate organ dysfunction in sepsis. Moreover, new knowledge is needed on the effects of post-hospital exercise on the long-term outcomes of sepsis. This knowledge can further our understanding of whether physical exercise can be a non-pharmacological treatment for sepsis.

In this review, we outlined the potential mechanisms of the beneficial effects of physical exercise on sepsis (**Figure 2**). We illustrated that mitochondrial biogenesis, mitochondrial fusion and fission, mitophagy, systemic inflammation, redox balance, the gut microbiome, and noncoding RNA are involved. Despite existing investigations into these molecular mechanisms, many of the mechanisms associated with physical exercise and sepsis have not yet been revealed. There is a need for further research to systematically screen molecular mechanisms that are associated with physical exercise and sepsis.

AUTHOR CONTRIBUTIONS

XZ, ZF, and YW conceived this idea. All authors participated in writing and reviewing this manuscript. All authors approved the final version of the review.

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A Shorter-Bout of HIIT Is More Effective to Promote Serum BDNF and VEGF-A Levels and Improve Cognitive Function in Healthy Young Men

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Objective: The aim of this study was to investigate the effects of single bouts of high-intensity interval training (HIIT) with different duration on serum brain-derived neurotrophic factor (BDNF) and vascular endothelial growth factor-A (VEGF-A) levels and cognitive function in healthy young men.

Methods: Twelve healthy young men were participated in two HIIT treatments (20 min HIIT and 30 min HIIT) in a random order. BDNF, VEGF-A, cortisol, testosterone, blood lactic acid were measured and cognitive function was assessed by Stroop test (CWST) and Digital Span test (DST) before, immediately after, and 30 min after HIIT.

Results: 20 and 30 min HIIT increased BLa (both $p < 0.01$), cortisol (20 min HIIT: $p < 0.05$; 30 min HIIT: $p < 0.01$), and testosterone (both $p < 0.05$) levels immediately when compared with their baselines. While BLa and cortisol were significantly higher in 30 min HIIT group than in 20 min HIIT group. Moreover, BDNF concentration ($p < 0.01$), DST-F ($p < 0.01$) and DST-B ($p < 0.05$) were increased and response time of Stroop was decreased immediately after HIIT only in 20 min HIIT group. VEGF-A concentration was increased immediately after HIIT in both groups ($p < 0.01$), but after 30 min recovery, it was returned to the baseline in the 20 min HIIT group and was lower than the baseline in 30 min HIIT group ($p < 0.05$).

Conclusion: Twenty minutes HIIT is more effective than 30 minutes HIIT for promoting serum levels of BDNF and VEGF-A as well as cognitive function in healthy young men.

Keywords: high-intensity interval training, BDNF, VEGF-A, cognitive function, young men

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INTRODUCTION

A growing body of evidence indicates that physical activity promotes brain health including cognitive function (Leahy et al., 2020; Ai et al., 2021). In addition, recent findings have bolstered that physical fitness was positively correlated with brain health and a reduction in risk and progression rate of a number of neurological diseases (Weaver et al., 2021). It is widely accepted that lack of time is the most common barrier for people to persistent in regular exercise, especially for young people (Fisher et al., 2015). In recent years, high-intensity interval training (HIIT), characterized by repeated bouts of high-intensity exercise interspersed by passive recovery or low-intensity exercise, has attracted

growing attention as a time-efficient manner (MacInnis and Gibala, 2017; Li et al., 2021). It is recognized to improve cardiopulmonary fitness, vascular function, skeletal muscle metabolism and other metabolic processes in young people (Karlsen et al., 2017; Grace et al., 2018; Su et al., 2019; Chin et al., 2020). Nevertheless, only a few studies have investigated the brain adaptations induced by HIIT in young adults. According to these studies, we know the effects of HIIT on brain health in young people are mainly focused on the expression of neurotrophins and cognitive function (Jiménez-Maldonado et al., 2018; Oberste et al., 2018; García-Suárez et al., 2020; Inoue et al., 2020).

Brain-derived neurotrophic factor (BDNF), abundantly expressed in the nervous system, is one of the proteins that contribute to the neuron survival and growth, maintenance of synaptic connections between neurons, and improvement of brain function (Figurov et al., 1996; Li et al., 2008). Vascular endothelial growth factor-A (VEGF-A), a signaling protein stimulating angiogenesis, plays a crucial role in cognitive function by improving neural regeneration (Morland et al., 2017). Current studies indicate

that HIIT is more beneficial than moderate-intensity continuous training (MICT) on elevating serum BDNF and VEGF-A levels in healthy young men (Ferris et al., 2007; Saucedo Marquez et al., 2015; Weaver et al., 2021). Ferris et al. (2007) reports that a bout of graded exercise test (GXT) of VO_{2max} training induced higher serum BDNF level in healthy youth than one bout of 20% below the ventilatory threshold (VTh-20) training or 10% above the VTh (VTh+10) training. Similarly, Saucedo Marquez et al. (2015) show that a bout of HIIT (1-min 90%W_{max} interspersed 1-min passive rest) trigger higher serum BDNF level in healthy youth than a bout of 70%W_{max} MICT. Furthermore, Weaver et al. (2021) indicate that a bout of 4 × 30s 200%W_{max} sprint interval training (SIT) cause higher serum BDNF and VEGF-A levels in healthy youth than a bout of 65%VO_{2peak} MICT.

Cognitive function including attention, executive function, visuospatial skills and memory has been considered as one of the most essential higher functions of the human brain (Aridi et al., 2017). Several studies indicate that the effect of acute HIIT on cognitive function in healthy young adults is related to the intensity of HIIT (Hashimoto et al., 2018; Ludyga et al., 2019; Schwarck et al., 2019; Tian et al., 2021). Ludyga et al. (2019) suggests that a bout of 20 min HIIT (30s training, 30s recovery, recovery ratio = 1:1) results in significant improved inhibitory control in adolescents compared with a bout of 20 min HIIT (60s training, 30s recovery, recovery ratio = 2:1). Whereas, Schwarck et al. (2019) demonstrates that a single bout of 25 min HIIT protocol at 90% of VO_{2max} for 2 min alternating with 3 min of rest (2:3) has no impact on cognitive function in healthy males. But Hashimoto et al. (2018) observes a significant improvement of executive functions in healthy males after a bout of 28 min HIIT (4 × 4 min training at 80–90% VO_{2max} with 3 min active recovery at 50–60% VO_{2max}). Furthermore, Tian et al. (2021) demonstrates that 20 min HIIT (10 × 1 min training at 85–90% HR_{max} with 1 min self-paced walking) protocol significantly improves inhibitory control of healthy youth immediately compared with 20 min MICT and resting, and the improved inhibitory control elicited by HIIT can be sustained for 90 min after exercise.

Improving healthy and avoiding lifestyle-related diseases is the aim of most people to take part in physical exercise. However, it does not mean that the prolonged duration HIIT brings people more benefits. On the contrary, the longer-duration HIIT may be ineffective or even harmful. Indeed, Farias-Junior et al. (2019) shows that prolonged-intense exercise is neurotoxic and leads to damage on cognitive function. Generally, a prescription of acute exercise involves modality, intensity, and duration. Despite relatively well described dose–response relations between exercise intensity and cognitive function, the effect of exercise duration has not yet been well examined (Chang and Etnier, 2009). A systematic review indicates that positive effects of acute HIIT on executive function are observed after exercise with total time between 11 and 20 min or between 21 and 30 min, but those with total time of less than 10 min or more than 30 min did not considerably have positive effects on executive function (Ai et al., 2021). So, regarding to a single HIIT with total time of 20 and 30 min, it is unclear which is more beneficial for promoting brain health.

As such, the aim of the present study was to compare the effects of a HIIT protocol with different durations (20 and 30 min) on serum BDNF and VEGF-A levels as well as cognitive function in healthy young men. Our results will be beneficial to provide scientific exercise guidance for young adults aimed to promote health including cognitive performance. We hypothesize that 20 min HIIT would elicit more improvement in cognitive function and serum neurotrophin level than 30 min HIIT.

METHODS

Study Design and Participants

Twelve active men were recruited from a public university in Beijing with the following inclusion criteria: 1) 18–25 years of age; 2) BMI between 18.5 and 25 kgm^{-2}; 3) no any acute and chronic diseases; 4) no surgical history within 3 months prior to the study; 5) no smoking and alcohol history; 6) right hand dominant. All participants were informed about the purpose and risks of the study and signed the informed consent. Before conducting the experiments, a minimal sample size of 12 was determined by G*Power (version 3.1, Germany) using an $\alpha = 0.05$, a power1-β = 0.80, and an effect size = 0.40. We believe this sample size is feasible and realistic based on previous studies (Saucedo Marquez et al., 2015; Cabral-Santos et al., 2016).

All the participants were required to visit our laboratory three times with 1 week interval between each visit. During the first visit, anthropometric and body composition variables were measured by bioimpedance with an Inbody™ model 770 analyzer, and VO_{2peak} was measured by gas collection system (Moxus modular oxygen uptake system, AEI technologies, USA). During the second and third visit, they were assigned to perform a HIIT protocol for 20 and 30 min, respectively. Participants were instructed to avoid caffeine and alcohol within 48 h and avoid

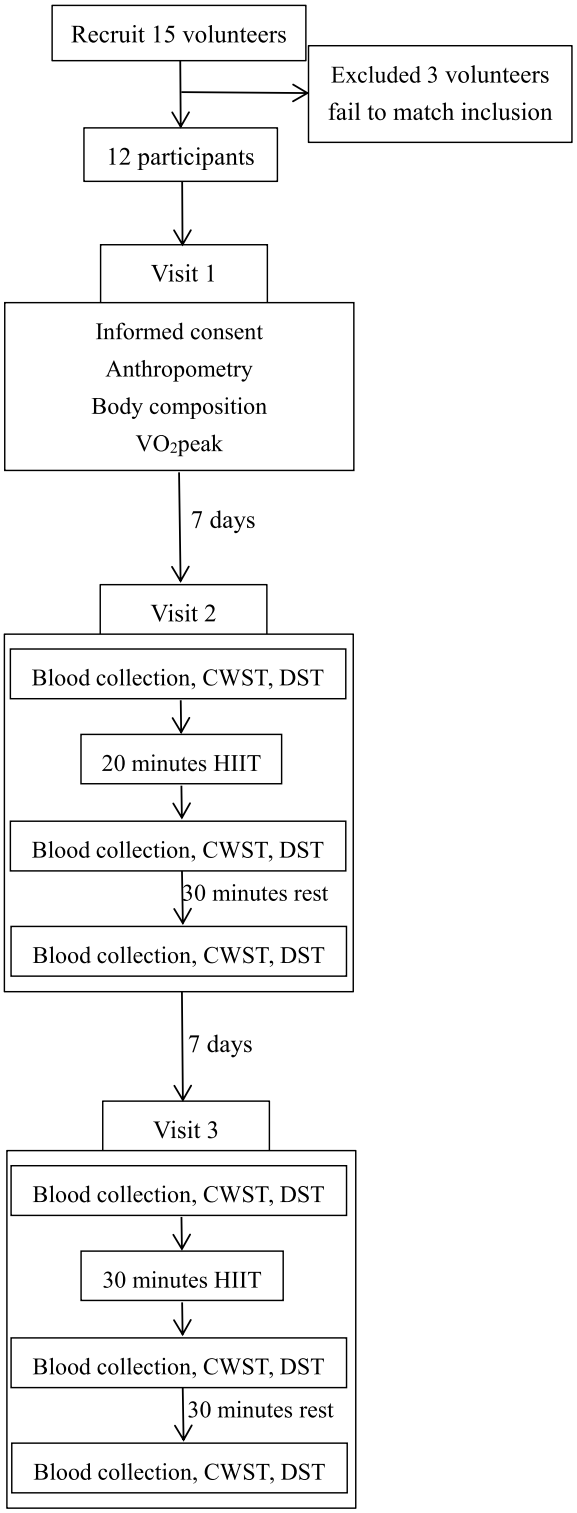

FIGURE 1 | Research study design.

ergogenic aids 10–12 h before every visit. During every HIIT session, participants were required to wear Polar (RS400, Finland) to record heart rate. All experimental procedures were performed between 7:00 a.m. and 12:00 p.m. A profile of the trial is shown in **Figure 1**. This research plan was approved by the Ethics Committee of Capital University of Physical Education and Sports (2021A32. Registered 1 September 2021). Baseline characteristics of the participants are reported in **Table 1**.

VO_{2peak} Measurement

During the first visit, all participants performed a graded incremental exercise test (GXT) on a cycle ergometer (ergoline100K, Germany) to determine peak oxygen uptake (VO_{2peak}). Briefly, participants warmed up for 5 min by pedaling at 1 W/kg body mass. After that, the workload was increased by 25 W every minute until voluntary exhaustion (the cadence was set at 60 rpm). During the GXT, breath-by-breath pulmonary gas-exchange data were collected by AEI moxus. Heart rate (HR) was measured continuously by Polar RS400, and the rating of perceived exertion (RPE) was assessed verbally by which participants were asked to rate their perceived exertion ranging from 6 (no exertion at all) to 20 (maximal exertion). VO_{2peak} was determined when at least three of the following criteria were satisfied: 1) the respiratory exchange ratio (RER) exceeded 1.05; 2) a plateau in the VO_2 despite increasing workload; 3) achievement of 90% of age-predicted peak HR (220-age); 4) an RPE of 19 or 20 (Suwabe et al., 2017).

High Intensity Interval Training

During the second and third visit, the participants completed two HIIT protocols (20 min HIIT and 30 min HIIT) on a cycle ergometer (the cadence was set at 60 rpm), separately. Each HIIT session was started with a 5 min warm-up at 50 W followed by repetitive cycles composed of a 1 min bout at 85% VO_{2peak} (high-load) and 1 min active recovery at 25% VO_{2peak} (low-load) and finally finished with a 5 min cooldown at 50 W. Participants performed 15cycles in 30 min HIIT group and 10cycles in 20 min HIIT group.

Cognitive Function

Cognitive function was assessed at baseline (pre-HIIT), immediately after training (post-HIIT) and 30 min after training (30 min post-HIIT) using color-word matching Stroop test (CWST) and Digital Span Test (DST).

The CWST consisted of 60 trials, including 20 congruent tests, 20 neutral tests and 20 incongruent tests, presented in a random order (**Figure 2**). For congruent tasks, the upper row contained the words 'RED', 'YELLOW', 'BLUE', or 'GREEN' printed in the congruent color (e.g., GREEN was printed in green) and the lower row contained color words printed in black. For neutral tasks, the

TABLE 1 | Basic characteristics of the participants (M ± SD n = 12).

Variables	M±SD
Age (year)	24.33 ± 1.65
Height (m)	1.77 ± 0.06
Weight (kg)	72.38 ± 6.03
BMI (kg/m^2)	22.18 ± 1.34
VO_2peak (ml/kg*min)	45.33 ± 5.62
Body fat mass (%)	16.41 ± 3.58

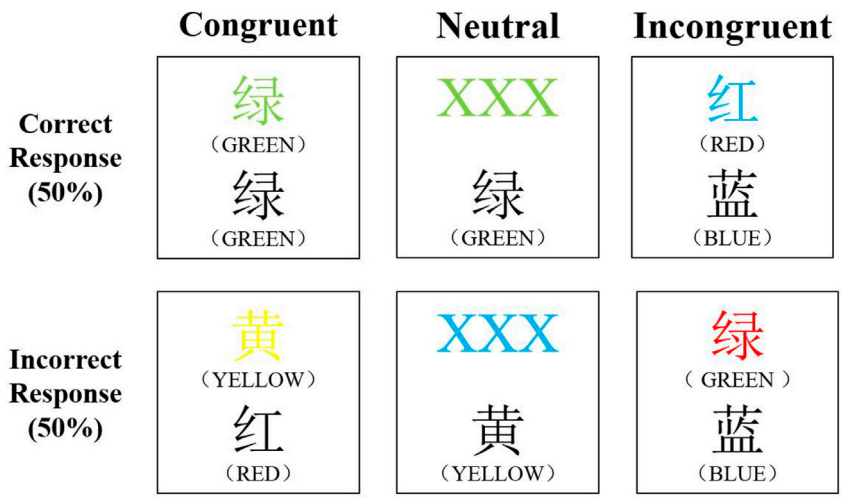

FIGURE 2 | The color-word matching Stroop task.

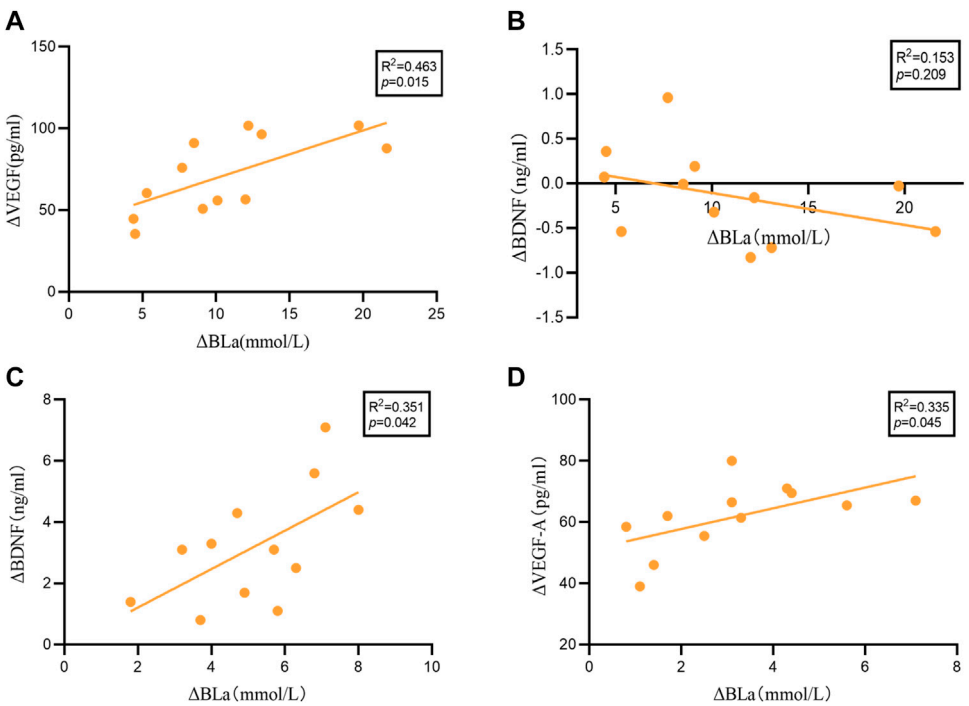

FIGURE 3 | The results of the linear regression analysis. **(A)**: ΔBLa and ΔVEGF-A in 30 minutes HIIT intervention. **(B)**: ΔBLa and ΔBDNF in 30 minutes HIIT intervention. **(C)**: ΔBLa and ΔVEGF-A in 20 minutes HIIT intervention. **(D)**: ΔBLa and ΔBDNF in 20 minutes HIIT intervention.

upper row consisted of 'XXX' printed in red, yellow, blue, or green and the lower row contained the words 'RED', 'YELLOW', 'BLUE', or 'GREEN' printed in black. For incongruent tasks, the color words in the upper row were printed in an incongruent color (e.g., RED was printed in blue) and the lower row contained color words printed in black. In each trail, the target marker (+) was first presented in the center of the screen, followed by the stimulus, the stimulus remained on the screen until the response was given (Kujach et al., 2018). We instructed participants to decide whether the color of the upper word was consistent to the color name of the lower word by choosing the correct key on the keypad (F key or J key representing yes or no). The correct-answer ratio assigned to yes and no was 50%. After pressing the key, the computer recorded the participant's response accuracy (RA) and response time (RT). All words were written in Chinese and all participants underwent three practice sessions prior to the experiment.

The DST from the Wechsler Adult Intelligence Scale (WAIS) consisted forward DST (DST-F) and backward DST (DST-B) in which the participants were requested to correctly repeat a series of increasingly length of random number sequences presented to them in the same order and reversed order, respectively. Both DST-F and DST-B were started with a four-number test at a rate of one digit per second. Correct repetition in the correct order allowed the participant to take a five-number test and so on. The task was stopped when the participant failed to repeat the last sequence correctly or recall at least two strings of the same length and score was considered to be the previous sequence length (Lavner and Rabinowitz, 2015).

Blood Sample Collection and Analysis

The whole blood (15 ml) was drawn from an antecubital vein and the capillary blood was drawn from fingertip by a skilled nurse at pre-HIIT, post-HIIT, and 30 min post-HIIT. Blood lactate (BLa) level was measured immediately after fingertip blood collection using a digital portable lactate analyzer (SYL115Lacate-Scout, Germany). The whole blood samples were allowed to clot at room temperature for 30 min, then centrifuged at 3,000 rpm for 10 min at 4°C. The separated serum samples were frozen and kept at −80°C. The serum levels of VEGF-A, BDNF, testosterone and cortisol were measured using the following ELISA kits: human BDNF (ml900214, mlbio, China), human VEGF-A (ml060752, mlbio, China), human testosterone (ml064301, mlbio, China), human cortisol (ml711149, mlbio, China). The intra-assay coefficient of variation for the kits was <10%. All analyses were performed on an automatic microplate reader (E 601, Germany).

Statistical Analyses

The statistical analyses were performed using SPSS26.0. All the results are presented as mean ± standard deviation (SD). The normality of data distribution was verified using the Shapiro-Wilk test. Statistical significance was set at $p \leq 0.05$. Differences of baseline variables between two groups were conducted by independent t-tests. Factorial 2 × 3 (groups: 20 min HIIT, 30 min HIIT × time points: pre-HIIT, post-HIIT, 30 min post-HIIT) repeated measures ANOVA were used to compare the concentration of BLa, serum levels of VEGF-A, BDNF, cortisol, and testosterone as well as RT and RA between two groups at the three time points chosen. Significant main effects and interactions were assessed using the Tukey post-hoc test. η^2 values will be reported as effect size, η^2 was considered small if $\eta^2 < 0.04$, and large if $\eta^2 > 0.36$. Linear regression analysis was used to determine the relationship between changes from baseline variables. Figures were plotted by the GraphPad Prism8.0.

RESULTS

BLa Responses to HIIT

As shown in **Table 2**, significant group by time of measurement interaction (F = 18.258, $p < 0.001$, $\eta^2 = 0.785$), time (F = 32.080, $p < 0.001$, $\eta^2 = 0.865$) and group (F = 39.997, $p < 0.001$, $\eta^2 = 0.784$) main effects were found in BLa analysis. The level of BLa was significantly increased immediately after the 20 min HIIT ($p < 0.001$) and 30 min HIIT ($p = 0.001$). Although BLa level was significantly decreased at 30 min post-HIIT compared with that measured at post-HIIT ($p < 0.001$), it was still significantly higher than baseline ($p < 0.001$). In addition, 30 min HIIT produced considerably higher BLa level than 20 min HIIT at both post-HIIT ($p < 0.001$) and 30 min post-HIIT ($p = 0.005$).

Serum BDNF and VEGF-A Responses to HIIT

As shown in **Table 2**, significant group by time of measurement interaction (F = 43.159, $p < 0.001$, $\eta^2 = 0.797$), time (F = 68.826, $p < 0.001$, $\eta^2 = 0.862$) and group (F = 9.082, $p = 0.012$, $\eta^2 = 0.452$) main effects were found in BDNF analysis. The BDNF level was significantly elevated immediately after 20 min HIIT ($p < 0.001$) and returned to the baseline 30 min later. In contrast, 30 min HIIT did not immediately affect the BDNF level, but the BDNF level was significantly decreased after 30 min recovery ($p = 0.045$) and was even lower than its baseline ($p = 0.013$). Moreover, 20 min HIIT produced significant higher BDNF level than the 30 min HIIT at post-HIIT ($p < 0.001$).

As shown in **Table 2**, a significant time (F = 217.955, $p < 0.001$, $\eta^2 = 0.978$) main effect was found in the analysis of VEGF-A. Compared with baseline, the VEGF-A level was significantly elevated immediately after both 20 min HIIT ($p < 0.001$) and 30 min HIIT ($p < 0.001$), and it returned to the baseline 30 min after 20 min HIIT. Whereas, the VEGF-A was significant decreased after 30 min recovery ($p < 0.001$) and was even lower than the baseline ($p < 0.001$) in 30 min HIIT group. Furthermore, 20 min HIIT induced considerably higher level of VEGF-A than 30 min HIIT at 30 min post-HIIT ($p = 0.014$).

Testosterone, Cortisol, T/C and RPE Responses to HIIT

As shown in **Table 2**, a significant time (F = 17.780, $p = 0.001$, $\eta^2 = 0.781$) main effect was found in the analysis of serum testosterone. The level of testosterone was significantly increased immediately after both 20 min HIIT ($p = 0.012$) and 30 min HIIT ($p = 0.045$) compared with their baselines. The 20 min HIIT induced increased testosterone level was declined back to the baseline after 30 min recovery, while the 30 min HIIT induced increased testosterone level was significantly decreased after 30 min recovery ($p = 0.003$) and was even lower than the baseline ($p = 0.006$). Group comparison indicated that the serum testosterone level measured 30 min after training was considerably higher in 20 min HIIT group than in 30 min HIIT group ($p = 0.012$).

As shown in **Table 2**, significant group by time of measurement interaction (F = 9.762, $p = 0.004$, $\eta^2 = 0.661$) and time (F = 18.955, $p < 0.001$, $\eta^2 = 0.791$) main effects were found in serum cortisol. Both 20 min HIIT ($p = 0.044$) and 30 min HIIT ($p = 0.006$) significantly upregulated the serum cortisol level immediately. After 30 min recovery, the serum cortisol returned

TABLE 2 | Hematology and cognitive function values for subjects in the study (M ± SD).

	20 minutes HIIT			30 minutes HIIT			Time	Group	Group × Time
	Pre-HIIT	Post-HIIT	30 minutes Post-HIIT	Pre-HIIT	Post-HIIT	30 minutes Post-HIIT	F(p-value)[η^2]	F(p-value)[η2]	F(p-value)[η^2]
BLa(mmol/L)	0.90 ± 0.36	4.10 ± 1.82$^{**\hat{}}$	1.63 ± 0.43$^{**\#\#\hat{}}$	0.86 ± 0.19	11.54 ± 5.48**	3.15 ± 1.38$^{**\#\#}$	32.080(<0.001)[0.865]	39.997(<0.001)[0.784]	18.258(<0.001)[0.785]
BDNF(ng/ml)	21.29 ± 1.17	26.66 ± 1.32**	21.27 ± 2.26$^{\#\#}$	21.83 ± 1.35	21.70 ± 1.54	20.86 ± 1.68$^{*\#}$	68.826(<0.001)[0.862]	9.082(0.012)[0.452]	43.159 (<0.001)[0.797]
VEGF-A(pg/ml)	549.44 ± 110.56	611.28 ± 107.28**	493.29 ± 154.67$^{\#}$	550.82 ± 85.81	622.37 ± 83.43**	367.01 ± 91.84$^{**\#\#}$	217.955(<0.001)[0.978]	1.765(0.211)[0.138]	3.549(0.068)[0.415]
T(ng/ml)	8.93 ± 2.44	10.74 ± 3.09*	8.67 ± 2.73$^{\#\#}$	8.23 ± 2.64	9.56 ± 3.64*	5.92 ± 1.51$^{**\#\#}$	17.780(0.001)[0.781]	2.563(0.138)[0.189]	2.482(0.133)[0.332]
C(ng/ml)	213.14 ± 37.77	254.26 ± 64.20*	207.05 ± 53.76$^{\#\#}$	217.57 ± 46.73	317.06 ± 95.27**	297.97 ± 78.39**	18.955(<0.001)[0.791]	3.702(0.081)[0.252]	9.762(0.004)[0.661]
T/C	0.042 ± 0.011	0.043 ± 0.011	0.044 ± 0.014$^{\hat{}}$	0.038 ± 0.013	0.032 ± 0.015	0.021 ± 0.006$^{*\#}$	8.860(0.002)[0.446]	7.346(0.020)[0.400]	9.002(0.001)[0.450]
RPE	6.833 ± 0.835	14.417 ± 1.165**	6.417 ± 0.515$^{\#\#}$	6.667 ± 0.651	16.417 ± 0.996**	6.500 ± 0.522$^{\#\#}$	621.792(<0.001)[0.985]	25.634(<0.001)[0.700]	18.293(<0.001)[0.624]
DST-F	8.25 ± 1.06	10.25 ± 1.144**	10.17 ± 1.19**	8.92 ± 1.24	9.00 ± 1.04	9.17 ± 1.03	10.599(0.003)[0.679]	1.715(0.217)[0.135]	11.585(0.002)[0.699]
DST-B	6.42 ± 1.24	7.33 ± 1.37*	7.08 ± 1.08	5.83 ± 1.53	6.50 ± 1.93	6.58 ± 1.31	5.377(0.013)[0.328]	5.711(0.036)[0.342]	0.2339(0.790)[0.021]
Congruent task									
RT(ms)	1071.10 ± 166.90	855.04 ± 232.34**	841.46 ± 201.72**	973.44 ± 251.78	928.05 ± 282.12	984.21 ± 211.48	4.006(0.033)[0.267]	0.866(0.372)[0.073]	3.094(0.065)[0.220]
RA(%)	96.90 ± 3.96	98.44 ± 3.88	97.42 ± 4.86	96.86 ± 4.05	95.85 ± 6.86	95.83 ± 5.37	0.078(0.925)[0.015]	2.014(0.184)[0.155]	0.771(0.488)[0.134]
Neutral task									
RT(ms)	1046.40 ± 205.85	766.10 ± 158.67**	745.01 ± 104.30**	1003.49 ± 113.56	977.92 ± 155.88	1081.90 ± 238.47	15.946(0.001)[0.761]	8.910(0.012)[0.448]	18.026(<0.001)[0.783]
RA(%)	99.36 ± 2.22	97.81 ± 3.25	97.32 ± 4.12	98.31 ± 3.08	98.16 ± 4.75	98.20 ± 3.28	1.161(0.332)[0.095]	0.008(0.930)[0.001]	0.302(0.743)[0.027]
Incongruent task									
RT(ms)	1152.08 ± 177.27	958.09 ± 204.45*	1052.51 ± 229.63	1071.19 ± 322.49	1016.68 ± 257.68	1073.83 ± 267.60	2.018(0.157)[0.155]	<0.001(0.996)[<0.001]	0.498(0.615)[0.043]
RA(%)	94.36 ± 6.26	96.35 ± 4.77$^{\hat{}}$	94.18 ± 6.61$^{\hat{}}$	91.52 ± 4.79	86.85 ± 3.92*	87.17 ± 4.34	1.242(0.308)[0.101]	30.323(<0.001)[0.734]	2.183(0.137)[0.166]

BLa, Blood lactate; BDNF, Brain-Derived neurotrophic factor; VEGF-A, Vascular endothelial growth factor-A; T, Testosterone; C, Cortisol; RPE, Rating of Perceived Exertion; DST-F, Digital Span test-forward; DST-B, Digital Span test-backward; RT, Response time; RA, Response accuracy.

*p < 0.05; **p < 0.01 vs. pre-HIIT; $^{\#}$p < 0.05, $^{\#\#}$p < 0.01 vs. post-HIIT; $^{\hat{}}$p < 0.05, $^{\hat{}}$p < 0.01vs. 30 minutes HIIT intervention.

to baseline in the 20 min HIIT group, while the serum cortisol maintained increased level in the 30 min HIIT group. What is more, the serum cortisol concentration was considerably lower at 30 min post-HIIT in the 20 min HIIT group compared with that in the 30 min HIIT group ($p = 0.012$).

As indicated in **Table 2**, significant group by time of measurement interaction (F = 9.002, $p = 0.001$, $\eta^2 = 0.450$), time (F = 8.860, $p = 0.002$, $\eta^2 = 0.446$) and group (F = 7.346, $p = 0.020$, $\eta^2 = 0.400$) main effects were found in the testosterone/cortisol ratio (T/C). There were no significant differences in T/C among three time points in 20 min HIIT group. In 30 min HIIT group, the T/C did not change immediately after training but decreased significantly after 30 min recovery ($p = 0.020$) and was even lower than the baseline ($p < 0.001$). Group comparison indicated that the T/C in 20 min HIIT group was considerably higher than 30 min HIIT group at 30 min post-HIIT ($p < 0.001$).

CWST Responses to HIIT

As seen in **Table 2**, significant time (F = 4.006, $p = 0.033$, $\eta^2 = 0.267$) main effects were found in the RT of congruent tasks. RT for congruent tasks was significantly shorter immediately after the 20 min HIIT than pre-HIIT ($p < 0.001$), this shortened RT was maintained for 30 min following 20 min HIIT ($p < 0.001$). Significant group by time of measurement interaction (F = 18.062, $p < 0.001$, $\eta^2 = 0.783$), time (F = 15.946, $p = 0.001$, $\eta^2 = 0.761$) and group (F = 8.910, $p = 0.012$, $\eta^2 = 0.448$) main effects were found in the RT of neutral tasks. RT for neutral tasks was significantly shorter immediately after the 20 min HIIT than pre-HIIT ($p < 0.001$), and this shortened RT was also maintained for 30 min following 20 min HIIT ($p < 0.001$). Moreover, 20 min HIIT contributed to significant shorter RT at post-HIIT ($p = 0.005$) and 30 min post-HIIT ($p = 0.001$) than 30 min HIIT. No significant main effect was found in the RT of incongruent tasks. RT for incongruent tasks was significantly shorter immediately after the 20 min HIIT than pre-HIIT ($p = 0.043$).

Significant group by time of measurement interaction (F = 30.323, $p < 0.001$, $\eta^2 = 0.734$) effects were found in the RA of incongruent tasks. The RA of congruent and neutral tasks did not differ significantly between the 20 and 30 min HIIT protocols throughout the experimental sessions. The RA was significantly decreased only for incongruent task immediately after 30 min HIIT when compared to pre-HIIT ($p = 0.035$). Importantly, 30 min HIIT resulted in significant lower RA at post-HIIT ($p < 0.001$) and 30 min post-HIIT ($p = 0.014$) than 20 min HIIT.

DST Responses to HIIT

As seen in **Table 2**, significant group by time of measurement interaction (F = 11.585, $p = 0.002$, $\eta^2 = 0.699$) and time (F = 10.599, $p = 0.003$, $\eta^2 = 0.679$) main effects were found in the DST-F. The participants got significant higher averaged score right after 20 min HIIT ($p < 0.001$) and also got considerably higher score 30 min later ($p = 0.002$), compared with baseline. In contrast, there were no significant differences in DST-F scores among three time points in 30 min HIIT group. Group comparison indicated that the DST-F score was considerably higher right after the 20 min HIIT, compared with the 30 min HIIT intervention ($p = 0.024$).

As shown in **Table 2**, significant group (F = 5.711, $p = 0.036$, $\eta^2 = 0.342$) and time (F = 5.377, $p = 0.013$, $\eta^2 = 0.328$) main effects were found in the DST-B. The DST-B averaged score was significantly elevated only after the 20 min HIIT ($p = 0.026$) and returned to baseline 30 min later. Whereas, it was not changed after 30 min HIIT.

Relationship Between Changes From Baseline Variables (ΔBDNF, ΔVEGF-A and ΔBLa)

We performed linear regression analysis to examine the association between ΔBDNF (20 min HIIT = 5.17 ± 1.79; 30 min HIIT = -0.13 ± 0.51), ΔVEGF-A (20 min HIIT = 61.83 ± 11.11; 30 min HIIT = 71.56 ± 23.66) and ΔBLa (20 min HIIT = 3.20 ± 1.90; 30 min HIIT = 10.68 ± 5.50), (Δ = post-HIIT-pre-HIIT). As shown in **Figure 3** significant relationship was identified between ΔBLa and ΔVEGF-A ($R^2 = 0.463$, $p = 0.015$), while no significant relationship was found between ΔBLa and ΔBDNF ($R^2 = 0.153$, $p = 0.209$) in 30 min HIIT group. ΔBLa was statistically related to ΔBDNF ($R^2 = 0.351$, $p = 0.042$) and ΔVEGF-A ($R^2 = 0.335$, $p = 0.045$) in 20 min HIIT group.

DISCUSSION

The main finding in the present study is that the duration of HIIT affects both neurotrophins and cognitive function. In other words 20 min HIIT leads to more beneficial response of cognitive function as well as serum BDNF and VEGF-A levels than 30 min HIIT in healthy young men.

As a protein member of the neurotrophin family, BDNF is derived mainly from the brain, however, its receptors are also found in a wide variety of peripheral tissues such as liver, pancreas, adipose tissue, heart, endocrine system, reproductive system, smooth muscle and skeletal muscle (Hallböök et al., 1991; Scarisbrick et al., 1993; Bathina et al., 2016; Matsumoto et al., 2021). Previous studies have indicated that BDNF passes through the blood-brain barrier in a bidirectional transport manner (Rasmussen et al., 2009). Therefore, alterations in periphery BDNF levels could reflect the variation of brain BDNF (Klein et al., 2011). Previous studies have shown that BDNF is sensitive to exercise (Cabral-Santos et al., 2016). In the present study, 20 min HIIT intervention elevated BDNF level in the serum at post-HIIT and the BDNF level was returned to the baseline at 30 min post-HIIT. However, as the effect of 30 min HIIT intervention, there was no significant difference in the serum BDNF concentration at post-HIIT, but it was decreased significantly at 30 min post-HIIT. Moreover, we found a positive correlation between ΔBLa and ΔBDNF in 20 min HIIT group, but there was no correlation between ΔBLa and ΔBDNF in 30 min HIIT group. García-Suárez et al. (2020) have reported similar findings that serum cortisol and BLa of healthy women increased immediately after a bout of 12 min HIIT, while

serum BDNF levels did not change following HIIT. Moreover, Rojas Vega et al. (2006) found that serum BDNF of healthy male athletes elevated immediately following a bout of ramp incremental cycle ergometry, but serum cortisol levels did not change following HIIT.

It has been pointed out that BLa is a key factor inducing BDNF synthesis (El Hayek et al., 2019; Müller et al., 2020), however, cortisol is a key factor suppressing BDNF synthesis (Issa et al., 2010; Kino et al., 2010; García-Suárez et al., 2020). Therefore, we speculate that BLa and serum cortisol might contribute to the different effects of HIIT with different duration on BDNF. In the present study, we found that the increase of concentrations of BLa and serum cortisol occurred in a duration-dependent manner, as higher BLa and cortisol levels were produced by a longer-duration training in the same intensity. Thirty minutes HIIT maintained temporal differences that is induced higher BLa and cortisol levels.

Moreover, testosterone has been shown to have a wide range of neuroprotective effect by increasing levels of BDNF and VEGF within the brain (Spritzer and Roy, 2020). Both interventions significantly evaluated testosterone level at post-HIIT, but after 30 min recovery, 20 min HIIT maintained higher level of testosterone than 30 min HIIT. Since testosterone shows anabolic effects and cortisol promotes catabolic effects, the T/C has been considered as a signal of the training activity is too high and catabolic processes prevail (Ambroży et al., 2021). T/C is correlated with the duration and intensity of training, T/C decline indicates that the body is under high metabolic stress and accumulates fatigue (Turner et al., 2017). In this study the ratio of T/C declined gradually over time in the 30 min HIIT group, while there was no significant change in the 20 min HIIT group, and 30 min HIIT produced higher RPE than 20 min HIIT (20 min HIIT: 14.417 ± 1.165; 30 min HIIT: 16.417 ± 0.996) indicating that the body is under higher physiological strain from training in 30 min HIIT than 20 min HIIT.

It has been reported that high intensity exercise may provoke an increase in circulating cortisol level and therefore increase arousal, which might lead to impaired cognitive performance (Hill et al., 2008; Quintero et al., 2018). Thus, the inhibitory effect on the synthesis of BDNF induced by cortisol may be greater than the stimulative effect induced by BLa in 30 min HIIT group. On the contrary, the stimulative effect induced by BLa on the synthesis of BDNF may be greater than the inhibitory effect induced by serum cortisol in the 20 min HIIT group. Given the antagonistic effects on BDNF synthesis produced by exercise-induced BLa and serum cortisol, exercise-induced BLa and serum cortisol must maintain optimal levels to produce BDNF for improving cognitive function.

In the present study, both 20 min HIIT and 30 min HIIT elevated serum VEGF-A immediately following HIIT. The serum VEGF-A returned to baseline 30 min after HIIT in the 20 min HIIT group, while the serum VEGF-A significantly decreased in the 30 min HIIT group. Hebisz et al. (2019) reported a similar finding that the serum VEGF-A of cyclists was downregulated after a bout of sprint interval training (4 × 30s all-out repetitions interspersed with 90 s of rest). It has been reported that the serum VEGF-A plays a role in tissue repair (Tischer et al., 1989). It can be surmised that the body is under high metabolic stress and this downregulation is because of local tissue injury 30 min after HIIT. Moreover, the decreases of serum BDNF and VEGF-A after HIIT indicate their utilization for local tissue repair (Nofuji et al., 2008; Hebisz et al., 2019).

The current study indicated that HIIT-induced BLa was positively correlated with the increased peripheral level of VEGF-A in both HIIT groups. Previous literature has demonstrated that serum VEGF-A is highly correlated with BLa. Kujach et al. (2019) observed a significant elevation across time in VEGF-A and BLa in young males after 6 × 30s sprint interval training, and BLa was positively correlated with increased peripheral level of VEGF-A. Morland et al. (2017) showed that L-lactate subcutaneous injection and HIIT leading to the increases in BLa levels, increases brain VEGF-A expression and capillary density in wild-type mice, but not in knockout mice lacking HCAR1 (a receptor of lactate).

Recently, a meta-analysis suggests that participation in HIIT can improve adolescents' cognitive function and mental health. Previous studies have suggested a dose-response relation between exercise intensity and cognitive performance after exercise (Kamijo et al., 2004; Chang and Etnier, 2009; Chang et al., 2011). Our results showed that there was also a dose-response relation between exercise duration and cognitive performance after exercise, that is, 20 min HIIT stimulated executive function, improving CWST performance by a short response time and elevating DST score, but 30 min HIIT could not. Importantly, the findings of this study suggest that exercise duration of 20 min, with 5-min warm-up and cool-down as recommended by the ACSM, results in the largest benefits to cognitive function. On the other hand the facilitative effect of 20 min acute moderate-intensity exercise on cognitive performance partly attributes to the expression of neurotrophins, and acute exercise induces greater attentional allocation, more efficient information processing speed, and optimal physiological and psychological arousal occurs (Tsai et al., 2014; Hwang et al., 2016; Bettio et al., 2019; Chen et al., 2019; Tian et al., 2021). We observed that the RA of incongruent tasks performed immediately after 30 min HIIT was significantly reduced compared to that at pre-HIIT. Moreover the incongruent condition resulted in longer RT and less RA than the congruent condition in CWST. This finding is consist with a previous study, Tsukamoto et al. found that RA was significantly decreased for incongruent task immediately after 28 min HIIT (4 × 4 min training interspersed with 3 min of active recovery) (Tsukamoto et al., 2016), suggesting that prolonged-intense exercise (30 min HIIT) might leads to damage on higher-order aspects of cognition in general. Hwang er al. (2016) found that shorter response times in Trail Making Test Part-B were significantly correlated with an increase in ΔBDNF after 20 min high-intensity exercise in healthy adults. Unfortunately, our study did not show the cognitive function was correlated with BDNF and VEGF-A. The heterogeneous results might be due to the differences in

methodologies involving exercise protocol, cognitive task type, test time points, participant's cardiorespiratory fitness level and other confounding factors.

Limitations

There are some limitations to be considered: First, CWST and DST are adopted to evaluate inhibition and updating of executive function respectively in this study. Whereas, executive function is a comprehensive cognitive area that consists of several subcomponents of cognitive performance, namely inhibition, updating, and shifting. Although a meta-analysis suggest that acute HIIT generally tends to have not positive effect on shifting (Ai et al., 2021), future studies are necessary to explore how effects are similar or different depending on the particular type of cognitive task. Second, the potential neurobiological mechanisms of acute exercise promoting cognitive function include exercise-induced neurotrophins and increases in general physiological arousal or neural activation (Hwang et al., 2016). This study focuses on neurotrophins, HIIT may lead to activation of different brain regions, so further studies will use neuroelectric techniques to explore the relationship between neurotrophins and regional neural activity. Furthermore, our subjects are right-handed young man, more heterogeneous subjects in a larger sample size will be necessary to be recruited in further study to assess the broad applicability of our findings.

CONCLUSION

Twenty minutes HIIT is more effective than 30 minutes HIIT for promoting serum levels of BDNF and VEGF-A, and cognitive function in healthy young men. In addition, both serum ΔBDNF and ΔVEGF-A in 20 min HIIT group were positively associated with ΔBLa, while only serum ΔVEGF-A was positively associated with ΔBLa in the 30 min HIIT group.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee of Capital University of Physical Education and Sports. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

SB conceived and designed research. QL, LZ and ZZ conducted experiments. QL analyzed data and wrote the manuscript. SB, YW and CZ revised the manuscript. All authors read and approved the manuscript.

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Immunological mechanisms of exercise therapy in dyslipidemia

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Numerous studies demonstrated the strong link between dyslipidemia and the cardiovascular risk. Physical activity and exercise represent effective prevention and therapy strategies for dyslipidemia and at the same time counteract numerous comorbidities that often accompany the disease. The physiological mechanisms are manifold, and primary mechanisms might be an increased energy consumption and associated adaptations of the substrate metabolism. Recent studies showed that there are bidirectional interactions between dyslipidemia and the immune system. Thus, abnormal blood lipids may favor pro-inflammatory processes, and at the same time inflammatory processes may also promote dyslipidemia. Physical activity has been shown to affect numerous immunological processes and has primarily anti-inflammatory effects. These are manifested by altered leukocyte subtypes, cytokine patterns, stress protein expression, and by reducing hallmarks of immunosenescence. The aim of this review is to describe the effects of exercise on the treatment dyslipidemia and to discuss possible immunological mechanisms against the background of the current literature.

KEYWORDS

inflammation, immunosenescence, sports therapy, metabolism, cardiovascular disease

Introduction

One of the key risk factors for the development of cardiovascular disease is hyper- and dyslipidemia. Both terms denote lipid metabolism disorders which are not quite uniformly defined, as in some cases the transported blood lipids, individual lipoprotein fractions or a combination of blood lipids and lipoproteins are used for clinical diagnosis. Hyperlipidemia mainly describes hypercholesterolemia (cholesterol >200 mg/dl or 5.2 mmol/L), hypertriglyceridemia (elevated triglycerides >150 mg/dl or 1.7 mmol/L) and combined hyperlipidemia (elevated cholesterol and elevated triglycerides). Altered lipoproteins mean hyperlipoproteinemia (mostly increased LDL), hypolipoproteinemia (mostly decreased HDL), and dyslipoproteinemia (high LDL and low HDL levels). Hyperlipidemia is suggested to be a major risk factor for the development of atherosclerosis. In particular, an increase in LDL is associated with an increased risk of cardiovascular disease, including coronary heart disease (CHD) and stroke (Pressler, 2017).

Lipid metabolism disorders as a risk factor for cardiovascular diseases

Cholesterol-rich LDL and other apolipoprotein B (ApoB)-containing lipoproteins, including very low-density lipoproteins (VLDL), intermediate density lipoproteins (IDL) and lipoprotein(a), m[Lp(a)], have a direct impact on the development of atherosclerosis and its cardiovascular consequences. The increased concentrations of these lipid fractions in the blood lead to functional changes in endothelial barrier function because lipids travel freely between the vessel lumen and the vessel wall. Hence, LDL and other ApoB-containing lipoproteins enter and leave the arterial intima especially at sites vulnerable for plaque formation. This subsequently leads to a retention and accumulation of cholesterol-rich lipoproteins within the intima. It is suggested that the higher the LDL-C concentrations, the higher the probability of retention, what creates the basis for the development of an arteriosclerotic plaques (Goldstein and Brown, 2015). In endothelial cells, enzymatic systems are activated, which induce an overproduction of reactive oxygen species (ROS). The increased oxidative stress in turn exhibits pro-atherogenic effects and induces pro-inflammatory signal cascades. The activated endothelium starts to express various inflammatory cytokines, chemokines and to express adhesion molecules, causing leukocytes adherence to the vascular endothelium. Depending on the degree of activation, immune cells transmigrate and infiltrate the vessel wall. Increased ROS formation and inflammation inhibits the production and bioavailability of nitric oxide (NO), which further exacerbates endothelial dysfunction (van Diepen et al., 2013).

Inflammatory processes interact with dyslipidemia

Migrating monocytes play a special role in these processes, because these cells differentiate into macrophages and transform into foam cells after taking up modified LDL *via* scavenger receptors (Sorci-Thomas and Thomas, 2016). Macrophage foam cells (MFCs) play a crucial role in the initiation and progression of atherosclerosis. At the same time, LDL cholesterol directly contributes to activation of the NLRP3 inflammasome, a cytosolic multiprotein complex of the innate immune system, which enhances the endothelial inflammatory response (Grebe and Latz, 2013). The multiple local inflammatory activities lead to an increased systemic production of C-reactive protein (CRP), tumour necrosis factor-alpha (TNF-α), interleukin (IL)-6 and IL-1, which are also thought to play a causal role in the development and progression of atherosclerosis. Conversely, systemic inflammatory processes, which are promoted by obesity, nicotine abuse, psychological stress or various autoimmune diseases, can promote local endothelial dysfunctions (Alack et al., 2019).

The subclinical inflammatory process is bi-directionally linked to lipid metabolism. On the one hand, inflammatory activities lead to increased cholesterol reverse transport and thus to increased formation of VLDLs (Khovidhunkit et al., 2004). On the other hand, circulating LDL has an increased tendency to oxidize, which explains the increased plasma levels of oxidized LDL (oxLDL) in patients with chronic inflammatory diseases (García-Gómez et al., 2014). OxLDL has a strong atherogenic effect, can promote inflammatory processes at the endothelium and even have a toxic effect on endothelial cells (Roma et al., 1992). Conversely, many immune cells interact with different classes of lipids and thus control their differentiation. In particular, the development of inflammatory leukocyte subtypes is favored (Hubler and Kennedy, 2016).

Immune ageing as a driver of atherosclerotic processes

Physiological aging is frequently accompanied by a progressive increase in the concentration of circulating inflammatory cytokines. In particular, T cell senescence appears to have a bidirectional relationship with the development of chronic low-grade inflammation (Franceschi, 2007). On the one hand, chronic inflammation is a driver of cellular senescence by constantly activating immune cells. On the other hand, senescent cell types often present a more pro-inflammatory phenotype and thus become a potent source for the secretion of pro-inflammatory cytokines (Davalos et al., 2010).

While the described immune aging processes are part of the physiological remodeling of the immune system in old age, they are accelerated by lifestyle factors, such as overnutrition, inactivity and the resulting overweight. Dyslipidemia may also accelerate the immune aging process. Data suggests that the metabolism of immune cells, especially T cells, is influenced by the altered lipid concentration in the environment. In particular, cholesterol metabolism of T cells is disturbed which was demonstrated to inhibit their proliferation and favor the development of pro-inflammatory phenotypes (Boßlau et al., 2021; Kim et al., 2021). Progressive "inflammaging", which is bi-directionally accelerated by lipid changes, leads to a vicious circle of dyslipidemia and maladaptive immune-aging. Inflammatory signaling pathways are continuously activated and initiate atypical cytotoxic activity toward endogenous structures such as the endothelium, which may ultimately favor the development of cardiovascular diseases (CVDs) (Nakajima et al., 2002).

Typical inflammatory markers, produced by many types of senescent cell in atherogenesis, are IL-1a, IL-1β, IL- 6, IL-8, IL-18 and TNF-a (Coppé et al., 2010; Freund et al., 2010; Prattichizzo et al., 2016). These cytokines promote inflammation locally in a

paracrine manner and perhaps at a systemic level (Stojanović et al., 2020). The chronic inflamed endothelium tends to become dysfunctional and allow deposits to build up, which also recruit adaptive immune cells by way of their inflammatory process (Libby et al., 2002). Besides MFCs, T cells play a curricular role as major regulators of atherogenesis. They can either act as positive or negative modulators of plaques. In particular, $CD4^+$ cells are considered to be an important cell type (Daugherty and Rateri, 2002; Hansson, 2005). Activated effector memory (EM) and central memory (CM) subtypes accumulate and thus stimulate the progression of atherogenesis. Inside the atherosclerotic plaques the cells release pro-inflammatory cytokines and bind to antigens of cholesterol-rich lipoproteins (Ammirati et al., 2012). After feeding a hypercholesterolemia-inducing diet, antigen-specific T cell clones actively expand in the plaque (Centa et al., 2018). $CD4^+$ cells in plaque are specific for oxLDL since LDL is a relevant autoantigen that could drive the autoimmune response against intrinsic proteins in the atherosclerotic plaque (Stemme et al., 1995) (Wolf and Ley, 2019).

These findings are in agreement with our own data demonstrating that T effector memory re-expressing CD45RA (T-EMRA cells) are highly associated with body fat (Boßlau et al., 2021). T-EMRA cells are also strong producers of inflammatory cytokines and exhibit cytotoxic activity toward the endothelium, probably contributing to plaque erosion. Therefore, they tend to be associated with unstable plaques and with severe CVD, and are considered a predictor of mortality in the elderly (Stojanović et al., 2020). In addition to the pro-atherogenic properties of highly differentiated $CD4^+$ T cells, also $CD8^+$ cells are involved in atherosclerotic remodeling, although little is known about their role in pathogenesis (Carrasco et al., 2022). It is suggested that $CD8^+$ cells contribute to inflammatory processes in the plaque, which might favor its instability. However, the antigen specificity of these CD8 lymphocytes is poorly understood (Kolbus et al., 2010; Kyaw et al., 2013).

Interaction of stress proteins with dyslipidemia

Heat shock proteins (HSPs) are expressed when cells are exposed to cellular stress factors such as hypoxia or infection. In addition, there are data showing that HSPs are also increased intracellularly and extracellularly in endothelial cells during atherosclerosis, and increased extracellular levels are associated with systemic inflammation (Xu et al., 2012). It is well known that proteins like HSP 70 can modulate the inflammatory response in the context of cellular stress reactions (Noble and Shen, 2012). Already in early stages of atherosclerosis, an increased expression and release of HSPs was shown. It is suggested that the HSP-induction results from one or a combination of factors, such as hyperlipidaemia, diabetes, smoking, and hypertension (Zhu et al., 2003). Increased oxidative stress might be the primary trigger which leads to the induction of HSP expression in vascular smooth muscle cells and in serum (Liao et al., 2000). It was further demonstrated that HSP60 serum levels correlate with the total cholesterol, LDL, and ApoB and negatively with adiponectin, and the intensity of HSP expression also correlates positively with the severity of atherosclerosis (Pockley, 2002). Another source of HSP60 are endothelial cells which are stressed by dyslipidemia. HSP60 synthesis and release modifies endothelial cells to targets of HSP60 specific T cells, as they express increased adhesion molecules. This favors the formation of macrophage-derived foam cells (Hashikawa et al., 2021). Mechanistically, HSP60 has been shown to contribute directly to the development of arteriosclerosis due to an increased synthesis of E-selectin andVCAM-1 within the endothelial cell (Kol et al., 1999). In response of chronic upregulation, these adhesion molecules participate in monocyte accumulation in the arterial intima. These results are supported by studies indicating that HSP 60 is selectively located in atherosclerotic lesions rather than non-atherosclerotic areas of the arterial wall (Guisasola et al., 2009).

The physiological processes triggered by increased HSP expression inside and outside the cell are divergent. While HSPs exhibit protective and anti-inflammatory activity intracellularly, increased extracellular levels results in pro-inflammatory signals (De et al., 2000). This divergence appears to be based in the fact that increased extracellular appearance of HSPs is often the result of a chronic and marked HSP expression, which is a consequence of cardiovascular stress, such as chronic exposure to oxidative stress. Then, the protective function is partially lost, and HSPs may promote atherosclerosis (Krüger et al., 2019).

Treatment of lipid levels is a key to risk reduction

The clear links between lipid metabolism disorders and cardiovascular morbidity and mortality already make it clear that there is, conversely, also a causal relationship between a therapeutically reduced LDL level, increased HDL and a reduction in the cardiovascular risk profile. Scientifically, this is undisputed, so that the treatment of elevated lipid levels aimed at individual targets represents a key component of risk modification in the primary and secondary prevention of cardiovascular disease (Pressler, 2017). The steadily increasing prevalence of dyslipidemia requires the progressive use of lifestyle measures in addition to drug therapy. Many randomized controlled trials and meta-analyses have convincingly demonstrated that regular physical activity is effective in both the prevention and treatment of hyperlipidemia and dyslipidemia. Conversely, this is

underpinned by the fact that a sedentary lifestyle is a major cause of dyslipidemia and cardiovascular disease (Albarrati et al., 2018).

Exercise therapy and sports for dyslipidemia

Epidemiological studies and prospective intervention studies provide clear evidence that physical activity (in the sense of total activity) and also exercise training can reduce cardiovascular morbidity and mortality by improving lipid profiles (Rhee et al., 2019). The best relationship can be established between the activity level and increased HDL values as well as reduced triglyceride values. Regarding HDL levels, a meta-analysis with 19 included studies showed that HDL2-C levels increased significantly through regular endurance training. It was interesting to note that this effect also occurred independently of changes in body weight and BMI (Kelley and Kelley, 2006). There are contradictory data regarding the effect of physical activity on LDL and its subfractions. However, with regard to the small LDL particles, which are classified as particularly atherogenic, there are indications that these are reduced by physical training (Varady et al., 2005).

The mechanisms of these effects are manifold. The primary one is certainly the metabolic effect of exercise, as the increased energy metabolism of active people increases both the metabolism due to more muscular activity and, in the long term, the resting metabolism. Accordingly, an increased calorie intake can be at least partially compensated for by exercise. Due to the increased energy turnover and the addressed substrate utilization pathways, different systems of the body adapt functionally and structurally to the corresponding metabolic challenge. For example, the reduction in plasma triglyceride concentration is primarily due to the upregulation of lipoprotein lipase (LPL) activity and quantity in skeletal muscle. Moderate endurance exercise in particular uses intramuscular triglycerides as a primary source of energy, depending on the duration (Watt et al., 2002). In addition to enzyme systems of the musculature, other processes of lipid metabolism are also promoted by physical activity. For example, exercise increases the activity of hormone-sensitive lipases (HSLs) in adipose tissue and muscle, allowing triglycerides to be more efficiently converted to free fatty acids and mobilized from the tissues. The expression of plasma membrane fatty acid binding proteins, such as FABPPM, also increases with regular activity, allowing fatty acids to enter the muscle cell more efficiently. The intramuscular capacity to bind free fatty acids in the cytosol and transport them to the mitochondria for β-oxidation is also improved by regular activity (Ringseis et al., 2011). The mechanism of HDL increase through exercise is only partially understood. One cause seems to be the increase in adiponectin secretion through exercise. Adiponectin is a protein hormone that is predominantly produced by adipocytes and is involved in the regulation of glucose levels and fatty acid breakdown. Adiponectin concentrations could be positively correlated with HDL-C levels in men and women in several studies. Thus, as an anti-inflammatory adipokine, adiponectin could also have a metabolic mediator role, since an influence of adipokine on HDL synthesis in the liver has been shown (Greene et al., 2012). Furthermore, regular activity reduces hepatic triglyceride secretion. Since peripheral HDL levels increase at the same time, both processes could be related in the context of activity (Couillard et al., 2001).

Immune-regulating effects of exercise on dyslipidemia

In addition to affecting blood lipids, physical exercise has a positive immune-regulating effect, which can foster anti-inflammation and thus can promote metabolic and cardiovascular health. Two mechanisms are important here. Exercise seems to positively influence cellular immune senescence, in addition to the release of pro- and anti-inflammatory cytokines. Thus, exercise affects both systems, which, as described above, are in a bidirectional relationship with chronic low-grade inflammation (Rosa-Neto et al., 2022).

Exercise affects T cell aging

With regard to T cell differentiation and expression of pro-inflammatory subtypes, research shows that training status and the starting of exercise training are related to senescent hallmarks (Duggal et al., 2019; Weyh et al., 2020). Cross-sectional data show that trained individuals or those with a long history of exercise have fewer senescent $CD4^+$ as well as $CD8^+$ $CD28^-CD57^+$ cells. Additionally, they had fewer differentiated $CD4^+$ CM cells, $CD8^+$ CM and EM cells and fewer highly differentiated $CD4^+$ and $CD8^+$ T-EMRA cells (Spielmann et al., 2011; Minuzzi et al., 2018). As described above, these cell types also play an important role in the development of atherosclerosis and plaque formation, in addition to being associated with antigens of cholesterol-rich lipoproteins. In parallel, a higher proportion of naïve T cells is observed. The beginning of regular exercise in previously inactive subjects can also affect hallmarks of T-cell aging. After only 3 weeks of endurance training in prediabetic subjects, a proportional increase in naïve and central memory T cells was found, while at the same time the proportion of senescent $CD8^+$ T-EMRA cells decreased (Philippe et al., 2019). Six weeks of a combined strength and endurance training increased the CD4+/CD8+ cell ratio in the elderly (Despeghel et al., 2021). However, further studies need to confirm these results as well as recommendations for the type, intensity, and duration of exercise.

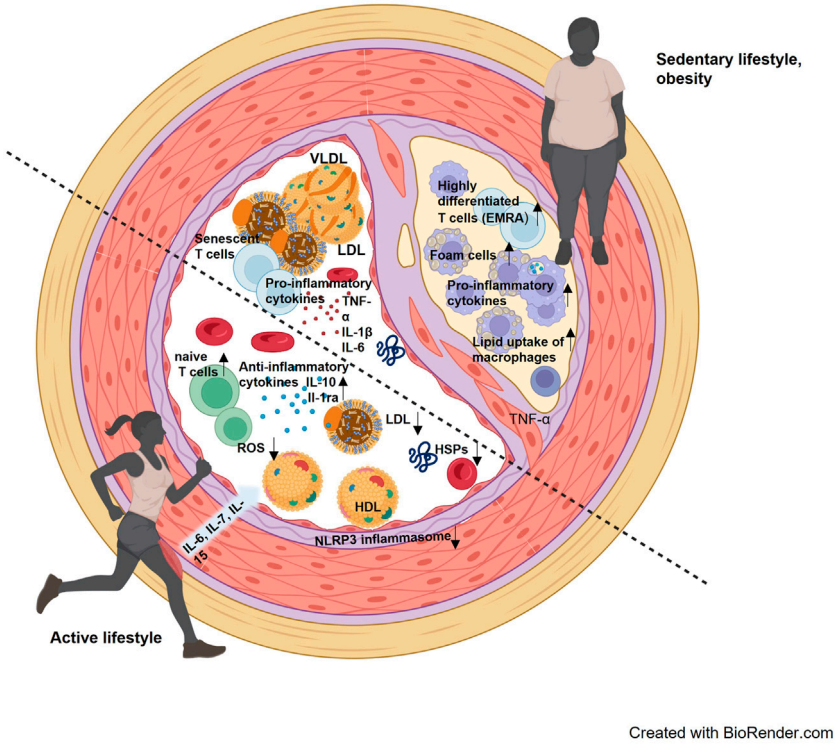

FIGURE 1
Interaction of blood lipids, cellular and molecular components of the immune system and stress proteins in an inactive lifestyle and an active lifestyle. The lower left half of the vessel visualizes the consequences of an active lifestyle, where reactive oxygen species (ROS) are effectively reduced, a more anti-inflammatory environment prevails, and blood lipids are present with a rather low LDL and higher HDL. The contrast is the upper right half of the vessel, where a more proinflammatory environment prevails, with higher LDL levels and accumulated senescent cells. An arteriosclerotic plaque has also already formed here, containing foam cells and infiltrated by more inflammatory leukocyte subpopulations.

Exercise affects polarization of macrophages and systemic inflammation

Regular exercise also affects components of the innate immune system. Accordingly, it was shown in animal experiments that regular treadmill exercise induced the conversion of M1 to M2 macrophages, which corresponds to a change from a classical, more pro-inflammatory type to an alternative, more immunoregulatory type. This altered polarization is associated with a change in the expression profile of cytokines towards more anti-inflammatory messengers (Gleeson et al., 2011; Oliveira et al., 2013). An important mechanism shows that anti-inflammatory signals occur from the active skeletal muscle itself. Thus, during muscle contraction, increased IL-6 is released, described as myokine. IL-6 has an anti-inflammatory effect through exercise by stimulating the production of immune regulatory mediators such as IL-10 and the IL-1 receptor antagonist (Steensberg et al., 2003) as well as the downregulation of TNF-α by monocytes and macrophages (Starkie et al., 2003). IL-7 (Haugen et al., 2010) and IL-15 (Rinnov et al., 2014) are also myokines that may stimulate lymphocyte proliferation. IL-7 is assumed to exert a protective effect on the thymus. IL-15 appears to have effects on the induction of better survival of naïve T cells. Both cytokines were increased in subjects who were physically active throughout their lives, compared to their inactive controls (Duggal et al., 2018). In contrast to endurance training, which can reduce systemic IL-6 and TNF-α levels (Zheng et al., 2019), resistance training seems to have no or marginal effect on chronic inflammation (Rose et al., 2021). A combination of endurance and resistance exercise seems to affect basal levels of pro- or anti-inflammatory cytokines like IL-6, -8 and -10, even in the presence of inflammatory comorbidities (Despeghel et al., 2021). Similarly, it was also shown that exercise in postmenopausal obese women with dyslipidemia reduces systemic levels of TNF-α, which in turn has positive effects on metabolic health (Biteli et al., 2021).

Exercise-effects on stress proteins

Long-term exercise training improves the intracellular HSP response, which was demonstrated in muscle tissue and

leukocytes (Fehrenbach et al., 2001; Yamada et al., 2008). Specifically, the expression of intracellular Hsp72 is suggested to be important to stabilize intracellular processes during conditions of increased oxidative stress which occurs in the context of systemic inflammation and dyslipidemia. (Morimoto, 1993; Febbraio and Koukoulas, 2000). Accordingly, we suggest that physical activity can strengthen cellular resilience by means of increased intracellular HSP induction, but at the same time prevent excessive release of HSPs into the extracellular space (Figure 1).

Practical implementation of exercise training

According to the S1 Guideline "Screening in sports", a sports medical screening should be carried out in the sense of a health examination to detect latent or pre-existing diseases that may pose a risk. Previous data indicate that targeted sporting activities have greater effects on changes in the lipid profile than a non-sport-based increase in everyday activity. Nevertheless, even more active daily life, which includes walking and strolling, climbing stairs and various physical activities of daily living, can contribute to the positive modification of plasma lipids. If we look at controlled exercise training, greater effects can be expected, especially through regular moderate and endurance activities. Current data recommend exercising for at least 30 min (or more) on as many days as possible. These amounts can also be started at a reduced level (e.g., shorter units of 10 min duration) and increased over time.

Practically, such activities can be achieved as walking and Nordic walking, jogging runs or cycling rides, by swimming or by participating in "cardio courses" in fitness facilities (Pressler, 2017). Intensities in the basic endurance range, i.e., below the individual anaerobic threshold, are recommended for such training units. Patients can be told as a rule of thumb for moderate-intensity aerobic training that you are active at a level that increases breathing and heart rate but still allows you to maintain yourself. In the area of endurance training, numerous health effects of High-Intensity Interval Training (HIIT) have also been demonstrated in recent years. This involves short-term intensive interval units that are usually performed on an ergometer or in running. At present, there is not enough data to assess the long-term effects of this type of training on a given lipometabolic disorder. Nevertheless, the studies show that HIIT also has significant effects on fat metabolism. Strong adrenergic innervation can improve the sensitivity of HSLs in adipose tissue, reduce metabolic stress in visceral fat and induce anti-inflammatory effects via stimulation of steroid hormone biosynthesis pathways (Sun et al., 2020). Accordingly, so-called "polarized training", which is a combination of basic and HIIT training, can certainly be an effective training for dyslipidemia when individually designed. The effectiveness of pure strength training on the lipid profile is contradictory in the literature. Nevertheless, strength training is always considered useful in the context of general health promotion in order to provide repeated stimuli to maintain muscle mass and thus functionally maintain a certain "everyday athleticism", prevent sarcopenia and promote the resilience of the musculoskeletal system (Barajas-Galindo et al., 2021). Here, the recommendations are 8–15 repetitions per exercise, which should be performed at least twice a week. This includes a 5–10 min warm-up with light aerobic activity. Each strengthening exercise should also be technically prepared so that it is performed correctly.

If statin therapy is indicated, it can be combined with activity and sports programs without any problems. If patients follow the activity recommendations in the long term, effects on blood lipids, especially on HDL and triglycerides, can already be expected after a few weeks. Finally, it should be mentioned that most patients with lipometabolic disorders have multiple morbidities that are influenced by physical activity. Here, it is particularly important to clarify the patients' fitness for sports by means of a sports medicine stress examination, depending on the indication.

Author contributions

KK did the literature reseach, developed the idea, written and edited the manuscript. PT did the literature reseach, written and edited the manuscript. CW the literature reseach, developed the idea, written and edited the manuscript.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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PERSPECTIVE
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Exercise as an Aging Mimetic: A New Perspective on the Mechanisms Behind Exercise as Preventive Medicine Against Age-Related Chronic Disease

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Age-related chronic diseases are among the most common causes of mortality and account for a majority of global disease burden. Preventative lifestyle behaviors, such as regular exercise, play a critical role in attenuating chronic disease burden. However, the exact mechanism behind exercise as a form of preventative medicine remains poorly defined. Interestingly, many of the physiological responses to exercise are comparable to aging. This paper explores an overarching hypothesis that exercise protects against aging/age-related chronic disease because the physiological stress of exercise mimics aging. Acute exercise transiently disrupts cardiovascular, musculoskeletal, and brain function and triggers a substantial inflammatory response in a manner that mimics aging/age-related chronic disease. Data indicate that select acute exercise responses may be similar in magnitude to changes seen with +10–50 years of aging. The initial insult of the age-mimicking effects of exercise induces beneficial adaptations that serve to attenuate disruption to successive "aging" stimuli (i.e., exercise). Ultimately, these exercise-induced adaptations reduce the subsequent physiological stress incurred from aging and protect against age-related chronic disease. To further examine this hypothesis, future work should more intricately describe the physiological signature of different types/intensities of acute exercise in order to better predict the subsequent adaptation and chronic disease prevention with exercise training in healthy and at-risk populations.

Keywords: preventive medicine, exercise physiology, physiological mechanisms, stress adaptation, aging

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INTRODUCTION

Age-related chronic diseases (e.g., cardiovascular disease, chronic kidney disease, Alzheimer's Dementia, Type II Diabetes, etc.) are among the most common causes of mortality and account for a majority of global disease burden (Yach et al., 2004; Kennedy et al., 2014; Murphy et al., 2018). Preventive lifestyle strategies such as exercise have emerged as potent, cost-effective means of reducing chronic disease risk (Sallis, 2009; Sepanlou et al., 2011; Bauer et al., 2014). Exercise has a critical role in disease prevention (Booth et al., 2012; Pedersen and Saltin, 2015; Sallis, 2015; Bennie et al., 2020) and has been proposed by the American College of Sports Medicine as a form of "medicine" (Church and Blair, 2009; Sallis, 2009, 2015). The protective effects of exercise on chronic disease risk are ultimately accumulated over time through physiological adaptations to the stress of exercise.

Acute exercise causes widespread physiological disruptions that require a complex, integrated response from the major physiological systems (autonomic, cardiovascular, metabolic, musculoskeletal, etc.) to meet the substantial requirements of human locomotion (Hawley et al., 2014, 2018). Repeated exposure to the physiological disruptions incurred by acute exercise (through exercise training) stimulate physiological adaptations that act to attenuate stress during subsequent exercise bouts (Małkiewicz et al., 2019). These exercise adaptations provide the foundation through which individuals can adapt and improve their ability to perform physical work (e.g., increase muscular power, endurance, aerobic capacity, etc.) and also prevent development of age-related chronic disease (Hawley et al., 2014, 2018). Thus, physiologic adaptations to exercise are the latent mechanisms through which exercise acts as medicine and reduces chronic disease risk. Despite seminal work that has identified several key mechanisms underlying the protective effects of exercise, there has yet to be an *overarching hypothesis* that explains broadly why or how it is that exercise protects against age-related chronic disease. We posit that exercise prevents age-related chronic disease because it acutely elicits physiological responses that mimic physiological changes seen with aging, the greatest contributing risk factor to all chronic disease (Bauer et al., 2014; Kennedy et al., 2014). Thus, we propose the hypothesis that exercise is "medicine" that protects against age-related chronic diseases because exercise can effectively simulate "aging." This paper is not intended to comprehensively review the physiological adaptations to exercise or their specific benefits on health/disease (see prior reviews; (Hawley et al., 2014, 2018; Green et al., 2017; Tanaka, 2019; McGee and Hargreaves, 2020), rather, we will examine this hypothesis by comparing age-related physiological changes with those induced during acute exercise and integrate these responses within the context and implications of stress-induced adaptation. This is not a systematic review, rather, we conducted a literature search of original data and reviews (when appropriate) examining the physiological effects of acute exercise on the brain (cognitive, brain-blood-barrier), cardiovascular, neuroendocrine, inflammation/oxidative stress, metabolic, and musculoskeletal systems and then aligned those observations with literature describing changes seen with aging and age-related chronic disease.

EFFECTS OF AGING AND EXERCISE ON THE BRAIN

Aging is accompanied by natural reductions across multiple domains of cognitive function (memory, reasoning abilities, executive function, and processing speed) (Carlson et al., 1995; Hayden and Welsh-Bohmer, 2012; Salthouse, 2012; Harada et al., 2013). Increasing age is also associated with inflammation and oxidative stress that damages the cerebral microvasculature and decreases blood-brain-barrier integrity (Verheggen et al., 2020). Ultimately, reductions in higher-order cognitive processing (memory/executive function) and blood-brain-barrier permeability are implicated in the underlying pathology and presentation of dementia and Alzheimer's disease (Salthouse, 2012; Harada et al., 2013; Gamba et al., 2015; Kirova et al., 2015).

Acute exercise imposes substantial stress on brain function and blood-brain-barrier integrity that parallel changes observed with age and cognitive disease. Acute exercise (particularly high intensity exercise) can impair higher order cognitive processing (e.g., executive function) through reallocation of mental resources (Audiffren et al., 2009) in an exercise intensity-dependent fashion (Lambourne and Tomporowski, 2010; Wohlwend et al., 2017). Exercise also acutely disrupts blood-brain-barrier integrity, with increased blood-brain-barrier permeability immediately following intense exercise (Sharma et al., 1991; Roh et al., 2017). This acute disruption in blood-brain-barrier integrity may be related to the effects of exercise on 1) oxidative-nitrosative stress (the origins of which are discussed further in subsequent sections) at the blood-brain-barrier interface that damages cells, reorganizes cytoskeletons, and increases inflammation (Sharma et al., 1991; Roh et al., 2017), 2) vasoactive effects of serotonin (Sharma et al., 1991), and 3) changes in cerebral blood flow patterns during exercise (e.g. increased pulsatile hemodynamics) (Armentano et al., 1991; Ogoh et al., 2005; Alwatban et al., 2020) which are linked with blood-brain-barrier damage and disruption (Jufri et al., 2015; Garcia-Polite et al., 2017; de Montgolfier et al., 2019).

EFFECTS OF AGING AND EXERCISE ON THE CARDIOVASCULAR SYSTEM

Aging is associated with an increase in mean blood pressure, resulting from a steady rise in systolic blood pressure and a slight decline in diastolic blood pressure (Franklin et al., 1997). Age-related increases in blood pressure may stem from, and simultaneously promote, large artery stiffening (Henskens et al., 2008; Najjar et al., 2008; Kaess et al., 2012; Mitchell, 2014; Tarumi et al., 2014; Zhou et al., 2018), which amplifies the magnitude of forward traveling energy waves and increases pulsatile blood pressure and flow (Mitchell, 2014; Tarumi et al., 2014; Lefferts et al., 2020). Age-related increases in large artery stiffness may be due, in part, to endothelial dysfunction wrought by oxidative stress and subsequent reductions in nitric oxide bioavailability (Donato et al., 2015; LaRocca et al., 2017). Ultimately, age-related vascular dysfunction increases cardiac work (i.e., afterload) and results in left ventricular hypertrophic remodeling (Lovic et al., 2017; Yildiz et al., 2020) and diastolic dysfunction (Strait and Lakatta, 2012; Abdellatif et al., 2018). Cumulatively, age-related vascular and cardiac dysfunction are intrinsically linked with the risk and development of cardiovascular disease (Lakatta and Levy, 2003; Abdellatif et al., 2018).

The cardiovascular response during acute exercise is markedly similar to the detrimental, chronic changes in cardiovascular function seen with aging. Exercise produces a substantial blood pressure response [systolic pressures >190 mmHg in young adults (Sabbahi et al., 2018)] and increase in heart rate that stiffens the large arteries (Armentano et al., 1991; Studinger et al., 2003; Townsend et al., 2015). Increases in large artery stiffness

during exercise (Studinger et al., 2003; Sharman et al., 2005; Pomella et al., 2018) are accompanied by increased forward wave energy (Jiang et al., 1995; Heckmann et al., 2000; Stock et al., 2021) and decreased wave reflection (Stock et al., 2021), ultimately contributing to greater pulsatile hemodynamics (Armentano et al., 1991; Ogoh et al., 2005; Alwatban et al., 2020). Additionally, exercise-induced acute increases in blood pressure may transiently impair endothelial function through a combination of mechanical distension/dilation of the artery, reductions in nitric oxide bioavailability, and endothelin-1 release during exercise (Millgård and Lind, 1998; Jurva et al., 2006; Gonzales et al., 2011; Morishima et al., 2020). This acute vascular response during exercise is further accompanied by a substantial (2–5-fold) increase in cardiac work (Channer and Jones, 1989; Rowland et al., 2002; Vega et al., 2017) that over time can stimulate ventricular remodeling in a similar manner to aging.

EFFECTS OF AGING AND EXERCISE ON NEUROENDOCRINE SYSTEM

Aging impacts various neuro/endocrine regulatory systems throughout the body. Serum cortisol increases 20–50% throughout the adult lifespan (Chahal and Drake, 2007; Feller et al., 2014) owing to hormonal changes in the hypothalamic-pituitary-adrenal axis (Corazza et al., 2014). Aging is also associated with autonomic nervous system dysfunction manifesting as increased sympathetic and decreased parasympathetic nervous system activity (Pfeifer et al., 1983; Jandackova et al., 2016). Higher cortisol levels over time are associated with increased cardiometabolic disease risk and may compromise immune function in older adults (Corazza et al., 2014; Feller et al., 2014), whereas shifts in autonomic balance favoring sympathetic activity is an independent risk factor for cardiovascular disease (de Jonge et al., 2010; de Lucia et al., 2018). Both of these neuroendocrine responses to aging are mimicked by acute exercise. Cortisol levels increase during acute exercise in an intensity-dependent manner (Brandenberger and Follenius, 1975; Kanaley et al., 2001). Similarly, sympathetic nerve activity increases in exercising muscle and cardiac autonomic balance shifts to favor sympathetic over parasympathetic activity (Rowell, 1997; Michael et al., 2017).

EFFECTS OF AGING AND EXERCISE ON INFLAMMATION AND OXIDATIVE STRESS

There is a well-established relationship between age and chronic low-level systemic inflammation (Ferrucci et al., 2010; Liberale et al., 2020). Circulating inflammatory markers increase with age in-part owing to increased chronic activation of the immune system (Liberale et al., 2020). Chronic inflammation with aging increases production of reactive oxygen (ROS)/nitrogen species (RNS) (Sergiev et al., 2015; Davalli et al., 2016). Higher levels of ROS/RNS promote cellular oxidative damage (cell membrane breakdown, protein modification, DNA damage) (Davalli et al., 2016) which can be further exaggerated by additional oxidative stress independent of ROS/RNS (Kudryavtseva et al., 2016). Ultimately, elevated markers of oxidative stress and systemic inflammation are strongly associated with increased risk of neurodegenerative, cardiovascular, and kidney disease, cancer, and dementia (Verbon et al., 2012; Marseglia et al., 2014; Kudryavtseva et al., 2016; Coen et al., 2018; Ferrucci and Fabbri, 2018; Senoner and Dichtl, 2019; Liberale et al., 2020).

Circulating inflammatory markers and oxidative stress also increase with acute exercise (Peake et al., 2005; Tsao et al., 2021). Acute exercise has been shown to increase pro-inflammatory cytokines such as interleukin (IL)-6 (Fischer, 2006), IL-7 (Małkiewicz et al., 2019), IL-10, C-reactive protein, and tumor necrosis-factor alpha (TNF-α) (Bernecker et al., 2013; Cerqueira et al., 2019; Fonseca et al., 2021) and initiate an inflammatory cascade (Powers and Jackson, 2008; McDonagh et al., 2014; Luca and Luca, 2019; Powers et al., 2020; Aragón-Vela et al., 2021). Additionally, exercise increases skeletal muscle ROS/RNS production *via* 1) electron leakage during oxidative phosphorylation within the mitochondria and NAD(P)H oxidase, 2) nitric oxide synthase activity within the skeletal muscle, 3) catecholamine and prostanoid release, and 4) ischemia/reperfusion-induced changes in xanthine oxidase activity, which ultimately contributes to oxidative stress, and subsequent cellular damage (Fisher-Wellman and Bloomer, 2009; McDonagh et al., 2014; Bouzid et al., 2015; Davalli et al., 2016; Powers et al., 2016; Petriz et al., 2017). As such, acute exercise can act as a pro-inflammatory stimulus that increases oxidative stress and damage in a manner similar to aging.

EFFECTS OF AGING AND EXERCISE ON METABOLISM

Advancing age is accompanied by alterations in both the metabolic pathways of energy production and mitochondrial function. Aging results in a steady rise in blood glucose concentration (Ko et al., 2006), driven in part by insulin resistance, exaggerated hepatic glucose production, and increasing cortisol levels (Satrústegui et al., 1986; Magnusson et al., 1992; Ko et al., 2006; Rizza, 2010). Similarly, aging and insulin resistance promote unrestrained lipolysis, which could contribute to systemic inflammation by increasing circulating free fatty acids (Reaven et al., 1989; Wende et al., 2012). Mitochondrial function is also impaired with aging, resulting in 1) increased sensitivity to ROS, 2) impaired oxidative metabolism, and 3) compromised mitochondrial membrane integrity (Shigenaga et al., 1994; Balaban et al., 2005). Mitochondrial dysfunction increases generation of oxidative byproducts (e.g. ROS/RNS) within the electron transport chain (Bratic and Larsson, 2013; Quinlan et al., 2013; Davalli et al., 2016) and thus accelerates age-related cellular damage (Genova and Lenaz, 2015). Taken together, these aspects of metabolic and mitochondrial dysfunction are associated with obesity, type II diabetes mellitus, obesity, fatty liver disease, cancer, sarcopenia and Alzheimer's disease (Kim et al., 2001; Wende et al., 2012; Girousse et al., 2018; Yoo et al., 2019; Spitler and Davies, 2020; Paliwal et al., 2021).

Acute exercise also perturbs metabolic pathways, increases mitochondrial ROS production, and alters mitochondrial

membrane permeability (Tonkonogi and Sahlin, 2002; Powers and Jackson, 2008). Acute exercise stimulates adipose tissue lipolysis, with low/moderate exercise eliciting a 2 to 5-fold increase in circulating free fatty acids for use in substrate metabolism (Havel et al., 1963; Ahlborg et al., 1974; Wolfe et al., 1990; Romijn et al., 1993; Ranallo and Rhodes, 1998; Burguera et al., 2000). Moreover, acute exercise also increases liver gluconeogenesis and hepatic glucose output *via* catecholamine release and sympathetic activity (Dibe et al., 2020) which aligns with changes in glucose production with aging.

EFFECTS OF AGING AND EXERCISE ON THE MUSCULOSKELETAL SYSTEM

Skeletal integrity begins to decrease around 35–40 years of age, with postmenopausal women experiencing bone loss at a rate of approximately 1–2% annually (Riggs et al., 2008; Curtis et al., 2015) owing to disproportionate increases in bone breakdown versus buildup. Similarly, aging is also often accompanied by 1) muscle atrophy from imbalances between muscle protein synthesis and degradation in response to anabolic stimuli (Reynolds et al., 2002; Koopman and van Loon, 2009; Wall et al., 2015) and 2) reduced muscular force production (Zizzo, 2021). Age-related shifts in protein synthesis/degradation and reductions in force production may stem from free radical accumulation/oxidative stress and inflammation that activate proteolytic pathways, damage the muscle, and impair mitochondrial function (Guo et al., 2013; McDonagh et al., 2014; Fernando et al., 2019; Zizzo, 2021). Taken together, these musculoskeletal changes contribute to dyna-/sarco-penia and osteoporosis which have a profound impact on health and longevity with aging (Tagliaferri et al., 2015; Prawiradilaga et al., 2020).

Though exercise has long been known to stimulate bone mineralization and promote increased bone density, the initial response following any mechanical stimulus such as exercise is the resorption/breakdown of bone (Feng and McDonald, 2011). Similarly, exercise may acutely suppress muscle protein synthesis and increase protein degradation (Tipton and Wolfe, 1998; Kumar et al., 2009). Muscle force production also decreases following a bout of acute exercise (Howatson and van Someren, 2008) owing to, 1) inflammatory damage *via* increased mitochondrial reactive oxygen/nitrogen species within the working muscle (Powers and Jackson, 2008), and 2) structural damage (i.e., filament disintegration/misalignment, z-band streaming, excitation-coupling failure) incurred within the straining muscle (Fridén and Lieber, 1992). These effects of acute exercise ultimately contribute to initial reductions in voluntary force production following exercise (Howatson and van Someren, 2008).

IMPLICATIONS OF ADAPTATIONS TO EXERCISE AS AN "AGING STIMULUS"

As outlined above, there is substantial evidence that the acute physiological response to exercise mimics physiological responses that occur with aging and age-related chronic disease (**Figure 1**).

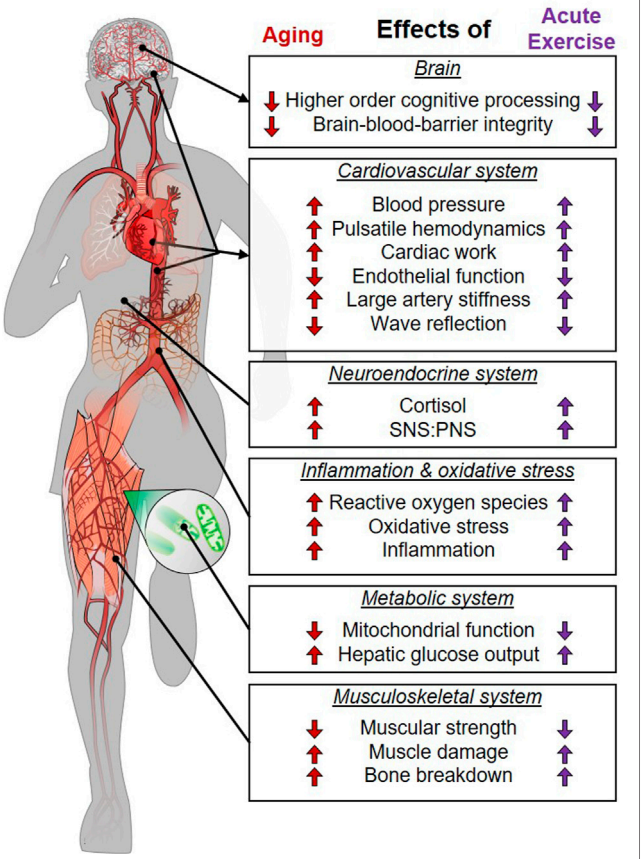

FIGURE 1 | Parallels between the stress of aging/chronic disease and acute physical exercise. SNS, sympathetic nervous system; PNS, parasympathetic nervous system.

As such, acute exercise could be conceptualized as a transient bout of "aging." The body naturally adapts to any stress (such as exercise) that disrupts homeostasis (**Figure 2A**). Proper adaptation to transient stimuli reduces stress during subsequent stressors (e.g., the next bout of exercise; see diminishing size of exercise-induced dysfunction in **Figures 2A,B**). For example, 1) exercise-induced increases in inflammation are attenuated following exercise training (Orlander et al., 1977; Fonseca et al., 2021), 2) increases in cardiac work are attenuated (e.g., lower heart rate) at a given workload following exercise training (Orlander et al., 1977), and 3) exercise training enhances antioxidant defense against exercise-induced oxidative stress (Bouzid et al., 2015). Parallels between the physiological stress of acute exercise and age-related chronic disease support the notion that repeated exposure to an exercise stimulus and the subsequent adaptations would protect against the physiological stress of aging and age-related chronic disease (**Figure 2B**).

If exercise is viewed as an aging mimetic, then more intense exercise should elicit a larger "aging" stressor and subsequent adaptation and protection against age-related chronic disease. Indeed, observational data suggest a dose-response

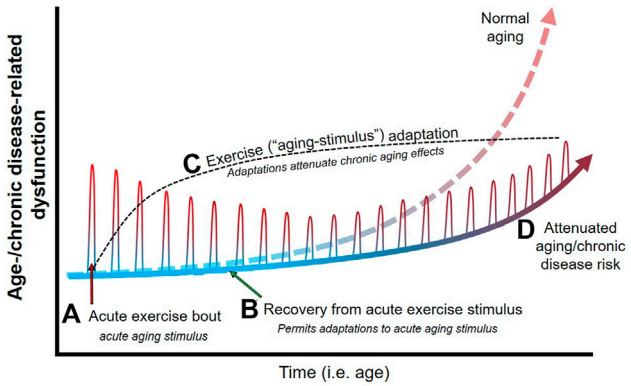

FIGURE 2 | Theoretical effects of physical exercise as an aging stimulus on age-/chronic disease-related physiological dysfunction. Age-related dysfunction (e.g., cardiovascular, metabolic, muscular) generally increases steeply around middle-age into older age, and results in an increase in chronic disease risk. An acute bout of exercise **(A)** acts as an aging stimulus and elicits responses during exercise that mimic that of age-related dysfunction (e.g., increased large artery stiffness, inflammation, etc.). Cessation of exercise (i.e., removal of the acute aging stimulus) and proper recovery between exercise bouts/stimuli **(B)** permits adaptations **(C)** that serve to reduce the physiological stress during successive exercise (i.e., aging) bouts. Since acute exercise elicits physiological responses that parallel aging, exercise adaptations essentially prepare the body to endure less physiological stress and dysfunction when exposed to the effects of aging over time. As such, regular exposure to transient aging stimuli (i.e., regular physical exercise) elicits physiological adaptations that attenuate age-/chronic disease-related dysfunction **(D)**, and thus attenuates many of the detrimental physiological effects of age and protects against chronic disease development.

relationship between exercise and physiological/health benefits, such that larger doses of exercise generally elicit greater protection (Ekelund et al., 2019; Shi et al., 2020; Aune et al., 2021). The protection afforded by exercise and the stress-adaptation cycle are maximized when the stress is transient and adequate recovery is allowed for adaptation (**Figure 2C**) (Booth and Laye, 2009). In the case of exercise, some data indicate extreme exercise volumes (e.g., marathons, ultramarathons) may be accompanied by pathological changes and a loss of health benefits, although this remains an area of debate (Eijsvogels et al., 2018; O'Keefe et al., 2020). Indeed, the line between physiological and pathological adaptations become blurred with high volumes of exercise being linked with risk of arrhythmias (Claessen et al., 2011; Andersen et al., 2013), cardiac dysfunction (O'Keefe et al., 2012; Rajanayagam and Alsabri, 2021), and myocardial injury (Neilan et al., 2006). Our hypothesis links to these observations since exposure to 1) extreme aging stimuli or 2) too frequent of exposure to an aging stimulus (preventing adequate recovery and adaptation) could contribute to negative (i.e., pathological) adaptations, accelerate physiological "aging," and attenuate health benefits (Eijsvogels et al., 2018; O'Keefe et al., 2020). As such, the notion that exercise mimics aging provides insight into how exercise can both protect against age-related chronic disease and potentially give way to pathological changes under extreme exercise volumes.

ALTERNATIVE PERSPECTIVES AND LIMITATIONS

We openly acknowledge that the actual cellular/molecular mechanisms driving acute and training responses to exercise may differ from those contributing to physiological changes with aging/age-related chronic disease (e.g., exercise and the cardiovascular demands required to meet metabolic output for musculoskeletal movement are fundamentally different mechanisms than those governing increases in blood pressure with aging such as degradation of elastin, microvascular rarefaction, endothelial dysfunction etc.). Many examples demonstrate the phenomenon of cross-tolerance, in which, despite diverse mechanisms, one stressor [e.g., exercise, environment (heat stress)] can confer protective benefits across other *different* stressors (Bond et al., 1999; Heled et al., 2012; Corbett et al., 2014; White et al., 2014; Wang et al., 2021). Consistent with this concept, our hypothesis is that the stimulus (e.g., an increase in blood pressure) for adaptation is similar between acute exercise and aging/age-related chronic disease and thus exercise adaptations may be mutually beneficial for both reducing the stress of subsequent exercise stimuli and aging/chronic disease pathways that involve that particular signal (e.g., blood pressure and cardio-/cerebro-vascular/cognitive disease).

Data indicate that lower intensity exercise/physical activity (e.g., walking) can confer mortality benefits in the absence of detectable physiological adaptations (Wasfy and Baggish, 2016). This raises the possibility that acute low intensity exercise 1) offers protection without adequately disrupting homeostasis and subsequent physiologic adaptations (contrary to our hypothesis), or 2) benefits age-related chronic disease burden through accumulation of diffuse, modest physiological adaptations that reflect a more modest exercise stimulus. Indeed, activities of daily living often viewed as "low" intensity (e.g., walking) are actually considered moderate intensity among older/deconditions populations (Sundquist et al., 2004; McPhee et al., 2016) and result in modest increases in energy expenditure (Maciejczyk et al., 2016), ventilation (Fusi et al., 2005), and cardiovascular stress (Renzi et al., 2010; Sugawara et al., 2015; Carter et al., 2018). Thus, even low-intensity exercise/physical activity may elicit similar directional physiological changes as "aging" and moderate-to-vigorous intensity exercise (as discussed above), albeit of smaller magnitude. This supports the idea that lower intensity activity patterns may need to be continued for longer periods of time to accumulate physiological benefits and reduce chronic disease risk (Carnethon, 2009). In the context of our hypothesis low-intensity exercise/physical activity likely elicits a smaller homeostatic disruption that represents a smaller "aging" stimulus, and thus more modest adaptations and benefits (in line with the dose-response literature). It is not surprising to see more sizeable benefits wrought from moderate and vigorous exercise intensities since these intensities can acutely elicit physiological responses comparable in magnitude to +10–50 years of aging (see **Table 1**) (Franklin et al., 1997; Hilbert et al., 2003; Ogoh et al., 2005; Ferrucci et al., 2012; Keith et al., 2013; Alwatban et al., 2020; Lefferts et al., 2020), and that exercise-trained older adults can be

TABLE 1 | Comparison of magnitude of acute exercise response with observed changes in the context of aging from select available literature.

Variable	Type of exercise	Acute exercise response	Aging	References
Cerebral pulsatility (MCA PI)	Moderate AE	+0.30au	+0.08/10 years from 45–85 years (totaling +0.30au across 40 years)[a]	Alwatban et al. (2020) Lefferts et al. (2020)
Pulse pressure	Mild AE	+10 mmHg	+22 mmHg[b] from 30–84 years	Ogoh et al. (2005)
	Moderate AE	+24 mmHg	+35 mmHg[c] from 30–84 years	Keith et al. (2013)
	Heavy AE	+37 mmHg		Franklin et al. (1997)
	Light AE	+21 mmHg		
Mean arterial pressure	Moderate AE	+7 mmHg	+7 mmHg[b] from 30–64 years	Ogoh et al. (2005)
	Heavy AE	+18 mmHg	+12 mmHg[c] from 30–64 years	Keith et al. (2013)
	Light AE	+14 mmHg		Franklin et al. (1997)
Aortic stiffness (cfPWV)	Light AE	+1.1–1.5 m/s	+1.1–2.0 m/s per +10 years from 40–70 years	Keith et al. (2013) Reference Values for Arterial Stiffness' Collaboration (2010)
Cortisol	Vigorous AE	+70–300% peakΔ	+20–50%	Kanaley et al. (2001) Van Cauter et al. (1996)
Inflammation (IL-6)	Vigorous AE	+0.20 pg/ml	+0.16 pg/ml per +10 years from 45–64 years	Tsao et al. (2021) Hager et al. (1994)
Strength	Peak torque[d]	−15–20%	−10–15% every 10 years from 45–84 years	Hilbert et al. (2003) Ferrucci et al. (2012)

MCA PI, middle cerebral artery pulsatility index; cfPWV, carotid-femoral pulse wave velocity; IL, interleukin.

[a]*secondary regression analysis calculated from Lefferts et al., 2020 data.*

[b]*for adults with systolic blood pressure between 120–139 mmHg.*

[c]*for adults with systolic blood pressure >160 mmHg.*

[d]*peak torque achieved following muscle damaging leg exercise. Data approximated from the following references (Hager et al., 1994; Van Cauter et al., 1996; Franklin et al., 1997; Kanaley et al., 2001; Hilbert et al., 2003; Ogoh et al., 2005; Reference Values for Arterial Stiffness' Collaboration, 2010 (Boutouyrie, corresponding author); Ferrucci et al., 2012; Keith et al., 2013; Alwatban et al., 2020; Lefferts et al., 2020; Tsao et al., 2021).*

phenotypically similar to adults 40 years younger (Bhella et al., 2014; Fragala et al., 2019).

In this paper we presented an amalgam of acute exercise literature, including aerobic and resistance exercise across a spectrum of exercise intensities. It is currently unclear whether one specific type of exercise is a better "aging-mimetic" and thus more protective against age-related disease. This gap in understanding reflects methodological limitations [challenges of assessing outcomes during discontinuous exercise (resistance/high-intensity exercise)], and greater attention paid to continuous aerobic over discontinuous aerobic/resistance exercise in the literature. We posit that the exact exercise type is less important than the response it elicits since 1) epidemiological evidence indicates both aerobic and resistance exercise are associated with reduced disease risk (Ross et al., 2016; Bennie et al., 2020) and 2) all forms of exercise disrupt homeostasis (e.g., running, resistance, and high-intensity interval exercise can induce inflammation, increase blood pressure, increase artery stiffness, load bones, damage muscles etc.), and thus may contribute to beneficial adaptations that attenuate physiological aging and reduce disease risk.

The acute effects of exercise are highly variable and may depend, in part, on age. Data indicate that the given response to acute exercise may be preserved (Hogan et al., 2013; Lavin et al., 2020a, 2020b; Luttrell et al., 2020; Rosenberg et al., 2020; MacNeil et al., 2021), exaggerated (Fleg et al., 1985; Fragala et al., 2019; Rosenberg et al., 2020), or blunted (Nordin et al., 2014; Jakovljevic, 2018; Fragala et al., 2019; Rosenberg et al., 2020) with aging, and that these conflicting responses could occur simultaneously depending on the physiological systems in question. This variable effects of age on acute exercise responses may alter the physiological stimulus that elicits adaptations to repeated exercise in older adults. It is possible that either 1) the attenuated physiological response to exercise (e.g., blunted stimulus), or 2) reduced plasticity/sensitivity (Slivka et al., 2008; Greig et al., 2011; Haran et al., 2012) to a similar or exaggerated exercise response could render exercise somewhat less potent or less beneficial among older adults. Indeed, it appears that greater exercise stimuli is required to elicit measurable physiological adaptations among older adults (Fujimoto et al., 2010). Despite reductions in plasticity and altered exercise responses among older adults, exercise training can elicit physiological adaptations in aged individuals (improved muscular, metabolic, cardiovascular function) (Fujimoto et al., 2010; Carrick-Ranson et al., 2014; Vigorito and Giallauria, 2014; Fragala et al., 2019; Green et al., 2021; Grevendonk et al., 2021) that may even be similar to benefits in young adults (Stratton et al., 1994) and ultimately increase cardiorespiratory fitness (Stratton et al., 1994; Woo et al., 2006; Fujimoto et al., 2010). Taken together, data are clear that despite potentially different acute responses and degree of adaptation to exercise, the cumulative effects of exercise are beneficial in older adults and contribute to reduced disease/mortality risk (Bijnen et al., 1998; Sundquist et al., 2004; Carrick-Ranson et al., 2014; Osawa et al., 2021). It should be underscored that the benefits of exercise are wrought over a lifetime of repeated exposure and thus engaging in regular exercise throughout life elicits greater physiological adaptations and health benefits than exercise initiated only

later in life (Russ and Kent-Braun, 2004; Fujimoto et al., 2010; Seals et al., 2019; Lavin et al., 2020a).

FUTURE DIRECTIONS, APPLICATIONS, AND CONCLUSION

Future work should seek to leverage technological advances and innovative methods to further explore acute cellular/physiological responses during exercise. The following recommendations are suggested to fill knowledge gaps surrounding the idea of exercise as an aging mimetic and the protective effects of exercise on age-related chronic disease risk: 1) examine mechanisms behind the beneficial effects of low-intensity exercise on age-related chronic disease, which remains under explored owing to more optimal signal-to-noise ratio observed with moderate-to-vigorous intensity exercise; 2) better identify and understand the phenotypic "signature" and physiological disruption caused by discontinuous exercise types (e.g., resistance, high-intensity interval) compared to continuous aerobic exercise; and 3) better understand the role of individual characteristics (age, sex, health status) in governing acute exercise responses and subsequent exercise-induced adaptation. Additionally, research often interrogates acute exercise to gain insight into training-induced adaptations under the guise that responses following acute exercise should be positive and contribute to beneficial long-term adaptations (Dawson et al., 2018; Voss et al., 2020). In reality, it is important to recognize that exercise is a potent disruption of homeostasis that mimics responses seen with aging and age-related chronic disease (i.e., exercise is disruptive and not necessarily immediately beneficial for physiological systems). It is this insult to homeostasis that primes adaptations to protect against chronic, age-related changes and reduce disease risk *over time* (**Figure 2D**). If research shifts to focus on the homeostatic disruption incurred *during* exercise, we may better understand the stimulus for adaptation and thus the mechanisms that govern adaptations to exercise and prevent age-related chronic disease.

Ultimately, we posit that regular exercise protects against aging and age-related chronic disease because each bout of exercise is, at its essence, an aging mimetic. The resilience and plasticity of the human body permit adaptations to these repeated exercise-induced "aging" stimuli and ultimately prepares the body's defenses against the stress of aging and age-related chronic disease.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

WL conceived, drafted, edited, and revised the paper/figures; and MD and RV drafted, edited, and revised the paper. All authors approved the final version of the paper.

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The effect of regular aerobic exercise on renal function in patients with CKD: A systematic review and meta-analysis

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Objective: To evaluate the effect of regular aerobic exercise on the improvement of renal function in patients with chronic kidney disease through meta-analysis and to provide targeted exercise recommendations for patients with CKD.

Methods: PubMed, Web of Science, EBSCO, China National Knowledge Infrastructure (CNKI), and other databases were searched, and randomized controlled trials on the effects of regular aerobic exercise on renal function-related indexes in patients with CKD were collected according to the inclusion and exclusion criteria. The methodological quality of the included literature was evaluated using the Cochrane evaluation tool second generation, and statistical analysis was performed using R analysis software.

Results: A total of 12 randomized controlled trials (RCTs) with a total of patients with CKD were included, and the results of the meta-analysis showed that regular aerobic exercise significantly improved the estimated glomerular filtration rate SMD = 0.65, 95% CI [0.30, 1.00], serum creatinine SMD = -0.63, 95% CI [-0.86, -0.40], 24-h urine protein volume in patients with CKD SMD = -0.41, 95% CI [-0.70, -0.11], and serum urea nitrogen SMD = -0.66, 95% CI [-1.20, -0.12]. Single exercise session longer than 30 min significantly improved the estimated glomerular filtration rate in CKD patients ($p < 0.01$), and walking and running as exercise modalities significantly improved CKD patients' SCr levels were significantly improved by walking and running as exercise modalities ($p < 0.05$), and the improvement effect was not significant when cycling was selected as an exercise modality.

Conclusion: Regular aerobic exercise has a significant effect on the estimated glomerular filtration rate, serum creatinine, 24-h urine protein amount, and blood urea nitrogen in CKD patients. Aerobic exercise with a single exercise duration longer than 30 min has a more significant effect on the estimated glomerular filtration rate, and aerobic exercise by walking or running can more effectively improve the serum creatinine in CKD patients.

KEYWORDS

aerobic exercise, chronic kidney disease, kidney function, meta-analysis, systematic review

1 Introduction

Chronic kidney disease (CKD) refers to the structural and functional disorders of the kidney caused by various reasons, usually manifested as a decrease in the glomerular filtration rate (GFR) and a decrease in renal function. At the beginning of the disease, patients only have mild symptoms such as weakness, lumbago, and loss of appetite, while entering in the early stages of the disease, patients only have mild symptoms such as weakness, lumbago, loss of appetite, etc., but after entering CKD stage 3, kidney failure may be accompanied by hypertension, heart failure, hyperkalemia, and other adverse symptoms and may even be life-threatening. Current epidemiological studies show that the incidence of CKD is increasing year by year worldwide, and the mortality rate of the affected population reaches 40 times that of the normal population (Collins et al., 2003), making CKD one of the major diseases threatening public health.

Since the initial symptoms of CKD are not obvious, many patients often miss the critical time for early intervention and control of the disease process after diagnosis, and once they enter the end stage, that is, end-stage renal disease (ESRD), they can only be treated by hemodialysis and kidney transplantation, which is costly and has large side effects on the organism. Therefore, prevention and intervention before or during chronic disease are essential to reduce the prevalence and slow down the disease process, and physical activity is a common non-pharmacological intervention that plays an active role in improving cardiovascular health, increasing aerobic capacity and muscle strength, reducing the inflammatory response, and improving immunity (Leehey, Moinuddin, Bast, Qureshi, & Collins, 2009; Headley et al., 2010; Kosmadakis et al., 2010; Meuwese et al., 2011; Watson et al., 2018). Relevant meta-analysis has shown that the incidence of CKD is lower in people with higher physical activity levels (Kelly et al., 2021), among which aerobic exercise is the most commonly used exercise modality in middle-aged and elderly people, but there is a lack of comprehensive quantitative evaluation studies on the effect of aerobic exercise on renal function intervention in CKD patients. Therefore, this study intends to select randomized controlled trials of aerobic exercise on CKD patients at home and abroad and use meta-analysis to evaluate the studies. Therefore, this study is intended to evaluate the consistency of the results among the studies by using meta-analysis to quantitatively evaluate the effects of different aerobic exercise programs on the improvement of renal function in CKD patients of different ages and to provide a theoretical basis and targeted exercise recommendations for the selection of exercise intervention programs for CKD patients.

2 Methods

2.1 Search strategy

The English search terms used in this article were “kidney,” “kidney function,” “kidney physiology,” “renal function,” “aerobic training,” “aerobic exercise,” and “endurance training.”

The Chinese search terms used in this article were “kidney,” “renal function,” “aerobic exercise,” “aerobic training,” “endurance training,” and “exercise.”

A combination of subject terms and free terms were used to search each database in combination, and the Chinese databases used were China National Knowledge Infrastructure (CNKI) and Wanfang Data Knowledge Service Platform; English databases included PubMed, Web of Science, and EBSCO to collect all literature on the effect of aerobic exercise on the improvement of kidney function. The search time frame was from the date of database construction to September 2021.

2.2 Literature inclusion and exclusion criteria

2.2.1 Literature inclusion criteria

The inclusion criteria of the literature were based on the PICO guidelines for evidence-based medicine, and the criteria developed were as follows: 1) study type: all included studies were randomized controlled trials (RCT); 2) literature type: full-text literature of randomized controlled trials of aerobic exercise as an intervention to improve renal function in patients with CKD at home and abroad, in Chinese and English; 3) study subjects: patients with diagnosed CKD, age ≥18 years, age, gender, race, and nationality were not restricted; 4) outcome indicators, including glomerular filtration rate, serum creatinine, urine protein, and blood urea nitrogen.

2.2.2 Exclusion criteria of the literature

The exclusion criteria of the literature were as follows: 1) animal studies; 2) cross-sectional studies; 3) data or articles were incomplete and fruitless after requesting from the authors; 4) non-English and Chinese literature.

2.3 Literature screening

Step 1: Import the retrieved literature into the reference management software Endnote. Step 2: Eliminate duplicate literature. Step 3: Read the title and abstract for the first round of screening. Step 4: Download the full text for the

second round of screening and determine whether the inclusion criteria are met.

2.4 Data extraction

Two authors, Qirui Ma and Gao Ye, extracted the literature data separately, and the data were cross-checked and then included in the analysis process. If the extracted data were different, they were discussed with the third author, Xinhong Liu, and then included. When the literature was incomplete, the original authors were contacted to improve the relevant data.

The extracted data included first author, year of publication, study site, sample size, baseline characteristics of patients (age, male/female ratio, and physical condition), intervention modality (intervention content, intervention period, intervention frequency, single intervention duration, and intervention intensity), and outcome evaluation index.

2.5 Literature quality evaluation

The included studies were evaluated using the revised Cochrane risk-of-bias tool for randomized trials (RoB 2) in terms of risk of bias arising from the randomization process, risk of bias due to deviations from the intended interventions (effect of assignment to intervention), risk of bias due to missing outcome data, risk of bias in the measurement of the outcome, risk of bias in the selection of the reported result were assessed in five main parts. Finally, an overall evaluation of the article was performed.

2.6 Data analysis

The meta-analysis was performed according to the guidelines for systematic evaluation and meta-analysis (PRISMA). All study data were continuous variables and measured in the same units, so SMD and 95% confidence interval (CI) were used for statistics. Heterogeneity among studies was quantified using I2. When $I^2 >$ 40%, indicating a high and unacceptable risk of heterogeneity. A random-effects model was used for analysis, and sensitivity analysis or subgroup analysis was performed depending on the possible sources of heterogeneity.

3 Results

3.1 Literature search and screening results

The initial literature search yielded 5,361 articles, including 5,055 articles in English and 306 articles in Chinese. Using Endnote software to remove duplicate literature 944, read the title of the literature after the initial screening to obtain the literature 404, read the abstract after screening to obtain the literature 51, including the exclusion of nine articles cannot get the full text, the remaining 42 full-text reading to eliminate 31, and finally included 11 RCT, including eight English literature studies and three Chinese literature studies. The process of including the literature is shown in Figure 1.

3.2 Basic characteristics of the included studies and evaluation of methodological quality

Of the 12 included studies (Leehey et al., 2009; Straznicky, Lambert, Mcgrane, Dawood, & Schlaich, 2010; Toyama, Sugiyama, Oka, Sumida, & Ogawa, 2010; Aoike et al., 2014; Shi, Wen, Liu, & Yao, 2014; Craenenbroeck et al., 2015; Feng, Min, & Zun, 2016; Afifi et al., 2016; Fang, Zhigang, & Lei, 2017; Miele et al., 2017; Feng et al., 2018), a total of 410 subjects were included, and the basic information of the included studies is shown in Table 1.

Nine of the 11 included articles were at low risk for the randomized design of the experiments, indicating more reasonable randomization in the grouping process. One article did not mention randomized grouping, and two others were uncertain; 12 studies were free of bias in both full presentation and selective reporting of outcome indicators, as detailed in Figure 2.

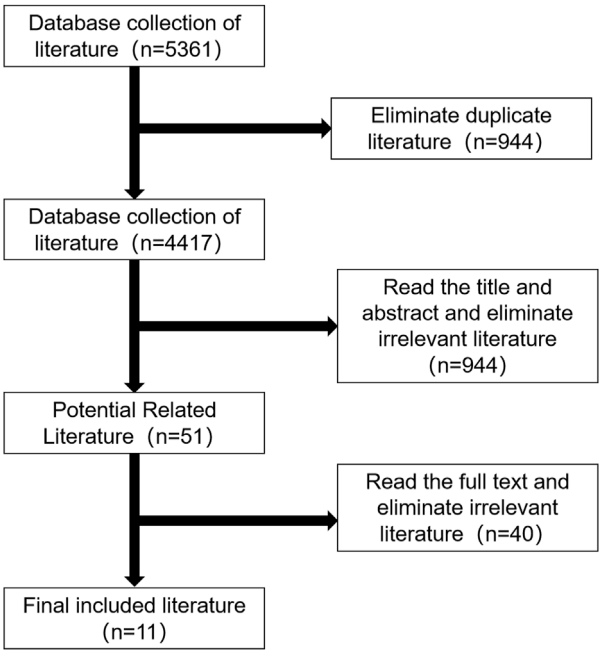

FIGURE 1
| Flow chart of literature screening.

TABLE 1 Basic information table of included literature.

Inclusion of studies and years	Age (years)	Gender	Sample characteristics	Stage of CKD	Sample size		Duration (weeks)	Type	Frequency (times/w)	Time	Intensity	Indicators[&]
					Control group	Exercise group						
Leehey et al. (2009)	Average age is 66	All male	CKD patients; obesity; diabetes mellitus; persistent proteinuria.	2-4	4	7	24	6 weeks (supervised walking on a treadmill), followed by 18 weeks of home exercise.	3	30–40 min	25–84% VO2 peak	1, 2, 3, and 4
Leehey et al. (2009)	Average age is 66	All male	CKD patients; obesity; diabetes mellitus; persistent proteinuria.	2-4	4	7	6	Walking	3	30–40 min	25–84% VO2 peak	1, 2, 3, and4
Straznicky et al. 2010	55±1	No mention	MetS* patients; nonsmoking; men and postmenopausal women	1-2	13	13	12	Cycling	Once in two days	40 min	65% HRmax	1, 2, and 3
Toyama et al. (2010)	71.7±11.0	Male: 89%	CKD patients with CVD**	3	9	10	12		Cycling (once a week) + walking (every day)	30 min	Borg RPE grade 12–13	1
Shi et al. (2014)	69.4±7.7	Male/ female = 14/7	CKD patients with CVD	3	10	11	12	Tai chi	3–5	30 min	—	1, 2, and 4
Liang et al. 2016	Average age is 48	Male/ female = 30/28	CKD patients	2-3	29	29	12	Cycling	3	30 min	50% VO2 peak	1, 2, 3, and 4
Liang et al. (2018)	Male: 49.2±6.3; Female: 47.5±5.6	Male/ female = 21/19	CKD patients	2-3	20	20	12	Cycling	3	30 min	50% VO2 peak	1, 2
Zhou et al. (2017)	Average age is 51	Male/ female = 37/33	CKD patients	2-3	35	35	12	Cycling	3	30 min	50% VO2 peak	2 and 3
Miele et al. (2017)	35–70	No mention	CKD patients	3	21	25	16	Cycling	3	55 min	50-60% VO2peak	1
Craenenbroeck et al. (2015)	Average age is 51	Male/ female = 22/18	CKD patients	3-4	21	19	12	Cycling	Every day	40 min	90% of heart rate at anaerobic threshold	1
Afifi et al. (2016)	45–55	Male/ female = 29/21	CKD patients	3-4	20	30	12	Walking	3	15–20 min	Borg RPE grade 14	1, 2, and 4
Aoike et al. (2014)	Average age is 55	Male/ female = 19/10	CKD patients	3-4	15	14	12	Walking	3	30 min	40–60% VO2max	1, 2, and 3

*MetS, metabolic syndrome; ** CVD: cardiovascular disease; [&]indicator: 1 - eGFR, 2 - SCr, 3 - 24UP, and 4 - BUN.

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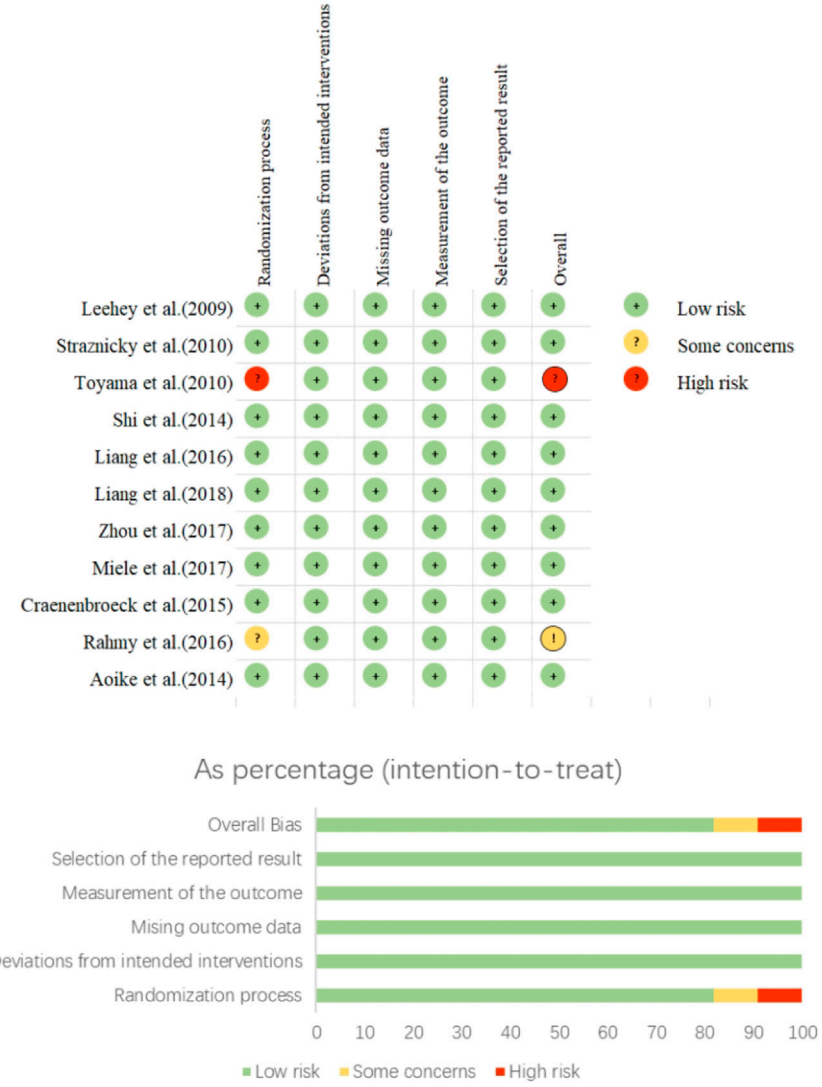

FIGURE 2
Risk bias diagram.

3.3 Publication bias test

Funnel plots initially scatter plots using the treatment effect estimates for each study as the *x*-axis and the sample size as the *y*-axis to observe publication bias in articles. Funnel plots are not suitable if the literature is small, and publication bias is usually performed when the number of studies in the meta-analysis is 10 or more. In this study, there were 11 studies reporting eGFR, which could be tested for publication bias. From Figure 3, we know that the distribution of funnel plots about eGFR on the *x*-axis is symmetrically distributed on the left and right, indicating that there is no significant publication bias.

4 Meta-analysis results

4.1 Effect of aerobic exercise on eGFR in patients with CKD

Eleven studies included the eGFR index with a total of 340 subjects (178 in the exercise group and 162 in the control group). Meta-analysis results showed high heterogeneity among the 11 studies ($I^2 = 57\%$, $p = 0.01$), using a random-effects model with a combined effect size of SMD = 0.65 and a 95% CI of [0.30, 1.00] ($p < 0.01$), indicating a significant difference between the two groups in terms of eGFR (Figure 4).

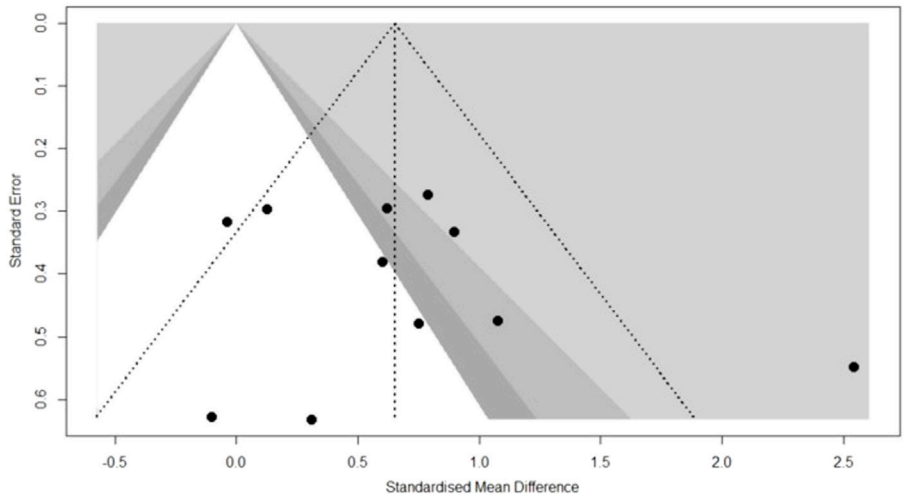

FIGURE 3
eGFR publication bias graph.

Study	Exercise Mean	Exercise SD	Exercise Total	Control Mean	Control SD	Control Total	Weight	Std. Mean Difference IV, Random, 95% CI
Leehey et al., 2009	39.00	22.00	7	41.00	5.30	4	5.5%	-0.10 [-1.33; 1.13]
Leehey et al., 2009	45.00	31.00	7	36.00	14.00	4	5.4%	0.31 [-0.93; 1.55]
Straznicky et al., 2010	109.80	1.00	13	106.90	1.20	13	6.6%	2.54 [1.47; 3.62]
Toyama et al., 2010	55.20	16.90	10	44.60	8.20	9	7.7%	0.75 [-0.19; 1.69]
Shi et al., 2014	51.00	6.50	11	43.00	7.80	10	7.8%	1.07 [0.15; 2.00]
Liang et al., 2016	69.65	11.23	29	60.68	11.22	29	12.2%	0.79 [0.25; 1.32]
Liang et al., 2018	66.80	6.40	20	61.00	6.30	20	10.7%	0.90 [0.24; 1.55]
Miele et al., 2017	52.40	19.10	25	50.10	16.20	21	11.7%	0.13 [-0.45; 0.71]
Craenenbroeck et al., 2015	38.60	14.20	19	39.20	15.20	21	11.1%	-0.04 [-0.66; 0.58]
Rahmy et al., 2016	31.47	10.62	30	24.57	11.39	20	11.7%	0.62 [0.04; 1.20]
Aoike et al., 2014	31.90	13.70	14	23.90	12.20	15	9.6%	0.60 [-0.15; 1.35]
Total (95% CI)			**185**			**166**	**100.0%**	**0.65 [0.30; 1.00]**

Heterogeneity: $Tau^2 = 0.1859$; $Chi^2 = 23.02$, df = 10 (P = 0.01); $I^2 = 57\%$
Test for overall effect: Z = 3.65 (P < 0.01)

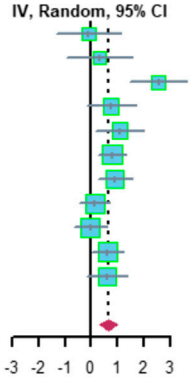

FIGURE 4
Meta-analysis of the effect of aerobic exercise on eGFR.

Subgroup analysis was performed according to the sources that may cause heterogeneity (Figure 5). All studies were grouped according to the intervention duration of each intervention, with a total of five studies included in the subgroup of >30 min per intervention and six studies included in the subgroup of ≤30 min. The results showed that exercise with an intervention duration >30 min significantly improved eGFR ($p < 0.01$), while exercise with an intervention duration ≤ 30 min indicated that there was no significant difference between the exercise group and the control group ($p = 0.96$).

Sensitivity analysis was performed using a literature-by-literature exclusion approach (Figure 6) to find sources of heterogeneity, and it was found that the exclusion of any one literature did not result in a significant decrease in heterogeneity, indicating that the results of this part of the meta-analysis were more robust.

4.2 Effect of aerobic exercise on SCr in patients with CKD

Nine studies included SCr indicators with a total of 316 subjects (166 in the exercise group and 150 in the control group). Meta-analysis showed low heterogeneity between the nine studies ($I^2 = 26\%$, $p = 0.21$), using a fixed-effects model with a combined effect size of SMD = -0.63 and a

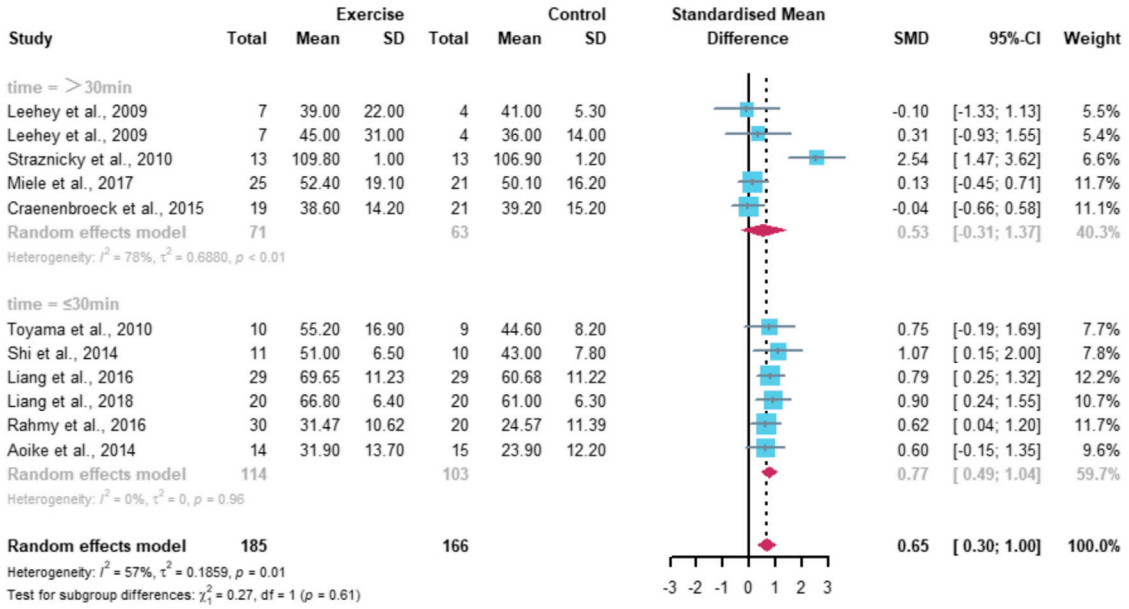

FIGURE 5
Subgroup analysis of the effect of aerobic exercise on eGFR.

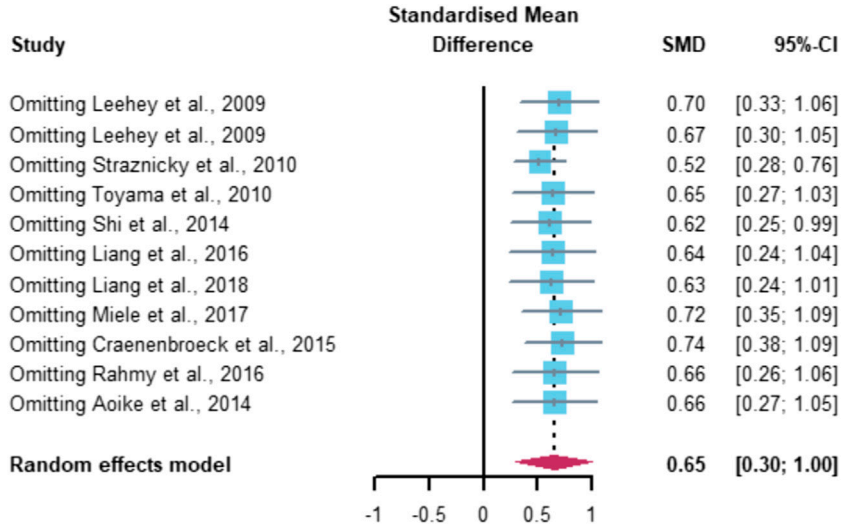

FIGURE 6
Sensitivity analysis of aerobic exercise affecting eGFR.

95% CI of [-0.86, -0.40] (p < 0.01), indicating a significant difference in SCr between the two groups (Figure 7).

A subgroup analysis of factors that may affect the experimental results (Figure 8) was performed, and the grouping was based on the exercise modality, with a total of four studies using cycling as the exercise modality and a total of five studies included in the subgroup of other exercise modalities. The results showed that there was no significant difference in SCr between the two groups at the intervention with cycling as the exercise modality (p = 0.95), while performing other modalities of exercise such as walking and running was able to significantly improve the SCr levels in CKD patients (p < 0.05).

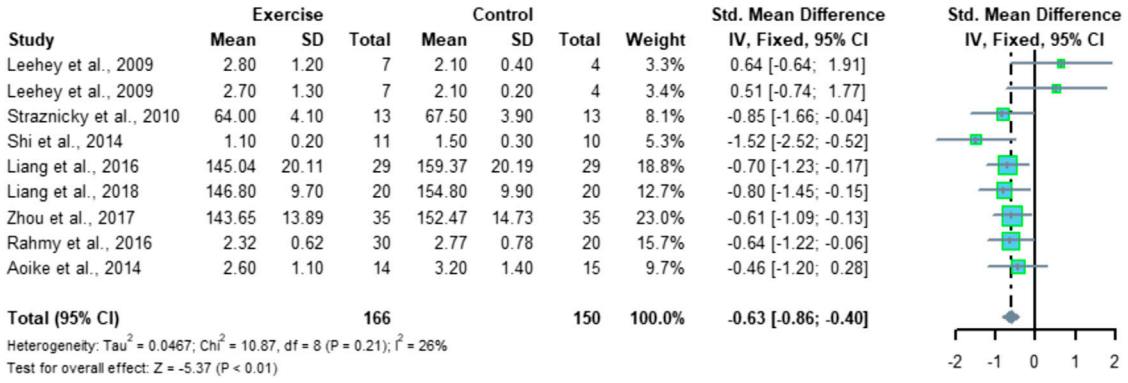

FIGURE 7
Meta-analysis of aerobic exercise affecting SCr.

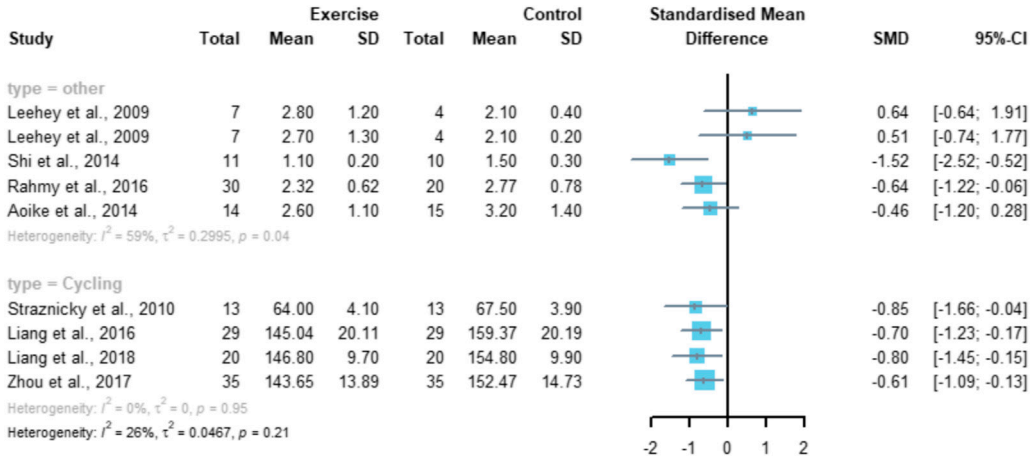

FIGURE 8
Subgroup analysis of the effect of aerobic exercise on SCr.

4.3 Effect of aerobic exercise on 24UP in patients with CKD

Six studies included 24UP indicators with a total of 191 subjects (96 in the exercise group and 95 in the control group). Meta-analysis showed low heterogeneity among the six studies (I^2 = 17%, p = 0.30), using a fixed-effects model with a combined effect size of SMD = -0.41 and a 95% CI of [-0.70, -0.11] ($p < 0.01$), suggesting that exercise significantly improved 24UP (Figure 9).

4.4 Effect of aerobic exercise on BUN in patients with CKD

Five studies included BUN indicators with a total of 151 subjects (84 in the exercise group and 67 in the control group), and the results of meta-analysis showed high heterogeneity among the five studies (I^2 = 51%, p = 0.08), using a random-effects model with a combined effect size of SMD = -0.66 and a 95% CI of [-1.20, -0.12] ($p < 0.05$), indicating that aerobic exercise significantly improved BUN levels in patients with CKD (Figure 10).

Sensitivity analysis was performed using a literature-by-literature exclusion (Figure 11) to find the source of heterogeneity, and it was found that the heterogeneity was significantly lower after excluding the study by Shi et al. (2014) (I2 = 27.4%, p = 0.0599) with a 95% CI of [-0.98 -0.07] (p = 0.0241), indicating that the heterogeneity originated from the study by Shi et al. (2014).

5 Analysis and discussion

In recent years, the proportion of CKD patients in the elderly population has been increasing, and the global

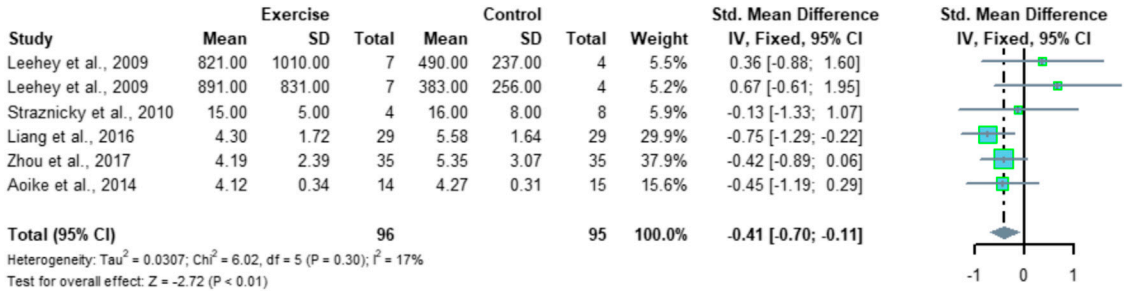

FIGURE 9
Meta-analysis of aerobic exercise affecting 24UP.

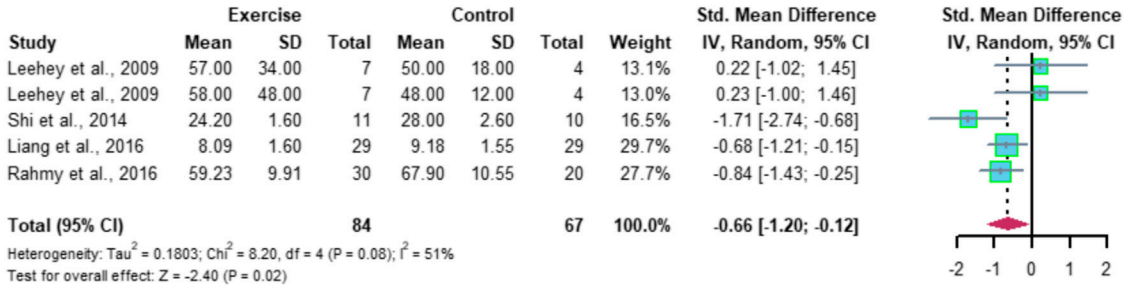

FIGURE 10
Meta-analysis of aerobic exercise affecting BUN.

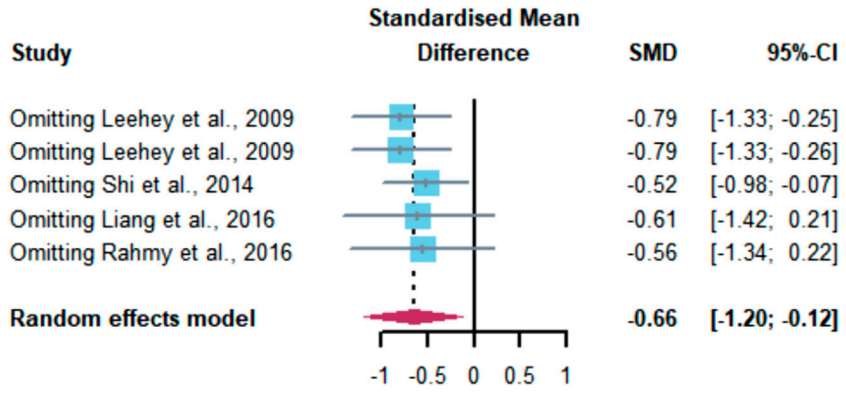

FIGURE 11
Meta-analysis of aerobic exercise affecting BUN.

incidence is also increasing year by year. The current interventions for CKD are mostly achieved through dialysis treatment or surgery, which are expensive and have large side effects on the body. The quality of life is greatly reduced. With the development of the concept of "exercise is medicine," the research direction of intervening in the process of chronic diseases through exercise has gradually come into public view (Barcellos, Santos, Umpierre, Bohlke, & Hallal, 2015). Meta-

analyses exploring the improvement of the health status of CKD patients through physical exercise have also gradually emerged, but most of the current studies on the effects of exercise intervention in CKD patients have focused on exploring the positive effects of physical exercise on the locomotor capacity and some basic physical functions of the body (Heiwe & Jacobson, 2014; Clarkson, Bennett, Fraser, & Warmington, 2019; Villanego et al., 2020), while systematic evaluation studies directly analyzing the effects of exercise on renal function in CKD patients are still lacking. Therefore, the present study will focus on the effects of exercise on renal health.

Aerobic exercise is a recognized form of exercise that can improve the health of the organism, and the effects on the kidney have received widespread attention. Aerobic exercise ultimately has an impact on kidney function, mainly through the improvement of related proteins and kidney structure (Wang & Liu, 2009). It has been shown that aerobic exercise improves renal oxidative stress levels and enhances the renal antioxidant capacity. The adaptive changes in renal tissues are most pronounced at moderate intensity (Liu et al., 1999); it also improves body nitrogen reserves and serum protein levels, reduces body lipids, and avoids damage to renal filtration function from lipid peroxidation (Chen et al., 2000). In addition, aerobic exercise may inhibit renal fibrosis and improve renal structure by modulating the TGF-β1/Smad signaling pathway, thus improving renal function (Peng et al., 2021). As a chronic disease that endangers public health, whether aerobic exercise can play a role in the prevention and control of the disease course is a research direction worthy of attention. A 10-year follow-up study by Pechter et al. (Pechter, Raag, & Ots-Rosenberg, 2014) showed that the incidence of CKD was significantly lower in people who performed long-term aerobic exercise than in sedentary people. However, it is uncertain whether it can play a role in improving the renal function of CKD patients and whether factors such as the amount of exercise performed, the duration of exercise, and the basic condition of the subjects at the time of exercise can have an impact on the intervention effect. This study aimed to verify the effect of aerobic exercise on renal function in CKD patients. Meta-analysis of the included literature revealed that aerobic exercise had a significant improvement in estimated glomerular filtration rate, serum creatinine, 24-h urine protein, and blood urea nitrogen in CKD patients compared with controls ($p < 0.05$). Aerobic exercise with a single exercise duration >30 min had a significant improvement in eGFR ($p < 0.01$). Cycling as a means of aerobic exercise did not have a significant effect on the improvement of SCr in CKD patients ($p > 0.05$), while performing other forms of exercise such as walking and running could significantly improve the SCr level in CKD patients ($p < 0.05$).

5.1 Effect of aerobic exercise on eGFR

The amount of filtrate produced by both kidneys per unit of time is called glomerular filtration rate (GFR). The normal range for adult males is 125 ± 15 ml/min, and the normal value for adult females is about 10% lower than that for males. eGFR is mostly used in practical applications to estimate the kidney rate situation by estimating the index of glomerular filtration rate (eGFR). eGFR, as a traditional biological marker (Ntrinias et al., 2019), which can visually reflect the level of renal function, can be used in CKD to measure the degree of renal function and loss of functional renal units.

Meta-analysis of this study showed that aerobic exercise was able to have a significant positive effect on eGFR in CKD patients, SMD = 0.65, 95% CI [0.30, 1.00] ($p < 0.01$). After further subgroup analysis, the results showed that aerobic exercise of longer than 30 min per exercise session was required to significantly improve eGFR ($p < 0.01$), and exercise less than or equal to 30 min had no significant effect on this index, which indicates that aerobic exercise of longer single duration has a better effect on the improvement of eGFR in CKD patients. The study by Vanden Wyngaert et al. (2018) also presented similar findings, which may be related to the choice of aerobic exercise intensity. The study subjects in the literature included in this study were all CKD patients. Considering the subjects' own conditions, the choice of the exercise intensities were all relatively small. Most of the exercise methods used were walking and cycling, which were less stimulating to the organism, so a longer exercise duration was required to achieve an effective exercise volume. From this, it can be inferred that aerobic exercise with a single duration of 30 min or more may play a positive intervention role in the disease process of CKD patients, but the selection and development of an effective specific exercise program still need further exploration.

5.2 Effect of aerobic exercise on blood renal function indexes

In this study, the blood indicators reflecting renal function were serum creatinine (SCr) and blood urea nitrogen (BUN). SCr is one of the most common methods to detect renal function in clinical practice and is an important indicator of renal function. Although it has been suggested that the diagnostic and prognostic significance of SCr and BUN as biomarkers of kidney diseases in the whole disease process is not ideal and cannot be used as the gold standard (Waikar, Betensky, & Bonventre, 2009), no new biomarkers have received wide public recognition yet (Wasung, Chawla, & Madero, 2015). Therefore, these two blood indicators reflecting renal function are still of great research significance.

Meta-analysis conducted in this study for the SCr index showed that aerobic exercise significantly improved the SCr level in CKD patients, SMD = -0.63, 95% CI [-0.86, -0.40] ($p < 0.01$). After further subgroup analysis, the results showed that conducting an aerobic exercise with cycling as an exercise modality

did not significantly improve the SCr levels of CKD patients (p = 0.95), while performing other forms of exercise such as walking and running could significantly improve the SCr levels of CKD patients ($p < 0.05$), which indicates that performing whole-body aerobic exercise may have a better effect on improving the SCr levels of CKD patients and that CKD patients should also pay attention to the choice of exercise modality when performing exercise interventions, combining its effectiveness and their own conditions to carry out the exercise. This is not consistent with the findings of Zhang et al. (2019) that exercise therapy did not have a significant improvement on SCr, which may be due to the fact that there were too many types of exercise modalities included in the study to conclude the effectiveness of a certain form of exercise, and the results of this study can be integrated to infer that aerobic exercise may be one of the effective forms of exercise to improve SCr levels in CKD patients.

Meta-analysis conducted in this study for the BUN index showed that aerobic exercise significantly improved BUN levels in CKD patients, SMD = -0.66, 95% CI [-1.20, -0.12] ($p < 0.05$), which is similar to the results of animal experiments conducted by Duan et al. (2021), where aerobic exercise effectively reduced BUN levels in spontaneously hypertensive rats. In conclusion, aerobic exercise can play an effective role in improving all the indicators of renal function in the blood of CKD patients.

5.3 Effect of aerobic exercise on urine protein

The 24-h urinary protein (24UP) is measured by collecting all urine for 24 h to determine the amount of protein in it and then calculating the total amount of protein in 24 h. The amount of protein in normal urine is minimal, while the amount of urinary protein increases significantly when suffering from kidney disease or performing certain strenuous exercises. This index can be more. It is a marker of kidney injury and a predictor of the disease process in CKD (Ene-Iordache et al., 2016).

Meta-analysis of this study showed that aerobic exercise significantly reduced 24UP levels in CKD patients, SMD = -0.41, 95% CI [-0.70, -0.11] ($p < 0.01$), which is similar to the findings of Afshinnia et al. (Afshinnia, Wilt, Duval, Esmaeili, & Ibrahim, 2010). Proteinuria in the obese population was also significantly reduced after exercise intervention. Similarly, Yang et al.'s (2020) systematic review results suggest that exercise training in adult CKD patients does not aggravate proteinuria, but it is unclear whether there is a positive effect and that low to moderate intensity exercise may reduce proteinuria. The reason for this conclusion may be that CKD patients are usually older, and the included studies mostly selected lower intensity and milder forms of exercise for intervention. The body reacts more violently during high-intensity exercise. The results of this study suggest that aerobic exercise is an effective way to improve 24UP in CKD patients, and different forms of aerobic exercise such as running, swimming, and walking can be selected for an exercise intervention in practical applications.

6 Conclusion

Regular aerobic exercise had a significant improvement on the estimated glomerular filtration rate, serum creatinine, 24-h urine protein amount, and blood urea nitrogen in CKD patients ($p < 0.05$) and effectively alleviated the decline of renal function in CKD patients. Aerobic exercise with a single exercise duration of more than 30 min had a more significant improvement on the estimated glomerular filtration rate compared with cycling, walking, or running. Carrying out aerobic exercise can more effectively improve serum creatinine in CKD patients.

7 Limitations and their analysis

This study included literature on the length of the intervention cycle, which was mostly about 12 weeks, so it was not possible to compare the difference between the improvement effect of short-term and long-term exercise. Therefore, the conclusion of the study only explored the effect of regular aerobic exercise habits on the renal function of CKD patients. The effect of lifelong exercise habits on renal function still needs to be further investigated. Due to the lack of research in this field, this study is limited in exploring exercise modalities, and it cannot compare the specific differences between tai chi, swimming, square dance, and other exercise modalities preferred by the elderly population so as to give specific recommendations on exercise modalities. Due to the different designs of exercise programs in each study, it is difficult to unify the comparison of exercise intensity and exercise volume, and the comparison can only be made through exercise frequency and cycle length. Most of the study subjects included in this study in the literature were patients with stage 3–4 CKD. There is a lack of literature on the selection of patients with mild disease as study subjects in this field, so more researchers' attention and exploration are still needed in exploring the preventive aspects of aerobic exercise.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material; further inquiries can be directed to the corresponding author.

Author contributions

QM and YG designed the systematic review and supervised the entire program; QM and XL reviewed all the

studies and extracted the information from the eligible trials; JL and QM analyzed the data and prepared the figures and table; QM, YG, and YS wrote the manuscript; YG, JL, and HS revised the manuscript. All authors reviewed and approved the manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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