NUTRITIONAL AND PHYSICAL ACTIVITY STRATEGIES TO BOOST IMMUNITY, ANTIOXIDANT STATUS AND HEALTH

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PUBLISHED IN: Frontiers in Physiology, Frontiers in Cardiovascular Medicine and Frontiers in Psychiatry

frontiers Research Topics

ISSN 1664-8714
ISBN 978-2-88974-384-1
DOI 10.3389/978-2-88974-384-1

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NUTRITIONAL AND PHYSICAL ACTIVITY STRATEGIES TO BOOST IMMUNITY, ANTIOXIDANT STATUS AND HEALTH

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Citation: Korivi, M., Reddy, L. V., Mohammed, A., eds. (2022). Nutritional and Physical Activity Strategies to Boost Immunity, Antioxidant Status and Health. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-88974-384-1

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frontiers in Physiology

EDITORIAL
published: 18 March 2022
doi: 10.3389/fphys.2022.846261

Editorial: Nutritional and Physical Activity Strategies to Boost Immunity, Antioxidant Status and Health

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Keywords: dietary intake, inflammation, oxidative stress—related enzymes, exercise, physical health

Editorial on the Research Topic

Nutritional and Physical Activity Strategies to Boost Immunity, Antioxidant Status and Health

OPEN ACCESS

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Specialty section:
This article was submitted to Integrative Physiology, a section of the journal Frontiers in Physiology

Received: *30 December 2021*
Accepted: *21 February 2022*
Published: *18 March 2022*

Citation:
Korivi M, Reddy Lebaka V, Mohammed A and Ye W (2022) Editorial: Nutritional and Physical Activity Strategies to Boost Immunity, Antioxidant Status and Health. Front. Physiol. 13:846261. doi: 10.3389/fphys.2022.846261

SUMMARY

In this thematic collection, we intended to explore the role of nutritional supplements and physical activity on various forms of immunity, inflammatory response, redox signaling, and health. A study demonstrated that supplementation of Santé premium silver perch essence (SPSPE) delayed swimming fatigue and attenuated exhaustive swimming exercise-induced lipid peroxidation and myoglobin induction in rats (Chen C.-Y. et al.). The SPSPE is rich in proteins, collagen, trace elements, minerals, and branch chain amino acid (Chen C.-Y. et al.). Another study reported that "turtle oil," which is extracted from the fat of Chinese soft-shelled turtle (*Pelodiscus sinensis*), comprised a highest percentage of unsaturated fatty acids (UFAs), including ogema-3 poly UFAs (∼22%), and omega-9 mono-UFAs (∼30%) (Yang et al.). Feeding of this turtle oil to aging rats in combination with swimming exercise improved spatial memory, physical strength, antioxidant status (superoxide dismutase) and maintained stable blood pressure in aging rats (Yang et al.). The beneficial effect of combined intervention was further emphasized by Chen C.-N. et al. in middle-aged adults with obesity. In this randomized controlled trial, combination of high-protein diet and exercise intervention (12-week) resulted a significant decrease of fat mass and lipid profiles, and improvement of insulin sensitivity, glucose tolerance and inflammation, which indicates improved cardiometabolic health (Chen C.-N. et al.). Dietary intake of micronutrients (calcium, vitamin D, zinc, and selenium) also contributes to promote cardiovascular health (Narayanam et al.). However, due to rapid changes in lifestyle, people couldn't intake sufficient dietary nutrients that result in huge dependency on dietary supplements. Whilst, supplements can cause sudden rise in circulating micronutrients which may cause cardiovascular damage (Narayanam et al.). Therefore, dietary intake of sufficient nutrients with or without combination of physical activity could boost immunity for all ages of adults with or without metabolic disorders.

Conversely, insufficient intake of certain nutrients (iron, vitamins A, B12, D, E, folate, and copper) lead to low concentrations of hemoglobin, known as "nutritional anemia." A study on Malaysian men reported that body mass index (BMI) of individual is a potent anthropometric index to predict the anemia (Dutta et al.). Besides, malnutrition is closely associated with the

incidence of tuberculosis (TB), and treatment outcomes affected by protein calorie malnutrition in patients. Usually, pathogenesis of lung injury in TB patients is depends on persons' immune system and/or healthy bodyweight. A study claimed that BMI and total serum protein levels of TB drug addicts were normal, but hemoglobin and albumin levels were significantly lower compared with non-TB drug addicts (Jia et al.). These findings emphasize the importance of nutrition in disease treatment and prevention. An interesting animal study demonstrated that inflammatory lung injury induced by particulate matter 2.5 ($PM_{2.5}$) exposures was effectively attenuated by aerobic exercise training (Qin et al.). This was evidenced by alleviated airway obstruction, ultrastructural damage and inflammatory response in exercised rats against $PM_{2.5}$ exposure (Qin et al.). Furthermore, a meta-analysis concluded that exercise intervention together with calorie restriction improved inflammatory response in overweight and obese adults (Liu et al.). To be particular, the decreased C-reactive protein (CRP), interleukin-6 (IL-6), and tumor necrosis factor-α (TNF-α) levels with combination intervention was effective in overweight and obese adults who had active lifestyle (Liu et al.). Taken together, our Research Topic summarizes that the beneficial effects are greater with combination of nutritional supplements and exercise than either type of intervention alone, in improving the inflammatory response, immunity, and antioxidant status.

AUTHOR CONTRIBUTIONS

MK and VRL drafted, edited, and finalized the editorial. AM and WY organized the articles sequence and extracted essential information. All authors contributed to the article and approved the submitted version.

FUNDING

This study was partially supported by the Zhejiang Provincial Natural Science Foundation of China (LGF19H160021).

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.

frontiers in Physiology

ORIGINAL RESEARCH
published: 22 March 2021
doi: 10.3389/fphys.2021.651972

Anti-fatigue Effects of Santé Premium Silver Perch Essence on Exhaustive Swimming Exercise Performance in Rats

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Specialty section:
This article was submitted to Integrative Physiology, a section of the journal Frontiers in Physiology

Received: *11 January 2021*
Accepted: *26 February 2021*
Published: *22 March 2021*

Citation:
Chen C-Y, Yuen H-M, Lin C-C, Hsu C-C, Bernard JR, Chen L-N, Liao Y-H and Tsai S-C (2021) Anti-fatigue Effects of Santé Premium Silver Perch Essence on Exhaustive Swimming Exercise Performance in Rats. Front. Physiol. 12:651972. doi: 10.3389/fphys.2021.651972

Aim: Fish soup is a traditional Chinese food usually offered as a healthy supplement to elders, pregnant women and persons who just had surgery. Silver perch (Santé premium silver perch essence, SPSPE) extract contains various quality proteins, collagen, minerals, trace elements, and branch chain amino acids (BCAA) that could help individuals recover from exhaustion and control body weight. However, there are very limited studies exploring the effects of fish extracts on exercise performance and fatigue, and relevant physiological mechanisms. Therefore, the purpose of this study was to investigate the effects of chronic SPSPE administration on exhaustive exercise performance.

Method: Male Wistar rats weighing around 250 g were divided into 4 groups: Control, 1X SPSPE (6.2 ml/kg), 2X SPSPE (12.4 ml/kg) and 5X SPSPE (31.0 ml/kg). Rats were administrated SPSPE by oral gavage feeding every day for 33 days. Their body weight were measured every week. Before and after the exhaustive swimming test, the blood was collected for circulating lactate, glucose, ammonia, hormones, and myoglobin analysis. Rats were sacrificed after performing an exhaustive swimming exercise test. The liver tissues were collected for glycogen content and H&E staining.

Results: After the administration of 1X and 5X SPSPE, swimming fatigue was significantly delayed ($p = 0.024$). There was no difference in the hormone plasma level between the control and SPSPE groups. The induction of plasma corticosterone and TBARS by exhaustive swimming exercise could be decreased by SPSPE administration. The increased plasma myoglobin concentration from exhaustive swimming exercise was weakened by SPSPE supplementation. The higher glycogen sparing contained in liver tissue was observed in SPSPE-treated groups ($p < 0.05$).

Conclusion: SPSPE could efficiently delay swimming fatigue through sparing of liver glycogen and attenuation of plasma TBARS, myoglobin induction by exhaustive exercise. Our findings provide a scientific-based fundamental information and better understanding for developing a fish extract-based anti-fatigue supplement.

Keywords: exhaustive exercise, fish essence, myoglobin, liver glycogen, amino acids

INTRODUCTION

Exercise-induced fatigue is mainly associated with excessive exercise leading to fatigue and/or discomfort (Carroll et al., 2017). In highly intense exercise training or competitions, people receive energy regulated by the endocrinological and nervous systems, and the latter mainly controls muscle contraction. Carbohydrates are the main source of energy for sustaining endurance exercise (Gonzalez et al., 2016). The use of such energy is affected by exercise intensity, exercise duration, and nutrient status (Betts et al., 2008; Cermak and van Loon, 2013; Gonzalez et al., 2016). In humans, the main source of carbohydrates for muscles comes from the glycogen stored in muscle and liver during exhaustive exercise. The glycogen storage is closely related to fatigue induced by endurance exercise (Wei et al., 2019). The role of endocrine system plays during exercise metabolism is well-established, however, we are still learning about the exercise-induced changes to the endocrine system itself that are associated with exercise adaptation and fatigue recovery (Kraemer and Ratamess, 2005). For example, tissue damage caused by excessive exercise increases oxidative stress. When the body exhibits insufficient anti-oxidative capacity, it might not be able to counter the cytotoxic effect induced by the presence of excess free radicals (Droge, 2002). This situation begs the question of whether the endocrine system can be enhanced via the intake of specific nutrients to alleviate the fatigue caused by excessive exercise, thereby improving exercise performance.

In Eastern Asian countries, traditional foods such as broth made from pig bones, meat, or fish are used to accelerate the fatigue recovery of patients after surgery, in frail individuals, older adults, pregnant women, and breastfeeding mothers. Of these ingredients, fish flesh is composed of abundant unsaturated fatty acids, and its fat content is half that of pork. However, fish rots easily and generates substances that are harmful to the body. Therefore, an effective strategy that converts fish into easily preservable products such as fish protein hydrolysate and fish essence is necessary. Compared with whey protein hydrolysate, fish protein hydrolysate of equivalent weight exhibits higher total antioxidant capacity (Oliveira et al., 2020). Research on supplements created using fish extracts or essence has indicated that the ability of fish extracts to effectively enhance exercise performance and muscular strength is related to the presence of rich antioxidants (e.g., anserine and imidazole dipeptide carnosine), which reduce oxidative stress (Kikuchi et al., 2004).

Santé premium silver perch essence (SPSPE) is a supplement that is rich in protein and amino acids and is mainly constituted of branched-chained amino acids (BCAAs), a crucial nutrient for tissue synthesis, energy supply, and health maintenance (Li et al., 2009). Such amino acids also facilitate collagen repair and cell growth and affect exercise performance (Moore et al., 2005). Besides, a meta-analysis indicated that taking a BCAA-based supplement daily (>200 mg/kg of weight/day) for 10 days in a row can effectively alleviate mild and moderate muscular damage induced by exercise; this effect is particularly notable when the supplement is taken before high-intensity training (Fouré and Bendahan, 2017). Another recent study also reported that taking BCAA-based supplements 1 h before incremental treadmill exercise delays the occurrence of exhaustion (AbuMoh'd et al., 2020). However, few studies have explored the effects of fish extracts on exercise performance and fatigue, and relevant physiological mechanisms have yet to be fully clarified. Therefore, this study explores the effect of long-term perch essence supplementation on exercise performance and fatigue recovery in rats. The adequate supplement dose is also determined. In addition, we analyzed the physiological and biochemical mechanisms that potentially affect the benefits brought by perch essence.

MATERIALS AND METHODS

Materials

The perch extract (essence) used in this study was produced by Yilan Anyong Lohas Co., Ltd. (Yilan, Taiwan). The perch essence was stored in a refrigerator at 4°C to ensure the quality of storage. Herbiotek Co., Ltd. was commissioned to analyze the nutrient content of the perch essence. The analysis results are listed in **Table 1**. The essence was determined to contain 90 mg/mL protein and a total BCAA content of 7.14 mg/mL. The recommended daily perch essence intake for an adult weighing 60 kg is 60 mL (1 mL/kg of weight). According to the human equivalent dose proposed by the US Food and Drug Administration, the rat equivalent dose is ∼6.2 times that of humans. A conversion coefficient of 6.2 was therefore employed in this study to determine the perch essence dose for rats 6.2 mL/kg in weight (Wojcikowski and GobeShin, 2014).

Animal Care and Experimental Design

Six weeks-old Wistar rats (BioLASCO, Yilan, Taiwan) were given 2 weeks to adapt to the laboratory environment and then being assigned according to their weights into four *groups*, namely the control group ($n = 9$), low dose group (1X dose, or 6.2 mL/kg of weight; $n = 9$), medium-dose group (2X dose, or 12.4 mL/kg of weight; $n = 9$), and high dose group (5X dose, or 31.0 mL/kg of weight; $n = 9$). During this experiment, the rat drinking water was replaced daily. Rats were given access to food and water *ad libitum*. The room temperature was controlled between 21

TABLE 1 | Nutrition facts, hydrolyzed amino acid profiles and total branched-chain amino acids of fish essence.

Nutrition Facts	Content (/100 ml SPSPE)
Protein	9 g
Fat	0.033 g
Saturated fat	0.017 g
Trans fat	0 g
Cholesterol	0 mg
Carbohydrate	0 g
Sodium	98.33 mg
Total calories	36.1 Kcal
Hydrolyzed amino acid profiles	**mg/100 ml SPSPE**
Alanine	837.56
Phenylalanine	222.33
Cysteine	141.67
Aspartic acid	561.27
Glutamic acid	1,077.86
Glycine	1,539.95
Histidine	116.58
Leucine	294.97
Isoleucine	131.72
Lysine	339.63
Methionine	173.03
Proline	1,121.0
Arginine	612.22
Serine	243.06
Threonine	293.82
Valine	201.93
Tryptophan	11.09
Tyrosine	88.31
Total BCAA	**mg/100 ml SPSPE**
Valine, leucine and isoleucine	628.62

and 23°C and the humidity between 50 and 60%. The lights were switched on or off every 12 h to simulate a 12-h day-night cycle. The cage bedding was replaced twice a week, and the cages were cleaned weekly. All animal experiments performed in this study were approved by the Institutional Animal Care and Use Committee of the University of Taipei (UT108004).

As shown in **Figure 1**, after the rats were relocated to their respective cages (two rats per cage), they were given another week to become familiar with the cage environment and oral gavaging operation before perch essence supplementation was begun. Before all the experiments, the animals were familiarized with oral gavaging operation for at least 1 week to eliminate the possible stress interference for this procedure. The initial body weights of each group at the time of perch extract administration are shown as follow: the control group (control: 260.67 ± 1.76 g), low dose group (1X dose: 261.89 ± 2.66 g), medium-dose group (2X dose: 265.50 ± 3.92 g), and high dose group (5X dose: 270.44 ± 3.96 g). And all processes are operated by well-trained laboratory colleagues, and all animals were received the familiarization gavaging procedure for 7 days to eliminate the related stress response during acclimation period. After assigning animal treatment groups, the same gavaging operation was performed regardless of taking either placebo or treatment supplement to ensure the consistency of the experiment. The control group also received identical sham gavage as other animals with supplementation substance. We also followed the recommendation of ∼10 ml/kg for each gavaging supplement in rats. The total daily amount was delivered by separating to three times, and the individual providing doses were ranged between 2.1 ml/kg (1X) and 10.3 ml/kg (5X) for each gavaging operation. Although the amount was very close to the upper limit of the recommend gavaging amount, we still tried best to minimize the possible stress during our procedure. The experimental period spanned 33 days, during which the rats received a standard chow diet (5001, PMI Nutrition International, MI, USA).

In this study, the main purpose was to observe the anti-fatigue effects of fish extract to an exhaustive swimming exercise. After oral gavage feeding for 32 days, the rats were fasted for 12 h and underwent an exhaustive swimming exercise endurance test in which they were loaded with a weight equivalent to 3% of their body weight. On the day of the test, the rats began the test 60 min after receiving oral gavaging. Before and after the exhaustive swimming test, the venous blood samples were collected from the tail to measure the following circulating parameters: lactate, glucose, ammonia, thiobarbituric acid reactive substances (TBARS), corticosterone, testosterone, growth hormone, myoglobin, and blood urea nitrogen (BUN) concentrations. The rats were sacrificed immediately after the test to collect liver samples to analyze the glycogen content and perform histological staining.

Exhaustive Swimming Exercise Test

Herein we used the overnight fasting that has been commonly utilized in metabolic and exercise-related research to ensure that there are no interference factors (Wu et al., 2013). In this study, we thus carried out 12-h fasting to minimize the possible impacts of uncertain time food intake on metabolic changes and subsequent exercise performance, and the rats received their final corresponding doses of oral SPSPE gavaging at 60 min in prior to swimming test. One week before the formal test the rats swam for 10 min per day for 5 days in a row without any weight load to become accustomed to swimming. The pool was 53 cm in diameter and 60 cm in-depth, and the water temperature was set at 27 ± 1°C as the formal swimming exercise test. After 33 days of the perch essence intervention, the rats underwent the exhaustive swimming exercise endurance test, during which a load equivalent to 3% of their weight was tied to their tails. The water temperature was 27 ± 1°C. We recorded the time at which each rat began to swim and the time they could not sustain swimming and be completely exhausted. The following standards were used to determine whether a rat was completely exhausted: the rat could not (1) continue the swimming movement (2) or reach the water surface within 8 s after becoming submerged. After the test, tail venous blood samples were collected before the rats were sacrificed immediately.

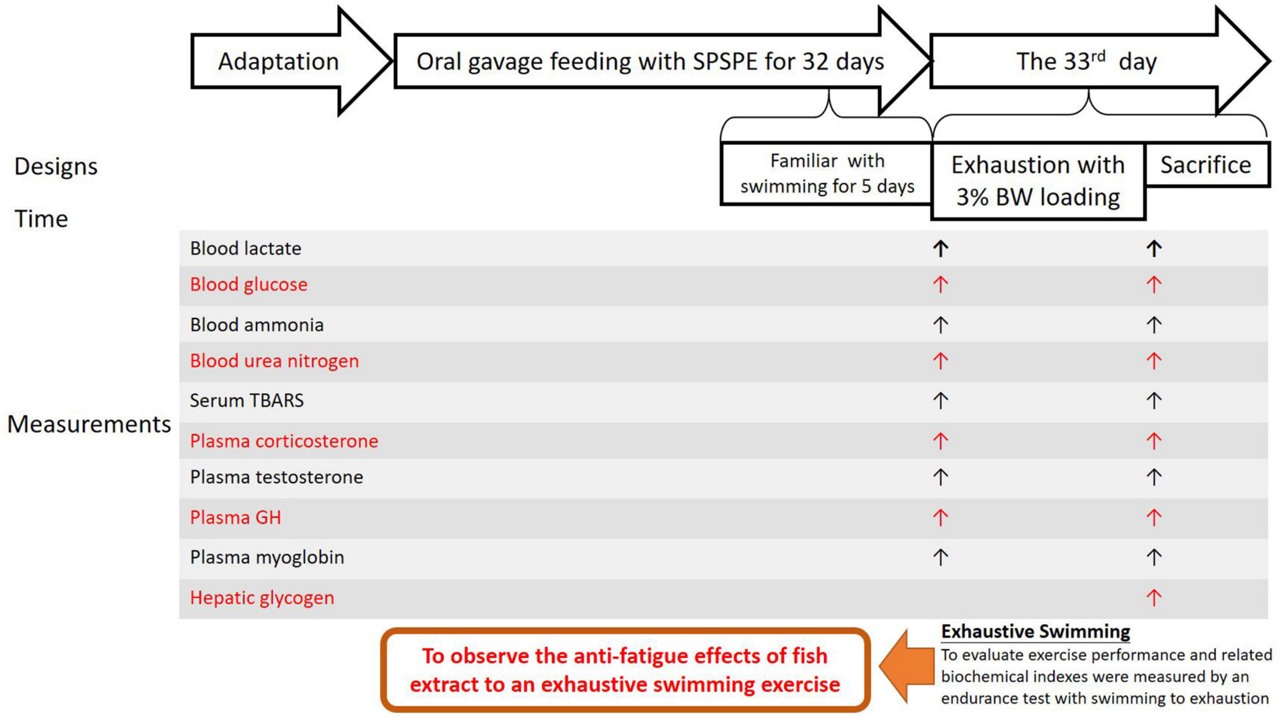

FIGURE 1 | Procedure and measurement of experimental design.

Biochemical Profiles and Plasma Hormone Content

The collected blood samples were tested using a blood glucose meter (GE100, Taiwan) and a blood lactate meter (The EDGE, Taiwan). Blood ammonia and blood urea nitrogen were determined using colorimetric analysis. The blood samples were also centrifuged at 4°C to acquire plasma samples. The enzyme-linked immunosorbent assay (ELISA) was used to determine the plasma corticosterone (Immuno-Biological Laboratories, Inc., NE, USA), testosterone (Cayman, MI, USA), growth hormone (Société de Pharmacologie et d'Immunologie-BIO, France), and myoglobin concentrations (Immunology Consultants Laboratory, Inc., OR, USA). Reaction agents were loaded into all samples according to the respective instructions from the suppliers, and an ELISA analyzer (TECAN Infinite R200 PRO, Switzerland) was used to determine the optical density (OD). The obtained values underwent standard regression plot standard curves, which were used to determine the sample concentrations through interpolation. The TBARS concentration was measured using a chemical kit (Cayman, MI, USA).

Tissue Glycogen Determination

The largest liver lobe and white gastrocnemius muscle from each rat was also preserved at −80°C to perform subsequent glycogen content analysis using a hydrolysis enzyme decomposition kit (Glycogen Colorimetric/Fluorometric Assay Kit; BioVision Incorporation, CA, USA). Specifically, 50 mg of a liver lobe sample and 50 mg of white gastrocnemius muscle sample were ground on an icy surface and mixed with 500 μL of cold water. The mixture was then boiled in a boiling water bath for 10 min to destroy the enzymes in the tissue sample. The mixture was then centrifuged to acquire the supernatant, which was mixed with reagents, reacted in the dark for 30 min, and measured at 570 nm. The concentration was determined using regression and interpolation.

Histological Tissue Staining

We collected liver tissue samples from the outer areas of the left liver lobe, fixed the samples in 10% formalin, and coated in paraffin wax. The coated samples were sliced into 4-μm-thick sections to perform hematoxylin and eosin staining. The sections were examined by veterinarians using optical microscopes with charge-coupled cameras (Olympus BX-51, Tokyo, Japan).

Statistical Analysis

All experimental data were used SPSS 22.0 software (SPSS, Chicago, IL, USA) to analyze and presented as mean ± standard errors mean. The Levene test was used to verify the hypothesis of homogeneity between groups, and the Kruskal-Wallis test was used to examine the mean between groups that did not conform to the normative distribution. Because the samples did not conform to the normative distribution, we used a more conservative statistical analysis for multiple tests (i.e., Kruksal-Walis with Bonferroni correction) to compare the means between groups. The significance level was set as $p < 0.05$.

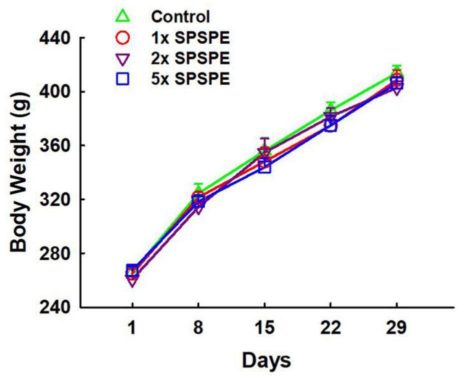

FIGURE 2 | The effects of SPSPE treatment on body weight. Each value represents Mean ± SEM, $n = 9$.

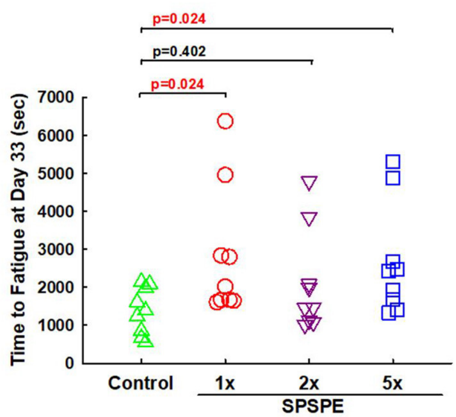

FIGURE 3 | Effect of SPSPE treatment on the results of swimming exhaustive test in rats. After treatment for 32 days, the rats were fasting for 12 h. The next day, the rats were administrated with different doses of SPSPE and stayed in their cages for 60 min, and then the rats performed a swimming exhaustive test.

RESULTS

The Body Weight

The changes in body weight in each group were measured weekly and shown in **Figure 2**. The body weight in each group increased from 250 to 400 g after treatments for 29 days. There was no significant difference among the control and SPSPE treated groups.

The SPSPE Supplementation Effects on Exercise Performance

On day 33, the rats received their final corresponding doses of oral SPSPE gavaging after an overnight fasting. After 60 min the rats began the swimming endurance test as an indicator of endurance performance. In the SPSPE treated groups the endurance performance was significantly higher than that of the control group (**Figure 3**). In the control group the longest swimming time was 2,149 s. The mean values were 1,394 s and the median value was 1,390 s. In the 1X SPSPE group the swimming time ranged from 1,610 to 6,381 s. The median value was 2,020 s which is significantly higher than that of the control group ($p = 0.024$). In the 2X SPSPE group the swimming time was distributed between 1,014 and 3,845 s. The median value was 1,467 s with no significant difference compared to the control group ($p = 0.402$). In the 5X SPSPE group the swimming time ranged from 1,331 to 5,314 s. The median value was 2,429 s which is significantly higher than the control group ($p = 0.024$).

SPSPE Effects on Circulating Biomakers to the Exhaustive Swimming Exercise Test

After the exhaustive swimming exercise test, the changes in serum TBARS after the exhaustive swimming test (as compared to the serum level of TBARS before the exhaustive swimming testing) are shown in **Figure 4**, although all other measured biomarkers (i.e., BUN, ammonia, blood glucose, and lactate) did not exhibit any statistical differences among treatments. The elevated serum TBARS decreased in the SPSPE treated groups, and a significant difference could be observed in the 2X SPSPE group ($p = 0.047$).

SPSPE Effects on the Exhaustive Swimming Exercise Test Induced Plasma Level of Hormones

Before and after the exhaustive swimming test, blood was collected and corticosterone, testosterone, and growth hormone were tested in serum or plasma by enzyme immunoassay. The changes in corticosterone concentration induced by exhaustive exercise were no significant difference among groups (**Figure 5A**). Likewise, the changes in testosterone concentration after swimming exercise also exhibited no significant differences among groups (**Figure 5B**). Additionally, the SPSPE treatment did not alter the changes in plasma growth hormone response after exhaustive swimming exercise (**Figure 5C**).

SPSPE Effects on Circulating Myoglobin Levels in Response to the Exhaustive Swimming Exercise Test

Exhaustive exercise could cause muscular damage and release myoglobin into the blood. The SPSPE treatment significantly attenuated the exhaustive exercise-induced changes in plasma myoglobin level (**Figure 6A**). Furthermore, we calculated the myoglobin releasing rate (pg/ml/second) and found that the myoglobin release rate in SPSPE supplemented groups were significantly lower than that in the control group (**Figure 6B**).

SPSPE Supplementation Effect on Liver and Muscle Tissue Glycogen Levels

After exhaustive exercise, the rats were sacrificed and the liver and white gastrocnemius muscle were extracted for the glycogen measurements. After oral gavaging SPSPE for 33 days, glycogen sparing in the liver was significantly increased compared with the control group (**Figure 7A**). However, the SPSPE supplementation did not exhibit any beneficial effects

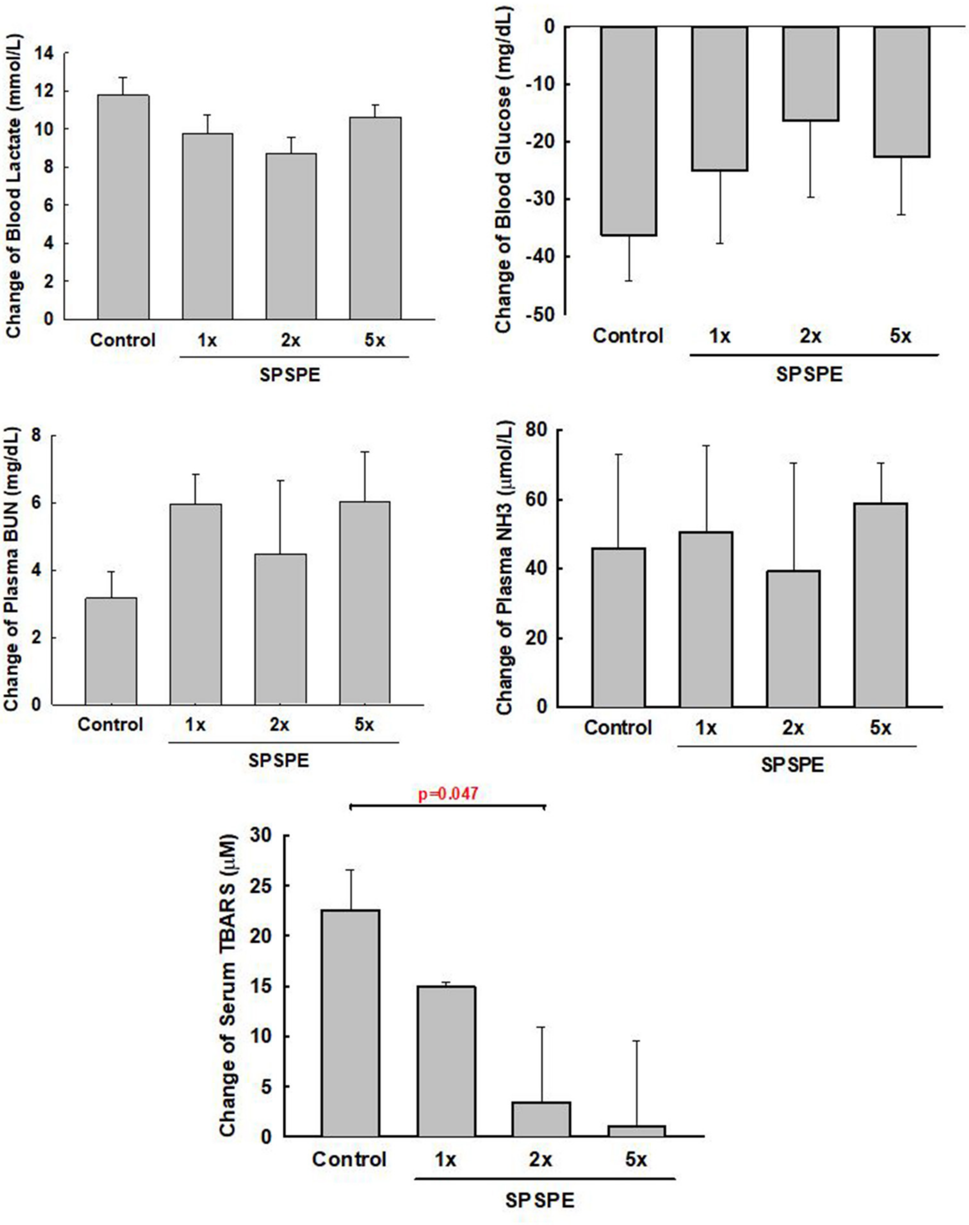

FIGURE 4 | The rats were administrated with different doses of SPSPE for 33 days. The change of circulating lactate, glucose, BUN, NH3, and TBARS after exhaustive swimming exercise test is shown each value represents Mean ± SEM, $n = 9$.

on sparing muscle glycogen after exhaustive swimming exercise (**Figure 7B**).

Correlation Analyses

For the correlation analyses, we pooled the data from all treatment groups to further analyze the correlations among certain anti-fatigue related parameters. The results showed that only the increase of exhaustive time was negatively correlated with the releasing rate of myoglobin per unit of time in response to the exhaustive swimming exercise ($r = -0.317$, $p = 0.023$). The correlation between exhaustive time and change of lactate concentration was approaching significant ($r = -0.276$, $p = 0.052$). There were no significant correlation between swim performance and TBARS ($r = -0.094$, $p = 0.283$), growth hormone ($r = -0.129$, $p = 0.214$), hepatic glycogen content ($r = 0.043$, $p = 0.398$).

Assessments of Histopathology

H&E stain was performed after the liver tissue was sliced. By observing the liver plates near the central vein (eyepiece 10 times,

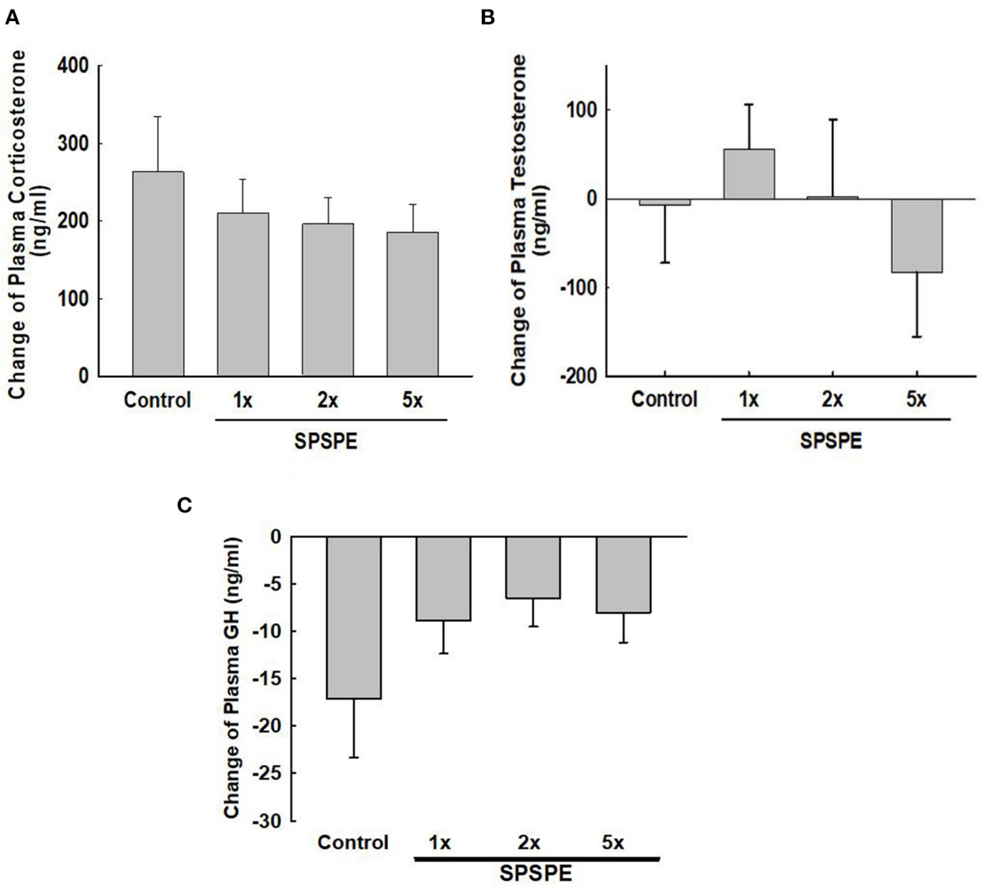

FIGURE 5 | The effects of SPSPE treatment on the plasma level of corticosterone **(A)**, testosterone **(B)**, and growth hormone **(C)** in rats after exhaustive swimming exercise test, each value represents Mean ± SEM, $n = 9$.

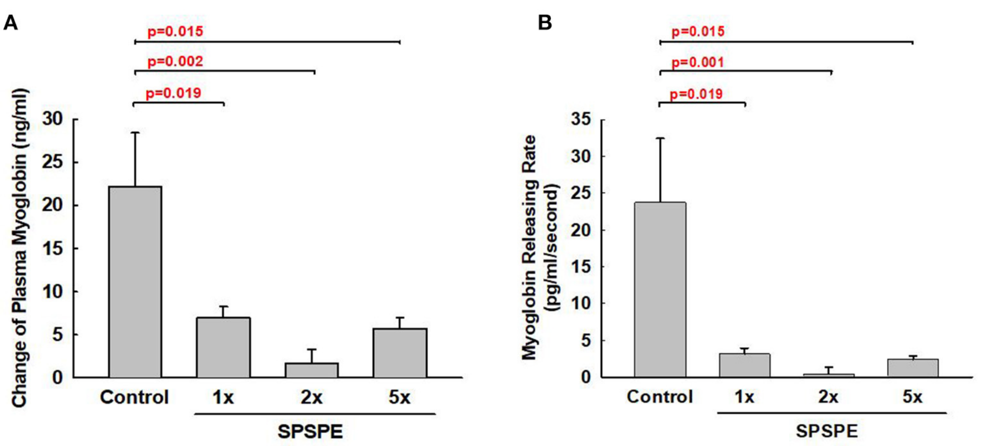

FIGURE 6 | The effects of SPSPE treatment for 33 days on the plasma level of myoglobin **(A)** and myoglobin releasing rate **(B)** after an exhaustive swimming exercise test, each value represents Mean ± SEM, $n = 9$.

objective lens 20 times), we found that the liver plates arranged in a radial pattern in the control and SPSPE treated groups. No pathological changes were shown after SPSPE administration (**Figure 8**).

DISCUSSION

The results revealed that after the rats were fed perch essence continually for 33 days, their swimming endurance performance

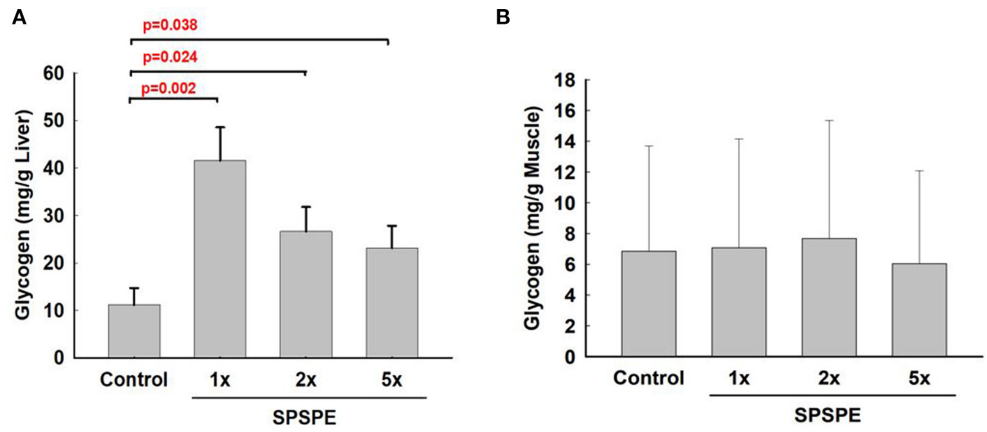

FIGURE 7 | The influence on glycogen content in liver tissue **(A)** and white gastrocnemius muscle **(B)** after treatment of SPSPE for 33 days, each value represents Mean ± SEM, $n = 9$.

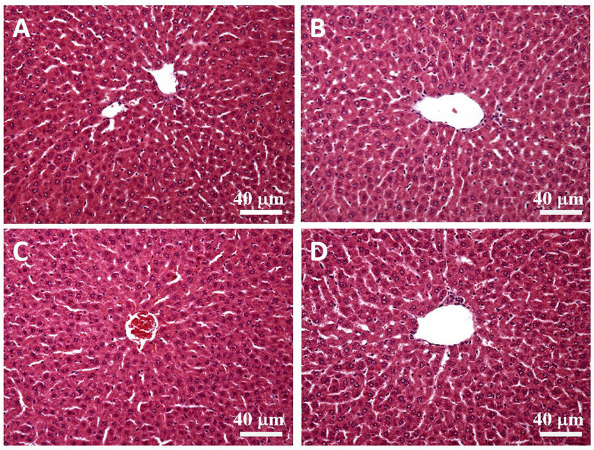

FIGURE 8 | The liver morphology in control **(A)** and SPSPE treated groups (**B**: 1X, **C**: 2X, **D**: 5X). The H & E stain was performed and the slices were observed under 200x magnification.

was significantly improved. The rats effectively maintained glycogen content following the swimming exercise. Plasma TBARS formation decreased with increasing perch essence intake, which also significantly reduced myoglobin formation per unit time of exhaustive exercise. The aforementioned results indicate that the perch essence effectively enhances exercise performance through several possible mechanisms, such as liver glycogen sparing, muscle damage reduction, and oxidative stress acceleration.

Carbohydrates and fats are the main sources of energy for humans when they perform endurance exercise (Gonzalez et al., 2016), and the similar responses are also well-recognized in rat (Baldwin et al., 1975; Fitts et al., 1975; Clark and Conlee, 1979; Dohm et al., 1983). The utilization ratio of these two substances depends on exercise intensity, exercise duration, and nutrient status (Betts et al., 2008; Cermak and van Loon, 2013; Gonzalez et al., 2016). The body usually stores carbohydrates as glycogen in the liver and muscles. Research has indicated that during moderate- and high-intensity endurance exercise that spans an extensive period, glycogen in the liver and muscles is the main source of energy. Therefore, glycogen storage in the body and fatigue during endurance exercise are closely related (Wei et al., 2019). Compared with the control group, the rats receiving 1X, 2X, and 5X doses of the perch essence exhibited significantly higher glycogen concentrations after the exhaustive swimming exercise. This hints at the possibility that perch essence facilitates effective energy utilization (i.e., comes from lipids and proteins) during exercise and induces a glycogen-sparing effect, which delays the occurrence of fatigue. On the other hand, we did not determine the initial glycogen levels for 1X, 2X, and 5X groups, thus we still cannot rule out the possibility whether the chronic administration would alter the initial hepatic glycogen levels.

During intense exercise or long-term training, biochemical variables might change considerably. Acute endurance exercise (e.g., intense swimming exercise) might substantially increase blood indicators, such as lactate dehydrogenase, aspartate aminotransferase, creatine kinase, myoglobin, and bilirubin (Wu et al., 2013). These indicators are associated with postexercise heart and muscle damage and often increase continually after exercise (Nie et al., 2011). Different experimental models have been adopted to identify multiple nutrient supplements that can effectively reduce these indicators. However, few studies have explored whether perch essence can exert long-term effects on these indicators when performing exercise challenges. Healthy participants were recruited to receive eccentric contraction-induced muscle damage. Their muscle strength deterioration and lactate dehydrogenase levels were reduced after consuming whey protein (Cooke et al., 2010). In our study, the exhaustive swimming exercise significantly increased the myoglobin level, a blood indicator associated with muscle damage (**Figure 6A**). This finding is consistent with those of relevant studies (Sabriá et al., 1983). Our study provides further evidence that long-term intake of perch essence can effectively reduce myoglobin formation per unit time of exhaustive exercise. The drastic increase in plasma myoglobin content might indicate oxidative damage, physical cellular damage, or other disease symptoms, which could cause myoglobin leakage from damaged muscle cells (Kiessling et al., 1981; Sabriá et al., 1983; Driessen-Kletter et al., 1990). Another study verified that long-term soy protein intake is conducive to reducing muscle damage caused by exhaustive exercise (reducing serum creatinine phosphate kinase concentration) and enhancing exercise capacity recovery (Wei et al., 2019). Similarly, we also observed that perch essence significantly reduced myoglobin level related to muscle damage. This indicates that perch essence can protect skeletal muscles from damage induced by intense exercise. This beneficial effect might also contribute to the improved swimming performance caused by perch essence.

Tissue damage caused by exhaustive exercise might lead to increased oxidative stress. When the endogenous anti-oxidative system of the body cannot react adequately, it might not be able to offset the cytotoxic effect caused by excess free radicals (Droge, 2002). The oxygen consumption of muscle tissues during exercise increases the formation of free radicals. The reduction of free radical removal ability might induce oxidative damage in cellular biomolecules, including those in cell membranes, cytoplasm, and DNA (Aoi et al., 2004; Bessa et al., 2016; Jamurtas, 2018). TBARS is metabolic byproducts of phospholipid peroxidation and is often used as a biological indicator to assess oxidative damage in organisms (Yada et al., 2020). Besides, several studies have observed significant increases in the plasma lipid peroxidation level after exhaustive aerobic exercise (Waring et al., 2003; Watson et al., 2005). In this study, the adopted exhaustive swimming exercise significantly increased oxidative stress in rats. Compared with other groups, the control group exhibited a greater increase in the plasma lipid peroxidation level after exercise (**Figure 4**). A 2-fold increase in the perch essence dose significantly reduced this increase in TBARS after the exhaustive swimming exercise ($p = 0.047$, **Figure 4**). Other studies verified that excess free radical or reactive oxygen species produced from intense exercise exacerbate muscle damage and fatigue affect skeletal muscle contraction (Alessio et al., 2000; Mastaloudis et al., 2001; Powers et al., 2004). Together with previous studies (Alessio et al., 2000; Mastaloudis et al., 2001; Powers et al., 2004) and our present findings, long-term perch essence intake is recommended before performing an intense high-volume exercise to reduce lipid peroxidation induced by the exercise.

Highly intense or long exercise periods affect endocrine system regulation. A study concluded that the changes in the endocrine system and adaptation responses following exercise are related to fatigue recovery (Kraemer and Ratamess, 2005). Previous studies have indicated that increasing the secretion of anabolic hormones following intense exercise can accelerate recovery from fatigue (Kraemer and Ratamess, 2005; Peake et al., 2005). Growth hormone and testosterone are anabolic hormones secreted by the human body and are conducive to muscle strength development, muscle growth, protein synthesis, and damaged tissue repair. Studies have discovered that exercise is a crucial factor in increasing the secretion of growth hormone and testosterone. After exercise, the secretion of these two hormones is increased, which in turn regulates the synthesis of muscle proteins, accelerates the recovery of muscle strength, and facilitates tissue repair (Ferrando et al., 2002; Saugy et al., 2006). In this study, we observed that the secretion of testosterone (**Figure 5B**) and growth hormone (**Figure 5C**) was not affected by perch essence intake, indicating that the perch essence-induced endurance enhancement did not mediated through further increasing exercise-induced anabolic hormones.

Herein we observed that the glycogen sparing effects of SPSPE supplementation were only existing in the liver tissue instead of skeletal muscle after exhaustive swimming exercise (**Figures 7A,B**). The reason we measured the white muscle fiber was considering that the white fibers are recruited when strenuous work is performed or when red fibers become fatigued, indicating that the glycogen levels in white fiber would better represent the energy substrate utilization and sparing at the end of exhaustive exercise (Baldwin et al., 1975; Fitts et al., 1975; Clark and Conlee, 1979; Dohm et al., 1983). According to the present findings, we speculate that the SPSPE spares liver glycogen content, thereby possibly better sustaining glucose availability during prolonged exercise. Subsequently, animals with SPSPE treatment had better sparing of liver glycogen which may indicate that fat oxidation is mainly during exercise rather than anaerobic metabolism of carbohydrates. But another possibility may also be due to the supplement of SPSPE before the exhaustive exercise. Since branched-chain amino acids are the main components in the SPSPE, it may help reduce the central fatigue state of experimental animals (AbuMoh'd et al., 2020). Previous reports have shown that there is no decline in the submaximal or maximal cycling performance after caloric restriction for 3 weeks combined with overnight fasting (Ferguson et al., 2009), suggesting that overnight fasting before our swimming test should not affect outcome measurements of this study. Furthermore, our study is mainly to explore the impact of long-term administration of fish extract on exercise performance and anti-fatigue ability. Therefore, we believed that the single overnight fasting should be required to eliminate possible confounding factors without affecting chronic treatment effects. Also, our findings that the changes in performance and related biomarkers should be mainly reflected by our treatment supplement and dosage.

When using specially processed food (e.g., nutrient supplements) as a health food, food safety is the most crucial consideration. Currently, no study has explored the safety of perch essence, which is primarily composed of protein-based amino acids. Nutrient supplements with protein or amino acids have been prevalently taken by athletes to improve their adaptation to exercise intensity. Moreover, studies have reported that no undesirable effect was observed when consuming as much as 3 $g \cdot kg^{-1} \cdot d^{-1}$ of protein (Dyer et al., 2008), and that food additive made of 2 $g \cdot kg^{-1}$ of protein hydrolysates do not increase the fatality rate (Anadón et al., 2013). In the present study, the 1X-5X doses given to the rats were equivalent to 0.56–2.70 $g \cdot kg^{-1} \cdot d^{-1}$ of protein, which did not exceed the safe dose range determined in previous research. Moreover, the liver tissue samples of rats in the 1X, 2X, and 5X dose groups did not exhibit any undesirable reactions (**Figure 8**). Therefore, the biochemical variables measured in this study can accurately reflect the physiological effect and fatigue alleviation caused by the perch essence.

Fish extract contains essential amino acids (EAAs) and 17–20% protein (Kietzmann et al., 1969; Ludorff and Meyer, 1973; Mol, 2005), and our fish extract analyses also exhibited a rich content of EAAs, especially a high percentage of BCAAs (714 mg/100 mL). In addition, fish muscle is also easily digestible due to its inherent connective tissue content (∼2%) (Kietzmann et al., 1969; Ludorff and Meyer, 1973). Moreover, some fish tissues contain fat-soluble vitamins and numerous fish products are rich in water-soluble vitamins (Ludorff and Meyer, 1973). Since previous studies have previously shown that protein or amino acid supplementation can significantly reduce exercise-induced fatigue, suppress muscle soreness, and promote recovery from high-intensity exercise (Cooke et al., 2010; Fouré and Bendahan, 2017; Wei et al., 2019; AbuMoh'd et al., 2020); furthermore, certain fat- and water-soluble vitamins have been reported to have antioxidant effects and be capable of reducing the degree of exercise-induced muscle damage (Evans, 2000; Thompson et al., 2001; Taghiyar et al., 2013). Although our nutrient analyses did not perform vitamin analyses, we speculated that the SPSPE supplementation reduces exercise-induced fatigue and muscle protective effects possibly mediated through the above possible benefits of fish-containing nutrients. Future investigations are warranted to analyze the vitamin contents to better understand the possible potential benefits of fish extract.

There are still several limitations in this study. Here a total of nine animals in each group were included to test the dose-response of SPSPE on the anti-fatigue effects during exercise. Although many animal studies investigating nutrients supplementation also used comparable number of animals as our current investigation, it is warranted to conduct experiments with a larger sample size in the future. Moreover, according to the findings in our measured biomarkers, we were not able to provide the explanation for our observations that only 1X and 5X doses were efficient at improving time to exhaustion but not 2X dose. Still, we could not exclude the possible benefits of this BCAA-enriched SPSPE on reducing the central fatigue state (AbuMoh'd et al., 2020), thus the role of central fatigue in this observation is needed to investigate for better understanding about the possible underlying mechanisms. Furthermore, it has to be noted that we here used a more conservative statistical analysis to compare the mean among groups when performing multiple tests (i.e., Kruksal-Walis with a Bonferroni correction). Therefore, there might be some existing possible benefits of SPSPE administration that could be masked using such conservative statistical analysis. In the future, more empirical studies are warranted to translate the applications in general or athletic human populations.

CONCLUSION

In summary, we examined the biochemical indicators related to fatigue and recovery in rats performing an exhaustive swimming exercise. Continual intake of perch essence for 33 days significantly extended their swimming duration. Moreover, perch essence supplementation effectively maintained their glycogen level after exhaustive exercise. According to our present results, the effective dose is 6.2–12.4 ml per kilogram of rat body weight/day, which is equivalent to 1–2 ml per kilogram of human body weight/day. Meanwhile, perch essence markedly reduced the formation of TBARS and myoglobin during exercise. Also, we observed that the increase in exhaustive time was negatively associated with the appearance rate of circulating myoglobin during the exhaustive swimming exercise. Furthermore, we performed pathological observation of the liver, and provided evidence regarding the safety of perch essence, verifying its feasibility as an alternative nutrient supplement for reducing exercise fatigue.

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 animal study was reviewed and approved by Institutional Animal Care and Use Committee of the University of Taipei.

AUTHOR CONTRIBUTIONS

C-YC, H-MY, Y-HL, and S-CT: conceptualization and writing—original draft preparation. C-CL, C-CH, and JB: methodology and data curation. C-YC, H-MY, and Y-HL: validation. S-CT: formal analysis and funding acquisition. C-YC, H-MY, C-CL, C-CH, and JB: investigation. S-CT and L-NC: resources. C-YC, Y-HL, and S-CT: writing—review and editing. Y-HL and S-CT: supervision. H-MY: project administration. All authors have read and agreed to the published version of the manuscript.

FUNDING

This research was supported by grant from Yilan Anyong Lohas Co., Ltd. (Yilan, Taiwan) (grant number: 108471) and Ministry of Science and Technology (MOST 102-2410-H-845-014-MY3).

ACKNOWLEDGMENTS

We sincerely appreciate all researchers for contributing to this study.

REFERENCES

AbuMoh'd, M. F., Matalqah, L., and Al-Abdulla, Z. (2020). Effects of oral branched-chain amino acids (BCAAs) intake on muscular and central fatigue during an incremental exercise. *J. Hum. Kinet*. 72, 69–78. doi: 10.2478/hukin-2019-0099

Alessio, H. M., Hagerman, A. E., Fulkerson, B. K., Ambrose, J., Rice, R. E., and Wiley, R. L. (2000). Generation of reactive oxygen species after exhaustive aerobic and isometric exercise. *Med. Sci. Sports Exerc*. 32, 1576–1581. doi: 10.1097/00005768-200009000-00008

Anadón, A., Martínez, M. A., Ares, I., Castellano, V., Martínez-Larrañaga, M. R., Corzo, N., et al. (2013). Acute and repeated dose (28 days) oral safety studies of ALIBIRD in rats. *J. Food Protoc*. 76, 1226–1239. doi: 10.4315/0362-028X.JFP-13-032

Aoi, W., Naito, Y., Takanami, Y., Kawai, Y., Sakuma, K., Ichikawa, H., et al. (2004). Oxidative stress and delayed-onset muscle damage after exercise. *Free Radic. Biol. Med*. 37, 480–487. doi: 10.1016/j.freeradbiomed.2004.05.008

Baldwin, K. M., Fitts, R. H., Booth, F. W., Winder, W. W., and Holloszy, J. O. (1975). Depletion of muscle and liver glycogen during exercise. *Pflugers Arch*. 354, 203–212. doi: 10.1007/BF00584644

Bessa, A. L., Oliveira, V. N., Agostini, G. G., Oliveira, R. J., Oliveira, A. C., White, G. E., et al. (2016). Exercise intensity and recovery: biomarkers of injury, inflammation, and oxidative stress. *J. Strength Cond. Res*. 30, 311–319. doi: 10.1519/JSC.0b013e31828f1ee9

Betts, J. A., Williams, C., Boobis, L., and Tsintzas, K. (2008). Increased carbohydrate oxidation after ingesting carbohydrate with added protein. *Med. Sci. Sports Exerc*. 40, 903–912. doi: 10.1249/MSS.0b013e318164cb52

Carroll, T. J., Taylor, J. L., and Gandevia, S. C. (2017). Recovery of central and peripheral neuromuscular fatigue after exercise. *J. Appl. Physiol*. 122, 1068–1076. doi: 10.1152/japplphysiol.00775.2016

Cermak, N. M., and van Loon, L. J. (2013). The use of carbohydrates during exercise as an ergogenic aid. *Sports Med*. 43, 1139–1155. doi: 10.1007/s40279-013-0079-0

Clark, J. H., and Conlee, R. K. (1979). Muscle and liver glycogen content: diurnal variation and endurance. *J. Appl. Physiol. Respir. Environ. Exerc. Physiol*. 47, 425–428. doi: 10.1152/jappl.1979.47.2.425

Cooke, M. B., Rybalka, E., Stathis, C. G., Cribb, P. J., and Hayes, A. (2010). Whey protein isolate attenuates strength decline after eccentrically-induced muscle damage in healthy individuals. *J. Int. Soc. Sports Nutr*. 7:30. doi: 10.1186/1550-2783-7-30

Dohm, G. L., Tapscott, E. B., Barakat, H. A., and Kasperek, G. J. (1983). Influence of fasting on glycogen depletion in rats during exercise. *J. Appl. Physiol. Respir. Environ. Exerc. Physiol*. 55, 830–833. doi: 10.1152/jappl.1983.55.3.830

Driessen-Kletter, M. F., Amelink, G. J., Bär, P. R., and van Gijn, J. (1990). Myoglobin is a sensitive marker of increased muscle membrane vulnerability. *J. Neurol*. 237, 234–238. doi: 10.1007/BF00314625

Droge, W. (2002). Free radicals in the physiological control of cell function. *Physiol. Rev*. 82, 47–95. doi: 10.1152/physrev.00018.2001

Dyer, A. R., Burdock, G. A., Carabin, I. G., Haas, M. C., Boyce, J., Alsaker, R., et al. (2008). In vitro and in vivo safety studies of a proprietary whey extract. *Food Chem. Toxicol*. 46, 1659–1665. doi: 10.1016/j.fct.2007.12.029

Evans, W. J. (2000). Vitamin E, vitamin C, and exercise. *Am. J. Clin. Nutr*. 72, 647S−652S. doi: 10.1093/ajcn/72.2.647S

Ferguson, L. M., Rossi, K. A., Ward, E., Jadwin, E., Miller, T. A., and Miller, W. C. (2009). Effects of caloric restriction and overnight fasting on cycling endurance performance. *J. Strength Cond. Res*. 23, 560–570. doi: 10.1519/JSC.0b013e31818f058b

Ferrando, A. A., Sheffield-Moore, M., Yeckel, C. W., Gilkison, C., Jiang, J., Achacosa, A., et al. (2002). Testosterone administration to older men improves muscle function: molecular and physiological mechanisms. *Am. J. Physiol. Endocrinol. Metab*. 282, E601–E607. doi: 10.1152/ajpendo.00362.2001

Fitts, R. H., Booth, F. W., Winder, W. W., and Holloszy, J. O. (1975). Skeletal muscle respiratory capacity, endurance, and glycogen utilization. *Am. J. Physiol*. 228, 1029–1033. doi: 10.1152/ajplegacy.1975.228.4.1029

Fouré, A., and Bendahan, D. (2017). Is Branched-Chain amino acids supplementation an efficient nutritional strategy to alleviate skeletal muscle damage? A systematic review. *Nutrients* 9:1047. doi: 10.3390/nu9101047

Gonzalez, J. T., Fuchs, C. J., Betts, J. A., and van Loon, L. J. (2016). Liver glycogen metabolism during and after prolonged endurance-type exercise. *Am. J. Physiol. Endocrinol. Metab*. 311, E543–E553. doi: 10.1152/ajpendo.00232.2016

Jamurtas, A. Z. (2018). Exercise-induced muscle damage and oxidative stress. *Antioxidants*. 7:50. doi: 10.3390/antiox7040050

Kiessling, W. R., Ricker, K., Pflughaupt, K. W., Mertens, H. G., and Haubitz, I. (1981). Serum myoglobin in primary and secondary skeletal muscle disorders. *J. Neurol*. 224, 229–233. doi: 10.1007/BF00313285

Kietzmann, U., Priebe, K., Rakou, D., and Reichstein, K. (1969). *Seefisch als Lebensmittel*. Berlin: Paul Parey Verlag.

Kikuchi, K., Matahira, Y., and Sakai, K. (2004). Separation and physiological functions of anserine from fish extract. *Dev. Food Sci*. 42, 97–105. doi: 10.1016/S0167-4501(04)80012-8

Kraemer, W. J., and Ratamess, N. A. (2005). Hormonal responses and adaptations to resistance exercise and training. *Sports Med*. 35, 339–361. doi: 10.2165/00007256-200535040-00004

Li, P., Mai, K., Trushenski, J., and Wu, G. (2009). New developments in fish amino acid nutrition: towards functional and environmentally oriented aquafeeds. *Amino Acids*. 37, 43–53. doi: 10.1007/s00726-008-0171-1

Ludorff, W., and Meyer, V. (1973). *Fische und Fischerzeugnisse*. Berlin: Paul Parey Verlag.

Mastaloudis, A., Leonard, S. W., and Traber, M. G. (2001). Oxidative stress in athletes during extreme endurance exercise. *Free Radic. Biol. Med*. 31, 911–922. doi: 10.1016/S0891-5849(01)00667-0

Mol, S. (2005). Preparation and the shelf-life assessment of ready-to-eat fish soup. *Eur. Food Res. Technol*. 220, 305–308. doi: 10.1007/s00217-004-1030-2

Moore, D. R., Phillips, S. M., Babraj, J. A., Smith, K., and Rennie, M. J. (2005). Myofibrillar and collagen protein synthesis in human skeletal muscle in young men after maximal shortening and lengthening contractions. *Am. J. Physiol. Endocrinol. Metab*. 288, E1153–E1159. doi: 10.1152/ajpendo.00387.2004

Nie, J., Tong, T. K., George, K., Fu, F. H., Lin, H., and Shi, Q. (2011). Resting and post-exercise serum biomarkers of cardiac and skeletal muscle damage in adolescent runners. *Scand. J. Med. Sci. Sports*. 21, 625–629. doi: 10.1111/j.1600-0838.2010.01096.x

Oliveira, G. V., Volino-Souza, M., Cordeiro, E. M., Conte-Junior, C. A., and Alvares, T. S. (2020). Effects of fish protein hydrolysate ingestion on endothelial function compared to whey protein hydrolysate in humans. *Int. J. Food Sci. Nutr*. 71, 242–248. doi: 10.1080/09637486.2019.1635090

Peake, J. M., Suzuki, K., Hordern, M., Wilson, G., Nosaka, K., and Coombes, J. S. (2005). Plasma cytokine changes in relation to exercise intensity and muscle damage. *Eur. J. Appl. Physiol*. 95, 514–521. doi: 10.1007/s00421-005-0035-2

Powers, S. K., DeRuisseau, K. C., Quindry, J., and Hamilton, K. L. (2004). Dietary antioxidants and exercise. *J. Sports Sci*. 22, 81–94. doi: 10.1080/0264041031000140563

Sabriá, M., Ruibal, A., Rey, C., Foz, M., and Domenech, F. M. (1983). Influence of exercise on serum levels of myoglobin measured by radioimmunoassay. *Eur. J. Nuclear Med*. 8, 159–161. doi: 10.1007/BF00252887

Saugy, M., Robinson, N., Saudan, C., Baume, N., Avois, L., and Mangin, P. (2006). Human growth hormone doping in sport. *Brit. J. Sports Med*. 40, i35–i39. doi: 10.1136/bjsm.2006.027573

Taghiyar, M., Darvishi, L., Askari, G., Feizi, A., Hariri, M., Mashhadi, N. S., et al. (2013). The effect of vitamin C and e supplementation on muscle damage and oxidative stress in female athletes: a clinical trial. *Int. J. Prev. Med*. 4:S16.

Thompson, D., Williams, C., McGregor, S. J., Nicholas, C. W., McArdle, F., Jackson, M. J., et al. (2001). Prolonged vitamin C supplementation and recovery from demanding exercise. *Int. J. Sport Nutr. Exerc. Metab*. 11, 466–481. doi: 10.1123/ijsnem.11.4.466

Waring, W. S., Convery, A., Mishra, V., Shenkin, A., Webb, D. J., and Maxwell, S. R. (2003). Uric acid reduces exercise-induced oxidative stress in healthy adults. *Clin. Sci*. 105, 425–430. doi: 10.1042/CS20030149

Watson, T. A., Callister, R., Taylor, R. D., Sibbritt, D. W., MacDonald-Wicks, L. K., and Garg, M. L. (2005). Antioxidant restriction and oxidative stress in short-duration exhaustive exercise. *Med. Sci. Sports Exerc*. 37, 63–71. doi: 10.1249/01.MSS.0000150016.46508.A1

Wei, L., Wen, Y. T., Lee, M. C., Ho, H. M., Huang, C. C., and Hsu, Y. J. (2019). Effects of isolated soy protein and strength exercise training on exercise performance and biochemical profile in postpartum mice. *Metabolism*. 94, 18–27. doi: 10.1016/j.metabol.2019.01.012

Wojcikowski, K., and GobeShin, G. (2014). Animal studies on medicinal herbs: predictability, dose conversion and potential value. *Phytother. Res*. 28, 22–27. doi: 10.1002/ptr.4966

Wu, R. E., Huang, W. C., Liao, C. C., Chang, Y. K., Kan, N. W., and Huang, C. C. (2013). Resveratrol protects against physical fatigue and improves exercise performance in mice. *Molecules*. 18, 4689–4702. doi: 10.3390/molecules18044689

Yada, K., Roberts, L. A., Oginome, N., and Suzuki, K. (2020). Effect of acacia polyphenol supplementation on exercise-induced oxidative stress in mice liver and skeletal muscle. *Antioxidants* 9:29. doi: 10.3390/antiox9010029

Conflict of Interest: S-CT is a Professor at University of Taipei, Taipei City, Taiwan, and she received the research funding from Yilan Anyong Lohas Co., Ltd. (Yilan, Taiwan) (grant number: 108471). Researchers at University of Taipei are responsible for the entire animal study and report.

The remaining 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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ORIGINAL RESEARCH
published: 28 May 2021
doi: 10.3389/fphys.2021.660552

Chinese Soft-Shelled Turtle Oil in Combination With Swimming Training Improves Spatial Memory and Sports Performance of Aging Rats

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Specialty section:
This article was submitted to Integrative Physiology, a section of the journal Frontiers in Physiology

Received: *29 January 2021*
Accepted: *16 April 2021*
Published: *28 May 2021*

Citation:
Yang C-E, Yeh T-M, Chang C-D and Shih W-L (2021) Chinese Soft-Shelled Turtle Oil in Combination With Swimming Training Improves Spatial Memory and Sports Performance of Aging Rats. Front. Physiol. 12:660552. doi: 10.3389/fphys.2021.660552

In this study, waste fat from the Chinese soft-shelled turtle (*Pelodiscus sinensis*) was used as the raw material, and soft-shelled turtle oil (SSTO) was extracted by water heating. Analysis of the fatty acid composition of SSTO revealed that unsaturated fatty acids (UFAs) comprised more than 70% of the oil, of which more than 20% were omega-3 poly-UFAs. DPPH radical scavenging and cellular ROS assays confirmed the reduction of oxidative stress by SSTO. In D-galactose-induced aging rats, SSTO feeding alone or in combination with swimming training resulted in improved memory and physical strength. In addition, SSTO feeding with swimming intervention significantly increased the SOD level and maintained better blood pressure in the aged rats. The serum DHEAS and soleus muscle glycogen level were also highly correlated with SSTO feeding and swimming training. In conclusion, the results of this study demonstrated that SSTO has the potential to be developed into a health food that exerts anti-aging effects, and those effects are stronger when combined with daily swimming exercise.

Keywords: soft-shelled turtle, ROS, DHEAS, swimming, aging

INTRODUCTION

Aging is an irreversible phenomenon in which organisms undergo progressive and systemic complex physiological processes at the end of life, which are related to the complex interactions between genetics, acquired lifestyles, environment and chronic diseases. With improvement in quality of life, increasing health consciousness and the rapid advancement of medical science, people aged 65 years and older will comprise 16% of the population in 2050. A combination of internal and external factors causes aging, which is inevitable, but can be delayed; many aging-associated diseases are also avoidable (Siedner, 2019; Sabatini et al., 2021).

Oxidative damage affects many organs, and the brain is particularly vulnerable, as it consumes 20% of the body's oxygen and contains large quantities of unsaturated fatty acids (UFAs) that are susceptible to oxidation (Barja, 2004). Studies have shown that oxidative stress causes neurodegenerative brain diseases, including Alzheimer's and Parkinson's diseases, which are directly related to free-radical damage (Song and Zhao, 2007; Calviello et al., 2013).

Increased free-radical reactions is known to promote the aging process, and inhibition of the accumulation of free radicals in cells can reduce the rate of aging and alter disease pathogenesis (Harman, 2003). Exercise is considered to be a beneficial factor in a healthy lifestyle, as physical activity can improve the antioxidant system and lower lipid peroxidation levels in the elderly (Bouzid et al., 2018). In addition to being able to delay aging, exercise is known to prevent cognitive decline; therefore, reducing antioxidative stress and increasing exercise levels are very important in old age. Sustained physical activity with antioxidant supplementation has been suggested to further shield the body from oxidative stress, which is especially beneficial in the elderly population (Simioni et al., 2018).

Chinese soft-shelled turtles (*Pelodiscus sinensis*) were popular in ancient times as a food and medicine, and are the most common farmed turtle in Asia. People in some areas of these countries still consume soft-shelled turtle meat (Yamanaka et al., 2016). Owing to people commonly believing that consumption of too much fat may lead to a high cholesterol level, along with turtle fat having a heavy fishy taste, it is normally discarded. Some companies have already developed health foods from soft-shelled turtle meat or eggs for use in humans and pets, but no scientific research has been conducted on the oil of the soft-shelled turtle in order to assess its health benefits.

Studies have shown that the benefits of swimming over other sports include reduced stress on bones and joints, a reduced risk of osteoporosis, improved cardiovascular health, maintenance of muscle mass and coordination, and more importantly, little to no injury, as no physical contact occurs (Jennings, 1997; Tanaka et al., 1997; Hart et al., 2001; Moreira et al., 2014). These factors make swimming especially suitable for the elderly. Therefore, in this study, we investigated whether rats fed with soft-shelled turtle oil (SSTO) had a better learning and memory performance in the Y-maze test and better physical endeavor in swimming training.

MATERIALS AND METHODS

Preparation of Soft-Shelled Turtle Oil

Soft-shelled turtle fat obtained from Sanhe Biotech (Ligang, Pingtung, Taiwan) was used to prepare SSTO. Briefly, 100 g of fat were placed in a steel pan, stirred and heated to 100, 200, 300, or 400°C in water. An infrared thermometer was used to determine the temperature of the material. After the target temperature had been reached, the fat was continually stirred for an additional 10 min to obtain SSTO. After cooling, the oil was centrifuged at 10,000 × *g* and filtered through Advantec No. 1 filter paper to remove solid impurities or precipitation from the oil. The purified turtle oil was stored at 4°C.

Cytotoxicity Assessment and Cellular Reactive Oxygen Species (ROS) Assay

Cytotoxicity analysis was performed using RAW264.7 cells (obtained from the Food Industry Development Research Institute) cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 4 mM L-glutamine, 4.5 g/L glucose, 1.5 g/L sodium bicarbonate and 10% fetal calf serum (FCS). Analysis was carried out on cells treated with various concentrations of emulsified SSTO (1, 5 and 10%) or vehicle control (0.04% Tween-20) for 24 h, and the cell viability was determined using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) cell viability and cytotoxicity assay.

In the cellular ROS assay, RAW264.7 cells were treated with various concentrations of SSTO for 2 h. Cells were then incubated with 2′,7′-dichloro-dihydrofluorescein diacetate (H2DCFDA) for 30 min, followed by treatment with H_2O_2 to induce ROS formation. The fluorescence intensities were determined as described in previous studies (Shih et al., 2018).

DPPH Radical Scavenging Assay

Different concentrations of SSTO or vehicle control were compared with butylated hydroxytoluene (BHT) as the positive control. In this assay, 0.1 mM DPPH in methanol was prepared, and 2.4 mL of this solution were mixed with 1.6 mL of SSTO in methanol. The reaction mixture was vortexed thoroughly and incubated for 30 min in the dark. The absorbance of the mixture was measured spectrophotometrically at 517 nm. The resting DPPH level was calculated as the percentage of absorbance using the following formula: % DPPH level = 1-($OD_{control}$ − OD_{sample})/$OD_{control}$ × 100%

Animal Experimental Protocol

The animal study was approved by the Experimental Animal Care and Use Committee of National Pingtung National University of Science and Technology (Project number NPUST-109-041). Forty male 8-week-old Sprague-Dawley (SD) rats were purchased from BioLESCO Taiwan Co. The animals were housed in individual ventilated cages (IVCs) under 25 ± 1°C and a 12:12-h light:dark cycle. The experiment was initiated 1 week after the animals had been allowed to adapt to their new environment. The rats were divided into five groups (n = 8 for each group) as follows: Group 1, no treatment; Group 2, subcutaneous (s.c.) D-galactose (120 mg/kg) injection to accelerate aging; Group 3, D-galactose-induced aging and SSTO feeding alone; Group 4, D-galactose-induced aging and SSTO feeding plus swimming training; Group 5, D-galactose-induced aging with swimming training only. D-galactose was injected for 42 days, and SSTO feeding and exercise training started on day 14 after the injections began and lasted for 28 days in total. The amount of SSTO used was based on the recommendation for fish oil daily consumption of the Food and Drug Administration, Ministry of Health and Welfare, Taiwan, which is 2 g/day for humans (Taiwan Food and Drug Administration, 2020). According to a drug usage conversion coefficient between humans and rats (US Foof & Drug Administration, 2005), this is equal to 175 mg/kg/day for rats (assume adult human body weight is 70 kg, and the conversion factor for human equivalent doses to rat dose is known as 6.2; the dosage calculation formula: (2 g/70 kg) × 6.2 = ∼175 mg/kg). The complete procedure of the animal experiment was as shown in **Figure 1**. SSTO was fed to the animals by gavage at 10 a.m. daily, and the animals were allowed to freely consume food.

Y-Maze Test

Rodents have the habit of exploring new environments. The Y-maze consists of three arms, labeled A, B, and C. At the start of the test, the C arm was closed off, and the animal was allowed to explore the start of arms A and B for 5 min, then placed back into the home cage and allowed to rest for 3 min. The maze was wiped with alcohol to remove any smell. Then, all three ends of the maze arms were opened, and the rat was allowed to explore freely for 10 min, during which the number of times and the times at which the rat entered the three arms, in addition to the speed of movement, were recorded (Dellu et al., 2000). Spontaneous alteration behavior was defined as (consecutive entries into three different arms/total number of arm entries – 2) ×100; a higher percentage showed improved memory and cognition (Muhammad et al., 2019).

Then, in order to evaluate the effects of SSTO and swimming on short-term spatial memory, with all three arms open, a food reward was placed at the end of arm C, and the animal was placed into arm A. After three sessions of training to enable the animal to recognize that the food was always placed in arm C, the animal was placed back in the home cage. After 1 day of rest and 8 h of starvation, the spatial memory of the animal was then tested in terms of its ability to remember that the food was located in arm C. The search for food was conducted 5 times, within a duration of 10 min. A digital camera was positioned above and software employed for analysis as described in the previous section.

Regular Swimming Training and Weight-Loaded Forced Swimming

A water tank was employed for swimming training, of a diameter of 54 cm and a height of 65 cm. The water temperature was maintained at 34 ± 1°C. Training began at 2 weeks after the start of accelerated aging induction (**Figure 1**) and was conducted at 11 AM every day. During the training, the rats voluntarily swam free of load, and the daily swimming duration was 10 min. After training, the rats were gently towel-dried and warm air-dried before being returned to their cages. Weight-loaded forced swimming was performed until exhaustion with a weight of 5% of the animal's body weight attached to the tail (da Silva et al., 2016; Lin et al., 2018). Animals were assessed as being fatigued when they failed to rise to the surface of the water to breathe within an 8-s period. The rats were gently towel-dried and warm air-dried immediately after the exercise.

Blood Pressure Measurement and Blood Sample Collection

The rats were placed in plastic restrainers, and a cuff with a pneumatic pulse sensor was attached to the tail and fixed with surgical tape. Blood pressure was recorded on a non-invasive rodent blood pressure monitor (MK-2000ST, Muromachi Kikai, Japan). Blood samples were collected from the tail vein and centrifuged at 1,000 × g for 10 min to separate the serum.

Biochemical Analysis of Serum

Commercial superoxide dismutase (SOD) and total antioxidant capacity (TAC) assay kits were used to measure the SOD and TAC levels in serum samples of the rats. The processes were as described in the instructions of the manufacturers. A dehydroepiandrosterone sulfate (DHEAS) ELISA kit (Wuhan Fine Biotech Co., Inc.) was used to measure the level of DHEAS.

Glycogen Level in the Soleus Muscle

The glycogen levels in soleus muscle samples were measured using a glycogen colorimetric assay kit (BioVision) according to the manufacturer's instructions. Soleus was selected as the target muscle for this study as studies have shown that soleus muscle is one the key muscle types that can be improved with swimming training in rat model (Forte et al., 2020; Moustafa and Arisha, 2020).

Statistical Analysis

The results of this study were analyzed using Excel 2010. The unpaired *t*-test was employed for statistical analysis to compare data from treated groups with those of the aged rats with saline treatment, and to compare data from the SSTO or swimming group with those of the SSTO+swimming group, using Prism software (v.6.0; GraphPad Software, San Diego, CA, United States). A p-value of <0.05 was considered as significant. Data are presented as mean ± SD.

RESULTS

SSTO Yield and Fatty Acid Analysis

The yields of SSTO extracted at different rendering temperatures indicated that the yield increased with increasing rendering temperature, being 30% at 100°C and 65% at 400°C. Fatty acid analysis was conducted by the Center for Agricultural and Aquacultural Products Inspection and Certification at NPUST. **Table 1** lists all the types of fatty acid that accounted for more than 1% of the total fatty acid content. The results showed that the SSTO extracted at 100°C, similar to fish oil, had high levels of UFAs, at approximately 70%, including 22% omega-3 poly-UFAs and 29.78% omega-9 mono-UFAs.

Assessment of Free-Radical Scavenging Activity of SSTO *in vitro* and in Cultured Cells

Our results showed that SSTO at concentrations of 25 and 50% had a significantly high antioxidant activity. SSTO prepared

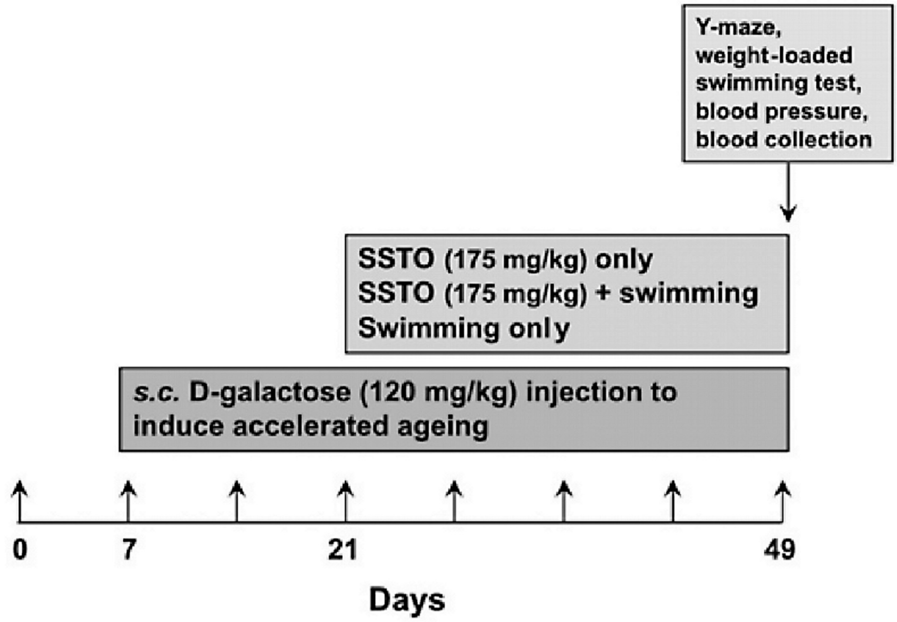

FIGURE 1 | Flowchart of the animal experiment. One week after the animals had been allowed to adapt to the environment, the rats were injected subcutaneously with D-galactose to induce accelerated aging for 42 days. SSTO feeding and/or swimming training were started on day 14 after the D-galactose injections began and continued for 4 weeks. The outcomes were assessed using the Y-maze for learning memory performance and weight-loaded swimming for physical fitness testing. Blood pressure was also recorded and blood samples were collected for further analysis.

at lower rendering temperatures had a better free radical scavenging effect than samples prepared at higher rendering temperatures (**Figure 2A**). In order to measure the ability of SSTO to reduce oxidative stress in cells caused by H_2O_2, an ROS assay using non-cytotoxic concentrations of SSTO was performed. The results showed that SSTO (extracted at 100°C) at 5 and 10% significantly reduced oxidative stress and had a high ROS scavenging activity (**Figure 2B**). As SSTO prepared under 100°C has the best antioxidant capacity, all subsequent experiments utilized SSTO rendered at that temperature.

TABLE 1 | Fatty acid composition of the SSTO.

	Types of fatty acid	Content (%)
omega-9 mono-UFAs	Oleic acid	27.98
omega-7 mono-UFAs	Palmitoleic acid	6.52
omega-6 poly-UFAs	Linoleic acid	8.50
	Arachidonic acid	2.12
omega-3 poly-UFAs	Docosahexaenoic acid (DHA)	12.97
	Eicosapentaenoic acid (EPA)	5.37
	Docosapentaenoic acid (DPA)	2.62
	Alpha-linoleic acid	1.04
SFAs	Palmitic acid	20.27
	Steric acid	5.94
	Myristic acid	3.55
	Total	96.88

Assessment of Aging in the Rats Treated With SSTO

As shown in **Figures 3A,B**, compared with the control rats, D-galactose-induced aging rats lost the desire to explore new environments, and exhibited poor mobility and spatial learning. The aging rats fed with SSTO alone, those with SSTO feeding in combination with swimming training, and those with swimming training alone all exhibited greater exploration of a new environment and longer stays in arm C in the Y-maze test, in addition to a greater speed of movement and a higher percentage of direction alteration behavior. Among the groups, the rats with SSTO feeding in combination with swimming training exhibited the best anti-aging response. These results indicated that SSTO together with swimming training had the best outcome in terms of improving cognition, which led to enhancement of the motivation and desire of the aging rats to explore new environments.

Further, using the Y-maze with food as a reward, the short-term spatial memory and sports performance in the different groups of rats were compared. As shown in **Figure 3C** (left *y*-axis), the aging rats required a greater amount of time to find the food than the normal rats, indicating that D-galactose injection caused deterioration in cognitive memory. All the rats in the treatment groups with SSTO feeding and/or swimming training exhibited improvement in short-term spatial memory, especially the rats with SSTO feeding in combination with

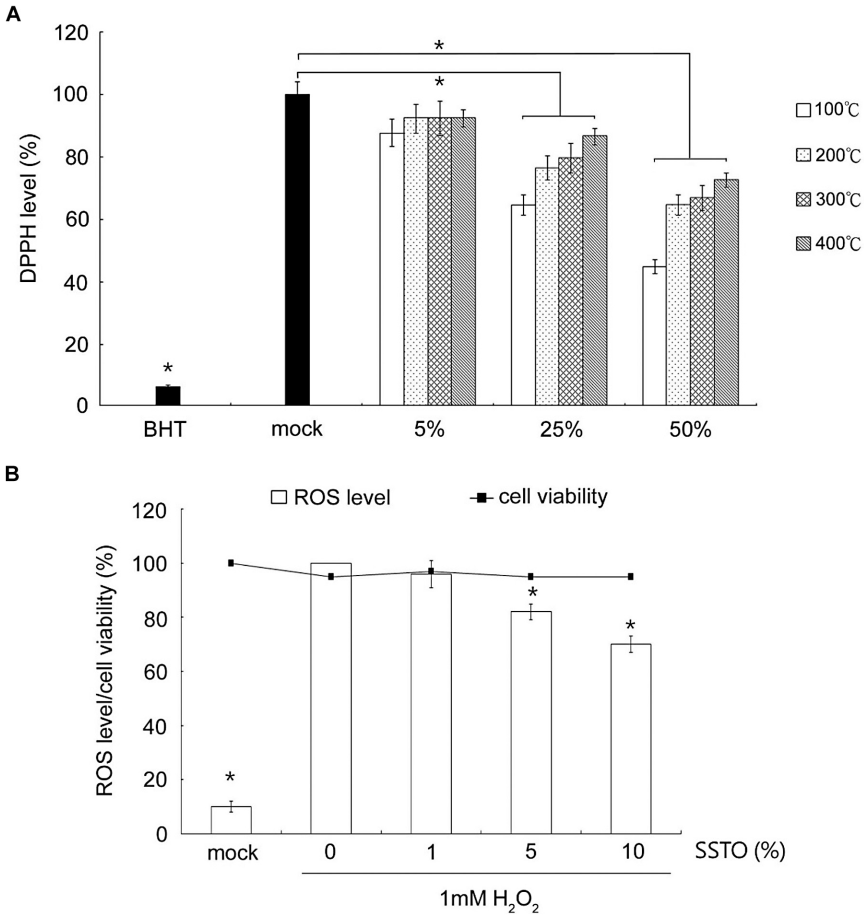

FIGURE 2 | Antioxidant activity of SSTO. **(A)** DPPH assay. The *y*-axis shows the relative percentage of the residual DPPH level as compared with butylated hydroxytoluene (BHT) as the positive control. The *x*-axis shows the positive and negative controls and SSTO samples at different concentrations prepared at various rendering temperatures. **(B)** Cellular ROS assay. RAW264.7 cells were incubated with SSTO, and the percentage of ROS reduction in the culture was measured. Cell viability was also monitored by MTT assay. *Statistically significant difference as compared with the non-SSTO treated group, but with 1 mM H_2O_2.

swimming training, in which the results were most similar to those of the normal rats. As shown in **Figure 3C** (right *y*-axis), the aging rats were less physically fit than the normal rats, and the duration of weight-loaded swimming was reduced. When the aging rats were fed with SSTO and/or completed swimming training, their swimming performance was improved. Combined SSTO feeding and swimming training improved the duration of weight-loaded swimming to a level that was four times that of the aged rats with no treatment or training.

Effects of SSTO and Swimming Training on the Regulation of Blood Pressure

In normal rats, the systolic blood pressure (SBP) ranges from 84–134 mmHg, and the diastolic blood pressure (DBP) is around 60 mmHg. In our study, the D-galactose-induced aging rats without treatment or intervention had significantly higher SBP and DBP values than the control group. Aged rats with SSTO feeding and/or swimming training all showed significant improvement in terms of a lowered blood pressure. In particular, in rats with SSTO feeding in combination with swimming training, the SBP and DBP values were lower, to near the ranges of normal rats (**Table 2**). Therefore, SSTO has the potential to be developed into a health food product for blood pressure control.

Serum Antioxidant Enzyme Activity and DHEAS Analysis

The antioxidant enzyme activities of SOD and TAC in the serum were compared between groups. As shown in **Figure 4A**, the rats with SSTO feeding alone did not have an increased serum SOD level as compared with the aging rats, while rats with swimming training alone or SSTO feeding in combination with swimming had significantly higher SOD levels. Additionally, SSTO feeding in combination with swimming resulted in the best outcome with regards to the TAC level in the aged rats. DHEAS is a metabolite of dehydroepiandrosterone (DHEA) that circulates in the blood, and measurement of DHEAS in the blood is a reliable approach by which to show the bioactive level of DHEA. DHEA is known to play important roles in neurological functions, and

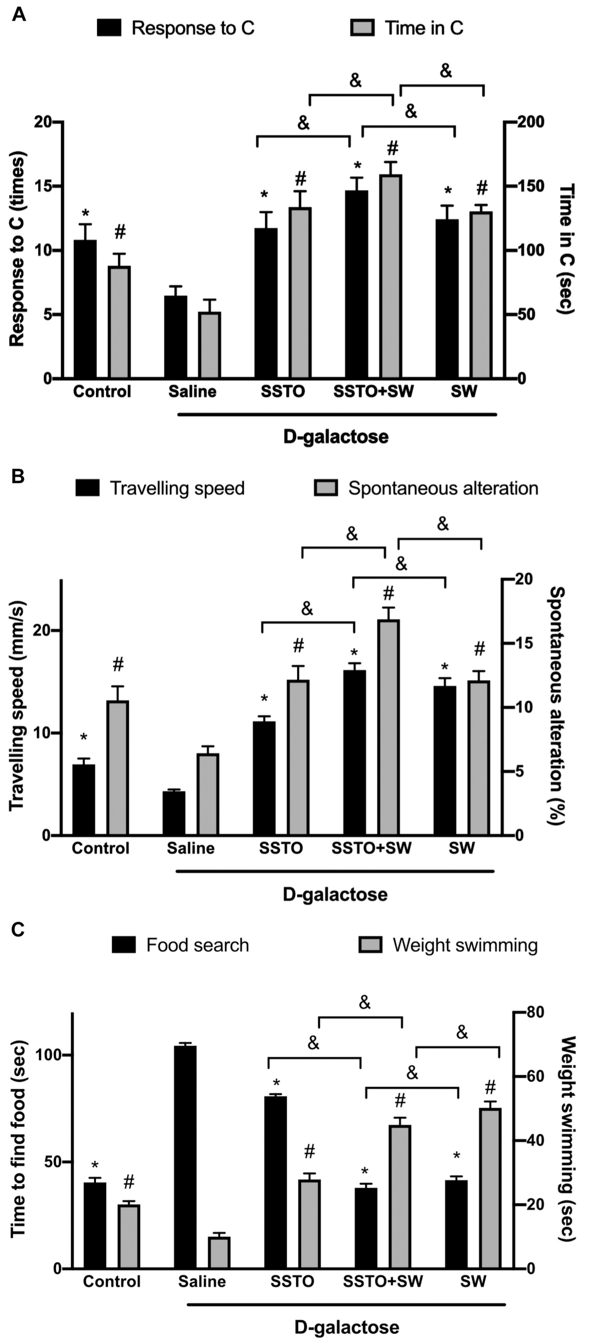

FIGURE 3 | Effects of SSTO and swimming training on aging rats. **(A)** Exploration evaluation. The left *y*-axis (black bars) shows the number of times that the rats entered the new environment (arm C) within 10 min, and the right *y*-axis (white bars) shows the average duration for which the animals stayed in arm C. **(B)** Mobility determination. The left *y*-axis (black bars) shows the traveling speed, and the right y-axis (white bars) indicates the spatial learning ability, as evaluated by the spontaneous alteration percentage. **(C)** SSTO and swimming training improved short-term spatial memory (black bars) and physical performance (white bars). Left *y*-axis: average time to find food; right *y*-axis: endurance measured as weight-loaded forced swimming duration. *[#]Statistically significant difference as compared with the aged rats with saline treatment; [&]statistically significant difference when compared with SSTO + swimming group.

TABLE 2 | Systolic and diastolic blood pressure and mean blood pressure of the rats in this study.

	Blood pressure		
Group	**SBP (mmHg)**	**DBP (mmHg)**	**Average (mmHg)**
Control	116.0 ± 15.4*	63.3 ± 21.5*	81.3 ± 24.6*
Aged rats	152.3 ± 10.5	115.8 ± 19.0	128.0 ± 15.2
Aged rats + SSTO	141.8 ± 20.1	73.2 ± 12.2*	96.0 ± 14.1*
Aged rats + SSTO + swimming	109.3 ± 35.3*	74.5 ± 23.2*	86.0 ± 25.8*
Aged rats + swimming	140.3 ± 27.3	78.0 ± 8.4*	98.8 ± 12.5*

**Statistically significant differences as compared with the aged rats without treatment. Data presented as mean ± SD. SBP, systolic blood pressure; DBP, diastolic blood pressure.*

has been suggested to be associated with the development of neurodegenerative diseases in humans. As shown in **Figure 4B** (right *y*-axis), rats with D-galactose-induced aging that did not receive treatment exhibited a significantly decreased DHEAS level, while swimming training with or without SSTO feeding resulted in increased DHEAS levels in the aged rats. Aged rats with SSTO feeding alone did not exhibit this effect.

Glycogen Level in the Soleus Muscle

Kinesiology study has shown that the soleus muscle is the primary muscle used in exercise in rats. This muscle is highly-correlated with exercise endurance, and therefore, strengthening of the soleus muscle with regular excise will improve physical performance and can decrease soreness, pain and spasm after exercise. The glycogen levels in the soleus muscle in the different groups were compared. As shown in **Figure 4B** (left *y*-axis), in the aging rats, the soleus muscle glycogen was significantly depleted, while the glycogen levels of the rats with swimming alone or swimming combined with SSTO feeding were significantly increased, suggesting that exercise is important in order to preserve a high level of glycogen in the muscles. SSTO feeding alone did not boost the glycogen level in the aged rats.

DISCUSSION

In this study, Chinese SSTO was extracted from waste fat after turtle slaughter, and the fat was transformed into a useful bioactive agent and a valuable aquacultural by-product. However, the strong fishy smell will need to be eliminated when it is developed as a product for human consumption. The results of the present study showed that a higher rendering temperature resulted in a higher oil yield, but a decreased antioxidant activity. A previous study compared the extraction of SSTO by water bath, frying, ethyl acetate extraction and n-hexane extraction, then measured the changes in the acid value, peroxide value and fatty acids after storing the oil samples for 60 days. The results showed that the yield of extraction by chemical solvents was significantly higher than that of traditional physical methods, and the oil obtained by high-temperature rendering turned rancid more quickly (Lee, 2017). Our present study also showed that the antioxidant activity of SSTO extracted at

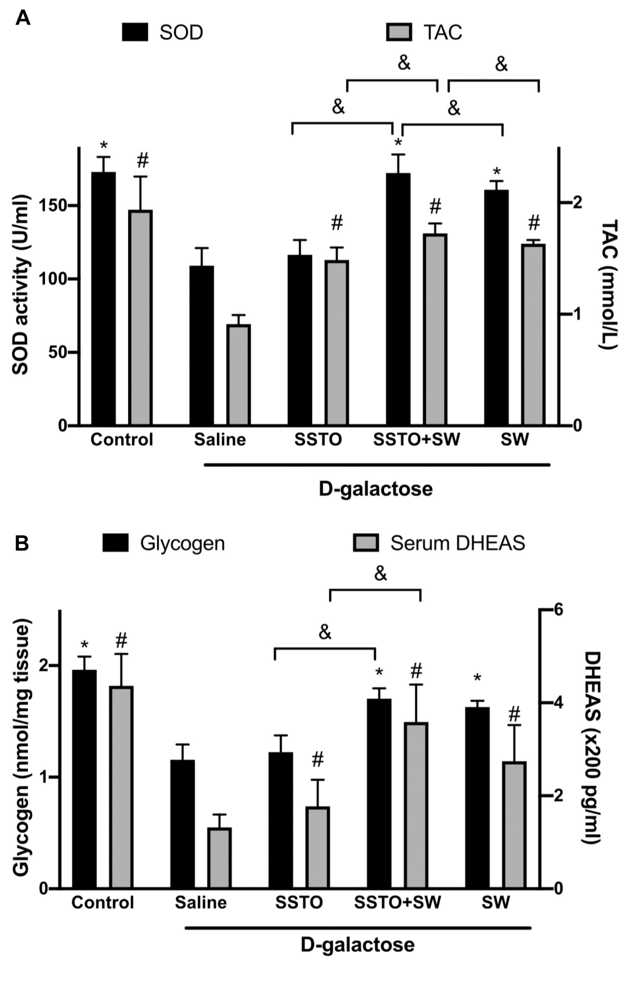

FIGURE 4 | Biochemical analysis of serum and muscle. **(A)** Effects of SSTO and/or swimming on serum SOD (black bars) and TAC (white bars) antioxidant enzyme activity. **(B)** Glycogen content (black bars; left *y*-axis) in the soleus muscle and serum DHEAS level (white bars; right *y*-axis). *#Statistically significant difference as compared with the aged rats with saline treatment; &statistically significant difference when compared with SSTO + swimming group.

higher rendering temperatures was poor, suggesting that a high rendering temperature may damage the fatty acids and other bioactive components.

The prevention and treatment of dementia has long been a major topic in research. A study performed previously, in which participants were followed-up for more than 20 years, showed that saturated fatty acid (SFA) intake is associated with dementia and Alzheimer's disease, while UFAs have a protective effect (Laitinen et al., 2006). Another long-term study showed that replacing 5% of the total calories with poly-UFAs and mono-UFAs reduced the risk of cardiovascular disease (Hu et al., 1997). The results of our study showed that UFAs comprise more than 70% of SSTO. Although this percentage is lower than that of vegetable oils, such as olive oil and bitter tea oil, a UFA content of 70% shows it to be a high-quality oil among animal oils, especially as it contains high levels of omega-3, -6 and -9, and eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) at 5.37 and 12.97%, respectively. As EPA and DHA have been shown to effectively reduce triglycerides (Klingel et al., 2019), our results suggested that SSTO is a good healthy oil.

Omega-3 fatty acids present in fish oil have been shown to be of therapeutic potential with regards to several neurodegenerative and neurological disorders (Dyall, 2015), and EPA and DHA have been demonstrated to have important roles in maintaining neuronal cell functions (Reimers and Ljung, 2019). Based on information from the US Department of Agriculture, per 100 g of fish oil, the contents of EPA, DPA and DHA are 6.898 g, 0.935 g and 10.968 g, respectively (Agriculture UDo, 2019). Our results showed that SSTO has higher DHA (12.97 g) and DPA levels (2.62 g), suggesting that EPA and DHA may be key to the anti-aging effects of SSTO. Additionally, SSTO has a high content of oleic acid (at 27.98%), an omega-9 fatty acid that has been demonstrated to lower the level of low-density lipoprotein (LDL) cholesterol and prevent arteriosclerosis. This further indicates that SSTO has a great potential to be developed as a health food supplement. Based on the results of our study and those reported in the literature, SSTO possesses the beneficial characteristics of both vegetable oil and high-quality animal oil, which render it suitable for development as an anti-aging supplement, SSTO being rich in omega-3 UFAs, which are most often lacking in the diet.

In studies of yeast and fruit flies, and in mouse models, it was found that superoxides may damage the genomic integrity and stability, contributing to acceleration of aging and shortening the lifespan (Villani et al., 2013). Antioxidant supplements are therefore thought to remove free radicals and boost the enzyme system within the body, which disarms free radicals and helps to delay aging. The most important antioxidant enzyme system in the body is located in the cytosol and mitochondrial intermembrane space. The system utilizes SODs to convert superoxide ($O2^-$) into two less-damaging species, H_2O_2 and oxygen. In the present study, we found that SSTO directly scavenged superoxide radicals from cultured cells and increased the endogenous SOD enzyme activity in an aging rat model, and its antioxidant effect was greater when combined with regular swimming training. Further studies are needed to determine whether the antioxidant effect of SSTO is mediated by regulation of cell signaling pathways, such as mitogen-activated protein kinases (MAPKs), antioxidant/electrophile response element (ARE/EpRE) and transcription factor Nrf2, in the host. Whether other antioxidant enzymes in the body, such as catalase (CAT) in peroxisomes and glutathione peroxidase (GP), which may scavenge both ROOH and H_2O_2 (Vecchione et al., 2007), are also enhanced after SSTO administration is also an area most worthy of investigation.

Many studies have shown that exercise has positive effects on cognitive and other brain functions (Cotman and Berchtold, 2002; Cotman et al., 2007). In studies of rodents, voluntary wheel-running was shown to increase the gene expressions of neurotrophic factors and other neuroplasticity-related genes in the hippocampus, such as calmodulin-dependent protein kinase II, MAPK, and CREB (Neeper et al., 1996; Tong et al., 2001).

Cellular factors that mediate the anti-aging effects of SSTO need to be elucidated.

The soleus muscle is one of the triceps surae muscles located at the calf. It starts at the upper portions of the fibula and tibia, and plays a major role in plantar flexion. It has more red muscle fibers than the gastrocnemius, hence it is also known as the "endurance muscle." Studies have shown that people with better endurance have lower mortality rates, and this same trend exists across all age groups (Montori-Grau et al., 2009). The main determinant of muscle endurance is glycogen, the main energy substrate that can produce anaerobic energy by releasing glucose to be used by the muscle fibers. The results of the present study demonstrated that SSTO feeding in combination with swimming training had a positive effect on the glycogen level in the soleus muscle.

DHEA and DHEAS are secreted by the adrenal glands. They are known to be multi-functional hormones that are associated with immunity, diabetes, growth of neurons, memory and cognition, and aging. A long-term follow-up study of 27 years' duration showed that the DHEAS level in the serum can predict longevity in men (Enomoto et al., 2008). So far, it is not clear what factors are linked to the serum DHEAS level, but it is relatively certain that an increase in the serum DHEAS concentration may extend the life span. The present study also confirmed that SSTO feeding and exercise attenuated DHEAS level decrease caused by D-galactose induced aging, although the level in the aged rats with SSTO feeding and swimming was still lower than that in normal young rats (**Figure 4B**).

Although forced exercise such as treadmill and swimming training, or voluntary roller exercise, are commonly used in experimental settings to train rodents, whether or not forced and voluntary exercise differentially affect brain functions is not yet known. However, the maze test is the most acceptable method by which to study cognition and memory in experimental animals. In this study, we used a simple Y-maze to study the effects of SSTO feeding and swimming in an aging rat model. In the future, we plan to use more advanced behavioral analysis methods to explore the mechanisms of SSTO involved in slowing the aging process, improving brain function, increasing physical fitness, and even prolonging lifespan in animal models.

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 animal study was reviewed and approved by the IACUC of National Pingtung University of Science and Technology.

AUTHOR CONTRIBUTIONS

C-EY and T-MY performed the cell culture-based analyses and animal tests, and drafted the manuscript. C-DC participated in the design and coordination of the study and helped to draft the manuscript. W-LS, the principal investigator of the study were responsible for the grant application, interpretation of the results, and manuscript editing. All authors read, commented on, and approved the final manuscript.

ACKNOWLEDGMENTS

We appreciate the assistance of OxBiosci for English editing.

REFERENCES

Agriculture UDo. (2019). *Fish Oil, Cod Liver*. Available online at: https://fdc.nal.usda.gov/fdc-app.html#/food-details/173577/nutrients (accessed March 15, 2021).

Barja, G. (2004). Free radicals and aging. *Trends Neurosci.* 27, 595–600.

Bouzid, M. A., Filaire, E., Matran, R., Robin, S., and Fabre, C. (2018). Lifelong voluntary exercise modulates age-related changes in oxidative stress. *Int. J. Sports Med.* 39, 21–28. doi: 10.1055/s-0043-119882

Calviello, G., Su, H. M., Weylandt, K. H., Fasano, E., Serini, S., and Cittadini, A. (2013). Experimental evidence of omega-3 polyunsaturated fatty acid modulation of inflammatory cytokines and bioactive lipid mediators: their potential role in inflammatory, neurodegenerative, and neoplastic diseases. *Biomed. Res. Int.* 2013:743171.

Cotman, C. W., and Berchtold, N. C. (2002). Exercise: a behavioral intervention to enhance brain health and plasticity. *Trends Neurosci.* 25, 295–301. doi: 10.1016/s0166-2236(02)02143-4

Cotman, C. W., Berchtold, N. C., and Christie, L. A. (2007). Exercise builds brain health: key roles of growth factor cascades and inflammation. *Trends Neurosci.* 30, 464–472. doi: 10.1016/j.tins.2007.06.011

da Silva, E., Natali, A. J., da Silva, M. F., Gomes Gde, J., da Cunha, D. N., Toledo, M. M., et al. (2016). Swimming training attenuates the morphological reorganization of the myocardium and local inflammation in the left ventricle of growing rats with untreated experimental diabetes. *Pathol. Res. Pract.* 212, 325–334. doi: 10.1016/j.prp.2016.02.005

Dellu, F., Contarino, A., Simon, H., Koob, G. F., and Gold, L. H. (2000). Genetic differences in response to novelty and spatial memory using a two-trial recognition task in mice. *Neurobiol. Learn Mem.* 73, 31–48. doi: 10.1006/nlme.1999.3919

Dyall, S. C. (2015). Long-chain omega-3 fatty acids and the brain: a review of the independent and shared effects of EPA. DPA andDHA. *Front. Aging Neurosci.* 7, 52. doi: 10.3389/fnagi.2015.00052

Enomoto, M., Adachi, H., Fukami, A., Furuki, K., Satoh, A., Otsuka, M., et al. (2008). Serum dehydroepiandrosterone sulfate levels predict longevity in men: 27-year follow-up study in a community-based cohort (Tanushimaru study). *J. Am. Geriatr. Soc.* 56, 994–998. doi: 10.1111/j.1532-5415.2008.01692.x

Forte, L. D. M., Rodrigues, N. A., Cordeiro, A. V., de Fante, T., Simino, L. A. P., Torsoni, A. S., et al. (2020). Periodized versus non-periodized swimming training with equal total training load: physiological, molecular and performance adaptations in Wistar rats. *PLoS One* 15:e0239876. doi: 10.1371/journal.pone.0239876

Harman, D. (2003). The free radical theory of aging. *Antioxid Redox Signal.* 5, 557–561.

Hart, K. J., Shaw, J. M., Vajda, E., Hegsted, M., and Miller, S. C. (2001). Swim-trained rats have greater bone mass, density, strength, and dynamics. *J. Appl. Physiol. (1985)* 91, 1663–1668. doi: 10.1152/jappl.2001.91.4.1663

Hu, F. B., Stampfer, M. J., Manson, J. E., Rimm, E., Colditz, G. A., Rosner, B. A., et al. (1997). Dietary fat intake and the risk of coronary heart disease in women. *N. Engl. J. Med.* 337, 1491–1499.

Jennings, G. L. (1997). Exercise and blood pressure: walk, run or swim? *J. Hypertens.* 15, 567–569. doi: 10.1097/00004872-199715060-00001

Klingel, S. L., Metherel, A. H., Irfan, M., Rajna, A., Chabowski, A., Bazinet, R. P., et al. (2019). EPA and DHA have divergent effects on serum triglycerides and lipogenesis, but similar effects on lipoprotein lipase activity: a randomized controlled trial. *Am. J. Clin. Nutr.* 110, 1502–1509. doi: 10.1093/ajcn/nqz234

Laitinen, M. H., Ngandu, T., Rovio, S., Helkala, E. L., Uusitalo, U., Viitanen, M., et al. (2006). Fat intake at midlife and risk of dementia and Alzheimer's disease: a population-based study. *Dement. Geriatr. Cogn. Disord.* 22, 99–107. doi: 10.1159/000093478

Lee, T.-M. (2017). *Effects of Extracting Methods on the Quality of Soft-Shelled Turtle Oil*, Ph.D.thesis. Taichung: Hongguang University of Science and Technology.

Lin, T. C., Wang, S. H., Huang, C. C., Lai, Y. C., Song, T. Y., and Tsai, M. S. (2018). Anti-fatigue, antioxidation, and anti-inflammatory effects of eucalyptus oil aromatherapy in swimming-exercised rats. *Chin. J. Physiol.* 61, 257–265. doi: 10.4077/cjp.2018.bag572

Montori-Grau, M., Minor, R., Lerin, C., Allard, J., Garcia-Martinez, C., de Cabo, R., et al. (2009). Effects of aging and calorie restriction on rat skeletal muscle glycogen synthase and glycogen phosphorylase. *Exp. Gerontol.* 44, 426–433. doi: 10.1016/j.exger.2009.03.005

Moreira, L. D., Oliveira, M. L., Lirani-Galvao, A. P., Marin-Mio, R. V., Santos, R. N., and Lazaretti-Castro, M. (2014). Physical exercise and osteoporosis: effects of different types of exercises on bone and physical function of postmenopausal women. *Arq. Bras. Endocrinol. Metabol.* 58, 514–522. doi: 10.1590/0004-2730000003374

Moustafa, A., and Arisha, A. H. (2020). Swim therapy-induced tissue specific metabolic responses in male rats. *Life Sci.* 262:118516. doi: 10.1016/j.lfs.2020.118516

Muhammad, T., Ikram, M., Ullah, R., Rehman, S. U., and Kim, M. O. (2019). Hesperetin, a citrus flavonoid, attenuates LPS-induced neuroinflammation, apoptosis and memory impairments by modulating TLR4/NF-kappaB signaling. *Nutrients* 11:648. doi: 10.3390/nu11030648

Neeper, S. A., Gomez-Pinilla, F., Choi, J., and Cotman, C. W. (1996). Physical activity increases mRNA for brain-derived neurotrophic factor and nerve growth factor in rat brain. *Brain Res.* 726, 49–56. doi: 10.1016/0006-8993(96)00273-9

Reimers, A., and Ljung, H. (2019). The emerging role of omega-3 fatty acids as a therapeutic option in neuropsychiatric disorders. *Ther. Adv. Psychopharmacol.* 9:2045125319858901.

Sabatini, S., Ukoumunne, O. C., Martyr, A., Ballard, C., Lamont, R. A., Collins, R., et al. (2021). Relationship between self-perceptions of aging and 'living well' among people with mild-to-moderate dementia: findings from the ideal programme. *Arch. Gerontol. Geriatr.* 94:104328. doi: 10.1016/j.archger.2020.104328

Shih, W. L., Chang, C. D., Chen, H. T., and Fan, K. K. (2018). Antioxidant activity and leukemia initiation prevention in vitro and in vivo by N-acetyl-L-cysteine. *Oncol. Lett.* 16, 2046–2052.

Siedner, M. J. (2019). Aging, health, and quality of life for older people living with HIV in Sub-Saharan Africa: a review and proposed conceptual framework. *J. Aging Health* 31, 109–138. doi: 10.1177/0898264317724549

Simioni, C., Zauli, G., Martelli, A. M., Vitale, M., Sacchetti, G., Gonelli, A., et al. (2018). Oxidative stress: role of physical exercise and antioxidant nutraceuticals in adulthood and aging. *Oncotarget* 9, 17181–17198. doi: 10.18632/oncotarget.24729

Song, C., and Zhao, S. (2007). Omega-3 fatty acid eicosapentaenoic acid. a new treatment for psychiatric and neurodegenerative diseases: a review of clinical investigations. *Expert. Opin. Investig. Drugs* 16, 1627–1638. doi: 10.1517/13543784.16.10.1627

Taiwan Food and Drug Administration (2020). *Draft "Restrictions on the Use of Fish Oil as a Food Material*, Taipei: Taiwan Food and Drug Administration.

Tanaka, H., Bassett, D. R. Jr., Howley, E. T., Thompson, D. L., Ashraf, M., and Rawson, F. L. (1997). Swimming training lowers the resting blood pressure in individuals with hypertension. *J. Hypertens.* 15, 651–657. doi: 10.1097/00004872-199715060-00012

Tong, L., Shen, H., Perreau, V. M., Balazs, R., and Cotman, C. W. (2001). Effects of exercise on gene-expression profile in the rat hippocampus. *Neurobiol. Dis.* 8, 1046–1056. doi: 10.1006/nbdi.2001.0427

US Foof & Drug Administration (2005). *Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers*, Rockville, MD: US Foof & Drug Administration.

Vecchione, C., Gentile, M. T., Aretini, A., Marino, G., Poulet, R., Maffei, A., et al. (2007). A novel mechanism of action for statins against diabetes-induced oxidative stress. *Diabetologia* 50, 874–880. doi: 10.1007/s00125-007-0597-0

Villani, A. M., Crotty, M., Cleland, L. G., James, M. J., Fraser, R. J., Cobiac, L., et al. (2013). Fish oil administration in older adults: is there potential for adverse events? A systematic review of the literature. *BMC Geriatr.* 13:41. doi: 10.1186/1471-2318-13-41

Yamanaka, M., Shirakawa, J., Ohno, R., Shinagawa, M., Hatano, K., Sugawa, H., et al. (2016). Soft-shelled turtle eggs inhibit the formation of AGEs in the serum and skin of diabetic rats. *J. Clin. Biochem. Nutr.* 58, 130–134. doi: 10.3164/jcbn.15-131

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.

frontiers in Cardiovascular Medicine

ORIGINAL RESEARCH
published: 16 August 2021
doi: 10.3389/fcvm.2021.705282

Effects of Combined High-Protein Diet and Exercise Intervention on Cardiometabolic Health in Middle-Aged Obese Adults: A Randomized Controlled Trial

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Specialty section:
This article was submitted to Cardiovascular Metabolism, a section of the journal Frontiers in Cardiovascular Medicine

Received: 10 May 2021
Accepted: 19 July 2021
Published: 16 August 2021

Citation:
Chen C-N, Hsu K-J, Chien K-Y and Chen J-J (2021) Effects of Combined High-Protein Diet and Exercise Intervention on Cardiometabolic Health in Middle-Aged Obese Adults: A Randomized Controlled Trial. Front. Cardiovasc. Med. 8:705282. doi: 10.3389/fcvm.2021.705282

Background: Obesity is the main risk factor of cardiovascular diseases (CVD) and metabolic diseases. The middle-aged population is the age group with the highest prevalence of obesity. Thus, improving cardiometabolic health is important to prevent CVD and metabolic diseases in middle-aged obese adults. The aim of this study was to examine the effects of exercise alone or in combination with a high-protein diet on markers of cardiometabolic health in middle-aged adults with obesity.

Methods: Sixty-nine middle-aged adults with obesity were assigned randomly to the control group (C; $n = 23$), exercise group (E; $n = 23$), or exercise combined with high-protein diet group (EP; $n = 23$). Individuals in the E and EP groups received supervised exercise training and individuals in the EP group received high-protein diet intervention. Body composition (assessed by dual-energy X-ray absorptiometry), oral glucose tolerance test (OGTT), lipid profiles, and inflammatory markers were determined before and after 12 weeks of intervention. Insulin sensitivity index ($ISI_{0,120}$) was calculated from values of fasting and 2-h insulin and glucose concentration of OGTT. Insulin-peak-time during the OGTT was recorded to reflect β-cell function. Analysis of covariance with baseline values as covariates was used to examine the effects of the intervention. The significant level was set at 0.05.

Results: After 12 weeks of intervention, the E group had a greater percentage of individuals with early insulin-peak-time during the OGTT than that in the C and EP groups ($p = 0.031$). EP group had lower total cholesterol and triglycerides than that in the C group ($p = 0.046$ and 0.014, respectively). Within-group comparisons showed that the 2-h glucose of OGTT and C-reactive protein decreased in the EP group ($p = 0.013$ and 0.008, respectively) but not in the E and C groups; insulin sensitivity improved in the EP group ($p = 0.016$) and had a trend to improve in the E group ($p = 0.052$); and abdominal fat mass and total body fat mass decreased in both intervention groups ($p < 0.05$).

Conclusion: Combined high-protein diet and exercise intervention significantly decreased fat mass and improved lipid profiles, insulin sensitivity, glucose tolerance, and inflammation in middle-aged adults with obesity.

Clinical Trial Registration: Thai Clinical Trials Registry, TCTR20180913003, 13-09-2018.

Keywords: obesity, glucose homeostasis, insulin, oral glucose tolerance test, aerobic exercise

INTRODUCTION

Obesity is the main risk factor of metabolic diseases, cardiovascular diseases (CVD), and some types of cancer (1–3). The middle-aged population is the age group with the highest prevalence of obesity (4). Therefore, establishing effective evidence-based strategies to decrease CVD risks in the middle-aged population with obesity is a major research priority.

Biomarkers of cardiometabolic health include insulin sensitivity, lipid profiles, and markers of inflammation (5, 6). Increased visceral fat of individuals with obesity causes glucose intolerance, impaired lipid profiles, and greater inflammatory cytokines than individuals with normal weight (7–9). A meta-analysis reported that the risk of CVD mortality increased 13% per 88.5 mg/dl triglycerides (TG) increment (5). Postmenopausal women with the highest levels of total cholesterol (CHOL) combined with the highest levels of C-reactive protein (CRP) were 5 times at risk for CVD than individuals with the lowest levels of CHOL combined with the lowest levels of CRP (10).

Exercise and nutrition are fundamental strategies to improve the cardiometabolic health of individuals. Exercise, especially aerobic exercise, decreases fat mass and inflammation and improves lipid profile and insulin sensitivity in overweight/obese individuals regardless of age (11–13). Calorie restriction is a commonly used diet intervention for individuals with obesity. However, long-term engagement of calorie restriction for most middle-aged individuals is challenging. Recently, increasing evidence demonstrated the benefit of high-protein diet in individuals with obesity. Studies found that high-protein diet (20–30% energy intake from protein) increased satiety and induced sustained reductions in appetite (14, 15). In addition, high-protein diets ($\geq$25% energy intake from protein) have been shown to decrease body weight and fat mass and improve insulin sensitivity and blood pressure in individuals with obesity and patients with diabetes (16, 17).

Collectively, obesity in the middle-aged population requires attention and management to prevent obesity-related diseases. Research has shown that both exercise and high-protein diets have potential to be beneficial for the cardiometabolic health of individuals with obesity (13, 17). While many studies investigating the effects of exercise and nutrition found that high-protein diets/protein supplementation combined with exercise have stronger beneficial effects on skeletal muscle functions and physical functions than exercise alone (18, 19), the knowledge about the effects of exercise alone or in combination with high-protein diet on markers of the cardiometabolic health is lacking. In this regard, this study aimed to examine the effects of exercise alone or in combination with high-protein diet on markers of cardiometabolic health in middle-aged adults with obesity.

METHODS

Participants

The complete description of participant recruitment and randomization has been previously published (19). Briefly, 69 middle-aged (range: 50–64 years old; mean: 58.3 $\pm$ 0.5 years old) individuals (63 females and 6 males) with obesity (body mass index (BMI): 27.7 $\pm$ 0.4 kg/m^2; body fat: 41.5 $\pm$ 0.6%; waist circumference: 90.9 $\pm$ 1.0 centimeters) were randomly assigned into the control group (C), the exercise group (E) or the exercise combined with high-protein diet group (EP) and received 12 weeks of intervention. The prevalence of hypertension and type 2 diabetes mellitus (T2DM) was similar among the 3 groups ($\sim$1/3 hypertension, and 1/5 T2DM). Participants in the EP group had a 4 times higher prevalence of dyslipidemia (35%) than participants in the C (9%) and E groups (9%). This study was approved by the Institutional Review Board of the National Yang-Ming University and all the participants signed the consent form (IRB number: YM-106064F-1).

Exercise Intervention

As shown in the **Figure 1**, individuals in the E and EP groups received supervised exercise training on the spin bikes in a fitness room. The duration of each session was 60 min, including 10 min of warm-up, 30 min of high-intensity interval training (HIIT), and 20 min of cool-down and stretching. High-intensity interval training consisted of 5 cycles of 3-min of high-intensity exercise and 3-min of low-intensity exercise. The high-intensity was set at 90% of HR_{peak} or at 15 of the Borg 6-to-20 rating of perceived exertion (RPE) scale. The low-intensity was set at 70% HR_{peak} or at 12 of the RPE scale. A graded exercise test on a cycle ergometer with a metabolic system (Cortex Metalyzer 3B, Germany) was used to determine the HR_{peak}. Heart rate monitors (Polar RS400, USA) were used to ensure participants exercised at the target exercise intensity. The average attendance rate of exercise training of the E and EP groups was 90 and 94%, respectively. The average HR at the high-intensity component of HIIT was 91% HR_{peak} and 90% HR_{peak} for the E and the EP groups, respectively. The attendance rates and training intensity were similar between the E and the EP groups.

Diet Intervention

The target protein intake of this study was 1.6 g/kg/day, which is higher than the current recommended dietary allowance (RDA)

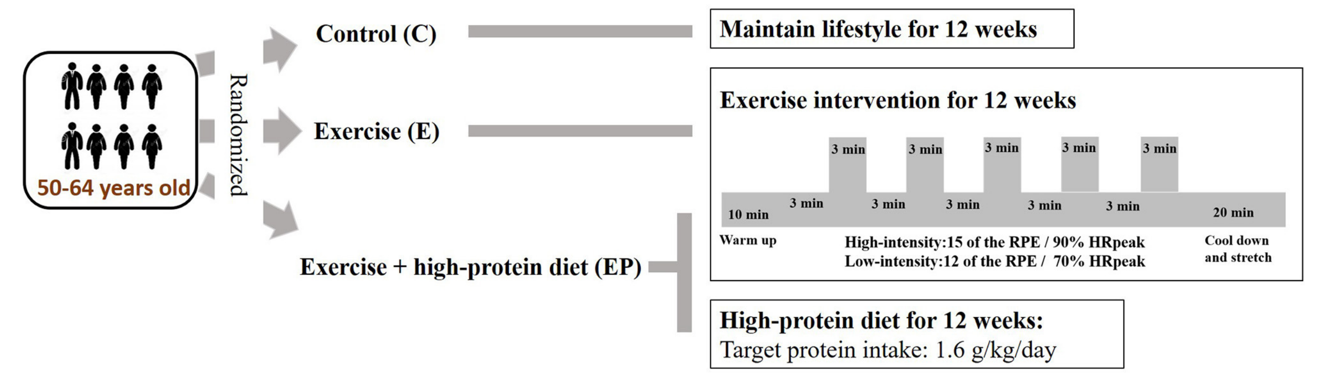

FIGURE 1 | Study design.

of protein for adults at 0.8 g/kg/day (20). Daily protein intake at 1.6 g/kg body weight (BW) was chosen because 1.6 g/kg/day of protein intake was shown to protect lean mass in response to exercise– and/or dietary-induced weight loss and to maximize the increase of muscle mass in healthy adults with exercise (21, 22). In the current study, whey protein powder supplements (M Power All WHEY plain, BriPower International Enterprise, Taiwan) was provided to participants to help them reach the target daily protein intake because our pilot study found that it was difficult for Asian middle-aged people to achieve 1.6 g/kg/day of protein intake with the traditional diet. The whey protein supplementation contained 50% of essential amino acid and 50% of non-essential amino acid. It provided 20 g of branch-chain amino acid (46% of leucine, 28% of isoleucine, and 26% of valine) per 100 g of protein. The target calorie intake was 25 kcal/kg body weight (Health Promotion Administration, Ministry of Health, and Welfare, Taiwan). Participants in the EP group were asked to record their daily food and beverage intake and upload the information to an application (Cofit, Cofit Healthcare Inc., Taiwan). A licensed dietitian analyzed the nutritional composition of the dietary intake of participants based on the report that participants uploaded and provided the diet education and consultation for participants. The dietitian also calculated the insufficient protein intake and gave the appropriate amount of protein powder so that participants could reach the target protein intake of the study. At baseline, the average daily energy and protein intakes were not significantly different among groups. Diet intervention increased the protein intake of participants in the EP group to the level of 1.5 ± 0.05 g/kg/day. In addition, individuals in the EP group had greater percentage of energy intake from protein and lower percentage of energy intake from carbohydrate than that in the C and the E groups (**Figure 2**).

Outcome Measurement

Body Composition

Body height and body weight were assessed before and after the 12-week intervention. Body composition (fat mass and muscle mass) was assessed by dual-energy X-ray absorptiometry (DXA) (Lunar iDXA, GE Medical System, Madison, WI).

Oral Glucose Tolerance Test

Oral glucose tolerance test (OGTT) challenges glucose homeostasis and reflects insulin sensitivity and β-cell function of individuals (23, 24). Oral glucose tolerance test was performed on participants without diabetes mellitus (DM) according to the recommendation of the World Health Organization. Specifically, individuals drank 300 ml of water with 75 g of glucose in 5 min after overnight fasting. Blood samples were taken at baseline and at 30, 60, and 120 min after drinking the glucose water. Plasma glucose and serum insulin levels were determined by the hexokinase glucose-6-phosphate dehydrogenase (G-6-PDH) method and the immunoradiometric assay, respectively. The area under curves (AUCs) of glucose and insulin levels at different time points were calculated by the trapezoid method.

The insulin-peak-time was determined as the time point that insulin concentration reached its highest value during the OGTT. The insulin-peak-time reflects the function of beta cells (25, 26). In addition, insulin sensitivity index$_{0,120}$ ($ISI_{0,120}$) was calculated by the following formula (27):

$$
\begin{aligned}
\text{m} &= [75,000\,\text{mg} + (\text{fasting glucose} \\
&\quad - 120\,\text{min glucose}) \times 0.19 \\
&\quad \times \text{BW}] \div 120 \\
\text{Mean plasma glucose(MPG)} &= (\text{fasting glucose} \\
&\quad + 120\,\text{min glucose}) \div 2 \\
\text{Mean serum insulin(MSI)} &= \log[(\text{fasting insulin} \\
&\quad + 120\,\text{min insulin}) \div 2] \\
\text{Metabolic clearance rate(MCR)} &= \text{m} \div \text{MPG} \\
\text{ISI}_{0,120} &= \text{MCR} \div \text{MSI}
\end{aligned}
$$

Insulin sensitivity index$_{0,120}$ has been shown to be a valid index of insulin sensitivity in various populations and has been used in many clinical studies (27–29). The higher values of the index indicate the better insulin sensitivity of individuals.

Lipid Profiles

Participants arrived laboratory at 8:00 a.m. after a 12-h overnight fast and 2 ml of venous blood was collected. Fasting serum

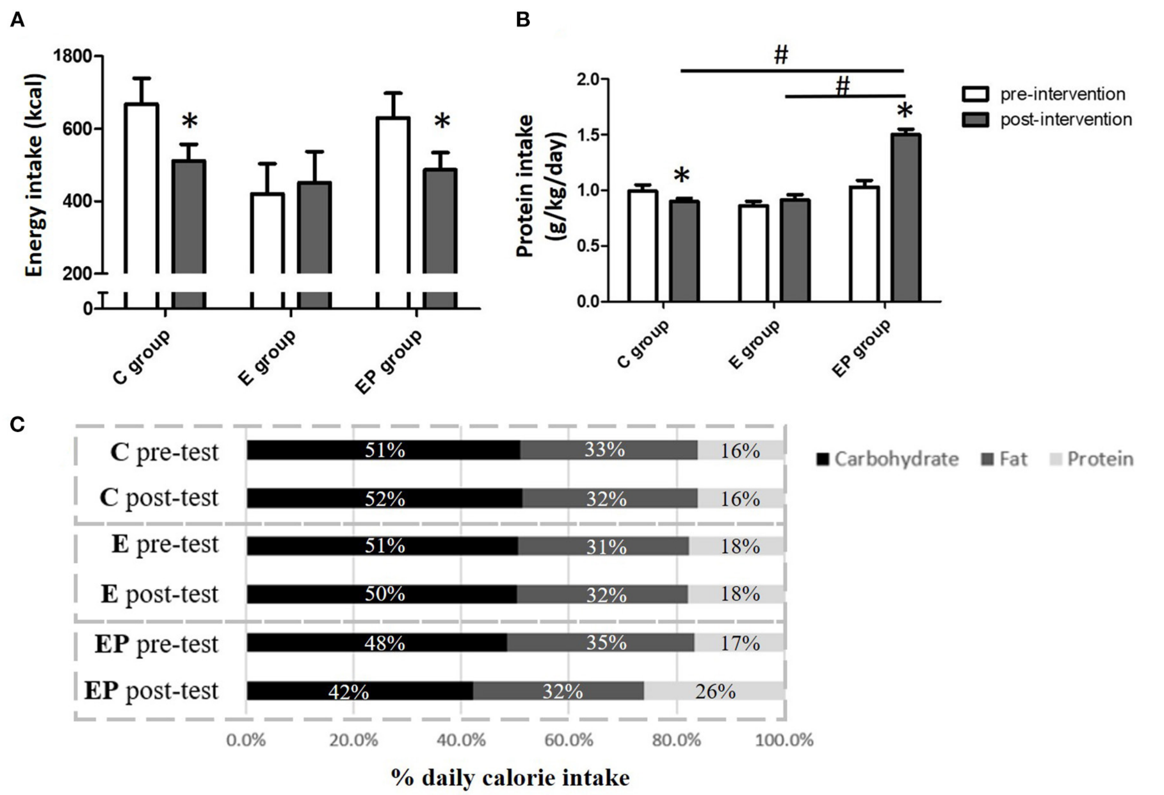

FIGURE 2 | Daily energy intake of participants before and after intervention. **(A)** Daily energy intake. **(B)** Daily protein intake. **(C)** The percentage of macronutrients of daily energy intake. Data were presented as mean ± SE or percentage. C, control group; E, exercise group; EP, exercise combined with high-protein diet group. *Significant change from the baseline. #Significantly different between groups.

CHOL, TG, low-density lipoprotein (LDL), and high-density lipoprotein (HDL) were analyzed at baseline and after the 12-weeks of intervention. All lipid content measurements were performed by the Beckman Coulter AU5820 (Beckman Coulter, Inc., Brea, CA, USA) with 300 μl of serum. Each test was performed according to the manufacturer's protocol (30, 31). The intra-assay coefficients of variation for TG, CHOL, LDL, and HDL were <2.9, <2.6, <4.7, and <5.0%, respectively. The CHOL to HDL ratio and the LDL to HDL ratio were calculated. Greater risk of cardiovascular diseases/events have been shown to be associated with higher ratio values of CHOL to HDL and LDL to HDL (32, 33).

Markers of Inflammation and Oxidative Stress

Plasma levels of C-reactive proteins were determined by the ELISA-kit (#378020 from Beckman Coulter, Brea, Calif., USA). Briefly, plasma samples (50 μl) were incubated with CRP Latex Reagent and analyzed on AU5820 auto-analyzer (Beckman Coulter, Inc., Brea, CA, USA). The intra-assay coefficients of variation (CV) for CRP was <3.0% (34).

Oxidative stress was determined by the superoxide dismutase activity in red blood cells (SOD-RBC). The RBC were lysed with four-times volumes of ice-cold HPLC-grade deionized water and centrifuge at 10000 g for 15 min at 4°C. The erythrocytes lysate was incubated in an assay medium containing phosphate buffer and the reaction was initiated by adding xanthine oxidase. The activity of SOD was determined spectrophotometrically at a wavelength of 450 nm for 20 min at 37°C. The optical density (OD) was determined by a spectrophotometer (Tecan, Männedorf, Switzerland) (35).

Statistical Analysis

Data were presented as mean ± standard error (SE). All outcome variables were tested for normal distribution by the Shapiro-Wilk test. Fasting insulin concentration, TG, LDL to HDL ratio, CHOL to HDL ratio, and CRP were log-transformed before the statistical analysis because data were not normally distributed. Differences in baseline characteristics among groups were determined by one-way analysis of variance (ANOVA) for continuous variables and by the chi-square test for categorical variables. Treatment effects were determined by analysis of covariance (ANCOVA) with baseline values as covariates. The Bonferroni test was used as the *post-hoc* test. Within-group comparisons were performed with paired *t*-test. An intention to treat analysis was used in this study where missing data were inputted with pre-test values based on the last-observation-carried-forward technique. Pearson's correlation coefficients were used to determine the relationship between daily protein intake and outcome variables.

TABLE 1 | Effects of interventions on anthropometry and body composition.

	C (n = 23; Male = 2, Female = 21)		E (n = 23; Male = 3, Female = 20)		EP (n = 23; Male = 1, Female = 22)	
	Pre	**Post**	**Pre**	**Post**	**Pre**	**Post**
Anthropometry						
Body height (cm)	157.4 ± 1.3		159.6 ± 1.9		156.9 ± 1.3	
Male	168.8 ± 5.8		173.5 ± 0.5		170 ± 0.0	
Female	156.4 ± 1.1		157.5 ± 1.7		156.1 ± 1.3	
BW (kg)	68.7 ± 2.2	68.4 ± 2.2	71.8 ± 2.5	71.0 ± 2.5	67.1 ± 2.3	66.0 ± 2.1*
Male	85.9 ± 11.6	83 ± 7.8	91.8 ± 4.7	91.4 ± 5.0	71.3 ± 0.0	68.5 ± 0.0
Female	67.1 ± 1.9	67.0 ± 2.1	68.8 ± 2.1	68.0 ± 2.0	66.9 ± 2.4	65.9 ± 2.2
BMI (kg/m^2)	27.6 ± 0.7	27.5 ± 0.7	28.1 ± 0.7	27.8 ± 0.7	27.3 ± 0.9	26.8 ± 0.8*
Male	30.0 ± 2.0	29.1 ± 0.8	30.5 ± 1.6	30.4 ± 1.7	24.7 ± 0.0	23.7 ± 0.0
Female	27.4 ± 0.7	27.4 ± 0.8	27.7 ± 0.7	27.4 ± 0.7	27.4 ± 0.9	27.0 ± 0.8
Percentage body fat (%)	41.7 ± 1.0	40.9 ± 1.0	42.7 ± 1.2	42.0 ± 1.1	40.3 ± 0.9	39.9 ± 1.1
Male	32.6 ± 0.3	30.7 ± 1.3	33.3 ± 2.1	33.2 ± 2.0	31.2 ± 0.0	27.6 ± 0.0
Female	42.6 ± 0.9	41.9 ± 0.8*	44.1 ± 1.0	43.3 ± 1.0*	40.8 ± 0.9	40.5 ± 1.0
Body composition						
AFM (kg)	2.4 ± 0.1	2.4 ± 0.1	2.6 ± 0.1	2.5 ± 0.1*	2.3 ± 0.1	2.2 ± 0.1*
Male	2.8 ± 0.4	2.4 ± 0.0	3.1 ± 0.4	3.0 ± 0.4	2.3 ± 0.0	1.8 ± 0.0
Female	2.4 ± 0.1	2.4 ± 0.1	2.6 ± 0.2	2.5 ± 0.1#	2.4 ± 0.2	2.3 ± 0.1*
TBF (kg)	27.7 ± 1.1	27.2 ± 1.1	29.8 ± 1.3	28.9 ± 1.2*	26.6 ± 1.4	25.8 ± 1.4*
Male	27.0 ± 3.6	24.4 ± 0.1	29.7 ± 2.8	29.4 ± 2.9	21.4 ± 0.0	18.2 ± 0.0
Female	27.8 ± 1.1	27.5 ± 1.2	29.8 ± 1.4	28.8 ± 1.4*	26.8 ± 1.4	26.1 ± 1.4*
ASM (kg)	16.8 ± 0.8	17.0 ± 0.7	17.4 ± 1.0	17.4 ± 1.0	16.7 ± 0.6	16.6 ± 0.6
Male	26.0 ± 4.0	25.5 ± 3.1	27.8 ± 0.8	27.5 ± 1.1	22.4 ± 0.0	22.5 ± 0.0
Female	15.9 ± 0.5	16.2 ± 0.5	15.9 ± 0.5	15.9 ± 0.5	16.4 ± 0.6	16.3 ± 0.6
TSM (kg)	38.9 ± 1.6	39.2 ± 1.5	40.0 ± 1.8	40.1 ± 1.8	38.8 ± 1.3	38.3 ± 1.2
Male	55.9 ± 8.2	55.6 ± 6.5	59.1 ± 2.9	59.0 ± 3.0	47.1 ± 0.0	47.6 ± 0.0
Female	37.3 ± 1.1	37.6 ± 1.1	37.2 ± 0.9	37.3 ± 0.9	38.4 ± 1.2	37.9 ± 1.1

Data were presented as mean ± SE. AFM, abdominal fat mass; ASM, appendicular skeletal muscle mass; BMI, body mass index; C, control group; E, exercise group; EP, exercise combined with high-protein diet group; TBF, total body fat mass; TSM, total skeletal muscle mass.
**Significant change from the baseline.*
#A trend to change significantly from the baseline ($0.1 > p \geq 0.05$).

The G*power was used to calculate the needed sample size and 22 participants were required for each group with a statistical power of 0.95, effects size of 0.25, and alpha value of 0.05. The significance level was set at $p < 0.05$. SPSS 24.0 (Statistical Package for the Social Sciences, SPSS, Chicago, USA) was used to perform statistical analyses.

RESULTS

Basic Characteristics of Participants

Overall, the average fasting glucose, fasting insulin, and $ISI_{0,120}$ of all participants was 94.2 ± 1.3 mg/dl, 13.4 ± 0.9 μIU/ml, and 19.9 ± 1.2, respectively. The average CHOL, TG, HDL, and LDL of all participants was 201.8 ± 4.2, 111.8 ± 7.8, 55.2 ± 1.4, and 123.0 ± 4.1 mg/dl, respectively. The average CRP and SOD-RBC of all participants was 0.22 ± 0.02 mg/dl and 94.1 ± 1.3 U/mg-protein. Baseline characteristics related to body composition, glucose homeostasis, lipid profiles, systematic inflammation, and oxidative stress were similar among groups, except for the 2-h glucose of OGTT. Two-hour glucose of OGTT in the E group was lower than that in the EP group ($p < 0.05$).

Effects of Interventions on Body Composition

Obesity is the main risk factor of metabolic and cardiovascular diseases (2). All parameters including BW, BMI, abdominal fat mass (AFM), total body fat mass (TBF), appendicular skeletal muscle mass (ASM), and total skeletal muscle mass (TSM) were similar among groups after intervention ($p > 0.05$). Within-group comparisons showed that all parameters of body composition did not change in the C group from the pre-test to the post-test. After the intervention, the average BW decreased 1.6% in the EP group; the average BMI decreased from 1.6% in the EP group; AFM decreased 3.6 and 3.7% in the E and EP groups, respectively ($p < 0.05$); TBF decreased 2.8 and 3.0% in the E and EP groups, respectively ($p < 0.05$). The changes of skeletal muscle mass before and after the intervention were not significant in all groups (**Table 1** and **Figure 3**).

Effects of Interventions on Glucose Homeostasis

Glucose homeostasis is a key marker of cardiometabolic health of individuals (36). We found that fasting glucose levels, fasting insulin levels, and AUCs of glucose and insulin were similar among groups after intervention ($p > 0.05$). Within-group comparisons showed that the 2-h glucose of OGTT decreased 10% in the EP group after intervention ($p < 0.05$), but the values did not change in the C and E groups after intervention ($p > 0.1$). The $ISI_{0,120}$ increased 11.5% in the EP group and had a trend to increase in the E group ($p = 0.052$) (**Table 2**). Regarding the insulin-peak-time, the percentages of individuals having insulin-peak-time at 30, 60, or 120 min of OGTT were different among groups after intervention ($p = 0.031$). In the E group, the percentage of individuals having insulin-peak-time at 30 min increased from 25 to 50%, and the percentage of individuals having insulin-peak-time at 120 min decreased from 37.5 to 12.5% after the intervention. The pattern of insulin-peak-time did not change in the C and EP groups (**Figure 4**).

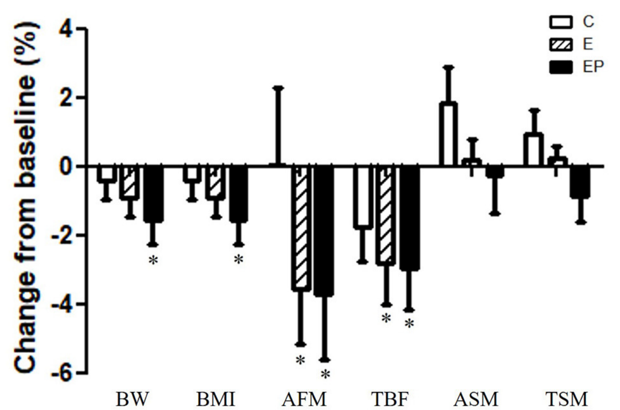

FIGURE 3 | Effects of intervention on body composition. Data were presented as mean ± SE. *Significant change from the baseline; AFM, abdominal fat mass; ASM, appendicular skeletal muscle mass; BMI, body mass index; BW, body weight; C, control group; E, exercise group; EP, exercise combined with high-protein diet group; TBF, total body fat mass; TSM, total skeletal muscle mass.

Effects of Interventions on Lipid Profiles, Systemic Inflammation, and Oxidative Stress

Dyslipidemia predicts the CVD risk (5, 10). After 12 weeks of intervention, the CHOL and TG of the EP group were significantly lower than that of the C group ($p < 0.05$). The CHOL in the EP group decreased from 204.1 ± 9.4 to 190.2 ± 10.7 mg/dl and it did not change in the C and E groups. The TG value in the C group increased from 101.2 ± 10.9 to 119.0 ± 10.3 mg/dl; whereas, it decreased from 132.4 ± 17.9 to 125.9 ± 17.6 mg/dl in the EP group. There were no group differences in HDL, LDL, the LDL to HDL ratio, and the CHOL to HDL ratio. Within-group comparisons showed that the HDL level decreased after intervention in the EP group (from 55.8 ± 2.8 to 51.1 ± 2.5 mg/dl) (**Figure 5**).

Inflammation and oxidative stress are associated with insulin resistance and vascular dysfunction (37). We found that CRP in the EP group decreased significantly ($p = 0.008$) from 0.22 ± 0.04 to 0.16 ± 0.03 mg/dl. The CRP values in the C and E group did not change significantly. The pre-test and post-test values of the CRP in the C group were 0.18 ± 0.04 and 0.19 ± 0.03, respectively. The pre-test and post-test values of the CRP in the E group were 0.25 ± 0.04 and 0.23 ± 0.04, respectively. In terms of antioxidant SOD-RBC, the level of SOD-RBC in the E group showed a tendency to increase ($p = 0.057$) after intervention. The levels of SOD-RBC in the C and EP group did not change significantly (**Figure 6**).

Correlation Between Daily Protein Intake and Outcome Measurements

We found that daily protein intake during the intervention was negatively correlated with BW ($r = -0.294$; $p = 0.015$), BMI ($r = -0.252$; $p = 0.038$), TBF ($r = -0.351$; $p = 0.003$) and AFM ($r = -0.362$; $p = 0.002$) at post-test. There were no significant correlations between daily protein intake and parameters of glucose homeostasis, lipid profiles, and CRP.

TABLE 2 | Effects of interventions on variables derived from oral glucose tolerance test.

	C		E		EP	
	Pre	Post	Pre	Post	Pre	Post
Fasting glucose (mg/dl)	94.5 ± 1.7	93.8 ± 1.8	92.8 ± 1.9	92.9 ± 1.8	95.5 ± 3.2	95.2 ± 2.5
Fasting insulin (μIU/ml)	13.1 ± 1.6	11.0 ± 0.7	15.0 ± 1.8	14.9 ± 1.2	11.8 ± 1.0	12.5 ± 1.3
2-h glucose (mg/dl)	186.5 ± 21.6	168.7 ± 17.2	133.8 ± 10.4	128.8 ± 10.7	182.9 ± 14.0	164.1 ± 15.6*
AUCs of glucose	19787.5 ± 1561.0	20332.5 ± 1736.6	16872.7 ± 925.1	16412.3 ± 881.5	20940.0 ± 1357.2	20579.3 ± 1176.0
AUCs of insulin	12761.0 ± 792.0	11557.5 ± 996.6	13340.4 ± 1754.2	12041.8 ± 1895.4	9433.8 ± 796.5	10033.5 ± 838.4
$ISI_{0,120}$	17.4 ± 3.5	19.0 ± 2.6	23.3 ± 1.9	25.7 ± 2.3#	18.3 ± 1.6	20.4 ± 1.9*

Data were presented as mean ± SE. 2-h, 2 hours. AUCs, area under curves; C, control group; E, exercise group; EP, exercise combined with high-protein diet group; ISI, insulin sensitivity index.

**Significant change from the baseline.*

#A trend to change significantly from the baseline (0.1 > p ≥ 0.05).

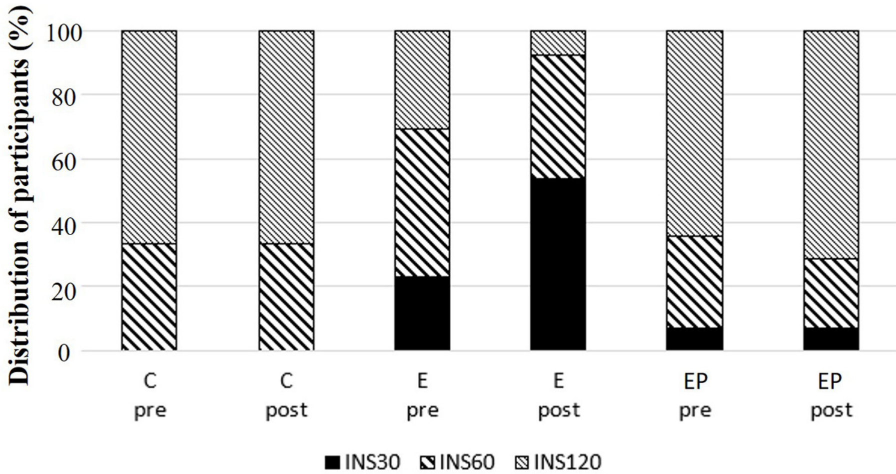

FIGURE 4 | Distribution of insulin-peak-time during oral glucose tolerance test (OGTT) among groups. C, control group; E, exercise group; EP, exercise combined with high-protein diet group. INS30: peak insulin level at 30 min of OGTT. INS60: peak insulin level at 60 min of OGTT. INS120: peak insulin level at 120 min of OGTT.

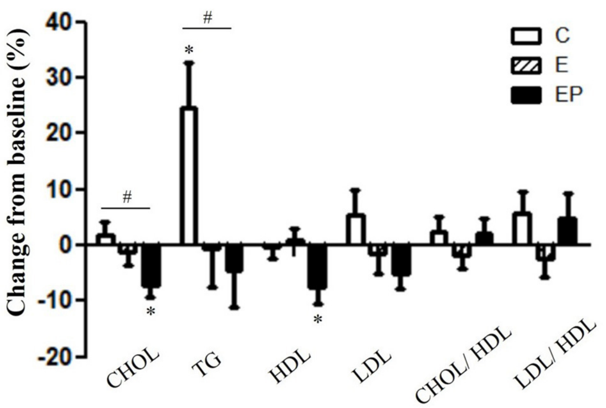

FIGURE 5 | Effects of intervention on lipid profiles. Data were presented as mean ± SE. # Significantly different between groups analyzed by ANCOVA and the Bonferroni test; *Significant change from the baseline. C, control group; CHOL, total cholesterol; E, exercise group; EP, exercise combined with high-protein diet group; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

DISCUSSION

This study examined the effects of exercise alone or in combination with high-protein diet on biomarkers of cardiometabolic health in middle-aged obese adults. The main finding is that exercise combined with high-protein diet significantly improved insulin sensitivity (IS) and decreased 2-h glucose of OGTT, CHOL, TG, and CRP levels in middle-aged obese adults. Exercise alone improved β-cell function characterized by the insulin-peak-time during the OGTT. Last, exercise reduced the fat mass of middle-aged obese adults with or without high-protein diet.

Appropriate insulin secretion and IS maintain the glucose homeostasis of individuals. In the current study, we found that IS improved significantly in the EP group and had a trend to improve in the E group. In addition, glucose levels at 2 h of the OGTT decreased 10.3% in the EP group after the intervention but the change in the E group was not significant. Both exercise and high-protein diet have been shown to improve the IS of individuals with obesity (17, 28, 38). However, mechanisms underlying the beneficial effects of exercise and high-protein diet on IS of obese individuals are different. Exercise increases IS by muscle contraction-induced increase of cellular glucose transporters (39). In contrast, high-protein diets increase IS *via* loss of body weight and insulinotropic effects of dietary protein (14, 40). Consistent with previous reports, we found daily energy intake decreased in our participants within the EP group. The BW and BMI also decreased significantly in the EP group. Our finding, the negative correlation between the daily protein intake and BW, BMI, TBF, and AFM, further supports the role of high-protein diet on loss of body weight. The high-protein diet associated greater satiety and reductions in appetite are likely the underlying mechanisms contributing to the reduced energy intake of participants in the EP group (14, 15). Thus, our findings together with others suggest that individuals can take advantage of the specificity of exercise and high-protein diet on the IS pathway to achieve the best outcomes of IS and metabolic health.

To our knowledge, this is the first study examining the effects of exercise and diet on the insulin-peak-time during the OGTT at the same time. Insulin-peak-time during the OGTT reflects β-cell function of individuals (9, 26, 41, 42). Impairment of early-phase insulin release suggests defects of insulin secretion in response to glucose. The delayed and blunted early-phase insulin response to glucose results in reduced suppression of hepatic glucose production and insufficient muscle glucose uptake (43). Praveen et al. (44) reported that individuals (with normal glucose tolerance) whose insulin–peak–time was at 60 min and at 120 min during OGTT had higher postprandial glucose levels than individuals whose insulin-peak-time was at 30 min. Sun et al. (25) followed individuals (with normal glucose tolerance

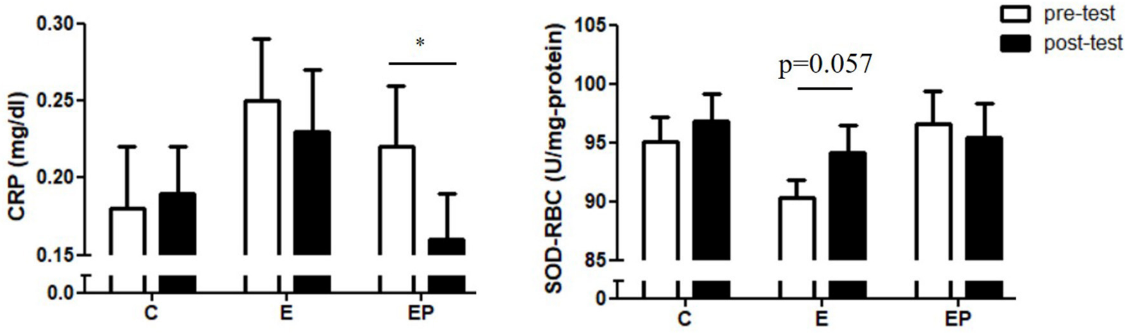

FIGURE 6 | Effects of intervention on inflammatory and oxidative stress markers. Data were presented as mean ± SE. C, control group; CRP, C-reactive protein; E, exercise group; EP, exercise combined with high-protein diet group; SOD-RBC, superoxide dismutase activity in red blood cells. $^{*}p < 0.05$.

at baseline) over a 6 years period and found that individuals with delayed insulin-peak-time during OGTT at baseline (120 and 180 min) were at a greater risk (7.3 times) for diabetes incidence compared to individuals with shorter insulin-peak-time (30 min) at baseline. In the current study where women were the majority (91%), we found that the number of participants having insulin peak at 30 min during the OGTT doubled in the E group after intervention. The beneficial effects of exercise on β-cell function were also reported in healthy men and men with pre-diabetes or T2DM (45). Although, clear mechanistic details of this phenomenon are still not understood, exercise-associated decreases of pancreatic fat are suggested as a mechanism underlying the exercise-induced benefits on β-cell function (45).

We did not see changes in the pattern of insulin-peak-time during OGTT in the EP group. This finding is unexpected. The non-significant change is likely due to a trend of poorer baseline glucose tolerance in participants of the EP group than that in the E group ($p = 0.057$). Supporting this explanation, Heiskanen et al. (45) demonstrated that exercise training increased early-phase insulin secretion rate in healthy men but not in pre-diabetic/T2DM men. Another possible explanation for the non-significant change of the insulin-peak-time during OGTT in the EP group is the protein source. Kahleova et al. (46) found that with matched energy and macronutrient composition, a meal with plant-based protein induced greater rate sensitivity (insulin secretion rate in response to the change of glucose) than a meal with meat-based protein in men with T2DM. Since the source of protein was not investigated and controlled in this study, the non-beneficial effects of exercise on insulin-peak-time during the OGTT in the EP group could be due to the increased intake of meat. Further, studies are needed to test the hypothesis.

We found that lipid profile did not significantly change in the E group, but CHOL and TG significantly reduced in the EP group. Triglyceride values have been shown to have a moderate and high association with coronary heart diseases (47). A meta-analysis of 160 randomized controlled trials (7,487 participants) showed that while exercise improved lipid profiles, the effects were gender-specific and dependent on the health condition of individuals (38). Greater exercise-related improvement in lipid profiles was reported in men than in women and in individuals with at least one health condition (T2DM, hypertension, hyperlipidemia, and metabolic syndrome) than individuals with no health condition stated above. Thus, there are two possible contributing factors to the non-significant change in lipid profile of the E group: (1) the composition of the study participants was 91% women, and (2) 61% of participants did not present with any of the identified health conditions stated above.

The decrease in TG level with high-protein diet is observed across many studies, including the current study. For instance, a meta-analysis of randomized controlled trials found that 12 weeks of high-protein diets (average protein intake: 1.3 g/kg/day; the average percentage calorie intake from protein: 30%) provided more favorable changes in TG than iso-calorically standard-protein diets (average protein intake: 0.7 g/kg/day; the average percentage calorie intake from protein: 18%) in overweight/obese adults (48). The mechanism underlying the decrease of TG with high-protein diets is likely due to the reduction of the intake of carbohydrates (CHO). In fact, it was shown that when the CHO intake accounts for more than 50% of total calorie intake, there is an elevation in blood TG level (49). Supporting the link between CHO intake and TG level, we demonstrated that the percentage of calorie intake from CHO in the EP group (42%) was significantly lower than that in the C (52%) and E group (50%). Similarly, in the meta-analysis study by Wycherley, the percentages of calorie intake from CHO in the high-protein-diets group and the iso-calorically standard-protein-diets group were 42 and 57% respectively (48). Thus, our findings together with others suggest that high-protein diet associated reduced CHO intake contributes to the reduction of TG levels.

Interestingly, we found HDL did not change in the E group and it decreased in the EP group after intervention. Most studies demonstrated an increase in HDL after exercise training (38, 50–52). A meta-analysis showed that aerobic exercise training with an average duration of 22 weeks significantly increased HDL level in women (50). Thus, the relatively short intervention period (12 weeks) might be a reason why we did not find the increase of HDL after exercise training. Another possible reason for the non-significant effects of exercise on HDL is that participants in this study had normal HDL levels at baseline (55.2 mg/dl). Most studies that found significant increase of HDL with exercise training had subjects with metabolic syndrome or low baseline HDL levels (53, 54). Similar to our finding, Keating et al. (55), showed that exercise training did not increase HDL in adults with normal HDL levels. It is unclear why HDL levels decreased significantly in the EP group, but it could be associated with the changes of HDL subpopulation profile. It was shown that large HDL particles are related with lower CVD risk, whereas, medium and small HDL particles are related with greater CVD risk (56, 57). Thus, further research that analyzes the HDL subpopulation profile is needed to clarify the effects of combined high-protein diet and exercise intervention on middle-aged obese adults. Importantly, although, we found HDL decreased in the EP group after intervention, the CHOL to HDL ratio and the LDL to HDL ratio (two better predictors for CVD morbidity than HDL alone) (32, 58, 59) did not change. This finding suggests that combined high-protein diet and exercise intervention does not increase the CVD risk.

We found that only exercise combined with high-protein diet decreased CRP of middle-aged obese adults; exercise alone did not significantly decrease CRP in this population. To date, the effects of exercise on inflammation are still inconclusive. A meta-analysis showed beneficial effects of exercise on the inflammatory status (CRP and interleukin-6) in adults with obesity (60). In contrast, another meta-analysis showed that exercise (both moderate- and vigorous-intensity) did not improve inflammatory status (CRP, tumor necrosis factor-alpha and interleukin-6) of sedentary healthy adults (38). Previous studies indicated that the decrease of systemic inflammation is associated with the degree of weight loss such that the more weight loss, the more decrease of systemic inflammation (61–63). Thus, the significant decrease of CRP in the EP group but not in the E group is likely associated with greater loss of BW in the EP group. Regarding the important role of the systemic inflammation in the cardiometabolic health, our finding implies that middle-aged obese adults could be suggested to have a high-protein diet in addition to exercise to help loss weight and improve the cardiometabolic health.

Some limitations exist in this study. First, we did not record the source of protein (plant or animal), thus, findings of this study can only suggest levels of daily protein intake. Second, only one oxidative stress marker was evaluated. A more comprehensive assessment including levels of multiple antioxidant enzymes (RBC-SOD, catalase, and glutathione peroxidase), free radical levels, and oxidative damage markers (such as serum malondialdehyde) is needed to provide solid evidence about the effects of intervention on oxidative stress of middle-aged obese adults. In summary, exercise combined with high-protein diet, but not exercise alone, improved IS, glucose tolerance, lipid profiles, and inflammation status in middle-aged obese adults. Thus, high-protein diet can be suggested to middle-aged obese adults who exercise to enhance cardiometabolic health.

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 Institutional Review Board of the National Yang-Ming University. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

C-NC and K-YC conceptualized the study, analyzed and interpreted the data, and wrote the manuscript. C-NC and K-JH coordinated and managed the project. K-JH and J-JC performed the intervention and collected the data. All authors reviewed and approved the manuscript.

FUNDING

This study was supported by the Ministry of Science and Technology, Taiwan [Grant Nos: MOST-106-2314-B-010−052 -MY3 (C-NC); MOST-109-2314-B-010−036 -MY3 (C-NC)] and partly supported by the University of Taipei for providing the DXA examination.

ACKNOWLEDGMENTS

We thank Shiow-Chwen Tsai for performing the DXA examination and helping data interpretation. We also thank the help of dietitian Ying-Fang Wang and Yun-Ru Chen in the diet intervention and Hua-Hung Yuan in the exercise intervention of this study. Last, we would like to thank all the individuals who volunteered to participate this study.

REFERENCES

1. Abdullah A, Amin F, Stoelwinder J, Tanamas S, Wolfe R, Barendregt J, et al. Estimating the risk of cardiovascular disease using an obese-years metric. *BMJ Open.* (2014) 4:e005629. doi: 10.1136/bmjopen-2014-005629
2. Nie J, Ardern C. Association between obesity and cardiometabolic health risk in Asian-Canadian sub-groups. *PLoS ONE.* (2014) 9:e107548. doi: 10.1371/journal.pone.0107548
3. Ortega FB, Lavie CJ, Blair SN. Obesity and cardiovascular disease. *Circ Res.* (2016) 118:1752–70. doi: 10.1161/CIRCRESAHA.115.306883
4. Collaborators GBDO, Afshin A, Forouzanfar MH, Reitsma MB, Sur P, Estep K, et al. Health effects of overweight and obesity in 195 countries over 25 years. *N Engl J Med.* (2017) 377:13–27. doi: 10.1056/NEJMoa1614362
5. Liu J, Zeng F, Liu Z, Zhang C, Ling W, Chen Y. Effects of blood triglycerides on cardiovascular and all-cause mortality: a systematic review and meta-analysis of 61 prospective studies. *Lipids Health Dis.* (2013) 12:159. doi: 10.1186/1476-511X-12-159

6. Sanghera DK, Bejar C, Sharma S, Gupta R, Blackett PR. Obesity genetics and cardiometabolic health: potential for risk prediction. *Diabetes Obes Metab.* (2019) 21:1088–100. doi: 10.1111/dom.13641
7. Pouliot M-C, Després J-P, Nadeau A, Moorjani S, Prud'homme D, Lupien PJ, et al. Visceral obesity in men: associations with glucose tolerance, plasma insulin, lipoprotein levels. *Diabetes.* (1992) 41:826–34. doi: 10.2337/diabetes.41.7.826
8. Monteiro R, Azevedo I. Chronic inflammation in obesity and the metabolic syndrome. *Mediators Inflamm.* (2010) 2010:289645. doi: 10.1155/2010/289645
9. Ying W, Lee YS, Dong Y, Seidman JS, Yang M, Isaac R, et al. Expansion of islet-resident macrophages leads to inflammation affecting beta cell proliferation and function in obesity. *Cell Metab.* (2019) 29:457–474 e455. doi: 10.1016/j.cmet.2018.12.003
10. Ridker PM, Hennekens CH, Buring JE, Rifai N. C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. *N Engl J Med.* (2000) 342:836–43. doi: 10.1056/NEJM200003233421202
11. Ross R, Hudson R, Stotz PJ, Lam M. Effects of exercise amount and intensity on abdominal obesity and glucose tolerance in obese adults: a randomized trial. *Ann Intern Med.* (2015) 162:325–34. doi: 10.7326/M14-1189
12. Marson EC, Delevatti RS, Prado AK, Netto N, Kruel LF. Effects of aerobic, resistance, and combined exercise training on insulin resistance markers in overweight or obese children and adolescents: a systematic review and meta-analysis. *Prev Med.* (2016) 93:211–8. doi: 10.1016/j.ypmed.2016.10.020
13. Sogaard D, Lund MT, Scheuer CM, Dehlbaek MS, Dideriksen SG, Abildskov CV, et al. High-intensity interval training improves insulin sensitivity in older individuals. *Acta Physiol.* (2018) 222:e13009. doi: 10.1111/apha.13009
14. Weigle DS, Breen PA, Matthys CC, Callahan HS, Meeuws KE, Burden VR, et al. A high-protein diet induces sustained reductions in appetite, ad libitum caloric intake, and body weight despite compensatory changes in diurnal plasma leptin and ghrelin concentrations. *Am J Clin Nutr.* (2005) 82:41–8. doi: 10.1093/ajcn/82.1.41
15. Westerterp-Plantenga MS, Lemmens SG, Westerterp KR. Dietary protein - its role in satiety, energetics, weight loss and health. *Br J Nutr.* (2012) 108(Suppl. 2):S105–112. doi: 10.1017/S0007114512002589
16. Dong JY, Zhang ZL, Wang PY, Qin LQ. Effects of high-protein diets on body weight, glycaemic control, blood lipids and blood pressure in type 2 diabetes: meta-analysis of randomised controlled trials. *Br J Nutr.* (2013) 110:781–9. doi: 10.1017/S0007114513002055
17. Yu Z, Nan F, Wang LY, Jiang H, Chen W, Jiang Y. Effects of high-protein diet on glycemic control, insulin resistance and blood pressure in type 2 diabetes: a systematic review and meta-analysis of randomized controlled trials. *Clin Nutr.* (2020) 39:1724–34. doi: 10.1016/j.clnu.2019.08.008
18. Liao CD, Tsauo JY, Wu YT, Cheng CP, Chen HC, Huang YC, et al. Effects of protein supplementation combined with resistance exercise on body composition and physical function in older adults: a systematic review and meta-analysis. *Am J Clin Nutr.* (2017) 106:1078–91. doi: 10.3945/ajcn.116.143594
19. Hsu KJ, Chien KY, Tsai SC, Tsai YS, Liao YH, Chen JJ, et al. Effects of exercise alone or in combination with high-protein diet on muscle function, aerobic capacity, and physical function in middle-aged obese adults: a randomized controlled trial. *J Nutr Health Aging*. (2021) 25:727–34. doi: 10.1007/s12603-021-1599-1
20. Joint WHO/FAO/UNU Expert Consultation. Protein and amino acid requirements in human nutrition. *World Health Organ Tech Rep Ser.* (2007) 2007:1–265.
21. Pasiakos SM, Cao JJ, Margolis LM, Sauter ER, Whigham LD, Mcclung JP, et al. Effects of high-protein diets on fat-free mass muscle protein synthesis following weight loss: a randomized controlled trial. *FASEB J.* (2013) 27:3837–47. doi: 10.1096/fj.13-230227
22. Morton RW, Murphy KT, Mckellar SR, Schoenfeld BJ, Henselmans M, Helms E, et al. A systematic review, meta-analysis and meta-regression of the effect of protein supplementation on resistance training-induced gains in muscle mass and strength in healthy adults. *Br J Sports Med.* (2018) 52:376–84. doi: 10.1136/bjsports-2017-097608
23. Stumvoll M, Mitrakou A, Pimenta W, Jenssen T, Yki-Järvinen H, Van Haeften T, et al. Use of the oral glucose tolerance test to assess insulin release and insulin sensitivity. *Diabetes Care.* (2000) 23:295–301. doi: 10.2337/diacare.23.3.295
24. Stumvoll M, Fritsche A, Häring H. The OGTT as test for beta cell function? *Eur J Clin Invest.* (2001) 31:380–1. doi: 10.1046/j.1365-2362.2001.00828.x
25. Sun Y, Han J, Lin Z, Song L, Wang C, Jia W. Delayed insulin secretion response during an OGTT is associated with an increased risk for incidence of diabetes in NGT subjects. *J Diabetes Complicat.* (2016) 30:1537–43. doi: 10.1016/j.jdiacomp.2016.07.029
26. Kim JY, Tfayli H, Bacha F, Lee S, Michaliszyn SF, Yousuf S, et al. Beta-cell function, incretin response, and insulin sensitivity of glucose and fat metabolism in obese youth: relationship to OGTT-time-to-glucose-peak. *Pediatr Diabetes.* (2020) 21:18–27. doi: 10.1111/pedi.12940
27. Gutt M, Davis CL, Spitzer SB, Llabre MM, Kumar M, Czarnecki EM, et al. Validation of the insulin sensitivity index (ISI(0,120)): comparison with other measures. *Diabetes Res Clin Pract.* (2000) 47:177–84. doi: 10.1016/S0168-8227(99)00116-3
28. Cederholm J, Wibell L. Insulin release and peripheral sensitivity at the oral glucose tolerance test. *Diabetes Res Clin Pract.* (1990) 10:167–75. doi: 10.1016/0168-8227(90)90040-Z
29. Hwu CM, Hsiao CF, Grove J, Hung YJ, Chuang LM, Chen YT, et al. Surrogate estimates of insulin sensitivity in subjects with hypertension. *J Hum Hypertens.* (2007) 21:246–52. doi: 10.1038/sj.jhh.1002137
30. Sung YC, Liao YH, Chen CY, Chen YL, Chou CC. Acute changes in blood lipid profiles and metabolic risk factors in collegiate elite taekwondo athletes after short-term de-training: a prospective insight for athletic health management. *Lipids Health Dis.* (2017) 16:143. doi: 10.1186/s12944-017-0534-2
31. Chmurzynska A, Muzsik A, Krzyzanowska-Jankowska P, Walkowiak J, Bajerska J. The effect of habitual fat intake, IL6 polymorphism, and different diet strategies on inflammation in postmenopausal women with central obesity. *Nutrients.* (2019) 11:1557. doi: 10.3390/nu11071557
32. Kinosian B, Glick H, Garland G. Cholesterol and coronary heart disease: predicting risks by levels and ratios. *Ann Intern Med.* (1994) 121:641–7. doi: 10.7326/0003-4819-121-9-199411010-00002
33. Kunutsor SK, Zaccardi F, Karppi J, Kurl S, Laukkanen JA. Is high serum LDL/HDL cholesterol ratio an emerging risk factor for sudden cardiac death? Findings from the KIHD Study. *J Atheroscler Thromb.* (2017) 24:600–8. doi: 10.5551/jat.37184
34. Goldberg L, Tirosh-Wagner T, Vardi A, Abbas H, Pillar N, Shomron N, et al. Circulating MicroRNAs: a potential biomarker for cardiac damage, inflammatory response, and left ventricular function recovery in pediatric viral myocarditis. *J Cardiovasc Transl Res.* (2018) 11:319–28. doi: 10.1007/s12265-018-9814-0
35. Ihara Y, Hayabara T, Sasaki K, Fujisawa Y, Kawada R, Yamamoto T, et al. Free radicals and superoxide dismutase in blood of patients with Alzheimer's disease and vascular dementia. *J Neurol Sci.* (1997) 153:76–81. doi: 10.1016/S0022-510X(97)00172-X
36. Lu MC, Fang WC, Li WC, Yeh WC, Shieh YH, Chen JY. The association between insulin resistance and cardiovascular disease risk: a community-based cross-sectional study among taiwanese people aged over 50 years. *Int J Environ Res Public Health.* (2020) 17:7195. doi: 10.3390/ijerph17197195
37. Heymsfield SB, Wadden TA. Mechanisms, pathophysiology, and management of obesity. *N Engl J Med.* (2017) 376:254–66. doi: 10.1056/NEJMra1514009
38. Lin X, Zhang X, Guo J, Roberts CK, Mckenzie S, Wu WC, et al. Effects of exercise training on cardiorespiratory fitness and biomarkers of cardiometabolic health: a systematic review and meta-analysis of randomized controlled trials. *J Am Heart Assoc.* (2015) 4:e002014. doi: 10.1161/JAHA.115.002014
39. Holloszy JO. Exercise-induced increase in muscle insulin sensitivity. *J Appl Physiol.* (1985) 99. (2005) 338–43. doi: 10.1152/japplphysiol.00123.2005
40. Rietman A, Schwarz J, Tome D, Kok FJ, Mensink M. High dietary protein intake, reducing or eliciting insulin resistance? *Eur J Clin Nutr.* (2014) 68:973–9. doi: 10.1038/ejcn.2014.123
41. Cersosimo E, Solis-Herrera C, Trautmann ME, Malloy J, Triplitt CL. Assessment of pancreatic β-cell function: review of methods and clinical applications. *Curr Diabetes Rev.* (2014) 10:2–42. doi: 10.2174/1573399810666140214093600
42. Cree-Green M, Xie D, Rahat H, Garcia-Reyes Y, Bergman BC, Scherzinger A, et al. Oral glucose tolerance test glucose peak time is most predictive

of prediabetes and hepatic steatosis in obese girls. *J Endocr Soc.* (2018) 2:547–62. doi: 10.1210/js.2018-00041

43. Mitrakou A, Kelley D, Mokan M, Veneman T, Pangburn T, Reilly J, et al. Role of reduced suppression of glucose production and diminished early insulin release in impaired glucose tolerance. *N Engl J Med.* (1992) 326:22–9. doi: 10.1056/NEJM199201023260104
44. Praveen EP, Chouhan S, Sahoo J, Goel SK, Dwivedi SN, Khurana ML, et al. Effect of different insulin response patterns during oral glucose tolerance test on glycemia in individuals with normal glucose tolerance. *Diabetes Technol Ther.* (2016) 18:316–26. doi: 10.1089/dia.2015.0379
45. Heiskanen MA, Motiani KK, Mari A, Saunavaara V, Eskelinen JJ, Virtanen KA, et al. Exercise training decreases pancreatic fat content and improves beta cell function regardless of baseline glucose tolerance: a randomised controlled trial. *Diabetologia.* (2018) 61:1817–28. doi: 10.1007/s00125-018-4627-x
46. Kahleova H, Tura A, Klementova M, Thieme L, Haluzik M, Pavlovicova R, et al. A plant-based meal stimulates incretin and insulin secretion more than an energy- and macronutrient-matched standard meal in type 2 diabetes: a randomized crossover study. *Nutrients.* (2019) 11:486. doi: 10.3390/nu11030486
47. Sarwar N, Danesh J, Eiriksdottir G, Sigurdsson G, Wareham N, Bingham S, et al. Triglycerides and the risk of coronary heart disease: 10,158 incident cases among 262,525 participants in 29 Western prospective studies. *Circulation.* (2007) 115:450–8. doi: 10.1161/CIRCULATIONAHA.106.637793
48. Wycherley TP, Moran LJ, Clifton PM, Noakes M, Brinkworth GD. Effects of energy-restricted high-protein, low-fat compared with standard-protein, low-fat diets: a meta-analysis of randomized controlled trials. *Am J Clin Nutr.* (2012) 96:1281–98. doi: 10.3945/ajcn.112.044321
49. Parks EJ, Parks EJ. Changes in fat synthesis influenced by dietary macronutrient content. *Proc Nutr Soc.* (2002) 61:281–6. doi: 10.1079/PNS2002148
50. Kelley GA, Kelley KS, Tran ZV. Aerobic exercise and lipids and lipoproteins in women: a meta-analysis of randomized controlled trials. *J Womens Health.* (2004) 13:1148–64. doi: 10.1089/jwh.2004.13.1148
51. Vanhees L, Geladas N, Hansen D, Kouidi E, Niebauer J, Reiner Z, et al. Importance of characteristics and modalities of physical activity and exercise in the management of cardiovascular health in individuals with cardiovascular risk factors: recommendations from the EACPR. Part II. *Eur J Prev Cardiol.* (2012) 19:1005–33. doi: 10.1177/1741826711430926
52. Pan B, Ge L, Xun YQ, Chen YJ, Gao CY, Han X, et al. Exercise training modalities in patients with type 2 diabetes mellitus: a systematic review and network meta-analysis. *Int J Behav Nutr Phys Act.* (2018) 15:72. doi: 10.1186/s12966-018-0703-3
53. Tjønna AE, Lee SJ, Rognmo, ø., Stølen TO, Bye A, Haram PM, et al. Aerobic interval training versus continuous moderate exercise as a treatment for the metabolic syndrome: a pilot study. *Circulation.* (2008) 118:346–354. doi: 10.1161/CIRCULATIONAHA.108.772822
54. Dieli-Conwright CM, Courneya KS, Demark-Wahnefried W, Sami N, Lee K, Buchanan TA, et al. Effects of aerobic and resistance exercise on metabolic syndrome, sarcopenic obesity, and circulating biomarkers in overweight or obese survivors of breast cancer: a randomized controlled trial. *J Clin Oncol.* (2018) 36:875–83. doi: 10.1200/JCO.2017.75.7526
55. Keating SE, Machan EA, O'connor HT, Gerofi JA, Sainsbury A, Caterson ID, et al. Continuous exercise but not high intensity interval training improves fat distribution in overweight adults. *J Obes.* (2014) 2014:834865. doi: 10.1155/2014/834865
56. Rye KA, Barter PJ. Predictive value of different HDL particles for the protection against or risk of coronary heart disease. *Biochim Biophys Acta.* (2012) 1821:473–80. doi: 10.1016/j.bbalip.2011.10.012
57. Fisher G, Brown AW, Bohan Brown MM, Alcorn A, Noles C, Winwood L, et al. High intensity interval- vs. moderate intensity- training for improving cardiometabolic health in overweight or obese males: a randomized controlled trial. *PLoS ONE.* (2015) 10:e0138853. doi: 10.1371/journal.pone.0138853
58. Lemieux I, Lamarche B, Couillard C, Pascot A, Cantin B, Bergeron J, et al. Total cholesterol/HDL cholesterol ratio vs. LDL cholesterol/HDL cholesterol ratio as indices of ischemic heart disease risk in men: the Quebec cardiovascular study. *Arch Intern Med.* (2001) 161:2685–92. doi: 10.1001/archinte.161.22.2685
59. Yokokawa H, Yasumura S, Tanno K, Ohsawa M, Onoda T, Itai K, et al. Serum low-density lipoprotein to high-density lipoprotein ratio as a predictor of future acute myocardial infarction among men in a 2.7-year cohort study of a Japanese northern rural population. *J Atheroscler Thromb.* (2011) 18:89–98. doi: 10.5551/jat.5215
60. Khalafi M, Symonds ME. The impact of high-intensity interval training on inflammatory markers in metabolic disorders: a meta-analysis. *Scand J Med Sci Sports.* (2020) 30:2020–36. doi: 10.1111/sms.13754
61. Forsythe LK, Wallace JM, Livingstone MB. Obesity and inflammation: the effects of weight loss. *Nutr Res Rev.* (2008) 21:117–33. doi: 10.1017/S0954422408138732
62. Christiansen T, Paulsen SK, Bruun JM, Pedersen SB, Richelsen B. Exercise training versus diet-induced weight-loss on metabolic risk factors and inflammatory markers in obese subjects: a 12-week randomized intervention study. *Am J Physiol Endocrinol Metab.* (2010) 298:E824–831. doi: 10.1152/ajpendo.00574.2009
63. Wright CS, Zhou J, Sayer RD, Kim JE, Campbell WW. Effects of a high-protein diet including whole eggs on muscle composition and indices of cardiometabolic health and systemic inflammation in older adults with overweight or obesity: a randomized controlled trial. *Nutrients.* (2018) 10:946. doi: 10.3390/nu10070946

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frontiers in Psychiatry

ORIGINAL RESEARCH
published: 18 August 2021
doi: 10.3389/fpsyt.2021.697508

Awareness and Mental Health of Male Drug Addicts With Tuberculosis During the COVID-19 Pandemic

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Introduction: At present, the COVID-19 pandemic remains the most pressing global health issue. Given the significant amount of public awareness, the infection rate and rehabilitation efforts are governed not only by the compliance of transmission mitigation strategies but also by the understanding of coexisting diseases and COVID-19 in patients with chronic infectious diseases. The main goal of this study was to evaluate the differences in demographics, as well as awareness, risk perception, and emotional reactions, among imprisoned drug addicts with and without tuberculosis (TB) regarding their perceptions of and feelings toward the COVID-19 pandemic. The secondary goal of the study was also to measure how the psychological health and nutritional indices of the drug addicts with TB changed during their ongoing rehabilitation.

Methods: A total of 265 male drug addicts, 45 of which were positive for TB and another 220 who were negative, were selected as subjects from a mandatory detoxification and rehabilitation center (MDRC). Data were collected through a combination of questionnaires (questions regarding COVID-19 awareness, emotional knowledge and responses, and SCL-90 tests), anthropometric examination, and laboratory blood tests, with which inferential and descriptive statistical analyses were performed.

Results: During a period of 1 week in early 2021, the differences in the accuracy of the responses from the questions probing the awareness of COVID-19 symptoms, transmission, susceptible populations, what kind of mask should be worn, and preventive measures between TB addicts to non-TB addicts were 11.11 vs. 60.45%, 57.78 vs. 77.27%, 66.67 vs. 78.64%, 97.98 vs. 97.73%, and 93.33 vs. 65.91%, respectively. In the risk perception and emotional reaction sections of the questionnaire, there was a significant difference in the responses to "What you were more worried about was?" ($p < 0.001$) and "Alteration in your mood since the outbreak?" ($p = 0.002$) between the two cohorts. In the section assessing the 10 dimensions of the SCL-90 scale, there were significant differences between the TB addicts and the Chinese norm. In addition, the BMI (21.06 ± 2.65 kg/m^2) and total serum protein (77.14 ± 6.12 g/L) levels of the TB addicts were normal, but the serum hemoglobin (117.02 ± 4.97 g/L) and albumin (42.08 ± 1.81 g/L) levels were significantly lower in the TB addicts compared to the norm ($p < 0.001$).

Conclusion: The COVID-19 pandemic we are facing is both an epidemiologic and a psychological crisis. However, while the COVID-19 epidemic will eventually disappear (or

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Edited by:
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Zhejiang Normal University, China

Reviewed by:
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Beijing Sport University, China
Bhaskar LVKS,
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Specialty section:
This article was submitted to Addictive Disorders, a section of the journal Frontiers in Psychiatry

Received: *21 April 2021*
Accepted: *09 July 2021*
Published: *18 August 2021*

Citation:
Jia D, Li H and Xu Y (2021) Awareness and Mental Health of Male Drug Addicts With Tuberculosis During the COVID-19 Pandemic. Front. Psychiatry 12:697508. doi: 10.3389/fpsyt.2021.697508

become manageable, similar to the flu), the TB epidemic may still persist. To avoid the deleterious consequences of multiple simultaneous epidemics, complementary response measures to COVID-19 and TB can help curb the exacerbation of both situations and, therefore, save lives. Imprisoned drug addicts, especially those with TB, can master relevant knowledge on COVID-19.

Keywords: awareness, tuberculosis, mental health, COVID-19, immunomodulation, drug addicts, rehabilitation

INTRODUCTION

Coronavirus disease 2019 (COVID-19), caused by the novel SARS-CoV-2 virus, emerged at the end of 2019 and caused a global pandemic. The disease mainly affects the respiratory system; however, there is growing evidence that COVID-19 is a multisystem inflammatory disease that also affects the immune system, among others (1). The pathogenesis of COVID-19 commences with a delayed antiviral response followed by an immunological overreaction that results in an excessive and prolonged proinflammatory response (2). All populations are generally susceptible to contracting COVID-19 regardless of age or sex (3), and many individuals with COVID-19 develop signs and symptoms, such as mild respiratory illness and persistent fever, an average of 5–6 days after infection with an average period of 1–14 days for symptom onset (4). After the cessation of symptoms, many COVID-19 survivors who required critical care developed psychological, physical, and cognitive impairments (5).

In 2020, the COVID-19 pandemic superseded tuberculosis (TB) as the world's leading cause of death derived from infectious diseases (6). Most TB infections are asymptomatic and latent, but approximately 5–10% of infected people with TB will develop the disease each year, with pulmonary TB (PTB) being diagnosed in the majority of these cases (7). PTB is the most common type of TB; due to the structural changes in the lungs and the suppression of cell-mediated immunity caused by long-term medication, PBT patients are prone to developing secondary lung infections, which exacerbates the PTB infection and can endanger the patient's life (8). The symptoms of PTB are similar to those of COVID-19, and patients suffering from PTB who are in close contact with COVID-19 patients have a risk of being infected with the SARS-CoV-2 virus and an even greater risk of developing serious respiratory complications from COVID-19 (9). In addition, the immune resistance of TB patients is lower compared to healthy people, and the main clinical symptoms of TB are fever, cough, and myalgia or fatigue (10), which can easily cause patients to believe they are suffering from COVID-19, thereby causing worry and fear. An observational study in Wuhan found that COVID-19 was a more-common comorbidity (36%) in patients infected with mycobacterium TB (M. TB) than diabetes (25%), hypertension (22%), ischemic heart disease (8%), and COPD (6%). They also found that M. TB coinfection was linked to more severe cases and a faster progression of COVID-19, raising concerns that latent TB infections (LTBI) represent a serious, independent risk factor that can increase the susceptibility of contracting the SARS-CoV-2 virus (11).

During the current global outbreak of COVID-19, PTB patients with compromised immune systems have a greater risk of developing complications from COVID-19, in addition to the increased exposure for hospitalized PTB patients. For the incarcerated population, the risk of infection and the incidence of TB was higher than for the general population—imprisonment and residential overcrowding being the determinants of an increased risk of latent tuberculosis infection (LTBI) among TB patients (12). In particular, more attention should be paid to older contacts to avoid infection and disease (13). There exists a higher potential for rapid TB transmission in situations where inmates actively infected with TB live in poorly ventilated custodial settings (typically in developing countries) (14). Malnutrition is another common health hazard in prisons that increases the risk of TB infection (15). In general, the link between TB and COVID-19 is likely to be bidirectional, meaning that temporary immunosuppression caused by TB infections may increase the susceptibility of patients to contracting COVID-19, and COVID-19 may in turn increase the susceptibility to TB infections (9). Among many vulnerable populations, modern imprisonment centers have a significant influence on the spread and clinical outcome of infectious diseases, making these a type of social institution that ultimately determines its own health status and outcome (16).

The state of lockdown in many parts of the world has negatively affected the physical, psychological, and emotional health of individuals (17). Despite strict measures taken by governments and communities, awareness of COVID-19 remains the most important factor in limiting its widespread transmission (18). Therefore, the best treatment against COVID-19 is by preventing it. The overall aim of this study was to investigate whether drug addicts with TB demonstrate better awareness and risk perception, as well as emotional and psychological health, related to COVID-19.

MATERIALS AND METHODS

Sample Size

The minimum sample size needed to conduct a statistically accurate questionnaire for this investigation was 99, assuming a 10% margin of error and a 95% confidence level. Assuming a 10% attrition rate, 257 participants (50 TB addicts and 207 non-TB addicts) needed to be recruited for an 85% statistical power ($\alpha = 0.05$). Meanwhile, to observe a statistically significant effect from the 45 TB addicts compared to the 220 non-TB addicts, the sample size was estimated based on an effect size (*Cohen's d*) of

0.5. Therefore, a slightly larger sample size of 265 (45 TB addicts and 220 non-TB addicts) was collected in this research study after eliminating any invalid questionnaires due to incorrect filling. This sample size was found to be sufficient based on the PASS V.15 sample size calculation software (NCSS, USA).

Participants

We carried out a cross-sectional observational study of drug addicts in a mandatory detoxification and rehabilitation center (MDRC) in the Hainan Province of China between February 25 and March 1 of 2021 during the COVID-19 pandemic. In accordance with the Anti-Drug Law of the People's Republic of China, newly found illicit drug users of Chinese ethnicity aged 18 to 65 years, who were diagnosed by DSM/ICD with illegal drug abuse/dependence, were sent to the hospital to assess the severity of addiction, after which they received detoxification treatment under the supervision of community social workers outside of the hospital. If the drug users relapsed, they were sent to the MDRC, where they participated in a combination of detoxification treatment, physical exercise, and manual labor for 2 years (19). The drug addicts were screened for active PTB, isolated for standardized treatment, and monitored and supervised throughout the period of rehabilitation treatment in the MDRC. LTBI was diagnosed through a tuberculin skin test (TST), and active PTB was diagnosed based on chest radiography images. Participants who either refused to accept the TST test or had contraindications against the TST test, who suffered from serious diseases that affected mobility, or who were taking any type of psychotropic medications for treating mental health issues were excluded from the study.

Protocol

The questionnaire was designed and developed based on literature precedent, expert opinion, and informal interviews with 18 drug addicts. We assured participants that the study was voluntary, anonymous, and confidential. Included in the questionnaire was a statement that the purpose of the study was to identify the participants' feelings or experiences related to the topic of investigation. In addition, some questions within necessitated only binary answers (yes or no), and there were no "neutral/don't know" answers. The questionnaire included three parts. The first part consisted of demographic information, such as age, marital status, education, employment status, as well as smoking, diet, and physical activity habits. The second part asked about the participants' level of awareness (Q1–Q5), as well as risk perception and emotional reaction (Q1–Q3), in accordance with the clinical guideline "Diagnosis and Treatment Protocol for Novel Coronavirus Pneumonia" (Trial Fifth Edition, 2020, China). The possible answers to these questions are shown in **Table 2**. In the third part, we used the Symptom Check List 90 (SCL-90, revised Chinese version) introduced in 1984 to assess the psychological health status of the participants. This scale produced 10 primary symptom dimensions: somatization (SOM), anxiety (ANX), obsessive-compulsive disorder (OCD), depression (DEP), interpersonal sensitivity (IS), psychoticism (PSY), paranoid ideation (PAR), hostility (HOS), phobic anxiety (PHOB), and general severity index (GSI). The participants were required to report their psychological health status in the week prior to the questionnaire. After completing the series of questions and obtaining written consent, blood samples (20 mL) were collected from each participant in the study, which were used to measure hemoglobin, total serum protein, and albumin levels as nutritional indices. In addition, the BMIs (body mass indices) of the participants were recorded.

Statistical Analyses

We hypothesized that awareness and mental health differences existed between the TB and non-TB groups. To test this hypothesis, inferential and descriptive statistical analyses were performed based on the data gathered from the drug addict participants in the study, including COVID-19 awareness, risk perception, and emotional reactions, as well as psychological health status, anthropometric measures, and results from the blood tests. All of the data were collected and organized using Excel 2016 (Microsoft Corporation, Redmond, WA, USA) and transferred to SPSS version 25.0 (IBM SPSS Statistics for Windows, IBM, Armonk, NY, USA, 2017) for statistical analysis. Descriptive statistics were used for all variables. The data reported herein were presented as the mean ± SD as either a rate or constituent ratio (%). Comparisons of the data corresponding to the demographics, level of awareness, risk perception, and emotional reactions of the drug addicts were performed using both the χ^2 and Fisher's exact tests. In contrast, comparisons of the SCL-90 dimensions, BMI, and blood test data between the TB addicts and the non-TB addicts were performed using Welch's t-test. In the bilateral test, the statistical significance was indicated as $p < 0.05$.

RESULTS

The demographics of the drug addicts in this study are shown in **Table 1**. The participants varied in age from 18 to 65 years (mean 36.27 ± 13.1 years), and 31.32% of the 265 participants were 35 years old and younger. There were no significant differences between the TB and non-TB addicts with respect to age ($p = 0.287$), marital status ($p = 0.267$), and employment status ($p = 0.060$); however, a significant difference in the education ($p = 0.002$) of the TB and non-TB addicts was observed. Compared to the non-TB addicts, the TB addicts had unhealthy lifestyle habits, such as a high-salt diet ($p < 0.001$) and lack of physical activity ($p < 0.001$), and almost all addicts (99.62%) smoked.

Table 2 presents the results of the questions regarding the awareness of the COVID-19 pandemic. The awareness accuracy rates of Q1–Q5 in the survey for the TB addicts and non-TB addicts were 11.11 vs. 60.45%, 57.78 vs. 77.27%, 66.67 vs. 78.64%, 97.98 vs. 97.73%, and 93.33 vs. 65.91%, respectively. There were no significant differences in the responses to Q3 (susceptible population) ($p = 0.168$) between the TB and non-TB addicts, and more than 97% knew that a surgical mask should be worn and would be effective at preventing transmission of COVID-19 (Q4). In addition, the non-TB addict group had adequate knowledge

TABLE 1 | Demographics of the study participants.

Characteristics	Total (265)	TB (45)	Non-TB (220)	χ^2	*P*
	N **(%)**	***N*** **(%)**	***N*** **(%)**		
Age (years)				2.500	0.287
18–34	83 (31.32)	11 (24.44)	72 (32.73)		
35–54	177 (66.79)	32 (71.11)	145 (65.91)		
≥55	5 (1.89)	2 (4.44)	3 (1.36)		
Marital status				2.638	0.267
Married	104 (39.25)	13 (28.89)	91 (41.36)		
Divorced	130 (49.06)	25 (55.56)	105 (47.73)		
Single	31 (11.70)	7 (15.56)	24 (10.91)		
Education				14.761	0.002
Primary school	117 (44.15)	30 (66.67)	87 (39.55)		
Junior high school	117 (44.15)	10 (22.22)	107 (48.64)		
Senior high school	18 (6.79)	3 (6.67)	15 (6.82)		
University and above	4 (1.51)	2 (4.44)	2 (0.91)		
Employment status				7.076	0.060
Unemployed	186 (70.19)	39 (86.67)	147 (66.82)		
Self-employed	25 (9.43)	1 (2.22)	24 (10.91)		
Entertainment industry	9 (3.40)	1 (2.22)	8 (3.64)		
Semi-employed	45 (16.98)	4(8.89)	41 (18.64)		
Smoking				0.092	0.762
Yes	264 (99.62)	44 (100.00)	219 (99.55)		
No	1 (0.38)	1 (0.00)	1 (0.45)		
High-salt diet				13.121	< 0.001
Yes	198 (74.72)	24 (53.33)	174 (79.09)		
No	67 (25.28)	21 (46.67)	46 (20.91)		
Physical activity				21.270	< 0.001
Inactive to light	221 (83.40)	26 (57.78)	195 (88.64)		
Moderate	40 (15.09)	17 (37.78)[b]	23 (10.45)		
Vigorous	4 (1.51)	2 (4.44)	2 (0.91)		

of Q1 (typical symptoms) ($p < 0.001$), Q2 (transmission routes) ($p = 0.003$), and Q5 (protective measures) ($p = 0.002$).

The results of the risk perception and emotional reaction questions are shown in **Table 3**. The survey questions in this section included multiple choices. In response to Q1 (What you were more worried about was?), significantly more non-TB addicts (55.00%) chose the answer "People around me are NOT well protected" compared to the TB addicts ($p < 0.001$), but the TB addicts were more worried about "New and confirmed local cases" (66.57%). In response to Q3 (Alteration in your mood since the outbreak?), the choice of "Nervous at first, but now I'm relieved" and "Worried about being infected because of health" had significant differences ($p = 0.002$). However, there were no significant differences between the two cohorts in the responses to Q2 ($p = 0.114$).

The comparisons of the SCL-90 data between TB addicts and Chinese norm are shown in **Table 4**. The t-test results showed that the scores of the 10 dimensions of the SCL-90 scale in the TB addict group ($n = 45$) were more significant than those of the Chinese norm ($n = 12{,}160$) (20), indicating that the mental health status of the TB addicts was clearly worse than the norm. Among them, the ANX ($t = 9.273$) and DEP ($t = 8.295$) dimensions had the largest deviations between the TB addicts and the Chinese norm.

The nutritional indices results are presented in **Table 5**. There was no significant difference in the BMI ($p = 0.633$) and total serum protein levels ($p = 0.487$) between the TB addicts and the Chinese norm, but the hemoglobin and albumin levels were significantly lower in the TB addict group compared to the norm ($p < 0.001$). The effect sizes related to the differences were generally large (*Cohen's* $d > 0.3$).

DISCUSSION

The aim of this study was to explore the feelings and experiences of drug addicts with TB toward the COVID-19 pandemic, which were evaluated by assessing the responses of the drug addicts to questions regarding various physical, psychological, and emotional health parameters, such as awareness of COVID-19, risk perception, emotional reactions, and SLC-90 scores, as well as the blood serum levels of certain proteins and BMIs (nutritional status). We found that awareness and mental health

TABLE 2 | COVID-19 awareness results.

Survey question (single-choice items)	TB (45)	Non-TB (220)	χ^2	*P*
	***N* (%)**	***N* (%)**		
Q1. Symptoms of COVID-19?			48.261	< 0.001
Fever	32 (71.11)	48 (21.82)		
Cough	4 (8.89)	15 (68.19)		
Myalgia or fatigue	4 (8.89)	24 (10.91)		
All above	5 (11.11)	133 (60.45)		
Q2. Transmission routes of COVID-19?			12.312	0.003
Contact	12 (26.67)[a]	16 (72.73)		
Droplet	7 (15.56)[a,b]	34 (15.45)		
Both	26 (57.78)[b]	170 (77.27)		
Q3. Susceptible population?			3.656	0.168
All people	30 (66.67)	173 (78.64)		
Only the elderly and children	5 (11.11)	22 (10.00)		
Except for young people	10 (22.22)	25 (11.36)		
Q4. What kind of mask should be worn?			0.000	1.000
General cotton mask	1 (2.22)	5 (2.27)		
Medical surgical mask	44 (97.78)	215 (97.73)		
Q5. Which protective measures should you choose?			17.262	0.002
Drink plenty of Radix Isatidis	1 (2.22)	25 (11.36)		
Steaming vinegar	1 (2.22)	9 (4.09)		
Wearing multi-layer mask	1 (2.22)	18 (8.18)		
Wash hands frequently, go out less, and do not gather	42 (93.33)	145 (65.91)		

TABLE 3 | Risk perception and emotional reaction results.

Survey question (multiple-choice items)	TB	Non-TB	χ^2	*P*
	***N* (%)**	***N* (%)**		
Q1. What you were more worried about was?			18.833	< 0.001
New and confirmed local cases	30 (66.57)	52 (23.64)		
Number of deaths	7 (15.56)	48 (21.82)		
Shortage of personal protective equipment	22 (48.89)	56 (25.45)		
People around me are NOT well protected	21 (56.67)	121 (55.00)		
I will be infected with COVID-19	26 (57.78)	94 (42.73)		
Q2. Your judgment on the current epidemic situation?			5.959	0.114
Government has strong control	40 (88.89)	167 (75.91)		
Try to get vaccinated	32 (71.11)	193 (87.73)		
Proper protective measures for the public	41 (91.11)	132 (60.00)		
Life is gradually back on track	38 (84.44)	152 (69.09)		
Q3. Alteration in your mood since the outbreak?			14.955	0.002
Nervous at first, but now I'm relieved	36 (80.00)	187 (85.00)		
Have been nervous, afraid of the rebound	2 (4.44)	30 (13.64)		
Worried about being infected because of health	19 (42.22)	34 (15.45)		
Always believe that if you do a good job of protection, you will not get infected	40 (88.89)	189 (85.91)		

of the drug addicts with TB were significantly influenced by COVID-19. Among the 265 drug addicts who participated in this study, the number of non-TB addicts ($n = 220$) who answered the questions regarding the symptoms of COVID-19 correctly was much higher than the number of TB addicts ($n = 45$). Most TB addicts chose fever symptoms, but this might have been influenced by their knowledge of the symptoms of fever due to TB. The accuracy of the answers to the question regarding the transmission of COVID-19 (i.e., transmitted through surface contact and fluid droplets) was very high in both groups. In

TABLE 4 | SCL-90 scores (mean ± SD).

Dimension	Norm (n = 12,160)	TB (n = 45)	*t*	*Cohen's d*	*p*
SOM	1.37 ± 0.46	1.83 ± 0.51	−6.693	−1.000	< 0.001
ANX	1.51 ± 0.55	2.12 ± 0.44	−9.273	−1.110	< 0.001
OC	1.66 ± 0.58	1.77 ± 0.30	−2.443	−0.190	0.019
DEP	1.45 ± 0.53	1.86 ± 0.33	−8.295	−0.774	< 0.001
IS	1.51 ± 0.55	1.73 ± 0.32	−4.587	−0.401	< 0.001
PSY	1.34 ± 0.44	1.81 ± 0.51	−6.174	−1.068	< 0.001
PAR	1.41 ± 0.50	1.49 ± 0.25	−2.131	−0.160	0.039
HOS	1.48 ± 0.57	1.68 ± 0.26	−5.115	−0.351	< 0.001
PHOB	1.23 ± 0.39	1.64 ± 0.42	−6.538	−1.051	< 0.001
GSI	1.51 ± 0.58	1.83 ± 0.24	−8.849	−0.553	< 0.001

TABLE 5 | Nutritional indices (mean ± SD).

Nutritional indices	Norm (ranges)	TB (n = 45)	*t*	*Cohen's d*	*p*
BMI (kg/m^2)	18.5–24.0	21.06 ± 2.65	0.481	0.072	0.633
Hemoglobin (g/L)	120.0–160.0	117.02 ± 4.97	31.017	4.618	< 0.001
Total protein (g/L)	66.0–87.0	77.14 ± 6.12	−0.702	−0.100	0.487
Albumin (g/L)	38.0–51.0	42.08 ± 1.81	8.969	1.341	< 0.001

addition, more than 97% of the participants correctly answered "medical surgical mask" to the question regarding "What kind of mask should be worn?" to prevent COVID-19 transmission. Among the "protective measures" question, the number of non-TB addicts who correctly responded with "wash hands frequently, go out less, and do not gather" was significantly lower than the corresponding accuracy of the TB addicts group; however, some non-TB addicts chose to exercise and get a decent amount of sleep, which was consistent with the options in the physical activity questions in the demographics section of the questionnaire (21). The total amount of physical activity of the non-TB addicts was significantly higher than that of the TB addicts, which was justifiable because physical activity typically declines as a result of TB infection due to the impairment of pulmonary function (22).

With regard to risk perception and emotional reactions, the TB addicts were more worried about "new and confirmed local cases" and "I will be infected with COVID-19," while non-TB addicts were more worried about "people around me are NOT well protected." This might have been attributed to TB drug addicts being more concerned about their poor physical condition compared to non-TB addicts (22). When asked about their judgment on the current epidemic situation, the non-TB drug addicts were more likely to pin their hopes on "getting vaccinated," but TB addicts more consistently chose the "proper protective measures for the public" response. However, both groups generally believed that the government has a strong control of the current situation. When caring for TB patients, the primary task is to provide anti-tuberculosis treatment, after which TB patients can be vaccinated when they recover or are cured (23). When asked about how the moods of the participants were altered since the coronavirus outbreak, most of the answers for all of the participants were either "nervous at first, but now I'm relieved" and "always believe that if you do a good job of protection, you will not get infected." This indicated that the participants had overall positive attitudes about current control measures in China.

Historically, pandemics have elicited a tremendous amount of fear. Ordinary citizens may experience some psychological changes, such as fear, depression, anxiety, psychological turbulence, or uncertainty (24). Comparably, drug abusers often endure excessive stress during compulsory detoxification treatment, which is the major drug rehabilitation modality in China currently, leading to a high prevalence of anxiety symptoms (25). A previous study in China showed that the SCL-90 scale (Chinese version) was used to evaluate the psychological health of eligible drug addicts to compare with normal adults, and the results showed that the psychopathological conditions of comorbidities based on the SCL-90 scale were more severe in all of the 10 dimensions assessed in the drug addicts compared to the normal adults (26). In our study, we also came to similar conclusions, with anxiety and depression being the two dimensions with the highest scores of the 10 that were assessed. China uses the media and the internet to disseminate information related to the epidemic to the public in a timely manner to help alleviate the negative reaction of the public.

The impact of SARS-CoV-2 on long-term diseases, such as TB, is of particular concern because TB is still a serious public health concern throughout the world, especially in emerging economies (23). In 2019, TB was the leading cause of death from a single infectious pathogen worldwide. In China, the number of people who developed TB in 2019 accounted for 8.4% of the global total. In some countries, TB was the leading cause of death in prisons, mainly due to poor physical conditions, overcrowding, and lack of proper treatment (22). The risk of TB exposure and transmission in the prison population is more serious than in other environments; therefore, there are many challenges that preclude its prevention and treatment. There is a variety of complex and unstable factors that govern the transmission of TB between drug users and ordinary social groups because of the two-way flow between these two populations, such that the normal population can enter detention centers, and drug addicts can also return to society. Meanwhile, staff, visitors, and drug abusers in detention centers also may come into direct contact with drug addicts with TB, resulting in increased risk of transmission (27).

In addition to other risks, malnutrition is closely related to the incidence of TB. Protein-calorie malnutrition (PCM) is a risk factor of TB and affects TB treatment outcome (16). Low BMIs are associated with a large percentage of people with TB in developing countries. One study found that the risk of developing TB in the low weight group (BMI < 18.5) was 12.4 times higher than in the normal weight group ($18.5 \leq$ BMI < 25.0) (28). In our study, the BMI and total protein levels of TB addicts were normal, while the levels of hemoglobin and albumin in the blood were lower than average for normal Chinese people. We speculated that a reasonable diet and different diet plans according to the clinical symptoms of drug abusers can effectively improve the BMI and total protein levels. Low serum albumin levels are typically a consequence of poor nutrition, but they can also be related to abnormal liver function, as plasma proteins are biosynthesized in the liver. In addition, excessive high-calorie and high-protein diets also increase the burden on the liver. Anemia is also a condition that plagues TB patients, the pathogenesis of which is related to the inhibition of erythropoiesis through nutritional deficiency, the expression of inflammation markers, and malabsorption syndromes (29). Given these perspectives, if the world expects to eliminate the public health threat of TB by 2035, it must address the main drivers of tuberculosis: malnutrition, diabetes, poverty, smoking, and household air pollution (6).

LIMITATIONS

Our study had several limitations that should be emphasized. TSTs are widely used for TB screening, but increased age, the use of immunosuppressive therapies, and high BMI were also associated with producing false negative TB diagnoses (30). This study paid more attention to the awareness, risk perception, and emotional reactions that drug addicts with TB may have regarding COVID-19 because any abnormalities in the dimensions assessed in the SCL-90 test may be associated with psychological phenomena that are common among drug abusers. However, whether the COVID-19 pandemic had adverse psychological effects on large populations was not studied in this paper. We will further increase the number of participants, including female drug addicts, to understand the psychological development of this cohort, and we will conduct in-depth research on the nutritional health and the consistency of the nutritional cognition and behavior of the TB addicts, which can be used to more-effectively promote the rehabilitation of TB addicts and prevent the omnipresent spread of TB.

CONCLUSION

This study supported the claim that the ongoing COVID-19 pandemic is both an epidemiologic and psychological crisis. The psychologic and social consequences caused by the virus may be as equally destructive as the infection itself (22). Imprisoned drug addicts, especially those with TB, can master relevant knowledge on COVID-19. The risk perception and emotional reactions among TB addicts toward COVID-19 have begun to gradually return to normal since the pandemic outbreak began in early 2020. Most TB addicts have poor mental health, with anxiety and depression being the most common mental health issues in these patients. Therefore, routine medical follow-ups, including physical and psychological evaluations and guidance, are significantly beneficial to improve patients' awareness and prevent not only psychological crises but also TB transmission.

The susceptible population with PTB consists of addicts with low immune function. In addition to routine drug therapy, detention centers (i.e., institutions, such as prisons and MDRCs) in China also perform nutritional management for TB addicts. Quarantining TB addicts in these institutions, to some extent, is a public health measure that can reduce the risk of infection and death from TB. To corroborate this measure, no healthcare staff have since been infected with COVID-19 in imprisonment centers, and no TB patients have been co-infected with COVID-19.

The Chinese government, the National Health Commission, and relevant departments must promptly and transparently announce any treatment progress, protective measures, and future outlooks regarding the COVID-19 pandemic to the public. To avoid another global pandemic, the current COVID-19 pandemic must disappear; however, the global TB epidemic will still exist, at least for the time being. If we utilize the same response measures for mitigating the spread of TB as we do for COVID-19, we can help curb the exacerbation of both diseases and, therefore, save lives. Although the COVID-19 pandemic has changed the way people live and behave, the measures that the global society implemented to fight it will provide useful information for managing the spread of TB infections and the exacerbation of TB epidemics in the future.

DATA AVAILABILITY STATEMENT

The raw data used for statistical analysis in this study will be made available by the authors without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the ethical commission of the Hainan Provincial Administration of Detoxification. The investigation was in accordance with the latest version of the Declaration of Helsinki.

The participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

DJ and HL designed the study, DJ conducted the statistical analyses and took the lead in writing the manuscript, and YX contributed significantly to the interpretation of the results. All authors contributed to the article and approved the submitted version.

FUNDING

This work was supported by the Hangzhou Medical Leading Academic Discipline Project (2020-2024-21).

ACKNOWLEDGMENTS

We would like to thank the study participants for their time and dedication to the research.

REFERENCES

1. Taghizadeh-Hesary F, Akbari H. The powerful immune system against powerful COVID-19: A hypothesis. *Med Hypotheses*. (2020) 140:109762. doi: 10.1016/j.mehy.2020.109762
2. Castelli V, Cimini A, Ferri C. Cytokine Storm in COVID-19: "When You Come Out of the Storm, You Won't Be the Same Person Who Walked in." *Front Immunol*. (2020) 11:2132. doi: 10.3389/fimmu.2020.02132
3. Wang Y, Wang Y, Chen Y, Qin Q. Unique epidemiological and clinical features of the emerging 2019 novel coronavirus pneumonia (COVID-19) implicate special control measures. *J Med Virol*. (2020) 92:568–76. doi: 10.1002/jmv.25748
4. Celayir MF. How should be the surgical treatment approach during Covid-19 pandemic in patients with gastrointestinal cancer? *SiSli Etfal Hastan Tip Bul*. (2020) 54:136–41. doi: 10.14744/SEMB.2020.93709
5. Barker-Davies RM, O'Sullivan O, Senaratne KPP, Baker P, Cranley M, Dharm-Datta S, et al. The Stanford Hall consensus statement for post-COVID-19 rehabilitation. *Br J Sports Med*. (2020) 54:949–59. doi: 10.1136/bjsports-2020-102596
6. Chakaya J, Khan M, Ntoumi F, Aklillu E, Fatima R, Mwaba P, et al. Global tuberculosis report 2020–Reflections on the Global TB burden, treatment and prevention efforts. *Int J Infect Dis*. (2021) 7:5190–5. doi: 10.1016/j.ijid.2021.02.107
7. Cavalcanti YVN, Brelaz MCA, Lemoine Neves JK de A, Ferraz JC, Pereira VRA. Role of TNF-Alpha, IFN-Gamma, and IL-10 in the development of pulmonary tuberculosis. *Pulm Med*. (2012) 2012:1–10. doi: 10.1155/2012/745483
8. Ding R-D, Zhang H-J. Effect of linezolid on serum PCT, ESR, and CRP in patients with pulmonary tuberculosis and pneumonia. *Medicine (Baltimore)*. (2018) 97:e12177. doi: 10.1097/MD.0000000000012177
9. Udwadia ZF, Vora A, Tripathi AR, Malu KN, Lange C, Sara Raju R. COVID-19 -Tuberculosis interactions: when dark forces collide. *Indian J Tuberc*. (2020) 67:S155–62. doi: 10.1016/j.ijtb.2020.07.003
10. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet*. (2020) 395:497–506. doi: 10.1016/S0140-6736(20)30183-5
11. Chen Y, Wang Y, Fleming J, Yu Y, Gu Y, Liu C, et al. Active or latent tuberculosis increases susceptibility to COVID-19 and disease severity. *medRxiv*. (2020) 2020.03.10.20033795. doi: 10.1101/2020.03.10.20033795
12. Aguilera XP, González C, Nájera-De Ferrari M, Hirmas M, Delgado I, Olea A, et al. Tuberculosis in prisoners and their contacts in Chile: estimating incidence and latent infection. *Int J Tuberc Lung Dis*. (2016) 20:63–70. doi: 10.5588/ijtld.15.0056
13. Zhang G, Wenliang W, Yuhua Z, Zhi Z. Analysis of tuberculosis infection and risk factors among prisoners in a prison in Tianjin. *Chinese J Tuberc Prev*. (2016) 38:569–75. doi: 10.3969/j.issn.1000-6621.2016.07.011
14. Noeske J, Kuaban C, Amougou G, Piubello A, Pouillot R. Pulmonary tuberculosis in the central prison of Douala, Cameroon. *East Afr Med J*. (2006) 83: doi: 10.4314/eamj.v83i1.9357
15. Liang-Fenton D. Failing to protect: food shortages and prison camps in North Korea. *Asian Perspect*. (2007) 31:47–74. doi: 10.1353/apr.2007.0021
16. Awofeso N. Prisons as Social Determinants of Hepatitis C Virus and Tuberculosis Infections. *Public Health Rep*. (2010) 125:25–33. doi: 10.1177/00333549101250S406
17. Roy D, Tripathy S, Kar SK, Sharma N, Verma SK, Kaushal V. Study of knowledge, attitude, anxiety & perceived mental healthcare need in Indian population during COVID-19 pandemic. *Asian J Psychiatr*. (2020) 51:102083. doi: 10.1016/j.ajp.2020.102083
18. Alahdal H, Basingab F, Alotaibi R. An analytical study on the awareness, attitude and practice during the COVID-19 pandemic in Riyadh, Saudi Arabia. *J Infect Public Health*. (2020) 13:1446–52. doi: 10.1016/j.jiph.2020.06.015
19. Du J, Sun H, Huang D, Jiang H, Zhong N, Xu D, et al. Use trajectories of amphetamine-type stimulants (ATS) in Shanghai, China. *Drug Alcohol Depend*. (2014) 143:44–50. doi: 10.1016/j.drugalcdep.2014.06.031
20. Liu Y, Wu S, Li Y, Shao F, Su J, Liu X. A survey of mental symptoms of Chinese population based on SCL-90. *Chinese Ment Heal J*. (2018) 32:437–41.
21. Helgadóttir B, Forsell Y, Ekblom Ö. Physical activity patterns of people affected by depressive and anxiety disorders as measured by accelerometers: a cross-sectional study. *PLoS ONE*. (2015) 10:e0115894. doi: 10.1371/journal.pone.0115894
22. Sharma SK, Ahluwalia G. Effect of antituberculosis treatment on cardiopulmonary responses to exercise in miliary tuberculosis. *Indian J Med Res*. (2006) 124:411–8. http://www.ncbi.nlm.nih.gov/pubmed/17159261
23. Sultana J, Mazzaglia G, Luxi N, Cancellieri A, Capuano A, Ferrajolo C, et al. Potential effects of vaccinations on the prevention of COVID-19: rationale, clinical evidence, risks, and public health considerations. *Expert Rev Vaccines*. (2020) 19:919–36. doi: 10.1080/14760584.2020.1825951
24. Kamara S, Walder A, Duncan J, Kabbedijk A, Hughes P, Muana A. Mental health care during the Ebola virus disease outbreak in Sierra Leone. *Bull World Health Organ*. (2017) 95:842–7. doi: 10.2471/BLT.16.190470
25. Yang X, Kovarik C, Wang Y, Yu S. A multi-site cross-sectional study of anxiety symptoms and the associated factors among Chinese drug users undergoing compulsory detoxification treatment. *Front Public Heal*. (2021) 9:524068. doi: 10.3389/fpubh.2021.524068
26. Wang Q-L, Liu Z-M. Characteristics of psychopathology and the relationship between routes of drug administration and psychiatric symptoms in heroin addicts. *Subst Abus*. (2012) 33:130–7. doi: 10.1080/08897077.2011.630945
27. Jiménez-Fuentes MA, Augé CM, Gómez MNA, Peiró JS, de Souza Galvao ML, Maldonado J, et al. Screening for active tuberculosis in high-risk groups. *Int J Tuberc Lung Dis*. (2014) 18:1459–65. doi: 10.5588/ijtld.14.0271
28. Cegielski JP, Arab L, Cornoni-Huntley J. Nutritional Risk Factors for Tuberculosis Among Adults in the United States, 1971–1992. *Am J Epidemiol*. (2012) 176:409–22. doi: 10.1093/aje/kws007

29. Mukherjee A, Kaeley N, Dhar M, Kumar S, Bhushan B. Prevalence, characteristics, and predictors of tuberculosis associated anemia. *J Fam Med Prim Care.* (2019) 8:2445. doi: 10.4103/jfmpc.jfmpc_311_19
30. Yamasue M, Komiya K, Usagawa Y, Umeki K, Nureki S, Ando M, et al. Factors associated with false negative interferon-γ release assay results in patients with tuberculosis: a systematic review with meta-analysis. *Sci Rep.* (2020) 10:1607. doi: 10.1038/s41598-020-58459-9

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frontiers in Physiology

MINI REVIEW
published: 09 September 2021
doi: 10.3389/fphys.2021.742425

The Impact of Micronutrients-Calcium, Vitamin D, Selenium, Zinc in Cardiovascular Health: A Mini Review

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The role of micronutrients in health and disease has increased the curiosity and interest among researchers. The prime focus of this review is the significance of trace elements-calcium, vitamin D, selenium and zinc with cardiovascular health. WHO identified cardiovascular diseases (CVD) as the leading cause of deaths globally. Identifying the risk factors that could be modified and creating new treatment strategies remains to be the main concern for CVD prevention. The data that showed the relationship between trace elements and various ways in which they may contribute to cardiovascular health and disease from clinical trials and observational studies were collected from databases such as PubMed and Embase. Based on these collected data, it shows that either high or low circulating serum levels can be associated with the development of cardiovascular diseases. Micronutrients through diet contribute to improved cardiac health. However, due to our current lifestyle, there is a huge dependency on dietary supplements. Based on the observational studies, it is evident that supplements cause sudden increase in the circulating levels of the nutrients and result in cardiovascular damage. Thus, it is advisable to restrict the use of supplements, owing to the potent risks it may cause. In order to understand the exact mechanism between micronutrients and cardiac health, more clinical studies are required.

Keywords: cardiac health, micronutrient, selenium, zinc, calcium, vitamin D

OPEN ACCESS

Edited by:
Lebaka Veeranjaneya Reddy, Yogi Vemana University, India

Reviewed by:
Ming Thong Ong, Universiti Sains Malaysia (USM), Malaysia
Young-Jung Wee, Yeungnam University, South Korea

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Specialty section:
This article was submitted to Integrative Physiology, a section of the journal Frontiers in Physiology

Received: *16 July 2021*
Accepted: *23 August 2021*
Published: *09 September 2021*

Citation:
Narayanam H, Chinni SV and Samuggam S (2021) The Impact of Micronutrients-Calcium, Vitamin D, Selenium, Zinc in Cardiovascular Health: A Mini Review. Front. Physiol. 12:742425. doi: 10.3389/fphys.2021.742425

INTRODUCTION

A staggering 31% of mortality worldwide can be primarily due to cardiovascular disease. Nearly 85% of these deaths are due to heart attacks and strokes. A combination of risk factors like unhealthy diet and obesity, physical inactivity, hyperlipidemia, stress, and hereditary factors contribute to developing cardiovascular diseases (CVD) (Roth et al., 2017; World Health Organization, 2021). Identifying the risk factors that can be modified and treatments remains to be a main concern for CVD prevention. The pivotal role micronutrients and trace elements play in health and disease has paved the way for numerous scientific research. Additionally, studies on how supplementation might improve health status have gained immense popularity (Gać et al., 2021). There seems to be an uncertainty in deciding if the supplements confer overall health or if they work only in parts. It cannot be said that all the health supplements are 100% safe to use.

The current review analyses the importance and effects of some micronutrients and trace elements and their supplements on cardiovascular health as illustrated in **Figure 1**.

Biological Role of Calcium in Bone Health and Cardiovascular System

Calcium plays an important role in the biological system but is a pivotal mineral in bone health and cardiovascular system (Cano et al., 2018). The daily dietary intake of calcium for both men and women is 1,200–1,500 mg. Milk and milk products, green leafy vegetables that include kale, spinach etc. and fortified flour are the major sources of calcium. To achieve the adequate serum levels through diet alone is quite challenging which is why many people opt for calcium supplements independently or in conjunction with vitamin D to achieve the adequate serum levels and enhance bone health (Michos et al., 2021). There have been mixed results on the role of calcium and its supplements in cardiovascular health based on numerous observational and clinical trial studies over the time. Concentration of extracellular calcium directly influences cell membrane potentials of all excitable tissues especially heart and nerves. Calcium is important for muscle contraction, including the myocardium (Elsevier, 2021). In a study conducted on patients admitted for acute coronary syndrome, it was observed that hypocalcaemia was independently correlated with poor long term cardiovascular outcomes (Wang et al., 2010). Similarly, Schmitz et al. (2021) have also postulated in their study that decreased serum calcium levels were independently linked with increased mortality after acute myocardial infarction, but no proof is available to show that calcium supplementation would benefit. In some animal studies, excess calcium intake lowered blood cholesterol level of rats by inhibiting the absorption of fatty acids. Similarly studies on humans showed a better regulation of blood pressure, decreased lipid levels and improved glycemic control all of which protect against development of CVD (Rautiainen et al., 2013). Calcium supplements proved beneficial and reduced mortality rate in a cohort study done on post-menopausal women (Shin et al., 2012). Lewis et al. (2015) in a meta-analysis study documented that increased calcium supplements did not significantly augment the risks for myocardial infarction, angina pectoris and acute coronary syndrome in elderly women. In their meta-analysis Wang et al. (2021) have reported that calcium intake either lesser or more than 800 mg per day was related with increasing risk of cardiovascular mortality and they did not find any evidence to associate calcium supplements with cardiovascular mortality. Mursu et al. (2011) and Prentice et al. (2013) in their cohort studies on women have reported that participants taking calcium exhibited lowered risk of developing cardiac diseases. Wang et al. (2021) reported that calcium supplements were not responsible for vascular calcification in either men or women.

On the contrary, increase in the levels of serum calcium is correlated with a potential risk of CVD like hypertension, myocardial infarction (MI) etc. and there are several meta-analysis reports in support (Kwak et al., 2014; Reid et al., 2016). Studies have shown that hypercalcemia promotes vascular calcification including coronary artery and aortic calcification and increases the formation of coronary artery plaque (Shin et al., 2012; Kwak et al., 2014). An analysis of 2,742 atherosclerotic adults revealed that increasing the dietary consumption of calcium decreased the risk of incident coronary atherosclerosis whereas substituting with calcium supplements increased the risk by 22% (Anderson et al., 2016). Hypercalcemia and hypercalciuria was reported by Gallagher et al. (2014) in participants who were taking the recommended calcium intake of 1,200 mg/day. In a meta-analysis of observational studies Chung et al. (2016) indicated that there is no association of dietary calcium on development of cardiovascular diseases including strokes and coronary heart disease (CAD) while supplement of calcium may surge the risk of coronary heart disease especially myocardial infarction. Lewis et al. (2015) in their meta-analysis of randomized controlled trials described that supplementing calcium either independently or along with vitamin D increased the incidence of coronary heart disease in older women. Larsson et al. (2017) have reported that a higher serum calcium level led to genetic susceptibility and was correlated with the increased threat of CAD and myocardial infarction. Calcium supplements cause a surge in the ionized and total serum calcium levels in a short interval of time after ingestion and this increases the levels of insoluble calciprotein particles which are calcification activators in the serum that may lead to vascular calcification and myocardial infarction. Whereas calcium coming from dietary sources results in a slow and controlled increase in calcium levels (National Institutes of Health, 2011).

Since there are studies reporting the downside of increased calcium levels, especially on cardiovascular health, there is some concern regarding calcium supplements. In view of the increasing concerns, it would be advisable to rely on the dietary sources of calcium to meet the daily recommended levels and limit the usage of supplements.

Correlation of Low Level of Vitamin D With the Risk of Cardiovascular Diseases

Cholecalciferol or vitamin D3 is a steroid hormone naturally available, and it can also be obtained from consumption of seafood especially salmon, tuna and eel, cow milk, and eggs which are rich sources of vitamin D. Vitamin D is absorbed in the small intestine. The skin naturally synthesizes cholecalciferol from 7-dehydrocholesterol when exposed to ultraviolet B rays (UVB). Cholecalciferol is hydroxylated to 25-hydroxyvitamin D [25(OH)D], the major metabolite that circulates in blood. It is transformed into 125-dihydroxyvitamin D (calcitriol) the active form in the kidneys by the 1α-hydroxylase enzyme.

Deficiency of vitamin D is a global problem and some of the causes included is diminishing synthesis in the skin due to aging, hyperpigmentation, low dietary intake, limited exposure to sun, pollution, smoking, obesity (Norman and Powell, 2014). The daily recommended dietary allowance of vitamin D for individuals aged between 1 and 70 years is 600 IU and for adults older than 70 years it is 800 IU/day. This value accounts to a serum 25-hydroxyvitamin D level of 20 ng/mL or greater based on bone health (National Institutes of Health, 2011).

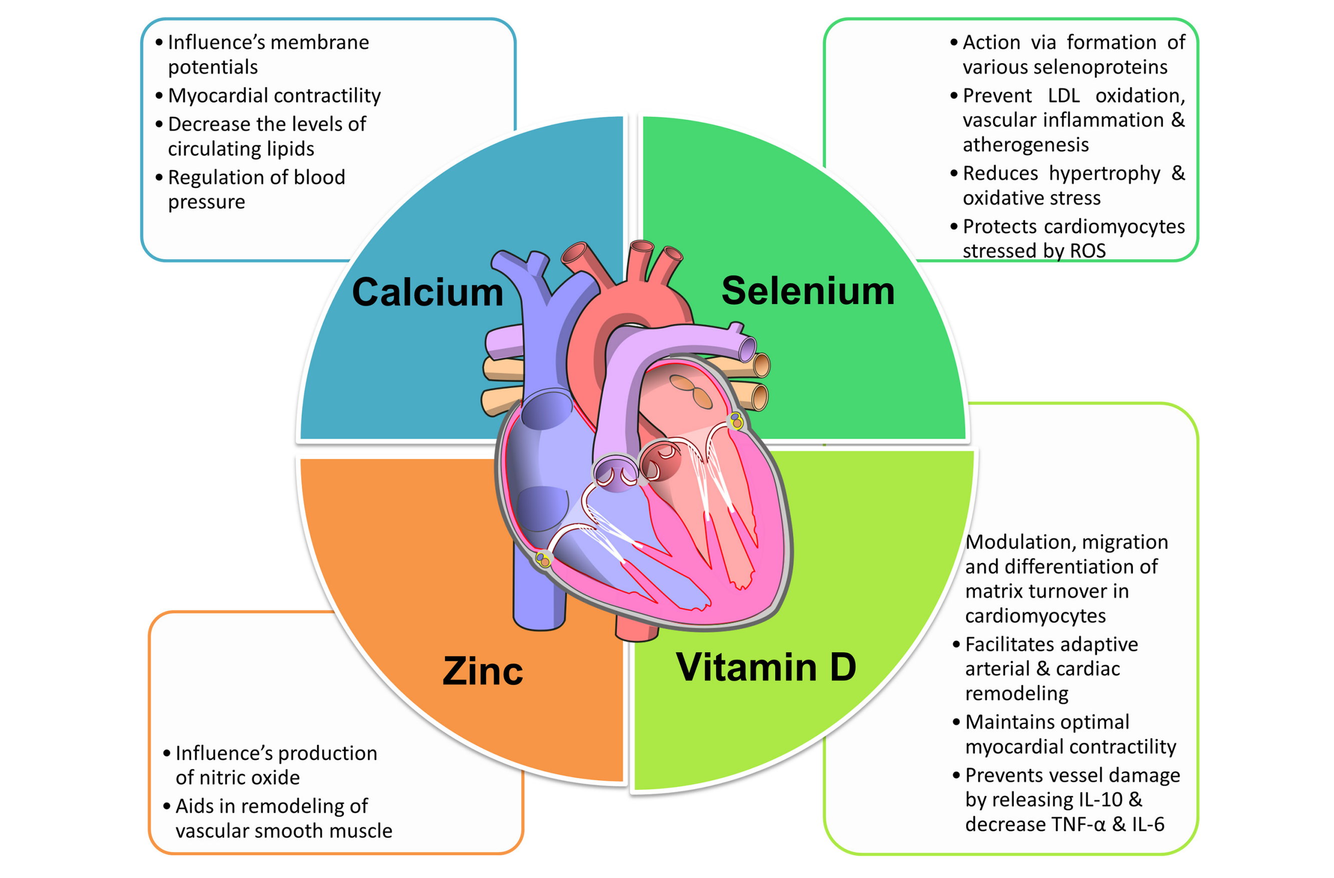

FIGURE 1 | The role of micronutrients in cardiovascular health. Image is adapted from https://commons.wikimedia.org/wiki/File:Diagram_of_the_human_heart_(no_labels).svg (Stenemo, 2017).

Lower levels of vitamin D in the blood has been linked with higher cardiovascular risk in numerous observational studies, epidemiological investigations, and laboratory studies (Al Mheid and Quyyumi, 2017). Several cells in the vascular system have the receptors for vitamin D. The enzyme 1α-hydroxylase, that converts 25-hydroxyvitamin D to calcitriol is produced in the vascular smooth muscle, endothelial cells, and cardiomyocytes (Danik and Manson, 2012). Calcitriol modulates proliferation, migration and differentiation and matrix turnover in the cardiomyocytes, modulates the osteoclastic gene expression in smooth muscle and thereby facilitates adaptive arterial and cardiac remodeling. It maintains optimal endothelial function and vascular tone, regulates calcium flux and sarcomere function in the cardiomyocytes to confer optimal myocardial contractility (Norman and Powell, 2014). Some studies have shown that vitamin D by its influence on PTH, indirectly on calcium metabolism has an important role in vascular calcification. Both hypo and hypervitaminosis of vitamin D can cause calcification of blood vessels (Zebger-Gong et al., 2011).

Low levels of vitamin D probably increase CVD risk by altering pre-determined cardiovascular risk factors like hypertension, diabetes, and inflammation (Chin et al., 2017). There were many observational studies that found a reverse relationship between blood pressure and serum vitamin D levels (Burgaz et al., 2011) but the findings were not very significant (Danik and Manson, 2012). The possible mechanism may involve diminishing the proliferative effects of renin-angiotensin-aldosterone system on vascular smooth muscle cells (Ajabshir et al., 2014). Calcitriol by binding to the promoter region in the gene *REN-1C* represses renin expression, and aids in lowering the probability of emerging hypertension. In addition, the renal arteries of individuals with diminished levels of vitamin D have low expressing angiotensin-l receptors (Mursu et al., 2011). In addition, evidence from some animal studies had related the direct effect of vitamin D on endothelium and vasculature and how it protects against endothelial dysfunction (Lubos et al., 2010).

Effect of vitamin D on parathyroid hormone (PTH) and calcium metabolism is also suggested as one of the mechanisms which alter the blood pressure. Minimal levels of vitamin D will increase PTH which in turn constricts the vasculature and leads to hypertension by increasing intracellular calcium levels (Fujii, 2018). Supplementation of vitamin D has been proven beneficial in treating high blood pressure in some ethnic and age groups as observed in some case-control studies (Judd et al., 2010) but it did not show any significant effect on non-hypertensive population. Based on this supposition the consumption of vitamin D supplements as an antihypertensive agent must not be advocated (Wang et al., 2010).

Many epidemiological studies have correlated lower serum vitamin D levels to increased incidence of type 2 diabetes mellitus. Diminished levels of vitamin D are assumed to increase insulin resistance, cause damage to the beta-cells of the pancreas, and consequently decrease secretion of insulin (Chin et al., 2017). There is conflicting data from various clinical studies and the valuable influence of vitamin D on treating or preventing diabetes cannot be ascertained due to lack of supporting evidence. Several systematic reviews and meta-analyses also established that HbA1c levels were not lowered even after supplementing vitamin D and there was no improvement in the function of pancreatic beta-cells and insulin resistance (Chin et al., 2017).

Vitamin D has shown some protective influence on vessel walls as observed in some research papers. Vessel walls are shielded against damage from inflammation by enhancing the expression of anti-inflammatory cytokines like IL-10 and by reducing the expression of pro-inflammatory proteins like, TNF-α and IL-6 (Zittermann et al., 2005). But there is not sufficient evidence to corroborate the findings.

Although vitamin D is dynamically involved in several pathways in the homeostasis of the cardiovascular health and low vitamin D levels are recognized as a menace for chief cardiovascular diseases including hypertension, atherosclerosis, strokes, heart failure etc. the supporting evidence available is limited. It is essential to prolong studying the outcomes of vitamin D on cardiovascular health with more emphasis on the pathology before advocating the use of vitamin D supplements for improving cardiovascular health to the public.

Trace Element Zinc in Development of Cardiovascular Diseases

Zinc has numerous functions in the human body and therefore is recognized as an important trace element. It is a vital antioxidant that prevents the formation and reactive response of free radicals which may damage the cells and lead to degenerative diseases (Chabosseau and Rutter, 2016). Daily allowance of zinc for men and women is 11 and 8 mg, respectively, and should never exceed a maximum amount of 40 mg/day (Ruz et al., 2019). The main dietary sources of zinc are meat that include beef, veal, pork, lamb, cereals, grains, fish, vegetables, nuts, milk, and dairy products (Olza et al., 2017). Zinc by being an important part in the metabolism of nucleic acids and protein biosynthesis warrants the proper course of cell growth, division, and functioning (Plum et al., 2010).

Deficiency of zinc is correlated with the development of cardiovascular diseases, mainly atherosclerosis (Chiu et al., 2004). Impaired function of superoxide dismutase causes oxidative stress which in turn increases the turnover of nitric oxide (NO) a potent vasodilator which can alter blood pressure. Also, increased oxidative stress can lead to atherosclerosis. A study conducted on the mice showed that when zinc intake was insufficient, there is increase in concentration of lipoproteins, augmented refashioning of vascular smooth muscle, augmented inflammation, and induced atherosclerotic plaque formation (Chiu et al., 2004). Zinc helps in maintaining adequate functioning of superoxide dismutase and keeps a check on NO generation (Ruz et al., 2019). However, studies on humans are still sparse and sufficient evidence is lacking to corroborate the findings.

Role of zinc in regulating blood pressure may be because of its antioxidant property and its role in regulating the flow of calcium ions. In a study conducted on mice, it was seen that zinc deficient diet increased the blood pressure in mice. Reduced zinc levels lead to hypertension, hypercholesterolemia and a higher BMI in a study conducted on human subjects. The association was statistically significant (Braun et al., 2018). Possible mechanism could be that deficiency of zinc stimulates retention of sodium in distal renal tubules (Williams et al., 2019). There have been controversial studies which showed an increase in hypertension with a rise in zinc levels and some studies showed no correlation between hypertension with either increase or decrease in the serum zinc levels (Gać et al., 2021).

Oxidative stress can trigger cellular and molecular destruction and aids in the development of insulin resistance which will lead to type 2 diabetes mellitus, which is a predetermined risk factor of cardiovascular disorders (Bao et al., 2010). Zinc is responsible for the production and maturation of insulin (Chabosseau and Rutter, 2016). Zinc can stifle the secretion of cytokines IL-1b, TNF-a, and IL-6 s from macrophages and monocytes, which

TABLE 1 | Recommended dietary allowance, normal serum concentration, dietary sources, and deficiency symptoms of calcium, Vitamin D, selenium, and zinc.

Micronutrient	Recommended dietary allowance	Normal serum concentration	Dietary sources	Deficiency symptoms
Calcium	1,200–1,500 mg/day	9–11 mg/dL	Milk, cheese, okra, spinach, kale, sardines, pilchards, breads made from fortified flour	Hypotension, heart failure
Vitamin D	15–20 μg/day	30–100 ng/mL	Salmon, sardines, herring, mackerel, red meat, liver, egg yolk, fat spreads, breakfast cereals	Atherosclerosis, hypertension
Selenium	55 μg/day	70–150 ng/mL	Brazil nuts, seafood, eggs, organ meat, red meat, grains, cereals	Atherosclerosis, hypertension, hypercholesterolemia
Zinc	8–11 mg/day	0.7–1.6 μg/mL	Mushrooms, spinach, broccoli, kale, garlic, chickpea, lentils, beans, pumpkin seeds, pine nuts	Myocardial infarction, acute coronary syndrome

may cause apoptosis of the pancreatic beta cells (Ruz et al., 2019). In a meta-analysis study, it was observed that zinc when supplemented at a low dose over prolonged period helped fasting blood glucose, improved insulin resistance, reduced triglycerides, total cholesterol, and LDL cholesterol (Pompano and Boy, 2021). But in another study women who ingested additional zinc doubled up their risk of developing cardiovascular occurrences (Gać et al., 2021). Although statistically significant results were seen with zinc supplementation on improving the blood cholesterol picture and on blood sugar levels nothing positively significant was reported on benefits of cardiovascular health (Khazdouz et al., 2020). Furthermore, some studies have reported a clear association between greater intake of zinc and the upsurge in total and cancer-related mortality (Shi et al., 2018).

Although there is some evidence showing the role and benefits of zinc in cardiovascular health, it is not enough to rationalize its supplementation. More research must be done, and more steadfast and consistent experimental data would be desired to arrive at a decision.

Selenium in Cardiovascular Health

Selenium is another important trace element which influences cardiovascular health. It has a vital role in the formation of selenoproteins, so far about 30 of them are identified (Tinggi, 2008). The recent interest in selenium is integrated into important amino acid derivatives, such as selenomethionine, selenocysteine, methylselenocysteine, and selenocystathionine. Selenoproteins have important role in the body some of which include reducing hydrogen peroxide and organic peroxides, control cellular proliferation and apoptosis, regulate thyroid hormone levels, adjusts the work of cardiovascular system (Gać et al., 2021). The recommended dietary allowance for selenium is 55 μg/day to meet the nutritional needs of all healthy adults. Plant foods, meat, cereals, and seafood are the major dietary sources of selenium (Flores-Mateo et al., 2006).

Deficiency of selenium has been associated with CVD from a long time and an example is the Keshan disease a type of cardiomyopathy seen in some parts of China that has soil low in selenium (Alexander et al., 2020). The underlying mechanism that explains how selenium deficiency causes deterioration of cardiomyocytes and increases the predisposition to damage is yet to be discovered (Sun et al., 2019). There are several other studies that have conveyed an association between decreased selenium and increased danger of MI (Suadicani et al., 1992), acute coronary syndrome (Lubos et al., 2010). A meta-analysis on pooled data indicated that a body with physiologically high selenium levels exhibited lessened risk of CVD and mortality when compared to low body selenium status. Analogous results were seen in other meta-analysis reports by Flores-Mateo et al. (2006), Alexander et al. (2020), Zhang et al. (2016) etc. Regions that had very low selenium levels in the soil exhibited the protective effect of selenium and benefited from its supplementation (Kuria et al., 2020). Jenkins et al. (2020) in the meta-analysis described that supplementing selenium along with antioxidant mixture proved to be beneficial for CVD.

A meta- analysis report by Xiang et al. (2020) validated that circulating selenium levels do not influence the mortality rate of coronary diseases and the supplements do not help much (Witham et al., 2009). Vinceti et al. (2019) in their study have shown that increased exposure to selenium resulted in increased blood pressure. Selenium if taken in excess (900 μg/day) can cause digestive system disorders, hair loss, skin lesions, along with disturbances of endocrine, respiratory, and nervous systems (Gać et al., 2021). Sufficient data from clinical studies is not available to show the beneficial effects of selenium supplementation in cardiovascular disease treatment or prevention. There is a strong need of more evidence, before prescribing supplements of selenium for cardiovascular health because the benefits of selenium supplementation are uncertain, and their arbitrary usage carries a probability of toxicity (Danik and Manson, 2012; Benstoem et al., 2015; Khazdouz et al., 2020).

Owing to the ill effects supplements have on the health of the individual, it is better to rely on naturally available sources. A summary of the daily requirement and dietary sources has been depicted in the **Table 1**.

CONCLUSION

Based on the large observational studies and available data, we conclude that, there definitely is an important role of the micronutrient's calcium, zinc, vitamin D, and selenium on cardiovascular health. However, high circulating levels of these micronutrients have a detrimental effect on the heart health as evidenced in few of the studies, although the underlying mechanism is not very clearly understood. Micronutrients taken in the form of supplements cause an immediate surge in the serum levels for a prolonged period and the increment is sustained for longer hours. Further clinical trials and studies are needed to establish the benefits of supplements and it is important to weigh the risks before taking them. It is, therefore, advisable to depend on dietary sources to meet the daily requirement of micronutrients.

AUTHOR CONTRIBUTIONS

HN and SC equally contributed in terms of conception of the work. HN performed database search, drafting of the manuscript. HN, SC, and SS edited and reviewed the manuscript. SC procured the grant for the publication. All the authors have given their consent for submission.

FUNDING

This work was supported by the FRGS, Malaysia, FRGS/1/2018/STG03/AIMST/02/1.

ACKNOWLEDGMENTS

We would like to acknowledge AIMST University, Manipal University College Malaysia.

REFERENCES

Ajabshir, S., Asif, A., and Nayer, A. (2014). The effects of vitamin D on the renin-angiotensin system. *J. Nephropathol.* 3, 41–43.

Al Mheid, I., and Quyyumi, A. A. (2017). Vitamin D and cardiovascular disease: controversy unresolved. *J. Am. Coll. Cardiol.* 70, 89–100.

Alexander, J., Alehagen, U., and Aaseth, J. O. (2020). Selenium-a trace element of clinical significance. *Tidsskr. Nor. Laegeforen.* 23:140.

Anderson, J. J. B., Kruszka, B., Delaney, J. A. C., He, K., Burke, G. L., Alonso, A., et al. (2016). Calcium intake from diet and supplements and the risk of coronary artery calcification and its progression among older adults: 10−year follow−up of the Multi−Ethnic Study of Atherosclerosis (MESA). *J. Am. Heart Assoc.* 5:e003815.

Bao, B., Prasad, A. S., Beck, F. W. J., Fitzgerald, J. T., Snell, D., Bao, G. W., et al. (2010). Zinc decreases C-reactive protein, lipid peroxidation, and inflammatory cytokines in elderly subjects: a potential implication of zinc as an atheroprotective agent. *Am. J. Clin. Nutr.* 91, 1634–1641. doi: 10.3945/ajcn.2009.28836

Benstoem, C., Goetzenich, A., Kraemer, S., Borosch, S., Manzanares, W., Hardy, G., et al. (2015). Selenium and its supplementation in cardiovascular disease—what do we know? *Nutrients* 7, 3094–3118. doi: 10.3390/nu7053094

Braun, L. A., Ou, R., Kure, C., Trang, A., and Rosenfeldt, F. (2018). Prevalence of zinc deficiency in cardiac surgery patients. *Heart Lung Circ.* 27, 760–762. doi: 10.1016/j.hlc.2017.07.009

Burgaz, A., Orsini, N., Larsson, S. C., and Wolk, A. (2011). Blood 25-hydroxyvitamin D concentration and hypertension: a meta-analysis. *J. Hypertens.* 29, 636–645. doi: 10.1097/hjh.0b013e32834320f9

Cano, A., Chedraui, P., Goulis, D. G., Lopes, P., Mishra, G., Mueck, A., et al. (2018). Calcium in the prevention of postmenopausal osteoporosis: EMAS clinical guide. *Maturitas* 107, 7–12. doi: 10.1016/j.maturitas.2017.10.004

Chabosseau, P., and Rutter, G. A. (2016). Zinc and diabetes. *Arch. Biochem. Biophys.* 611, 79–85.

Chin, K., Appel, L. J., and Michos, E. D. (2017). Vitamin D, calcium, and cardiovascular disease: "D" vantageous or "D"etrimental? An era of uncertainty. *Curr. Atheroscler. Rep.* 19:5.

Chiu, K. C., Chu, A., Go, V. L. W., and Saad, M. F. (2004). Hypovitaminosis D is associated with insulin resistance and beta cell dysfunction. *Am. J. Clin. Nutr.* 79, 820–825. doi: 10.1093/ajcn/79.5.820

Chung, M., Tang, A. M., Fu, Z., Wang, D. D., and Newberry, S. J. (2016). Calcium intake and cardiovascular disease risk: an updated systematic review and meta-analysis. *Ann. Intern. Med.* 165, 856–866. doi: 10.7326/m16-1165

Danik, J. S., and Manson, J. E. (2012). Vitamin D and cardiovascular disease. *Curr. Treat. Options Cardiovasc. Med.* 14, 414–424.

Elsevier (2021). *Guyton and Hall Textbook of Medical Physiology*, 14th Edn. Available online at: https://www.elsevier.com/books/guyton-and-hall-textbook-of-medical-physiology/hall/978-0-323-59712-8 (accessed July 14, 2021).

Flores-Mateo, G., Navas-Acien, A., Pastor-Barriuso, R., and Guallar, E. (2006). Selenium and coronary heart disease: a meta-analysis. *Am. J. Clin. Nutr.* 84, 762–773. doi: 10.1093/ajcn/84.4.762

Fujii, H. (2018). Association between parathyroid hormone and cardiovascular disease. *Ther. Apher. Dial.* 22, 236–241. doi: 10.1111/1744-9987.12679

Gać, P., Czerwińska, K., Macek, P., Jaremków, A., Mazur, G., Pawlas, K., et al. (2021). The importance of selenium and zinc deficiency in cardiovascular disorders. *Environ. Toxicol. Pharmacol.* 82:103553. doi: 10.1016/j.etap.2020.103553

Gallagher, J. C., Smith, L. M., and Yalamanchili, V. (2014). Incidence of hypercalciuria and hypercalcemia during vitamin D and calcium supplementation in older women. *Menopause (New York, NY)*, 21:1173.

Jenkins, D. J. A., Kitts, D., Giovannucci, E. L., Sahye-Pudaruth, S., Paquette, M., Blanco Mejia, S., et al. (2020). Selenium, antioxidants, cardiovascular disease, and all-cause mortality: a systematic review and meta-analysis of randomized controlled trials. *Am. J. Clin. Nutr.* 112, 1642–1652. doi: 10.1093/ajcn/nqaa245

Judd, S. E., Raiser, S. N., Kumari, M., and Tangpricha, V. (2010). 1,25-dihydroxyvitamin D3 reduces systolic blood pressure in hypertensive adults: a pilot feasibility study. *J. Steroid Biochem. Mol. Biol.* 121, 445–447. doi: 10.1016/j.jsbmb.2010.04.013

Khazdouz, M., Djalalinia, S., Sarrafi Zadeh, S., Hasani, M., Shidfar, F., Ataie-Jafari, A., et al. (2020). Effects of zinc supplementation on cardiometabolic risk factors: a systematic review and meta-analysis of randomized controlled trials. *Biol. Trace Elem. Res.* 195, 373–398. doi: 10.1007/s12011-019-01870-9

Kuria, A., Tian, H., Li, M., Wang, Y., Aaseth, J. O., Zang, J., et al. (2020). Selenium status in the body and cardiovascular disease: a systematic review and meta-analysis. *Crit. Rev. Food Sci. Nutr.* 7, 1–10. doi: 10.1080/10408398.2020.1803200

Kwak, S. M., Kim, J. S., Choi, Y., Chang, Y., Kwon, M.-J., Jung, J.-G., et al. (2014). Dietary intake of calcium and phosphorus and serum concentration in relation to the risk of coronary artery calcification in asymptomatic adults. *Arterioscler. Thromb. Vasc. Biol.* 34, 1763–1769. doi: 10.1161/atvbaha.114.303440

Larsson, S. C., Burgess, S., and Michaëlsson, K. (2017). Association of genetic variants related to serum calcium levels with coronary artery disease and myocardial infarction. *JAMA* 318, 371–380. doi: 10.1001/jama.2017.8981

Lewis, J. R., Radavelli-Bagatini, S., Rejnmark, L., Chen, J. S., Simpson, J. M., Lappe, J. M., et al. (2015). The effects of calcium supplementation on verified coronary heart disease hospitalization and death in postmenopausal women: a collaborative meta-analysis of randomized controlled trials. *J. Bone Miner. Res.* 30, 165–175. doi: 10.1002/jbmr.2311

Lubos, E., Sinning, C. R., Schnabel, R. B., Wild, P. S., Zeller, T., Rupprecht, H. J., et al. (2010). Serum selenium and prognosis in cardiovascular disease: results from the AtheroGene study. *Atherosclerosis* 209, 271–277. doi: 10.1016/j.atherosclerosis.2009.09.008

Michos, E. D., Cainzos-Achirica, M., Heravi, A. S., and Appel, L. J. (2021). Vitamin D, calcium supplements, and implications for cardiovascular health: JACC focus seminar. *J. Am. Coll. Cardiol.* 77, 437–449.

Mursu, J., Robien, K., Harnack, L. J., Park, K., and Jacobs, D. R. (2011). Dietary supplements and mortality rate in older women: the Iowa Women's Health Study. *Arch. Intern. Med.* 171, 1625–1633. doi: 10.1001/archinternmed.2011.445

National Institutes of Health (2011). "Dietary reference intakes for calcium and vitamin D," in *Institute of Medicine (US) Committee to Review Dietary Reference Intakes for Vitamin D and Calcium*, eds A. C. Ross, C. L. Taylor, A. L. Yaktine, and H. B. DelValle (Washington, DC: National Academies Press (US)). (The National Academies Collection: Reports funded by National Institutes of Health).

Norman, P. E., and Powell, J. T. (2014). Vitamin D and cardiovascular disease. *Circ. Res.* 114, 379–393.

Olza, J., Aranceta-Bartrina, J., González-Gross, M., Ortega, R. M., Serra-Majem, L., Varela-Moreiras, G., et al. (2017). Reported dietary intake and food sources of zinc, selenium, and Vitamins A, E and C in the Spanish population: findings from the ANIBES study. *Nutrients* 9:E697.

Plum, L. M., Rink, L., and Haase, H. (2010). The essential toxin: impact of zinc on human health. *Int. J. Environ. Res. Public Health* 7, 1342–1365. doi: 10.3390/ijerph7041342

Pompano, L. M., and Boy, E. (2021). Effects of dose and duration of zinc interventions on risk factors for type 2 diabetes and cardiovascular disease: a systematic review and meta-analysis. *Adv. Nutr.* 12, 141–160. doi: 10.1093/advances/nmaa087

Prentice, R. L., Pettinger, M. B., Jackson, R. D., Wactawski-Wende, J., Lacroix, A. Z., Anderson, G. L., et al. (2013). Health risks and benefits from calcium and vitamin D supplementation: Women's Health Initiative clinical trial and cohort study. *Osteoporos Int.* 24, 567–580. doi: 10.1007/s00198-012-2224-2

Rautiainen, S., Wang, L., Manson, J. E., and Sesso, H. D. (2013). The role of calcium in the prevention of cardiovascular disease–a review of observational studies and randomized clinical trials. *Curr. Atheroscler. Rep.* 15:362.

Reid, I. R., Gamble, G. D., and Bolland, M. J. (2016). Circulating calcium concentrations, vascular disease and mortality: a systematic review. *J. Intern. Med.* 279, 524–540. doi: 10.1111/joim.12464

Roth, G. A., Johnson, C., Abajobir, A., Abd-Allah, F., Abera, S. F., Abyu, G., et al. (2017). Global, regional, and national burden of cardiovascular diseases for 10 causes, 1990 to 2015. *J. Am. Coll. Cardiol.* 70, 1–25. doi: 10.1016/j.jacc.2017.04.052

Ruz, M., Carrasco, F., Rojas, P., Basfi-Fer, K., Hernández, M. C., and Pérez, A. (2019). Nutritional effects of zinc on metabolic syndrome and type 2 diabetes: mechanisms and main findings in human studies. *Biol. Trace Elem. Res.* 188, 177–188. doi: 10.1007/s12011-018-1611-8

Schmitz, T., Thilo, C., Linseisen, J., Heier, M., Peters, A., Kuch, B., et al. (2021). Low serum calcium is associated with higher long-term mortality in myocardial infarction patients from a population-based registry. *Sci. Rep.* 11:2476. doi: 10.1038/s41598-021-81929-7

Shi, Z., Chu, A., Zhen, S., Taylor, A. W., Dai, Y., Riley, M., et al. (2018). Association between dietary zinc intake and mortality among Chinese adults: findings from 10-year follow-up in the Jiangsu Nutrition Study. *Eur. J. Nutr.* 57, 2839–2846. doi: 10.1007/s00394-017-1551-7

Shin, S., Kim, K.-J., Chang, H.-J., Cho, I., Kim, Y. J., Choi, B.-W., et al. (2012). Impact of serum calcium and phosphate on coronary atherosclerosis detected by cardiac computed tomography. *Eur. Heart J.* 33, 2873–2881. doi: 10.1093/eurheartj/ehs152

Stenemo (2017). *English: Updated to Use SVG Version and Removed Arrows and Lines*. Available online at: https://commons.wikimedia.org/wiki/File:Diagram_of_the_human_heart_(no_labels).svg (accessed July 16, 2021).

Suadicani, P., Hein, H. O., and Gyntelberg, F. (1992). Serum selenium concentration and risk of ischaemic heart disease in a prospective cohort study of 3000 males. *Atherosclerosis* 96, 33–42. doi: 10.1016/0021-9150(92)90035-F

Sun, D., Sun, S., Feng, H., and Hou, J. (2019). "Keshan disease," in *Endemic Disease in China*, ed. D. Sun (Singapore: Springer), 175–221. (Public Health in China). doi: 10.1007/978-981-13-2529-8_6

Tinggi, U. (2008). Selenium: its role as antioxidant in human health. *Environ. Health Prev. Med.* 13, 102–108. doi: 10.1007/s12199-007-0019-4

Vinceti, M., Chawla, R., Filippini, T., Dutt, C., Cilloni, S., Loomba, R., et al. (2019). Blood pressure levels and hypertension prevalence in a high selenium environment: results from a cross-sectional study. *Nutr. Metab. Cardiovasc. Dis.* 29, 398–408. doi: 10.1016/j.numecd.2019.01.004

Wang, H., Li, J., Du, F., and Tian, J. (2021). *Correlation Between Serum Calcium and Cardiovascular Outcome Among Patients Suffering From Acute Coronary Syndrome*. Available online at: https://www.semanticscholar.org/paper/Correlation-between-Serum-Calcium-and-Outcome-among-Wang-Li/08d89152e94cf57c11665e80d894b1d2232064f7 (accessed July 12, 2021).

Wang, T. K. M., Bolland, M. J., van Pelt, N. C., Horne, A. M., Mason, B. H., Ames, R. W., et al. (2010). Relationships between vascular calcification, calcium metabolism, bone density, and fractures. *J. Bone Miner. Res.* 25, 2777–2785. doi: 10.1002/jbmr.183

Williams, C. R., Mistry, M., Cheriyan, A. M., Williams, J. M., Naraine, M. K., Ellis, C. L., et al. (2019). Zinc deficiency induces hypertension by promoting renal Na+ reabsorption. *Am. J. Physiol. Renal Physiol.* 316, F646–F653. doi: 10.1152/ajprenal.00487.2018

Witham, M. D., Nadir, M. A., and Struthers, A. D. (2009). Effect of vitamin D on blood pressure: a systematic review and meta-analysis. *J. Hypertens.* 27, 1948–1954. doi: 10.1097/HJH.0b013e32832f075b

World Health Organization (2021). *Cardiovascular Diseases (CVDs)*. Available online at: https://www.who.int/news-room/fact-sheets/detail/cardiovascular-diseases-(cvds) (accessed July 14, 2021).

Xiang, S., Dai, Z., Man, C., and Fan, Y. (2020). Circulating selenium and cardiovascular or all-cause mortality in the general population: a meta-analysis. *Biol. Trace Elem. Res.* 195, 55–62.

Zebger-Gong, H., Müller, D., Diercke, M., Haffner, D., Hocher, B., Verberckmoes, S., et al. (2011). 1,25-Dihydroxyvitamin D3-induced aortic calcifications in experimental uremia: up-regulation of osteoblast markers, calcium-transporting proteins and osterix. *J. Hypertens.* 29, 339–348. doi: 10.1097/HJH.0b013e328340aa30

Zhang, X., Liu, C., Guo, J., and Song, Y. (2016). Selenium status and cardiovascular diseases: meta-analysis of prospective observational studies and randomized controlled trials. *Eur. J. Clin. Nutr.* 70, 162–169.

Zittermann, A., Schleithoff, S. S., and Koerfer, R. (2005). Putting cardiovascular disease and vitamin D insufficiency into perspective. *Br. J. Nutr.* 94, 483–492. doi: 10.1079/BJN20051544

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ORIGINAL RESEARCH
published: 16 September 2021
doi: 10.3389/fphys.2021.731416

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Anthropometric Markers With Specific Cut-Offs Can Predict Anemia Occurrence Among Malaysian Young Adults

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OPEN ACCESS

Edited by:
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Reviewed by:
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Specialty section:
This article was submitted to Integrative Physiology, a section of the journal Frontiers in Physiology

Received: *27 June 2021*
Accepted: *13 August 2021*
Published: *16 September 2021*

Citation:
Dutta S, Karkada IR, Sengupta P and Chinni SV (2021) Anthropometric Markers With Specific Cut-Offs Can Predict Anemia Occurrence Among Malaysian Young Adults. Front. Physiol. 12:731416. doi: 10.3389/fphys.2021.731416

Objective: Anemia bears a high global prevalence with about 1.6 billion people living with this affliction. Malaysia carries the burden of 13.8% anemia prevalence which urges for extensive research directed to its prediction and amelioration. This is the first study that aims to (a) propose simple non-invasive predictive anthropometric markers and their specific cut-off values for early prediction of anemia among the young adults in Malaysia, (b) provide anemia prevalence based on both gender and ethnicity among young adults of Malaysia.

Method: The present cross-sectional study included 245 participants (113 men and 132 women) aged between 18 and 30 years. Anthropometric parameters were measured following the standard protocols. Blood samples were collected and hemoglobin levels were determined using the HemoCue haemoglobinometer (Hb 201+ System, Angelhom, Sweden) to detect the presence of anemia. The receiver operating characteristics (ROC) curve was employed to assess and compare the efficacy of anthropometric indices in the prediction of anemia. Data were analyzed using SPSS (v. 22.0, IBM, Chicago, IL, USA) and MedCalc (v. 19.05, Ostend, Belgium).

Result: The ROC analysis indicates that body mass index (BMI) is the best anthropometric marker with the highest area under the curve (AUC) and specificity (SP) for predicting the presence of anemia in young adults in Malaysia. Thus, the study proposes the optimal cut-off value of BMI for young men of Malaysia as 20.65 kg/m^2 (AUC: 0.889) and young women of Malaysia as 19.7 kg/m^2 (AUC: 0.904). The study also reports that Malaysian Indians have the highest prevalence of anemia (26.22%) followed by Malays (21.54%), "Others" (indigenous ethnic group) (20%), and Chinese (14.5%), with an overall higher prevalence of anemia in young adult women (21.96%) than in men (18.6%) of Malaysia.

Conclusion: The proposed anemia-predictive anthropometric markers with optimal cut-off values will aid early detection of anemia among young adults in Malaysia, and given its simple, inexpensive, and intelligible approach, it can be widely used. The ease of anemia prediction together with the reported distribution of anemia prevalence based on gender and ethnicity will facilitate in gauging the necessary extent of strategies of anemia management in the young adult population of Malaysia.

Keywords: anemia, anthropometry, body-mass index, hemoglobin, Malaysian adult

INTRODUCTION

Anemia afflicts one-fourth of the global population (WHO, 2011), and Malaysia holds the burden of 13.8% anemia prevalence with 20.1% women and 4.9% men population being anemic (Abdullah et al., 2020). As per the WHO, anemia is a condition marked by hemoglobin level lower than the physiological value, by two SDs, in age- and gender-matched populations. WHO stipulated reference hemoglobin values for defining anemia are 13 and 12 g/dL for adult men and women, respectively (WHO, 2011).

Anemia is a multideterminant condition among which the vastly reported determinants are the demographic factors, such as age, gender, ethnicity (Yusof et al., 2018), geographical location (Bharati et al., 2015), physical activity (Choudhary and Binawara, 2012), and nutritional status (Aigner et al., 2015). A large cohort of research has exploited multitudinous causative factors of anemia and its prevalence in Malaysia (Ishak and Hassan, 1994; Hassan et al., 2005; Haniff et al., 2007; Khambalia et al., 2011; Nik Rosmawati et al., 2012; Soh et al., 2015). As anemia is a common blood disorder affecting the mass population, a multiethnic nation, such as Malaysia, must direct its research to understand the pattern of anemia prevalence based on ethnicity to develop effective management strategies. Studies on the middle-aged and older population of Malaysia have reported that anemia is most prevalent in Indians, followed by Malays, and Chinese (Yusof et al., 2018; Abdullah et al., 2020). However, the relationship between ethnicity and anemia among young adult population in Malaysia is yet to be determined. On the global aspect, reports pertaining to prevalence of anemia based on the ethnicity are scanty, but some of the studies revealed significant variations of occurrence of anemia among various ethnic groups (Frith-Terhune et al., 2000; Guralnik et al., 2004; Gaskell et al., 2008; Yusof et al., 2018). This can be explained by the differences in lifestyles, customs, and beliefs among the ethnicities which influence their dietary choices (Gaskell et al., 2008; Hiza et al., 2013; Nohan et al., 2020).

Early detection of anemia may lead to its rapid amelioration. Blood hemoglobin estimation is not a feasible option to predict the occurrence of anemia in the entire large population of a nation affected by it, and the complexity of prediction of anemia among the mass hinders to sketch-out proper rapid remedial strategies. In this aspect, it may be considered that anemia holds a close association with body type and various anthropometric parameters (Micozzi et al., 1989; Smith et al., 1991; Collett-Solberg et al., 2007; Saxena et al., 2011; Chang et al., 2014; Sinha and Haldar, 2015; Virginia and Fenty, 2017; El-Shafie et al., 2020). Thus, anthropometric parameters can serve as surrogate predictors of anemia, which are simple, non-invasive, and rapid yet accurate. This will aid early speedy detection of anemia among a large population as the methods are inexpensive and do not mandate the presence of medical professionals. The most studied anthropometric parameters that find an association with hemoglobin levels include body mass index (BMI), height, weight, waist circumference (WC), waist-to-hip ratio (WHR), and waist-to-height ratio (WHtR) (Lee et al., 2004; Al-Hashem, 2007; Vuong et al., 2014). Nevertheless, to establish anthropometric parameters as predictors of anemia, it is needed to evaluate their definite predictive values. Thus, this study finds novelty in its aim to propose predictive anthropometric markers along with their specific cut-off values for early prediction of anemia among young adults in Malaysia and to provide the prevalence of anemia among adult men and women of Malaysia based on ethnicity.

MATERIALS AND METHODS

Ethical Considerations

The Institutional Research Ethics Committee of MAHSA University (RMC/EC05/2019) has approved the study proposal. The subjects were randomly selected from different faculties of the MAHSA University, Saujana Putra Campus, Jenjarom, Selangor, Malaysia within the duration from December 1, 2018, to June 30, 2019. All the respondents provided their written informed consent to participate in this study.

Study Population

This population-based cross-sectional study included 245 young adult men and women (comprised of 113 men and 132 women) aged between 18 and 30 years. The sample size was calculated considering the prevalence of anemia in Malaysia as 13.8% (Abdullah et al., 2020). The initial sample size, obtained using the formula, $n_1 = (z^2\text{PQ})/d^2$ (where, n_1 = initial sample size, z = statistic corresponding to the level of confidence, P = expected prevalence, Q = proportion of exception, d = precision corresponding to effect size), was 182.6 (~183). After including 20% of sampling error, using the formula, $n = n_1 + (n_1 \times 20/100)$ (where n = final sample size), we obtained the final sample size as 219.6 (~220), and thus, the sample size of 245 used in this study is sufficed to represent the young adult population of Malaysia.

Data collected were segregated based on the ethnic groups of Malaysia: Malays, Chinese, Indians, and a residual "Others" category including all indigenous populations of Malaysia. The exclusion criteria for this study were (a) citizens of other countries, (b) married individuals, (c) individuals with any kind of blood coagulation-related disorders, (d) individuals who had a medical history of depression, stroke, angina, hypertension, asthma, diabetes mellitus, hypercholesterolemia, tuberculosis, hepatitis chronic, bronchitis chronic, kidney diseases, and cancer, and (e) individuals with genetic disorders, such as Turner's

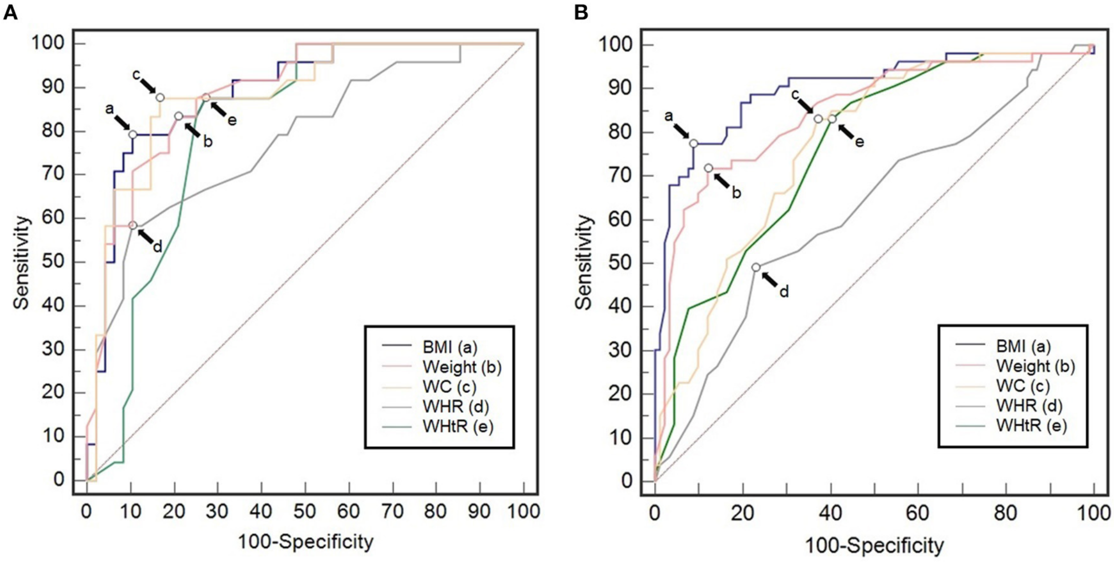

FIGURE 1 | Receiver operating characteristics curves of body mass index, body weight, waist circumference (WC), waist-to hip ratio (WHR), and waist-to to-height ratio (WHtR) in the prediction of anemia among young adults of Malaysia **(A)** men (n = 113); a = 20.65, b = 62, c = 76, d = 0.79, e = 0.45, and **(B)** women (n = 132); a = 19.7, b = 49.5, c = 71, d = 0.74, e = 0.45.

syndrome, primary hypopituitarism. Exclusion criteria also consist of subjects aged under 18 years and pregnant women.

Anthropometric and Biochemical Measurements

Height, body weight, WC, and buttock circumference (BC) were measured for every subject, and it was ascertained that the subjects would be in minimal garments and bare feet on a plane surface. Body weight was measured using portable electronic scales (measured to the nearest 0.1 kg) and prior to every measurement, the instrument was reset to "zero" to attain accuracy. Portable stadiometers were used to measure the height (measured to the nearest 1 cm), and the subjects were instructed to keep their feet together with their shoulder blades, hips, and heels in line with and touching the instrument stick, and head held straight on the horizontal plane. Measurement of WC and HC were done using inextensible anthropometric tape and the subjects stood erect with arms resting at their sides and feet placed together (Legro et al., 1999). The BMI (or Quetelet Index) was determined by the formula, "BMI = weight (kg) / (Height in m)^2" (WHO, 2006). Other anthropometric indices, WHR and WHtR were also calculated. The hemoglobin concentration was measured by following standard biochemical examinations. The capillary blood sample was tested for hemoglobin level using a HemoCue haemoglobinometer (HemoCue Hb 201+ System, Angelhom, Sweden).

Terms Definition

Anemia is diagnosed using the hemoglobin values and the levels given by the WHO, i.e., <12 g per dL in women and <13 g per dL in men (WHO, 2011). Several studies have employed these WHO stipulated diagnostic criteria for screening of anemia (Cesari et al., 2004; Semba et al., 2009; Yusof et al., 2018; Abdullah et al., 2020). The present study also employed the same WHO criteria to include men and women subjects in the anemic study group.

Statistical Analysis

Statistical analysis was performed considering a confidence level of 95%. The acquired data were analyzed using statistical software, namely, SPSS (v. 22.0, IBM, Chicago, IL, USA) and MedCalc (v. 19.05, Ostend, Belgium). For the continuous variables, the median (upper limit-lower limit) was determined as the frequency distribution of the parameters rejected normality. Descriptive statistics and the Students' independent sample t-test were employed, and a statistically significant level was set up at $P < 0.05$.

Receiver operator characteristic (ROC) analysis was done using body weight, BMI, WC, BC, WHR, and WHtR as continuous variables and anemia as the categorical variable. The area under the curves (AUCs), sensitivities (SS), specificities (SP), Youden's indices, and cut-off values for all continuous variables were obtained and compared. For all evaluations, $P < 0.05$ was deemed statistically significant.

RESULTS

Anthropometric Parameters as Predictors of Anemia

The ROC curves of the anthropometric parameters, i.e., BMI, body weight, WC, BC, WHR, and WHtR for prediction of anemia in the young adults of Malaysia are presented in **Figure 1**. The ROC curve analysis revealed a high-predictive value of BMI for anemia, which in men had AUC (CI 95%) of 0.889 with the optimal cut-off value of 20.65 kg/m^2 (sensitivity, SS: 79.17%; and

TABLE 1 | Comparison of receiver operating characteristics (ROC) curves of anthropometric indices in predicting anemia in young adult men of Malaysia.

Variables	Cut-off values	AUC (95% CI)	*P* value	SS (%)	SP (%)	Pairwise comparison of ROC curves		
						Variables	AUC difference	*P* value
BMI[b]	20.65	0.889	< 0.0001	79.17	89.80	BMI ~ WHtR	0.082	0.0709
						BMI ~ WHR	0.113	0.1144
BW[c]	62	0.886	< 0.0001	83.33	79.59	BMI ~ BW	0.003	0.8571
						BMI ~ WC	0.008	0.8307
WC[d]	76	0.883	< 0.0001	87.50	83.33	WHtR ~ WHR	0.031	0.6007
						WHtR ~ BW	0.079	0.0905
WHR	0.79	0.761	< 0.0001	58.33	87.76	WHtR ~ WC	0.074	0.0363[a]
						WHR ~ BW	0.110	0.1099
WHtR	0.45	0.793	< 0.0001	87.50	71.43	WHR ~ WC	0.105	0.0498[a]
						BW ~ WC	0.005	0.8829

[b]kg/m^2; [c]kg; [d]cm; BMI, body mass index; BW, body weight; WC, waist circumference; WHR, waist-to-hip ratio; WHtR, waist-to-height ratio; AUC, area under curve; SS, sensitivity; SP, specificity; [a]$P < 0.05$.

TABLE 2 | Comparison of ROC curves of anthropometric indices in predicting anemia in young adult women of Malaysia.

Variables	Cut-off values	AUC (95% CI)	*P* value	SS (%)	SP (%)	Pairwise comparison of ROC curves		
						Variables	AUC difference	*P* value
BMI[b]	19.7	0.904	< 0.0001	78.18	91.40	BMI ~ WHtR	0.137	0.0001[a]
						BMI ~ WHR	0.276	0.0001[a]
BW[c]	49.5	0.836	< 0.0001	69.09	88.17	BMI ~ BW	0.053	0.0225[a]
						BMI ~ WC	0.129	0.0003[a]
WC[d]	71	0.771	< 0.0001	83.02	63.04	WHtR ~ WHR	0.139	0.0001[a]
						WHtR ~ BW	0.084	0.0401[a]
WHR	0.74	0.624	0.0109	49.06	77.17	WHtR ~ WC	0.007	0.6138[a]
						WHR ~ BW	0.223	0.0001[a]
WHtR	0.45	0.764	< 0.0001	83.64	59.14	WHR ~ WC	0.147	0.0001[a]
						BW ~ WC	0.076	0.0354[a]

[b]kg/m^2; [c]kg; [d]cm; BMI, body mass index; BW, body weight; WC, waist circumference; WHR, waist-to-hip ratio; WHtR, waist-to-height ratio; AUC, area under curve; SS, sensitivity; SP, specificity; [a]$P < 0.05$.

specificity, SP: 89.80%), and in women, BMI had the AUC (CI 95%) of 0.904 with the optimal cut-off value of 19.7 kg/m^2 (SS: 78.18%; SP: 91.40%). Following BMI, the predictive value of body weight for anemia in men, showed an AUC (CI 95%) of 0.886 with the optimal cut-off value of 62 kg (SS: 83.33%; SP: 79.59%), while in women, it showed AUC (CI 95%) of 0.836 with the optimal cut-off value of 49.5 kg with (SS: 69.09%; SP: 88.17%). WC had the cut-off values of 76 cm (AUC: 0.883; SS: 87.5%; SP: 83.33%) and 71 cm (AUC: 0.771; SS: 83.02% SP: 63.04%) for men and women, respectively. WHR showed the predictive cut-off values of 0.79 (AUC: 0.761; SS: 58.33%; SP: 87.76%) for men and similarly in women, it yielded cut-off value of 0.74 (AUC: 0.624; SS: 49.06%; SP: 77.17%). For WHtR, the predictive cut-off value for anemia inmen was 0.45 (AUC: 0.793; SS: 87.50%; SP: 71.43%), and in women, it was also 0.45 (AUC: 0.764; SS: 83.64%; SP: 59.14%).

Body mass index stands out as the most potent anthropometric index to be used in the prediction of anemia in men based on the highest AUC and SP obtained. However, pairwise comparison of all the anthropometric variables in predicting anemia showed statistically significant differences only for WC vs. WHR and WHtR. It indicates that the efficacy of the anthropometric variables considered in the study is not distinctly different in detecting anemia in men (**Table 1**). However, in women, pairwise comparison of these anthropometric variables reflected the significant difference in all the cases (except WC vs. WHtR) and indicated the higher efficacy of BMI in predicting anemia among young adult women of Malaysia (**Table 2**).

Anthropometric Variables and Hemoglobin Concentration

In Malaysian Young Adult Men and Women

Comparison of anthropometric variables and hemoglobin concentration between age-matched young adult men and women of Malaysia are presented in **Table 3**. In this study, the numbers of total respondents were 113 for men and 132 for women, respectively. The group of men includes 30 Malays, 32

Chinese, 29 Indians, and 22 others. Similarly, the group of women includes 35 Malays, 37 Chinese, 32 Indians, and 28 others.

Table 4 represents a gender-wise comparison of these variables among different ethnic groups. One-way ANOVA was applied for the test at 95% CI, level of significance was considered when $P < 0.05$. Comparison of data of age-matched young men showed significantly lower hemoglobin in Indians and others. Anthropometric variables, such as WC and WHtR were also found to be significantly lower in other groups. The height of Chinese men was found to be slightly higher than other groups. In women, body weight, WC, and WHR were found to be lower in Chinese and others, whereas Indians have significantly higher BC and height than other ethnic groups.

Comparison Between Anemic and Non-anemic Groups of Young Adults of Malaysia

Among 113 men and 132 women, a total of 21 (18.6%) men and 29 women (21.96%) were found to be anemic following the WHO categorization (< 13 g per dL in men, < 12 g per dL in women) (WHO, 2011). **Figure 2** shows significantly lower hemoglobin and all the other anthropometric variables in anemic men compared with normal ($P < 0.0001$). All the anthropometric variables (except height) were found to be significantly different between anemic and normal men, for all the ethnic groups. While the variables were compared across the ethnic groups, body weight was found to be significantly lower in Chinese men and others, whereas BMI was found to be significantly lower in Indian men (**Table 5**). For women too, all the anthropometric variables differed significantly between anemic and normal women ($P <$ 0.0001) (**Figure 3**). Across the ethnic groups, Chinese women showed significantly higher body weight, but lower WHR ($P <$ 0.05) (**Table 6**).

TABLE 3 | Age, hemoglobin concentration, and anthropometric variables of respondents.

	Men (*N* = 113)	Women (*N* = 132)
Age[a]	21.0 (24.0–18.0)	19.0 (23.0–18.0)
Weight[b]	66.0 (97.0–49.0)	50.0 (80.0–40.0)
Height[c]	165.2 (175.0–161.0)	155.8 (171.0–148.0)
BMI[d]	22.4 (32.4–18.9)	20.9 (34.6–17.3)
Hemoglobin[e]	14.4 (16.4–10.5)	12.3 (14.5–7.9)
WC[c]	79.3 (108.7–64.0)	73.0 (97.0–59.0)
BC[c]	93.7 (117.4–82.2)	91.2 (115–75)
WHR	0.86 (0.93–0.77)	0.79 (0.89–0.69)
WHtR	0.49 (0.63–0.40)	0.47 (0.63–0.37)

Data are represented as median (upper limit–lower limit); [a]years, [b]kg, [c]cm, [d]kg/m^2, [e]g/dl; BMI, body mass index; BW, body weight; WC, waist circumference; BC, buttock circumference; WHR, waist-to-hip ratio; WHtR, waist-to-height ratio.

Prevalence of Anemia in Young Malaysian Adults Based on Gender and Ethnicity

The occurrence of anemia among the study groups of subjects is shown in **Table 7**, considering the hemoglobin concentration of ≥ 13 g per dL in men, ≥ 12 g per dL in women as normal. A total of 50 respondents (20.41%) of 245 were reported to have anemia. Among 113 men and 132 women, a total of 21 (18.6%) men and 29 women (21.96%) were found to be anemic. The gender-wise comparison showed a slightly higher prevalence of anemia in women. While comparing the anemia occurrence across the ethnic groups, it has been found that Indians have the highest prevalence of anemia (26.22%), followed by Malays (21.54%), others (20%), and Chinese (14.5%).

DISCUSSION

This is the first study that proposes cut-off values of the major anthropometric indices in predicting the occurrence of anemia in young male and female adults in Malaysia. The study has also evaluated and compared the anthropometric indices and occurrence of anemia among young adults of Malaysia belonging to different ethnicity.

Malaysia homes a large population with anemia with the current prevalence of being 13.8% (Abdullah et al., 2020). A non-invasive simple anthropometric predictor of anemia occurrence will significantly contribute to the early prediction of anemia and thereby aid its prevention. Earlier studies had suggested the essentiality of anthropometry in the prediction of hemoglobin

TABLE 4 | Comparison of various parameters between men and women of different ethnic groups.

	Malay (*N* = 65)		Chinese (*N* = 69)		Indian (*N* = 61)		Others (*N* = 50)	
	Men (*N* = 30)	Women (*N* = 35)	Men (*N* = 32)	Women (*N* = 37)	Men (*N* = 29)	Women (*N* = 32)	Men (*N* = 22)	Women (*N* = 28)
Age[b]	21.0 (24.0–18.0)	19.0 (23.0–18.0)	20.0 (25.0–16.0)	20.0 (23.0–17.0)	20.0 (23.0–17.0)	21.0 (26.0–17.0)	21.5 (24.0–20.0)	21.0 (24.0–18.0)
Weight[c]	66.0 (97.0–49.0)	50.0 (80.0–40.0)	66.0 (110.0–48.0)	53.0 (91.0–40.0)[a]	66.3 (92.0–41.0)	57.0 (118.0–34.0)[a]	64.0 (96.0–55.0)	52.0 (81.0–42.0)[a]
Height[d]	165.2 (175.0–160.0)	155.8 (171.0–148.0)	171.0 (185.0–160.0)[a]	160.0 (173.0–149.0)[a]	170.0 (187.0–157.0)	158.0 (168.0–150.0)	171.5 (179.0–162.0)	157.0 (168.0–145.0)
BMI[e]	22.4 (32.4–18.9)	20.9 (34.6–17.3)	21.9 (36.7–16.3)	19.9 (35.1–13.3)	21.7 (31.5–13.8)	21.7 (35.2–13.1)	21.7 (29.1–19.4)	19.9 (34.6–17.3)
Hemoglobin[f]	14.4 (16.4–10.5)	12.3 (14.5–7.9)	14.1 (17.2–7.3)	12.7 (14.8–7.6)	13.6 (16.9–9.2)[a]	12.5 (14.2–8.2)	13.5 (16.2–10.6)[a]	11.7 (14.1–10.0)
WC[d]	79.3 (108.7–64.0)	73.0 (97.0–59.0)	76.6 (101.0–63.0)	69.0 (96.0–59.0)[a]	76.8 (103.0–63.0)	74.8 (104.0–56.7)	75.9 (102.0–63.9)[a]	69.5 (92.5–57.0)[a]
BC[d]	93.7 (117.4–82.2)	91.2 (115.0–75.0)	92.7 (114.0–78.0)	90.1 (112.5–80.0)	93.7 (113.2–72.0)	95.0 (117.5–75.0)[a]	91.9 (113.0–79.9)	88.0 (109.5–82.0)
WHR	0.86 (0.93–0.77)	0.79 (0.89–0.69)	0.83 (0.99–0.74)	0.76 (0.92–0.67)[a]	0.87 (1.01–0.75)	0.80 (0.97–0.67)	0.83 (0.98–0.75)	0.74 (0.88–0.68)[a]
WHtR	0.49 (0.63–0.40)	0.47 (0.63–0.37)	0.45 (0.63–0.37)	0.43 (0.60–0.37)[a]	0.45 (0.61–0.36)	0.45 (0.69–0.36)	0.43 (0.63–0.34)[a]	0.45 (0.61–0.37)

Data are represented as median (upper limit–lower limit); [b]years, [c]kg, [d]cm, [e]kg/m^2, [f]g/dl; [a]compared to same gender, different ethnic group; $P < 0.05$; BMI, body mass index; BW, body weight; WC, waist circumference; BC, buttock circumference; WHR, waist-to-hip ratio; WHtR, waist-to-height ratio.

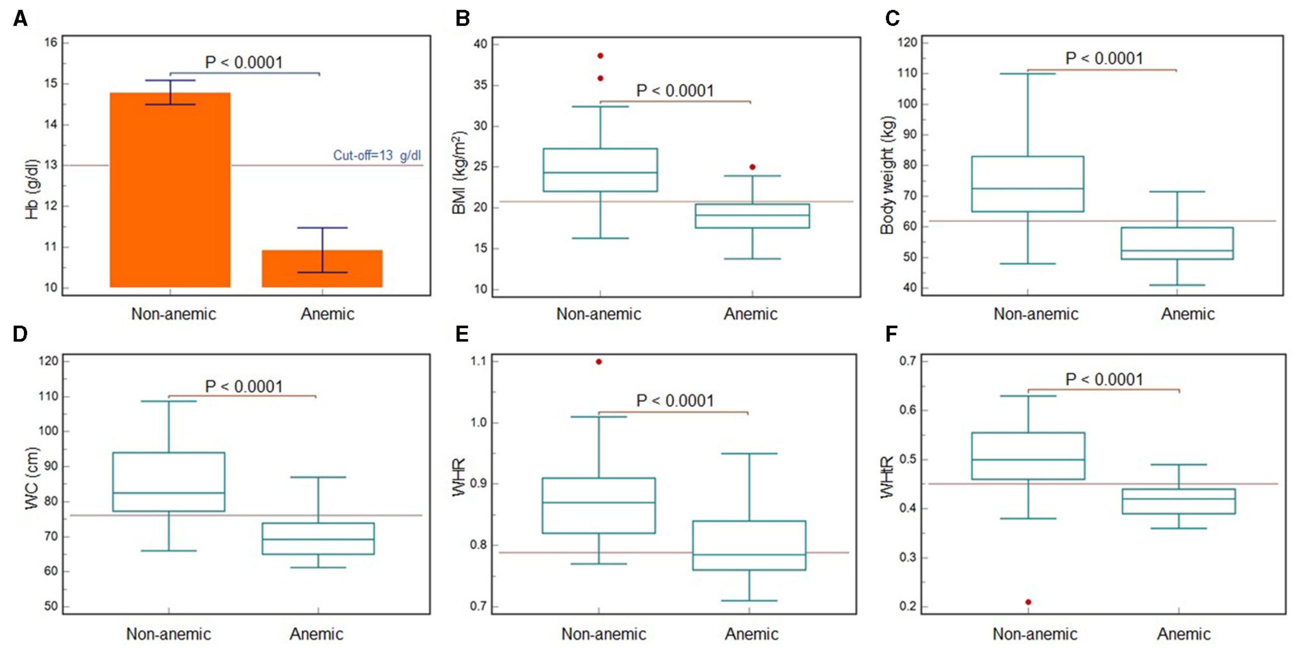

FIGURE 2 | Comparison of hemoglogin levels **(A)** and anthropometric variables **(B–F)** between Malaysian young adult men with and without anemia. Statistically significant differences ($P < 0.0001$) were recorded for hemoglobin concentration **(A)**, BMI **(B)**, body weight **(C)**, WC **(D)**, WHR **(E)** and WHtR **(F)**. The red lines are indicating the cut-off values. Hemoglobin cut-off is as per the WHO reference standard (WHO, 2011).

TABLE 5 | Comparison of various parameters among the men of different ethnic groups.

	Malay ($N = 30$)		**Chinese ($N = 32$)**		**Indian ($N = 29$)**		**Others ($N = 22$)**	
	Normal ($N = 24$)	**Anemic ($N = 6$)**	**Normal ($N = 28$)**	**Anemic ($N = 4$)**	**Normal ($N = 22$)**	**Anemic ($N = 7$)**	**Normal ($N = 18$)**	**Anemic ($N = 4$)**
Age[c]	21.0 (24.0–18.0)	19.3 (20.0–18.0)	20.0 (23.0–17.0)	20.0 (25.0–16.0)	20.0 (23.0–17.0)	22.0 (22.0–20.0)	21.0 (24.0–18.0)	21.3 (23.0–20.0)
Weight[d]	66.0 (97.0–55.0)	51.1 (53.0–49.0)[a]	76.5 (110.0–48.0)	56.0 (71.5–48.0)[ab]	69.0 (92.0–53.0)	51.0 (56.5–41.0)[a]	66.0 (96.0–56.0)	54.0 (66.0–55.0)[ab]
Height[e]	167.0 (175.0–161.0)	164.5 (166.0–160.0)	172.0 (185.0–162.5)	168.0 (181.0–160.0)	169.5 (187.0–161.0)	170.0 (177.0–157.0)	170.5 (179.0–166.0)	169.5 (170.0–162.0)
BMI[f]	24.9 (32.4–20.2)	19.2 (20.7–18.9)[a]	24.4 (36.7–16.3)	19.9 (25.0–17.4)[a]	24.1 (31.5–19.1)	16.9 (19.6–13.8)[ab]	24.7 (29.1–21.2)	18.9 (22.0–19.4)[a]
Hemoglobin[g]	14.9 (16.4–13.2)	11.2 (11.9–10.5)[a]	14.7 (17.2–13.2)	11.6 (12.3–7.3)[a]	15.0 (16.9–13.2)	10.1 (12.1–9.2)[a]	14.5 (16.2–14.0)	10.8 (12.3–10.6)[a]
WC[e]	81.3 (108.7–72.0)	68.5 (70.7–64.0)[a]	82.5 (101.0–66.0)	69.9 (87.0–63.0)[a]	92.0 (103.0–66.5)	69.5 (74.0–63.0)[a]	84.0 (102.0–67.0)	68.9 (75.0–63.9)[a]
BC[e]	94.7 (117.4–89.0)	83.6 (87.3–82.2)[a]	100.2 (114.0–80.0)	87.8 (104.0–78.0)[a]	98.0 (113.2–89.0)	85.8 (87.0–72.0)[a]	96.9 (113.0–88.0)	86.9 (88.0–79.9)[a]
WHR	0.87 (0.93–0.81)	0.79 (0.80–0.77)[a]	0.85 (0.99–0.77)	0.78 (0.90–0.74)[a]	0.89 (1.01–0.81)	0.84 (0.95–0.75)[b]	0.88 (0.98–0.81)	0.79 (0.85–0.75)[a]
WHtR	0.50 (0.63–0.44)	0.42 (0.43–0.40)[a]	0.48 (0.63–0.38)	0.43 (0.49–0.37)[a]	0.51 (0.61–0.43)	0.42 (0.44–0.36)[a]	0.50 (0.63–0.45)	0.42 (0.44–0.34)[a]

Data are represented as median (upper limit–lower limit); [c]years, [d]kg, [e]cm, [f]kg/m^2, [g]g/dl; [a]normal vs. anemic, [b]compared among normal/anemic across ethnic groups; $P < 0.05$; BMI, body mass index; BW, body weight; WC, waist circumference; BC, buttock circumference; WHR, waist-to-hip ratio; WHtR, waist-to-height ratio.

levels under various pathological conditions (Lee et al., 2004; Al-Hashem, 2007; Odagiri et al., 2014; Vuong et al., 2014). This study, using comparative ROC curve with the anthropometric parameters, namely BMI, body weight, WC, WHR, and WHtR as continuous variables and "anemia" as the categorical variable, has found BMI to be the best predictor of the occurrence of anemia among the young adult men ($n = 113$) and women ($n = 132$) of Malaysia (**Figure 1**). The result attributes to the highest AUC and SP of BMI among all the other anthropometric parameters (**Tables 1**, **2**). The present results are in line with some of the distinguished evidence in this realm. In the Korean population, it was reported that hemoglobin level associated significantly better with BMI and weight as compared with WC and WHtR (Lee and Kim, 2016). In the US population, several studies had reported that low BMI, minimal physical activities, and certain cardiac and renal diseases are among the best predictors of anemia (Frith-Terhune et al., 2000; Zakai et al., 2005; Ausk and Ioannou, 2008; Semba et al., 2009; Neymotin and Sen, 2011). Low BMI was also in the list of the best anemia predictors in the Italian population as described by Cesari et al. (2004). A study in the Chinese population also sinks with our prediction and it stated that obese women had less chances of being anemic than those with normal body weight (Qin et al., 2013). However, there are few studies that supported other anthropometric indices, such as WC (Al-Hashem, 2007), height, and WHR (Lee et al., 2004) over BMI to predict hemoglobin level and occurrence of anemia. In our

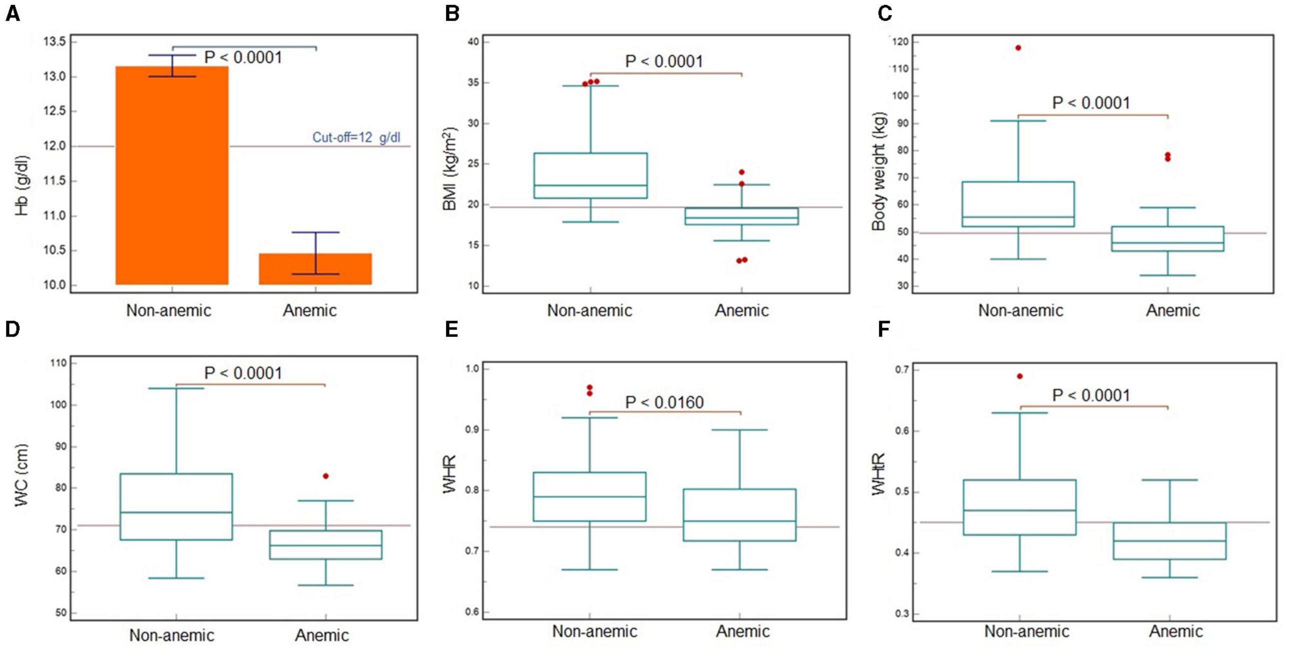

FIGURE 3 | Comparison of hemoglogin levels **(A)** and anthropometric variables **(B–F)** between Malaysian young adult women with and without anemia. Statistically significant differences (all variables: $P < 0.0001$; WHR: $P < 0.016$) were recorded for hemoglobin concentration **(A)**, BMI **(B)**, body weight **(C)**, WC **(D)**, WHR **(E)** and WHtR **(F)**. The red lines are indicating the cut-off values. Hemoglobin cut-off is as per the WHO reference standard (WHO, 2011).

ROC analysis, among the young adult men in Malaysia, other anthropometric parameters such as the body weight, WC, WHR, and WHtR scored slightly below BMI, all yielding high AUC, SP, and SS in predicting anemia (**Figure 1A**; **Table 1**). While, among the young adult women in Malaysia, the values of the anthropometric parameters in predicting anemia had significant differences and can be distinctly arranged in descending order of their efficacy in predicting anemia, which is, BMI > bodyweight> WC > WHtR > WHR (**Figure 1B**; **Table 2**). Overall, our findings commensurate with various previous reports (Cesari et al., 2004; Choi et al., 2004; Zakai et al., 2005; Qin et al., 2013) and, thus, it was proposed that BMI is a strong predictor of hemoglobin level and anemia with cut-off values of 20.65 kg/m^2 and 19.7 kg/m^2 in young adult men and women of Malaysia, respectively.

In general, anemia is more prevalent in women than in men, mostly because of menstruation, parasitic infestations, physical stress, and inadequate nutrient intake (Piammongkol et al., 2006; Pala and Dundar, 2008; Neymotin and Sen, 2011; Takeda et al., 2011; Abdullah et al., 2017). In this report, the study population comprises young adults who are far from the menopausal age of women of Malaysia, which is reportedly 50 years (Abdullah et al., 2017). It is known that during menstruation, young women normally may lose up to 80 ml of blood per cycle (Magnay et al., 2018). The food intake and dietary iron often fail to compensate for the loss of blood during heavy menstruation resulting in anemia (Mirza et al., 2018). This may explain the higher prevalence of anemia among women than in men in the study (**Table 1**). Moreover, Malaysia is a multi-ethnic nation, comprising of three main ethnic groups with a sizeable, diverse indigenous population. Based on ethnicity, culture, beliefs, lifestyle, dietary choices vary greatly among the population of Malaysia that deeply influences the physiological status (Gaskell et al., 2008; Hiza et al., 2013; Nohan et al., 2020). Thus, we segregated our data based on the ethnic groups of the young adults of Malaysia (**Tables 4–6**), and it was seen that the Indians comprised the greatest proportion of the anemic cohort (26.22%) followed by the Malays (21.54%), subjects with indigenous (other) ethnicity (20%), and the Chinese (14.5%) (**Table 7**). Very few previous reports are available that put forth the variation of anemia occurrence among the different ethnic groups in a multi-ethnic nation (Frith-Terhune et al., 2000; Guralnik et al., 2004; Lau et al., 2015). A study in Singapore, which evaluated anemia in a small number of subjects with chronic kidney disease, had considered ethnicity among its study variables. However, this study did not find any significant differences in anemia among Chinese, Malay, and Indian ethnicities (Lau et al., 2015). Whereas, in the US, a few large-scale studies suggested that the prevalence of anemia due to iron deficiency or low hemoglobin does differ with ethnicity (Frith-Terhune et al., 2000; Guralnik et al., 2004). The study by the National Health and Nutritional Examination Survey (NHANES) showed a significantly higher anemia prevalence among the non-Hispanic black population (Frith-Terhune et al., 2000). Moreover, two recent Malaysian studies also are in congruence with our findings

TABLE 6 | Comparison of various parameters among the women of different ethnic groups.

	Malay (*N* = 35)		Chinese (*N* = 37)		Indian (*N* = 32)		Others (*N* = 28)	
	Normal (N = 27)	Anemic (*N* = 8)	Normal (*N* = 31)	Anemic (*N* = 6)	Normal (*N* = 23)	Anemic (*N* = 9)	Normal (*N* = 22)	Anemic (*N* = 6)
Age[c]	19.0 (23.0–18.0)	20.0 (22.0–18.0)	20.0 (23.0–17.0)	21.0 (23.0–17.0)	20.0 (26.0–18.0)	21.0 (23.0–17.0)	21.0 (24.0–21.0)	20.0 (23.0–18.0)
Weight[d]	52.5 (80.0–44.0)	44.5 (53.0–40.0)[a]	54.5 (91–40.0)	49.3 (78.5–40.0)[ab]	68.0 (118.0–46.0)	44.5 (56.0–34.0)[a]	76.0 (81.0–49.0)	44.5 (77.0–42.0)[a]
Height[e]	155.3 (171.0–148.0)	156.5 (160.0–150.0)	159.5 (173.0–149.0)	160.3 (181.0–151.0)	158.0 (168.0–150.0)	157.0 (167.0–150.0)	159.0 (165.0–153.0)	156.0 (168.0–145.0)
BMI[f]	22.3 (34.6–17.9)	18.1 (20.7–17.3)[a]	21.8 (35.1–18.0)	18.7 (24.0–13.3)[a]	26.1 (35.2–19.9)	18.2 (20.1–13.1)[a]	27.9 (34.6–19.4)	19.2 (22.6–17.3)[a]
Hemoglobin[g]	12.8 (14.5–12.1)	9.4 (11.8–7.9)[ab]	13.1 (14.8–12.5)	10.9 (11.5–7.6)[a]	13.1 (14.2–12.0)	10.6 (11.6–8.2)[a]	13.2 (14.1–12.3)	11.2 (11.8–10.0)[a]
WC[e]	74.2 (97.0–64.0)	67.6 (74.0–59.0)[a]	71.0 (96.0–60.0)	66.0 (87.0–59.0)[a]	85.0 (104.0–65.0)	66.8 (74.9–56.7)[a]	82.0 (92.5–58.4)	66.8 (77.0–57.0)[a]
BC[e]	95.0 (115.0–75.0)	87.0 (93.0–83.0)[a]	92.8 (112.5–80.0)	88.0 (97.0–81.0)[a]	102.5 (117.5–91.0)	87.5 (92.0–75.0)[a]	106.0 (109.5–85.5)	87.9 (94.0–82.0)[a]
WHR	0.81 (0.89–0.71)	0.79 (0.85–0.69)	0.77 (0.92–0.67)	0.74 (0.90–0.67)[b]	0.82 (0.97–0.68)	0.75 (0.88–0.67)[a]	0.77 (0.88–0.68)	0.73 (0.88–0.70)[b]
WHtR	0.48 (0.63–0.42)	0.44 (0.47–0.37)	0.44 (0.60–0.37)	0.42 (0.52–0.37)[a]	0.54 (0.69–0.41)	0.41 (0.45–0.36)[ab]	0.50 (0.61–0.37)	0.43 (0.49–0.39)[a]

Data are represented as median (upper limit–lower limit); [c]years, [d]kg, [e]cm, [f]kg/m^2, [g]g/dl; [a]normal vs. anemic, [b]compared among normal/anemic across ethnic groups; $P < 0.05$; BMI, body mass index; BW, body weight; WC, waist circumference; BC, buttock circumference; WHR, waist–to-hip ratio; WHtR, waist-to-height ratio.

TABLE 7 | Distribution of respondents according to their gender, ethnicity, and prevalence of anemia.

	Total (*N* = 245)		Malay (*N* = 65)		Chinese (*N* =69)		Indian (*N* = 61)		Others (*N* = 50)	
	Normal	Anemic[a]	Normal	Anemic[a]	Normal	Anemic[a]	Normal	Anemic[a]	Normal	Anemic[a]
Men (*N* = 113)	92 (81.40)	21 (18.60)	24 (80)	6 (20)	28 (87.5)	4 (12.5)	22 (75.9)	7 (24.1)	18 (81.8)	4 (18.2)
Women (N = 132)	103 (78.04)	29 (21.96)	27 (77.14)	8 (22.86)	31 (83.78)	6 (16.22)	23 (71.8)	9 (28.2)	22 (78.57)	6 (21.43)
Total	195 (79.59)	50 (20.41)	51 (78.46)	14 (21.54)	59 (85.5)	10 (14.5)	45 (73.78)	16 (26.22)	40 (80)	10 (20)

Data are represented as n (%); [a]hemoglobin concentration < 13 g per dL in men, < 12 g per dL in women.

(Yusof et al., 2018; Abdullah et al., 2020). One of the concurrent studies conducted in the Malaysian population aged 35–70 years reported the highest prevalence of anemia among the Malaysian Indians (19.9%), with lower yet concerning prevalence of anemia among the Malays (13.1%) and Chinese (12.0%) (Abdullah et al., 2020). The second study, again based on Malaysian older population aged 60 years or above, showed a high prevalence of anemia in all the three ethnic groups, namely, Indians (42.1%), Malays (36.9%), Chinese (31.1%), and others (36.3%) (Yusof et al., 2018). This study stands unique also for being the first-ever report on the prevalence of anemia and its association with anthropometric indices among young adults in Malaysia.

In summary, this study has proposed specific anthropometric markers with optimal cut-off values to predict anemia among young adults of Malaysia. We envisage that the simple, inexpensive, and intelligible approach of using the anthropometric markers in the prediction of anemia will aid widescale early detection of this crucial health condition and help in the rapid development of its management policies. This analysis reveals that BMI is the most accurate anthropometric marker for the presence of anemia among young adult men and women in Malaysia having the optimal cut-off values of 20.65 kg/m^2 and 19.7 kg/m^2, respectively. This study has also shown the pattern of prevalence of anemia based on gender and ethnicity. Future wide-reaching studies in this population of young adults of Malaysia are encouraged that may encompass the association of menstrual parameters and the prevalence of anemia among women. Moreover, studies on the same population, young adults of Malaysia, may also focus on how socioeconomic factors may impact anemia prevalence.

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 Research Management Centre, MAHSA University. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

SD, IK, and PS have equally contributed to design the study, conceived the study, carried out the experiments, conducted the data analysis, and interpreted the data. SD, IK, PS, and SC have drafted, edited, and reviewed the manuscript. IK has procured the Institutional Ethical Approval for the study. SC has procured the grant for publication. All authors have given their consent for submission.

FUNDING

This work was supported by FRGS, Malaysia, FRGS/1/2018/STG03/AIMST/02/1.

ACKNOWLEDGMENTS

The authors would like to acknowledge all the participants who have participated in the study.

REFERENCES

Abdullah, B., Moize, B., Ismail, B. A., and Zamri, M. (2017). Prevalence of menopausal symptoms, its effect to quality of life among Malaysian women and their treatment seeking behaviour. *Med. J. Malaysia* 72:95.

Abdullah, N., Ismail, N., Abd Jalal, N., Radin, F. M., Othman, R., Arifin, A. S. K., et al. (2020). Prevalence of anaemia and associated risk factors amongst The Malaysian Cohort participants. *Ann. Hematol.* 99, 2521–2527. doi: 10.1007/s00277-020-04279-w

Aigner, E., Weiss, G., and Datz, C. (2015). Dysregulation of iron and copper homeostasis in nonalcoholic fatty liver. *World J. Hepatol.* 7, 177. doi: 10.4254/wjh.v7.i2.177

Al-Hashem, F. H. (2007). Is it necessary to consider obesity when constructing norms for hemoglobin or when screening for anemia using hemoglobin levels? *Saudi Med. J.* 28, 41–45.

Ausk, K. J., and Ioannou, G. N. (2008). Is obesity associated with anemia of chronic disease? A population-based study. *Obesity* 16, 2356–2361. doi: 10.1038/oby.2008.353

Bharati, S., Pal, M., Som, S., and Bharati, P. (2015). Temporal trend of anemia among reproductive-aged women in India. *Asia Pac. J. Pub. Health* 27, NP1193–NP1207. doi: 10.1177/1010539512442567

Cesari, M., Penninx, B. W., Lauretani, F., Russo, C. R., Carter, C., Bandinelli, S., et al. (2004). Hemoglobin levels and skeletal muscle: results from the InCHIANTI study. *J. Gerontol. Series A: Biol. Sci. Med. Sci.* 59, M249–M254. doi: 10.1093/gerona/59.3.M249

Chang, J.-S., Chen, Y.-C., Owaga, E., Palupi, K. C., Pan, W.-H., and Bai, C.-H. (2014). Interactive effects of dietary fat/carbohydrate ratio and body mass index on iron deficiency anemia among Taiwanese women. *Nutrients* 6, 3929–3941. doi: 10.3390/nu6093929

Choi, C. W., Lee, J., Park, K. H., Yoon, S. Y., Choi, I. K., Oh, S. C., et al. (2004). Prevalence and characteristics of anemia in the elderly: cross-sectional study of three urban Korean population samples. *Am. J. Hematol.* 77, 26–30. doi: 10.1002/ajh.20140

Choudhary, S., and Binawara, B. K. (2012). Effect of exercise on serum iron, blood haemoglobin and cardiac efficiency. *J. Postgrad. Med. Inst.* 26, 13–16.

Collett-Solberg, P. F., Fleenor, D., Schultz, W. H., and Ware, R. E. (2007). Short stature in children with sickle cell anemia correlates with alterations in the IGF-I axis. *J. Pediat. Endocrinol. Metab.* 20, 211–218. doi: 10.1515/JPEM.2007.20.2.211

El-Shafie, A. M., Kasemy, Z. A., Omar, Z. A., Alkalash, S. H., Salama, A. A., Mahrous, K. S., et al. (2020). Prevalence of short stature and malnutrition among Egyptian primary school children and their coexistence with Anemia. *Italian J. Pediat.* 46, 1–9. doi: 10.1186/s13052-020-00855-y

Frith-Terhune, A., Cogswell, M. E., Khan, L. K., Will, J. C., and Ramakrishnan, U. (2000). Iron deficiency anemia: higher prevalence in Mexican American than in non-Hispanic white females in the third National Health and Nutrition Examination Survey, 1988–1994. *Am. J. Clin. Nutr.* 72, 963–968. doi: 10.1093/ajcn/72.4.963

Gaskell, H., Derry, S., Moore, R. A., and McQuay, H. J. (2008). Prevalence of anaemia in older persons: systematic review. *BMC Geriat.* 8, 1–8. doi: 10.1186/1471-2318-8-1

Guralnik, J. M., Eisenstaedt, R. S., Ferrucci, L., Klein, H. G., and Woodman, R. C. (2004). Prevalence of anemia in persons 65 years and older in the United States: evidence for a high rate of unexplained anemia. *Blood* 104, 2263–2268. doi: 10.1182/blood-2004-05-1812

Haniff, J., Das, A., Onn, L. T., Nordin, N. M., Rampal, S., Bahrin, S., et al. (2007). Anemia in pregnancy in Malaysia: a cross-sectional survey. *Asia Pac. J. Clin. Nutr.* 16:527. doi: 10.6133/APJCN.2007.16.3.18

Hassan, R., Abdullah, W. Z., and Hussain, N. H. N. (2005). Anemia and iron status of Malay women attending an antenatal clinic in Kubang Kerian, Kelantan, Malaysia. *Southeast Asian J. Trop. Med. Pub. Health* 36:1304.

Hiza, H. A., Casavale, K. O., Guenther, P. M., and Davis, C. A. (2013). Diet quality of Americans differs by age, sex, race/ethnicity, income, and education level. *J. Acad. Nutr. Dietet.* 113, 297–306. doi: 10.1016/j.jand.2012.08.011

Ishak, R., and Hassan, K. (1994). The increasing importance of vitamin B12 deficiency as a contributing factor to anemia in Malaysia. *Southeast Asian J. Trop. Med. Pub. Health* 25, 457–457.

Khambalia, A. Z., Aimone, A. M., and Zlotkin, S. H. (2011). Burden of anemia among indigenous populations. *Nutr. Rev.* 69, 693–719. doi: 10.1111/j.1753-4887.2011.00437.x

Lau, B. C. V., Ong, K. Y., Yap, C. W., Vathsala, A., and How, P. (2015). Predictors of anemia in a multi-ethnic chronic kidney disease population: a case–control study. *SpringerPlus* 4, 1–9. doi: 10.1186/s40064-015-1001-z

Lee, B. J., and Kim, J. Y. (2016). Identification of hemoglobin levels based on anthropometric indices in elderly Koreans. *PloS ONE* 11:e0165622. doi: 10.1371/journal.pone.0165622

Lee, H. S., Kim, B. E., Cho, M. S., and Kim, W. Y. (2004). A study on nutrient intake, anthropometric data and serum profiles among high school students residing in Seoul. *Korean J. Comm. Nutr.* 9, 589–596.

Legro, R. S., Kunselman, A. R., Dodson, W. C., and Dunaif, A. (1999). Prevalence and predictors of risk for type 2 diabetes mellitus and impaired glucose tolerance in polycystic ovary syndrome: a prospective, controlled study in 254 affected women. *J. Clin. Endocrinol. Metab.* 84, 165–169. doi: 10.1097/00006254-199906000-00019

Magnay, J. L., O'Brien, S., Gerlinger, C., and Seitz, C. (2018). A systematic review of methods to measure menstrual blood loss. *BMC Women's Health* 18, 1–13. doi: 10.1186/s12905-018-0627-8

Micozzi, M. S., Albanes, D., and Stevens, R. G. (1989). Relation of body size and composition to clinical biochemical and hematologic indices in US men and women. *Am. J. Clin. Nutr.* 50, 1276–1281. doi: 10.1093/ajcn/50.6.1276

Mirza, F. G., Abdul-Kadir, R., Breymann, C., Fraser, I. S., and Taher, A. (2018). Impact and management of iron deficiency and iron deficiency anemia in women's health. *Expert Rev. Hematol.* 11, 727–736. doi: 10.1080/17474086.2018.1502081

Neymotin, F., and Sen, U. (2011). Iron and obesity in females in the United States. *Obesity* 19, 191–199. doi: 10.1038/oby.2010.112

Nik Rosmawati, N., Mohd Nazri, S., and Mohd Ismail, I. (2012). The rate and risk factors for anemia among pregnant mothers in Jerteh Terengganu, Malaysia. *J. Comm. Med. Health Educ.* 2, 2161–2165. doi: 10.4172/2161-0711.1000150

Nohan, A. F., Adznam, S. N. A., Jamaluddin, R., and Norazman, C. W. (2020). Diet quality and its associated factors among community dwelling older adults in urban district in Kuala Lumpur, Malaysia. *Malays. J. Med. Health Sci.* 16, 153–162.

Odagiri, K., Mizuta, I., Yamamoto, M., Miyazaki, Y., Watanabe, H., and Uehara, A. (2014). Waist to height ratio is an independent predictor for the incidence of chronic kidney disease. *PLoS ONE* 9:e88873. doi: 10.1371/journal.pone.0088873

Pala, K., and Dundar, N. (2008). Prevalence & risk factors of anaemia among women of reproductive age in Bursa, Turkey. *Indian J. Med. Res.* 128:282.

Piammongkol, S., Chongsuvivatwong, V., Williams, G., and Pornpatkul, M. (2006). The prevalence and determinants of iron deficiency anemia in rural Thai-Muslim pregnant women in Pattani Province. *Southeast Asian J. Trop. Med. Pub. Health* 37:553.

Qin, Y., Melse-Boonstra, A., Pan, X., Yuan, B., Dai, Y., Zhao, J., et al. (2013). Anemia in relation to body mass index and waist circumference among Chinese women. *Nutrition J.* 12, 1–3. doi: 10.1186/1475-2891-12-10

Saxena, Y., Shrivastava, A., and Saxena, V. (2011). Effect of gender on correlation of anaemia with body mass index in medical students. *Indian J. Physiol. Pharmacol.* 55, 364–369.

Semba, R., Ricks, M., Ferrucci, L., Xue, Q., Guralnik, J., and Fried, L. (2009). Low serum selenium is associated with anemia among older adults in the United States. *Eur. J. Clin. Nutr.* 63, 93–99. doi: 10.1038/sj.ejcn.1602889

Sinha, N. K., and Haldar, J. P. (2015). Correlation between haemoglobin level and anthropometric variables: a study on women of reproductive age group, West Bengal. *Anthropologist* 19, 185–192. doi: 10.1080/09720073.2015.11891653

Smith, B. B., Reed, P., Pearson, E., Long, P., Lassen, E., Watrous, B., et al. (1991). Erythrocyte dyscrasia, anemia, and hypothyroidism in chronically underweight llamas. *J. Am. Vet. Med. Assoc.* 198, 81–88.

Soh, K. L., Malaysia, U. P., Malaysia, U. P., Malaysia, U. P., Malaysia, U. P., Mewah, P., and Malaysia, U. P. (2015). Anemia among antenatal mother in urban Malaysia. *J. Biosci. Med.* 3:6. doi: 10.4236/jbm.2015.33002

Takeda, M., Tanaka, T., and Kudo, T. (2011). Gender difference in psychogeriatric disorders. *Geriatr. Gerontol. Int.* 11, 377–382. doi: 10.1111/j.1447-0594.2011.00736.x

Virginia, D. M., and Fenty, F. (2017). Correlation between anthropometric measurements and risk of anemia among rural community in Cangkringan, Sleman. *J. Pharm. Sci. Comm*. 14, 112–119. doi: 10.24071/jpsc.142712

Vuong, J., Qiu, Y., La, M., Clarke, G., Swinkels, D. W., and Cembrowski, G. (2014). Reference intervals of complete blood count constituents are highly correlated to waist circumference: should obese patients have their own "normal values?". *Am. J. Hematol*. 89, 671–677. doi: 10.1002/ajh.23713

WHO (2011). Haemoglobin Concentrations for the Diagnosis of Anaemia and Assessment of Severity. Vitamin and Mineral Nutrition Information System. Geneva: WHO. Available online at: https://www.who.int/vmnis/indicators/haemoglobin.pdf (accessed August 29, 2021).

WHO. (2006). *BMI Classification. Global Database on Body Mass Index*. Geneva: World Health Organization.

Yusof, M., Awaluddin, S. M., Omar, M., Ahmad, N. A., Abdul Aziz, F. A., Jamaluddin, R., et al. (2018). Prevalence of Anaemia among the elderly in Malaysia and its associated factors: does ethnicity matter? *J. Env. Pub. Health* 2018: 1803025. doi: 10.1155/2018/1803025

Zakai, N. A., Katz, R., Hirsch, C., Shlipak, M. G., Chaves, P. H., Newman, A. B., and Cushman, M. (2005). A prospective study of anemia status, hemoglobin concentration, and mortality in an elderly cohort: the Cardiovascular Health Study. *Arch. Intern. Med*. 165, 2214–2220. doi: 10.1001/archinte.165.19.2214

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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frontiers in Physiology

ORIGINAL RESEARCH
published: 26 October 2021
doi: 10.3389/fphys.2021.731594

Amelioration of Ambient Particulate Matter ($PM_{2.5}$)-Induced Lung Injury in Rats by Aerobic Exercise Training

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Ambient particulate matter ($PM_{2.5}$), as an inflammation-inducing factor, increases the prevalence of lung injury. The aim of this study was to examine the protective effect and mechanism of aerobic exercise on $PM_{2.5}$ exposure-induced lung injury. Forty Wistar rats were randomly divided into four groups: sedentary + $PM_{2.5}$ exposure, exercise + $PM_{2.5}$ exposure, sedentary, and exercise groups. All rats in the exercise-related groups underwent 8-week aerobic interval treadmill training (5 days week^{-1}, 1 h day^{-1}). PM-exposed rats were exposed to ambient $PM_{2.5}$ (6 h day^{-1}) for 3 weeks after the 8-week exercise intervention. Then, ventilation function, histopathological changes, and inflammation responses of pulmonary tissue were examined. Results showed that $PM_{2.5}$ exposure induced lung injury as manifested by decreased pulmonary function, abnormal histopathological changes, and increased pro-inflammatory cytokine levels (tumor necrosis factor-α and Interleukin-1α). Aerobic exercise alleviated the airway obstruction, reduced respiratory muscle strength, bronchial mucosal exfoliation, ultrastructure damage, and inflammatory responses induced by $PM_{2.5}$ in exercise-related groups. The benefits of exercise were related with the downregulation of p38-mitogen-activated protein kinase (MAPK), and the subsequent inhibition of the pathways of the cyclooxygenase 2 (COX-2) product, prostaglandin E_2 (PGE_2). Thus, pre-exercise training may be an effective way to protect against $PM_{2.5}$-induced lung inflammatory injury in rats.

Keywords: aerobic interval training, ambient particulate matter, lung injury, inflammation, p38–COX_2–PGE_2 pathways

OPEN ACCESS

Edited by:
Mallikarjuna Korivi, Zhejiang Normal University, China

Reviewed by:
Chandra Mouli Pavuluri, Tianjin University, China
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Specialty section:
This article was submitted to Integrative Physiology, a section of the journal Frontiers in Physiology

Received: *27 June 2021*
Accepted: *23 August 2021*
Published: *26 October 2021*

Citation:
Qin F, Fan Z, Xu M, Wang Z, Dong Y, Qu C, Cui S, Zhao L and Zhao J (2021) Amelioration of Ambient Particulate Matter ($PM_{2.5}$)-Induced Lung Injury in Rats by Aerobic Exercise Training. Front. Physiol. 12:731594. doi: 10.3389/fphys.2021.731594

INTRODUCTION

Air pollution, especially fine particulate matters with a diameter less than 2.5 μm ($PM_{2.5}$), has become a serious public health problem (Heck et al., 2017; Loxham et al., 2019). The mortality, morbidity, and risk factors in China published in the Lancet showed that particulate matter (PM) pollution is the top fourth risk factor for the number of deaths in 2017 (Zhou et al., 2019). Many epidemiological investigations suggested that $PM_{2.5}$ is associated with respiratory and other non-communicable diseases, and the increase in $PM_{2.5}$ is associated with increased hospital admissions for respiratory diseases (Qiu et al., 2012; Tian et al., 2019). An 18 years cohort study indicated that ambient air pollutants was significantly associated with declining lung function and increasing emphysema (Wang et al., 2019a). Additionally, $PM_{2.5}$ exposure could lead to lung injury characterized by pulmonary dysfunction, inflammatory cell infiltration, pulmonary edema, and pulmonary fibrosis (Feng et al., 2019; Li et al., 2019). These harmful effects of $PM_{2.5}$ are related to oxidative stress and inflammatory responses (Li et al., 2018; Zhang et al., 2019). Further researches showed that $PM_{2.5}$ may exacerbate inflammation in murine lung *via* a Toll-like receptor2 (TLR2)/Toll-like receptor4 (TLR4)/MyD88-signaling pathway (He et al., 2017). The activation of TLR4/Nuclear factor Kappa B (NF-κB) observed in $PM_{2.5}$-induced lung epithelial cells could lead to further inflammation infiltration (Gu et al., 2017b). Therefore, it is pivotal to develop therapeutic strategies to prevent the respiratory system injury caused by $PM_{2.5}$.

Regular exercise may be able to modulate the immune system to enhance resistance to respiratory infections (Pedersen and Hoffman-Goetz, 2000; Wang et al., 2020). Numerous researchers have indicated the protective effects of exercise against various diseases through its anti-inflammatory and antioxidant capability (De Sousa et al., 2017; Metsios et al., 2020). A related study reported that moderate-intensity aerobic physical training reduces oxidative stress and protects against the development of emphysema induced by cigarette smoke in mice (Toledo et al., 2012). Our previous work also found that aerobic interval training improves the pulmonary function and impedes the lesion progression induced by acute exposure to different $PM_{2.5}$ concentrations because of the effective inhibition of oxidative stress and inflammation (Qin et al., 2020b). However, the effects of exercise on lung injury induced by sub-chronic exposure to $PM_{2.5}$ are still uncertain, and the underlying mechanism of the protective effects of exercise training remains to be elucidated.

Cyclooxygenase 2 (COX-2) is a well-known inflammatory mediator that can regulate the conversion of arachidonic acid to prostaglandin E_2 (PGE_2; Tsai et al., 2017). COX-2/PGE_2 plays key roles in the pathogenesis of $PM_{2.5}$-induced inflammation (Fernando et al., 2019). PM exposure results in airway inflammation through the upregulation of COX-2/PGE_2 (Song et al., 2020). PM exposure stimulates COX-2/PGE_2 inflammatory signaling pathways in human fibroblast-like synoviocytes (Tsai et al., 2017). P38-mitogen-activated protein kinase (MAPK) plays a substantial role in proinflammatory responses and is closely related to the beneficial effect of exercise (Sur et al., 2008). p38-MAPK regulates COX-2 expression (Xu et al., 2008; Yu et al., 2014). Moreover, exercise remarkably inhibits COX-2 activity, which leads to the suppression of pro-inflammatory cytokines (Lee et al., 2015). However, whether p38–COX-2–PGE_2 signaling pathways are involved in the protective mechanism of exercise against the inflammation induced by $PM_{2.5}$ is unknown. Thus, the inhibition of p38–COX-2–PGE_2 pathway may provide a preventive approach for the inflammation induced by $PM_{2.5}$.

Here, we used a whole-body inhalation enrichment system to conduct $PM_{2.5}$ exposure toxicology research and exposed rats to real-time $PM_{2.5}$ inhalation for 3 weeks. We assessed the pulmonary function, histopathological characteristics, and inflammatory condition of rats to evaluate to whether aerobic interval training plays a protective role against the lung injury induced by $PM_{2.5}$. Additionally, we observed the p38–COX-2–PGE_2 signaling pathways involved in the protective effect of exercise on ameliorative inflammation. We hypothesized that exercise is related to the downregulated activation of p38-MAPK, which further inhibited the pathways of the COX-2 product, PGE_2.

MATERIALS AND METHODS

Animals

Male Wistar rats (age: 52 ± 3 days, weight: 247 ± 40 g) were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. All animals were separately raised in a ventilated caging system and exposed to a 12 h light – 12 h dark cycle (23 ± 1.0°C and 45–55% humidity). All the experimental procedures were approved by the Animal Ethical Committee of China Institute of Sports Science in accordance with the guidelines of experimental animal use (approval number: CISSLA-2017003).

Experimental Design

The animals were randomly assigned to four groups ($n = 10$ in each group): sedentary (S), exercise (E), sedentary + $PM_{2.5}$ exposure (S + PM), and exercise + $PM_{2.5}$ exposure (E + PM). All rats in the E-related groups underwent an 8-week aerobic interval treadmill training, and then all rats in the PM-related groups were exposed to $PM_{2.5}$ (**Figure 1**). The exposure time was set to 6 h per day, 7 days per week from October 15, 2018 to November 5, 2018, for a total duration of 3 weeks. Pulmonary function was examined 24 h after the final exposure. Finally, all rats were anesthetized with an intraperitoneal injection and blood was collected through the abdominal aorta. When the rats died, the bronchoalveolar lavage fluid (BALF) and lungs were collected.

Exercise Program

After treadmill adaptation (1 week), animal aerobic interval training was performed with a treadmill (DSPT-202, China) for 1 h per time and five times per week for 8 weeks. The training protocol consisted of a 5-min warm-up, a 6-min cool-down with 50–55% maximal oxygen uptake (VO_{2max}), and seven interval training periods (4 min intervals at 80–90% VO_{2max} interspersed with 3 min periods of 65–70% VO_{2max}). The VO_{2max} was measured through an incremental speed protocol (increased by 5 m/min every 3 min until the rats were exhausted) with a 0° slope treadmill and Columbus Oxymax Lab Animal Monitoring System (Columbus, United States; Qin et al., 2020a). Then the exercise intensity (80–90% VO_{2max} and 65–70% VO_{2max}) corresponding to different treadmill speeds were quantified, respectively.

$PM_{2.5}$ Exposure System

Rats in PM-related groups were exposed to a $PM_{2.5}$ concentration enrichment system (Beijing Huironghe Technology Co., Ltd., China), which can efficaciously concentrate ambient $PM_{2.5}$ (5–8-folds) without an apparent change in major physicochemical

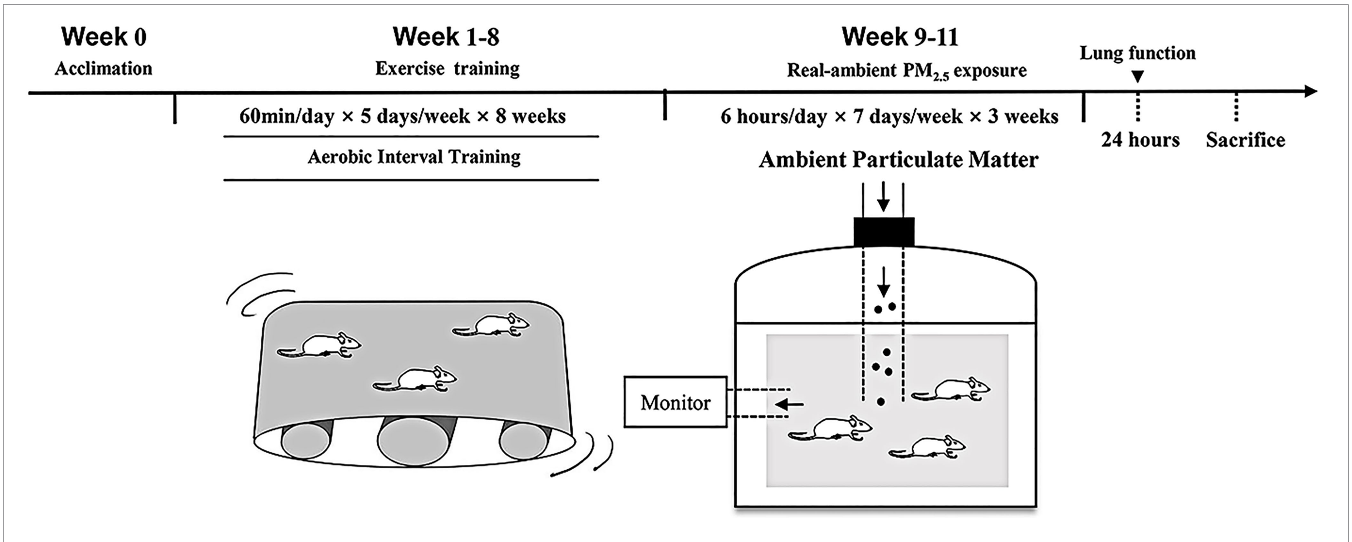

FIGURE 1 | Overview of the experimental procedure.

features. The exposure system is located at Zhongguancun Science and Technology Park, Tongzhou District, Beijing, China. The daily air quality index and the concentrations of particulate matters with a particle size below 10 µm (PM_{10}), sulfur dioxide (SO_2), nitrogen dioxide (NO_2), carbon monoxide (CO), and ozone (O_3) in ambient air are recorded in the website of the Ministry of Ecology and Environment of the People's Republic of China.[1] The particle components in the chamber were collected using a 47 mm Teflon filter, and the polycyclic aromatic hydrocarbons (PAHs) and metal were analyzed. The PM samples were extracted using a microwave accelerated reaction system and were purified using silica/alumina columns (Ma et al., 2015; Tala and Chantara, 2019). PAHs were analyzed by a gas chromatograph (Agilent 5890A, United States) coupled mass spectrometer (Agilent 5975C, United States). The 16 parent PAHs quantified include naphthalene (NAP), acenaphthene (ACE), acenaphthylene (ACY), fluorene (FLO), phenanthrene (PHE), anthracene (ANT), fluoranthene (FLA), pyrene (PYR), benzo(a)anthracene (BaA), chrysene (CHR), benzo(b)fluoranthene (BbF), benzo(k)fluoranthene (BkF), benzo(a)pyrene (BaP), dibenzo(a,n)anthracene (DanA), indeno (1,2,3-cd) pyrene (IcdP), and benzo(g,h,i)perylene (BghiP). For metal analysis, PM samples were digested using a mixture of HNO3 and HCl (Costas et al., 2010). The concentrations of Cr, Cu, Cd, Ni, Hg, Mn, Zn, Pb, and As were determined using Inductively coupled plasma mass spectrometry (ICP-MS, DIONEX, United States).

Pulmonary Function Test

Noninvasive small-animal whole-body plethysmography (Buxco, Inc., United States) was performed to detect the pulmonary function of the rats. The test protocol was performed as previously described (Qin et al., 2021). Finally, respiratory dynamics data, including minute ventilation (MV), tidal volume (TV), breathing frequency (*F*), relaxation time (Tr), expiration time (Te), inspiration time (Ti), pause (Pau), 50% expiratory flow (EF50), estimated peak expiratory flow (PEF), and estimated peak inspiratory flow (PIF), were measured and calculated. Pau is a unitless index that estimates bronchoconstriction and is calculated as: $Pau = (Te/Tr)^{-1}$ (Bosnjak et al., 2014).

Histopathological Analysis

Lung tissues were fixed, embedded in paraffin, and cut into 5 µm-thick sections. The tissue sections were deparaffinized, hydrated, stained with hematoxylin and eosin (HE), and then observed by optical light microscopy (100×, 200×, and 400×). An established scoring system that quantify pathological changes in lung tissues during acute lung injury (Matute-Bello et al., 2011; Li et al., 2019) was used, and the test protocol was performed as previously described (Qin et al., 2021).

Transmission Electron Microscopy Inspection

The lung tissues were immersed in 2.5% glutaraldehyde, then washed with phosphate buffer (PB) solution, and fixed with 1% OsO_4 for 1 h. Afterward, the tissues were washed with PB, then dehydrated in a graded series of ethanol, embedded in araldite, and polymerized for 24 h at 60°C. Additionally, ultrathin sections (60 nm) were cut and collected on 200-mesh copper grids, stained with lead citrate, and observed with a transmission electron microscope (JEM-2100, Japan).

Biomarker Estimation

The concentrations of PGE_2 (Abcam, United States, intra-CV: 5.8%; inter-CV: 5.1%, competitive ELISA), TNF-α (CUSABIO, China, intra-CV: 7.8%; inter-CV: 8.3%, sandwich ELISA), and IL-1α (CUSABIO, China, intra-CV: 7.3%; inter-CV: 7.9%, sandwich ELISA) in BALF supernatants were determined using enzyme-linked immunosorbent assay kits according to the manufacturers' instructions. Optical density was measured with

[1]https://air.cnemc.cn:18007/

an enzyme-linked analyzer (MultiskanAsc, Thermo, United States) within 10 min. The standard curve was constructed with the standard solution as the ordinate and OD value as the abscissa to help detect the sample concentration.

Western Blot

Total proteins were extracted from the lung samples by using a RIPA reagent kit. Protein concentrations were determined using the bicinchoninic acid (BCA) method. The proteins were transferred to a nitrocellulose membrane (loading 20 μg total protein per gel lane). The membranes for western blot analysis were incubated at 4°C overnight with the following primary antibodies against: Cox-2 (CST, United States; dilution: 1:2000), p38 (CST, United States; dilution: 1:2000), p-p38 (CST, United States; 1:1000), and β-actin (Immunoway, China; 1:500). Secondary antibodies (goat anti-rabbit/mouse IgG, TDYBIO, China, 1:10,000) were added for 40 min, and then the membranes were washed with TBST. Finally, immunoreactive bands were detected with an enhanced chemiluminescence (ECL) kit, and band density was analyzed by ImageJ software (National Institutes of Health, United States).

Statistical Analysis

All data are expressed as mean ± SD. Statistical analysis was performed using SPSS software (version 22.0, IBM SPSS Statistics, Chicago, IL, United States). The normality of data distribution was confirmed by Shapiro–Wilk test. Two-way ANOVA ($PM_{2.5}$ and exercise as factors) were used to compare the differences between groups. $p<0.05$ was considered significant. In addition, effect size estimates (Cohen's d) were calculated to assess and categorize efficacy as small ($d=0.2$), medium ($d=0.5$), or large ($d=0.8$; Lakens, 2013).

TABLE 1 | Average mass concentration of metals and polycyclic aromatic hydrocarbons (PAHs) in $PM_{2.5}$.

Metals	Mass concentration ($ng\,m^{-3}$ $PM_{2.5}$)	PAHs	Mass concentration ($\mu g\,m^{-3}$ $PM_{2.5}$)
Cr	11.41	Naphthalene	0.127
Mn	68.15	Acenaphthylene	0.033
Ni	4.80	Acenaphthene	1.108
Cu	21.91	Fluorene	1.145
Zn	216.38	Phenanthrene	2.195
As	12.32	Anthracene	0.145
Cd	2.94	Pyrene	0.293
		Fluoranthene	0.183
		Chrysene	0.052
		Benzo(a) anthracene	0.115
		Benzo(b) fluoranthene	0.207
		Benzo(k) fluoranthene	0.067
		Benzo(a)pyrene	0.102
		benzo(g,h,i) perylene	0.148
		Indeno(1,2,3,c,d) pyrene	0.153
		Dibenz(a,h) anthracene	0.052

RESULTS

$PM_{2.5}$ Concentration and Composition

During the 3 weeks of $PM_{2.5}$ exposure, the average $PM_{2.5}$ concentrations inside the concentrated $PM_{2.5}$ chamber was $237.01 \pm 206.41\,\mu g\,m^{-3}$, the maximum concentration of $651 \pm 70\,\mu g\,m^{-3}$, and the minimum concentration was $21 \pm 41\,\mu g\,m^{-3}$. The daily $PM_{2.5}$ concentration inside the chamber exceeded $150\,\mu g\,m^{-3}$ for 11 days from October 15, 2018 to November 5, 2018. The averages of other air components (SO_2, NO_2, CO, O_3, and PM_{10}) were not remarkably different between inside and outside the chambers. The concentrations of PAHs and metal inside the chamber are shown in **Table 1**. Phenanthrene was the most prevalent PAH, followed by fluorene and acenaphthene, and the top three prevalent metallic elements were Zn, Mn, and Cu.

$PM_{2.5}$ Exposure Decreased Lung Pulmonary Function, and Exercise Played a Protective Role in This Process

WBP was performed to evaluate the pulmonary function of rats. The F (**Figure 2A**) and Ti (**Figure 2F**) had no significant change in the S+PM2.5 compared with the S group. Obvious reduction in TV ($p<0.05$; ES = 1.52, **Figure 2B**), MV ($p<0.05$; ES = 1.48, **Figure 2C**), EF50 ($p<0.05$; ES = 1.53, **Figure 2D**), PIF ($p<0.05$; ES = 1.35, **Figure 2H**), and PEF ($p<0.05$; ES = 1.45, **Figure 2I**) was observed in the S + $PM_{2.5}$ group compared with the S group after 3 weeks of $PM_{2.5}$ exposure. Significant increase in PAU ($p<0.05$; ES = 1.69, **Figure 2E**) and Te ($p<0.05$; ES = 1.37, **Figure 2G**) were found in the S + $PM_{2.5}$ group compared with the S group. These results indicated that $PM_{2.5}$ induced a decrease in pulmonary ventilation function (TV and MV), promoted tract obstruction (EF50, PAU, and Te), and weakened respiratory muscle strength (PIF and PEF).

The TV ($p>0.05$; ES = 0.98, **Figure 2B**), MV ($p<0.05$; ES = 0.91, **Figure 2C**), EF50 ($p<0.05$; ES = 0.96, **Figure 2D**), PIF ($p<0.05$; ES = 1.17, **Figure 2H**), and PEF ($p>0.05$; ES = 0.75, **Figure 2I**) of E + PM rats were promoted compared with those of the S + PM rats after 8 weeks of aerobic pre-exercise. Moreover, the PAU ($p>0.05$; ES = 0.84, **Figure 2E**) and Te ($p<0.05$; ES = 1.18, **Figure 2G**) of the S + $PM_{2.5}$ group significantly declined compared with those in the S group. These results indicated that 8 weeks of aerobic interval training alleviated the pulmonary dysfunction caused by $PM_{2.5}$ in rats, especially pulmonary ventilation function, tract obstruction, and respiratory muscle strength.

Exercise and $PM_{2.5}$ Exposure Pathologically Affect the Characteristics of Lung Tissue

Morphological alterations in lung tissues were evaluated by HE staining as shown in **Figure 3**. Lung structures were almost normal in the S and E groups (**Figure 3A**). $PM_{2.5}$ exposure led to peribronchiolar neutrophil infiltration (**Figure 3A**), alveolar septal thickening (**Figure 3A**), bronchial mucosal exfoliation and lesions (**Figure 3A**), pulmonary arterial smooth muscle hypertrophy, and pulmonary vascular lumen stenosis (**Figure 3A**). Inflammatory

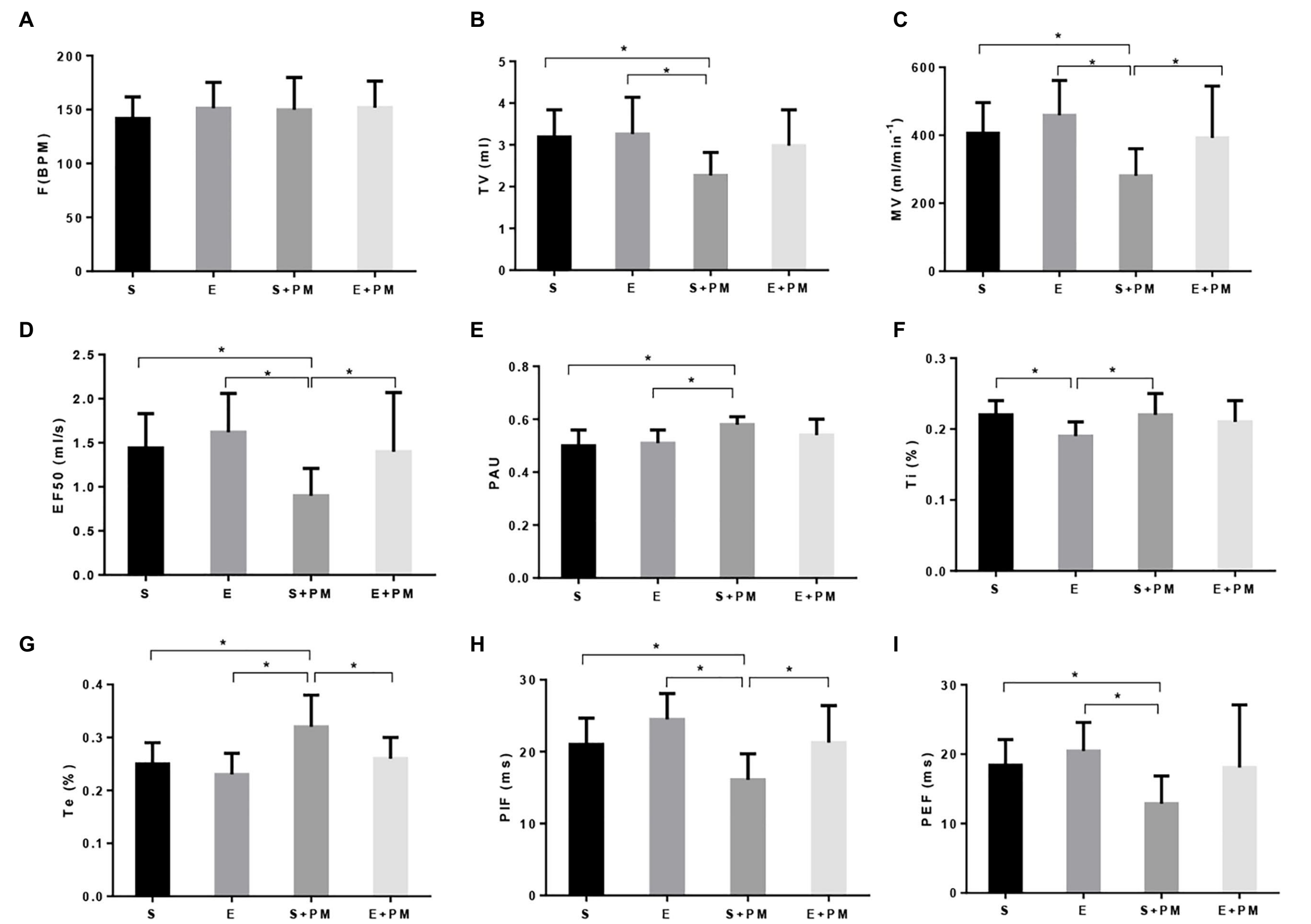

FIGURE 2 | Aerobic exercise protected rats from $PM_{2.5}$-induced pulmonary function decrease. **(A)** Breathing frequency (*F*). **(B)** Tidal volume (TV). **(C)** Minute ventilation (MV). **(D)** Forced expiratory flow-50% (EF50). **(E)** Pause (PAU). **(F)** Inspiration time (Ti). **(G)** Expiration time (Te). **(H)** Estimated peak inspiratory flow (PIF). **(I)** Estimated peak expiratory flow (PEF). Data represent mean ± SD. $^*p < 0.05$ between groups.

infiltration and the degree of lesions in the peribronchiolar and vascular walls were ameliorated in the E + PM group compared with those in the S + $PM_{2.5}$ group (**Figure 3A**). Meanwhile, the lung injury score of the E + $PM_{2.5}$ group was significantly lower than that in the S + $PM_{2.5}$ group ($p < 0.05$; ES = 4.23, **Figure 3B**), which means that the 8-week aerobic pre-exercise program has a preventive effect against lung tissue injury.

Furthermore, the ultrastructure of rat lung tissues was observed. Normal lung epithelial cells (type I and type II alveolar cells) and the tissue matrix of lung interval were observed in the S and E groups (**Figure 4**). However, more lamellar bodies and microvilli were found in the type II alveolar cells in the E group (**Figure 4**) compared with the S group (**Figure 4**). After 3-week $PM_{2.5}$ exposure, the ultrastructure of type II alveolar cells indicated obvious injury, such as mitochondrial swelling and vacuolization (**Figure 4**) and microvilli reduction or shedding (**Figure 4**). A part of the lumen was necrotic, and the basement membrane was fractured in a large area (**Figure 4**). Compared with the S + $PM_{2.5}$ group, the lamellar bodies in type II alveolar cells increased considerably and the degree of mitochondrial injury improved in E + $PM_{2.5}$ rats (**Figure 4**).

Exercise Suppressed $PM_{2.5}$-Induced Inflammation in the Lung

We examined proinflammatory cytokines in the BALF to characterize the inflammatory response *in vivo*. As shown in **Figures 5A1,A2**, the levels of IL-1α ($p < 0.05$; ES = 1.19) and TNF-α ($p < 0.05$; ES = 1.92) obviously increased in S + PM rats compared with those in S rats. Moreover, aerobic interval training resulted in a significant downregulation in the levels of TNF-α ($p < 0.05$; ES = 0.53) and IL-1α ($p < 0.05$; ES = 1.07) compared with those in the S + PM group.

The p38–COX-2–PGE_2 pathway is closely related to the pro-inflammatory effects of $PM_{2.5}$ exposure and the anti-inflammatory activity induced by exercise. Therefore, we further examined the expression levels of proteins related to the p38–COX-2–PGE_2 pathway (**Figure 5A3,B1–B3**. The protein levels of PGE_2 ($p < 0.05$; ES = 0.98, **Figure 5A3**), COX-2 ($p < 0.05$; ES = 1.34, **Figure 5B1**), and p-p38 ($p < 0.05$; ES = 0.75, **Figure 5B3**) showed an obvious increase in the S + PM group than in the S group after 3 weeks of $PM_{2.5}$ exposure. Apparently, the levels of PGE_2 ($p < 0.05$; ES = 0.89, **Figure 5A3**), COX-2 ($p < 0.05$; ES = 1.98, **Figure 5B1**), and p-p38 ($p < 0.05$; ES = 1.09,

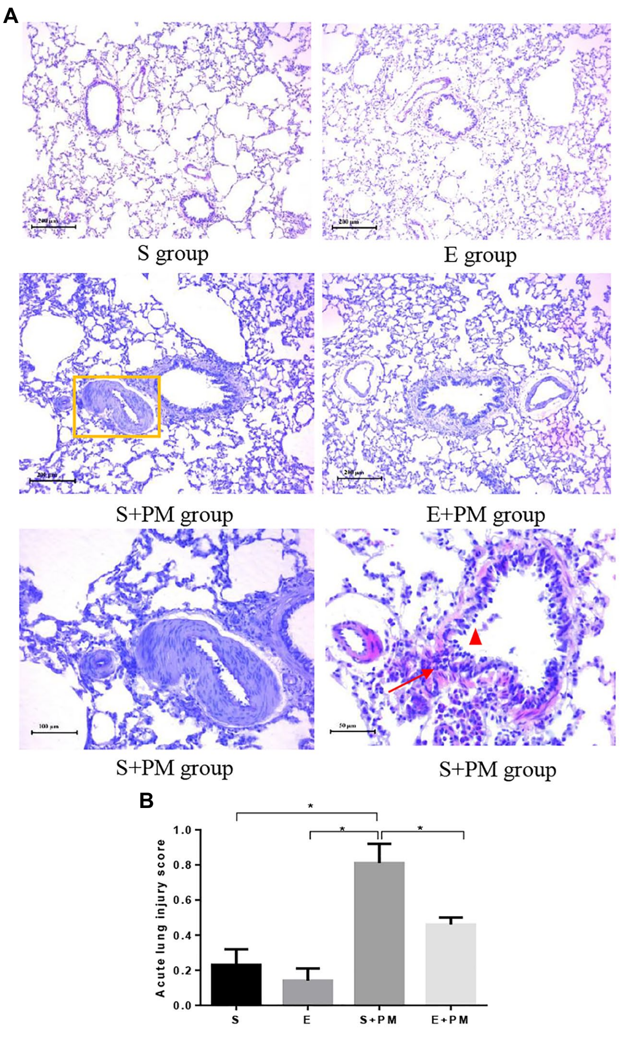

FIGURE 3 | Photomicrographs of rat lung (HE stain) in different groups. **(A)** Histologic analysis of lung tissues. Yellow rectangle indicated pulmonary artery; red triangle indicated mucous membrane exfoliation; red arrows indicated inflammatory infiltration. **(B)** Acute lung injury scores in different groups. Data represent mean ± SD. $^*p < 0.05$ between groups.

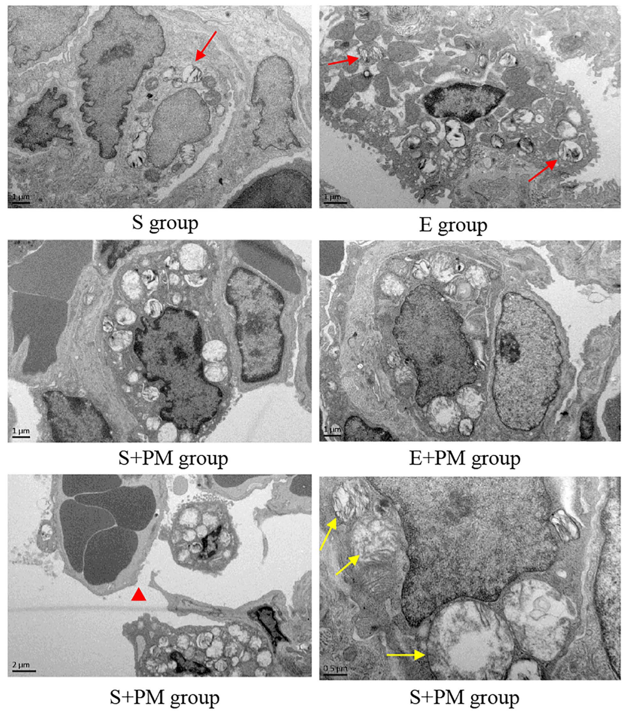

FIGURE 4 | Electron micrograph of lung tissue in different groups. Red arrows indicated lamellar bodies; red triangle indicated Basement membrane cracked after $PM_{2.5}$ exposure; yellow arrows indicated mitochondrial swelling and vacuolization in the S + PM group.

Figure 5B3) were effectively downregulated in the E + PM group compared with those in the S + PM group. The protein binding pattern determined by Western blot analysis were presented in **Figure 5C**.

Overall, aerobic exercise resulted in a remarkable decrease in pro-inflammatory cytokines and inflammatory pathway-related proteins. The results indicated that 8-week aerobic interval training could inhibit the inflammation induced by $PM_{2.5}$.

DISCUSSION

The major findings of the present study are that aerobic interval training may be an effective way to protect against $PM_{2.5}$-induced lung inflammation in rats, and the p38-COX-2-PGE2 signaling pathways might be involved in the protective effect of exercise on ameliorative inflammation. These findings implied that regular exercise training can effectively improve immune function, especially for the young individuals with high amounts of sedentary behavior. Therefore, developing regular exercise habits is essential for the young individuals to against the injury of smog weather. Our study suggests that exercise training may be as an adjuvant in the prevention of pulmonary disease due to $PM_{2.5}$ exposure.

In this study, a whole-body inhalation $PM_{2.5}$ enrichment system for rats was used to mimic the actual living environment of humans to the greatest extent possible (Chu et al., 2019; Su et al., 2020). The mean mass concentration of exposure chambers in our study was $237.01 \pm 206.41\,\mu g\,m^{-3}$, which is a relatively moderate pollution levels of air pollution according to the Air Quality Guidelines of China. The mean mass concentration of $PM_{2.5}$ represented the ambient air pollution in early winter in northern China. In our animal models, after 3 weeks subacute $PM_{2.5}$ exposure, the significant airway obstruction, declined of respiratory muscle strength, bronchial mucosal exfoliation, stenosis of the pulmonary vascular lumen, ultrastructure damages (mitochondrial swelling and microvilli shedding) were observed in S + $PM_{2.5}$ rats compared with the control. In a previous study, after $89.95\,\mu g\,m^{-3}$ $PM_{2.5}$ exposure for 3 weeks, there was increasingly inflammation characterized by alveolar capillary congestion and peribronchiolar neutrophils infiltration in mice (Li et al., 2019). Yang et al. (2018) reported that 2-week ($750\,\mu g\,m^{-3}$, 4 h day^{-1}, 5 days $week^{-1}$) $PM_{2.5}$ inhalation

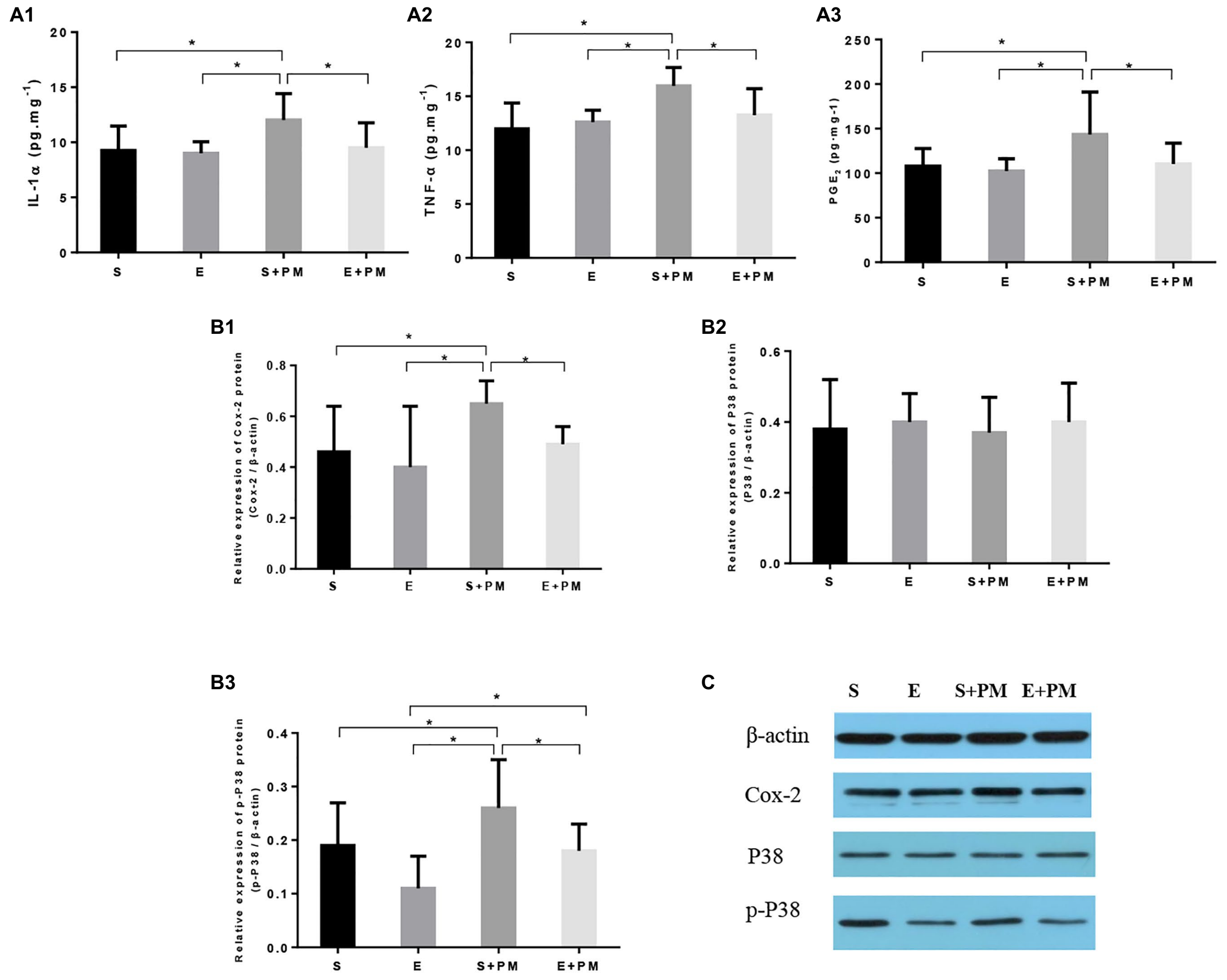

FIGURE 5 | Aerobic exercise suppressed $PM_{2.5}$-induced lung inflammation in rats. **(A1–A3)** Pro-inflammatory cytokines (IL-1α and TNF-α) and PGE_2 in bronchoalveolar lavage fluid (BALF). **(B1–B3)** Protein levels of Cox-2, p38, and p-p38 in the lung tissues of rats. **(C)** Protein binding pattern determined by Western blot analysis. Data represent mean ± SD. *$p < 0.05$ between groups.

results in lung edema and lesions, oxidative stress, and acute inflammatory responses, which subsequently result in lung tissue damage. The main factors of $PM_{2.5}$-induced lung injury are related to the concentration, duration, and composition of $PM_{2.5}$ (Ning et al., 2019). Moreover, in previous study, we observed a low tolerance of aging rats (16 months vs. 8 weeks) to high concentrations of $PM_{2.5}$. 2-week exposure duration of whole-body $PM_{2.5}$ inhalation led to lung injury, and the degree of lung injury in aging rats were more deleterious than that of present result in young adult rats (Qin et al., 2021). Meanwhile, the $PM_{2.5}$ exposure duration in aging rats were less than that of young adult rats (4 h/day; 2 weeks vs. 6 h/day; 3 weeks). It is also implied that aging individuals are susceptible to $PM_{2.5}$-induced lung injury.

Furthermore, we also analyzed the composition of $PM_{2.5}$ inside the exposure chambers. The prevalent toxic heavy metals, including Zn and Cr, and the common PAHs, including phenanthrene, can activate oxidative stress and inflammation in lung tissues (Maret, 2012; Han et al., 2019; Ma et al., 2020). Growing evidence suggests that these toxic elements in $PM_{2.5}$ are closely related to the development of lung injury (Ning et al., 2019; Qin et al., 2021). In addition, heavy metals, including Zn, Cu, and Cr, and PAHs, including acenaphthylene and phenanthrene, are associated with diesel and gasoline exhaust emissions (Valavanidis et al., 2006; Hu et al., 2016). Due to the exposure chambers of present study located near several high-speed road and urban trunk road in Tongzhou, Beijing, the primary point sources of ambient $PM_{2.5}$ were derived from motor vehicle exhaust, which further implied that traffic air pollution related to lung health. Thus, we should try to avoid rush-hour traffic when smog condition is serious.

Proverbially, physical activity is an effective nonpharmacological treatment for the prevention of chronic diseases and the enhancement of immunity; moreover, exercise type, intensity, and duration are the main factors that impact the intervention effect (MacInnis and Gibala, 2017; Nieman and Pence, 2020). In the present work, we selected aerobic interval training. Aerobic interval training can provide cardiorespiratory fitness and body fat reduction that are similar to or greater than those of traditional moderate-intensity continuous training (Molmen-Hansen et al., 2012; Wewege et al., 2017). Moreover, aerobic interval training has changeable exercise rhythm and challenging intensity, therefore, it has become an attractive approach for adults. In addition, we measured the VO_{2max} of rats to ensure an accurate initial exercise intensity, which was adjusted every 2 weeks to maintain satisfactory exercise effects (Qin et al., 2020a). Our results proved that the 8-week aerobic interval training alleviated the declined in ventilation function and respiratory muscle strength, as well as released tract obstruction; these effects may be related to the improvement of peribronchiolar and vascular wall lesions and inflammatory infiltration after regular exercise training. The findings are directly in line with previous findings (Rietberg et al., 2017). Notably, lamellar bodies and microvilli in type II alveolar cells increased after the 8-week aerobic interval training. Lamellar bodies are the specialized secretory organelles of type II alveolar cell that package phospholipid film and regulate its secretion (Mulugeta et al., 2002). The phospholipid film of dipalmitoyl phosphatidylcholine on the surface of lung alveoli reduces surface tension for optimal gas exchange and builds a hydrophobic protective lining as environmental barrier (Schmitz and Müller, 1991; Menon et al., 2018). One study indicated that lamellar bodies in A549 cells disappear compared with the control when exposed to 100 μg $PM_{2.5}$ (Peng et al., 2019). A similar change was also observed in our studies. However, research on the direct effect of exercise training on lamellar bodies is rare. Our results showed that the increase in lamellar bodies may be associated with the improvement of pulmonary ventilation and compliance function after the 8-week aerobic exercise training. Our research also provided direct *in vivo* evidence that the protective effects of aerobic interval exercise mediated the pulmonary dysfunction induced by $PM_{2.5}$ exposure.

The lung injury induced by $PM_{2.5}$ is linked to inflammatory responses (Feng et al., 2019), including peribronchiolar neutrophil infiltration, increased proinflammatory cytokines, and the activation of relative inflammatory pathway. Zhang et al. (2019) found that $PM_{2.5}$ induced Rac1and regulated AKT signaling associated with lung inflammation (Zhang et al., 2019). Gu et al. (2017a) indicated that $PM_{2.5}$ promotes the overactivation of the Notch signaling pathway and aggravates the immune disorder of COPD. Gu et al. (2017b) reported that $PM_{2.5}$ induced lung epithelial cells by the activation of TLR4/NF-kB leading to inflammation infiltration. Thus, $PM_{2.5}$ induced inflammatory responses may be an essential factor of lung injury. Similar results were obtained in present study. In addition, previous studies also investigated the effect of intermittent exercise on immune function. Interval training could modulate autoimmunity by decreasing the polarization of T cells into deleterious Th1 and Th17 cells (Goldberg et al., 2021). Ten weeks of low-volume, high-intensity interval exercise could improve neutrophil and monocyte function and enhance innate immune system in sedentary adults (Bartlett et al., 2017). Animal research also indicated that interval exercise training could reduce the inflammation induced by cisplatin nephrotoxicity and downregulate the TLR4/NF-κB signaling pathway (Leite et al., 2021). In the present study, we verified that aerobic interval training produces similar anti-inflammatory effects for $PM_{2.5}$-induced inflammation. Furthermore, p38–COX-2–PGE_2 signaling pathways were assessed to explore the putative mechanisms of protective effect of exercise against the lung injury induced by $PM_{2.5}$ exposure. After 3 weeks of $PM_{2.5}$ exposure, the high expression of p-p38, COX-2, and PGE_2 presented inflammatory response as induced by $PM_{2.5}$. However, we found that aerobic interval training could prevent the rise in p-p38 level and then hindered the activation of the COX-2-PGE_2 inflammatory pathway. Consequently, our data indicated that exercise could alleviate the lung injury induced by $PM_{2.5}$ possibly through p38–COX-2–PGE_2 pathway. Currently, the detailed mechanisms of the p38–COX-2–PGE_2 pathway involved in inflammation prevention by exercise is still uncertain and needs further verification.

This study has several limitations. First, we only used male rats. The deleterious effects of $PM_{2.5}$ and physiological characteristics are different between sexes (Kampa and Castanas, 2008; Wang et al., 2019b). However, we only selected male rats without considering the estrous cycle. Second, only the protective effects of exercise on lung injury induced by subacute PM exposure (3 weeks) were observed. Whether the aerobic exercise training has a protective effect on long-term exposure to smog weather will be studied in the future. Lastly, the detailed mechanisms of the p38–COX-2–PGE_2 pathway in preventing inflammation needs additional analysis. The critical regulatory molecules underlying these interactive effects warrant further investigation.

In summary, we showed that 8-week aerobic interval exercise may be an effective way to protect against $PM_{2.5}$-induced lung inflammation in rats. Aerobic exercise alleviated the airway obstruction, weakened respiratory muscle strength, bronchial mucosal exfoliation, ultrastructure damage, and inflammatory responses induced by $PM_{2.5}$ in exercise-related rats. These benefits of exercise were related with the downregulated activation of p38 and MAPK, which further inhibited COX-2-PGE_2 pathways. In future, the guidelines in different age groups on exercise promotion behavior combined with environmental factors are essential. It requires further researches to elucidate the relevant mechanism of exercise on preventing the injury induced by $PM_{2.5}$ exposure, which will offer an effective measure for health promotion and diseases prevention.

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.

ETHICS STATEMENT

The animal study was reviewed and approved by Animal Ethical Committee of the China Institute of Sports Science.

AUTHOR CONTRIBUTIONS

FQ, ZF, and MX contributed equally in the ideas, writing of the manuscript, and drafted the manuscript. FQ and JZ conceived and designed the research and edited and revised the manuscript. MX, ZF, ZW, YD, and FQ performed the experiments. FQ, SC, and ZF analyzed the data. FQ, JZ, and CQ interpreted the experimental results. CQ, SC, and LZ prepared the figures. JZ approved the final version of the manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

This work is supported by the China Postdoctoral Science Foundation (2018T110076), the National Natural Science Foundation of China (31900845 and 11775059), and the Fundamental Research Foundation of the China Institute of Sport Science (20-18).

REFERENCES

Bartlett, D. B., Shepherd, S. O., Wilson, O. J., Adlan, A. M., Wagenmakers, A. J. M., Shaw, C. S., et al. (2017). Neutrophil and monocyte bactericidal responses to 10 weeks of low-volume high-intensity interval or moderate-intensity continuous training in sedentary adults. *Oxidative Med. Cell. Longev.* 2017:8148742. doi: 10.1155/2017/8148742

Bosnjak, B., Tilp, C., Tomsic, C., Dekan, G., Pieper, M. P., Erb, K. J., et al. (2014). Tiotropium bromide inhibits relapsing allergic asthma in BALB/c mice. *Pulm. Pharmacol. Ther.* 27, 44–51. doi: 10.1016/j.pupt.2013.09.004

Chu, C., Zhang, H., Cui, S., Han, B., Zhou, L., Zhang, N., et al. (2019). Ambient $PM_{2.5}$ caused depressive-like responses through Nrf2/NLRP3 signaling pathway modulating inflammation. *J. Hazard. Mater.* 369, 180–190. doi: 10.1016/j.jhazmat.2019.02.026

Costas, M., Lavilla, I., Gil, S., Pena, F., De La Calle, I., Cabaleiro, N., et al. (2010). Evaluation of ultrasound-assisted extraction as sample pre-treatment for quantitative determination of rare earth elements in marine biological tissues by inductively coupled plasma-mass spectrometry. *Anal. Chim. Acta* 679, 49–55. doi: 10.1016/j.aca.2010.09.004

De Sousa, C. V., Sales, M. M., Rosa, T. S., Lewis, J. E., De Andrade, R. V., and Simões, H. G. (2017). The antioxidant effect of exercise: a systematic review and meta-analysis. *Sports Med.* 47, 277–293. doi: 10.1007/s40279-016-0566-1

Feng, S., Duan, E., Shi, X., Zhang, H., Li, H., Zhao, Y., et al. (2019). Hydrogen ameliorates lung injury in a rat model of subacute exposure to concentrated ambient $PM_{2.5}$ via aryl hydrocarbon receptor. *Int. Immunopharmacol.* 77:105939. doi: 10.1016/j.intimp.2019.105939

Fernando, I. P. S., Jayawardena, T. U., Kim, H. S., Lee, W. W., Vaas, A., De Silva, H. I. C., et al. (2019). Beijing urban particulate matter-induced injury and inflammation in human lung epithelial cells and the protective effects of fucosterol from Sargassum binderi (Sonder ex J. Agardh). *Environ. Res.* 172, 150–158. doi: 10.1016/j.envres.2019.02.016

Goldberg, Y., Fainstein, N., Zaychik, Y., Hamdi, L., Segal, S., Nabat, H., et al. (2021). Continuous and interval training attenuate encephalomyelitis by separate immunomodulatory mechanisms. *Ann. Clin. Transl. Neurol.* 8, 190–200. doi: 10.1002/acn3.51267

Gu, X. Y., Chu, X., Zeng, X. L., Bao, H. R., and Liu, X. J. (2017a). Effects of $PM_{2.5}$ exposure on the notch signaling pathway and immune imbalance in chronic obstructive pulmonary disease. *Environ. Pollut.* 226, 163–173. doi: 10.1016/j.envpol.2017.03.070

Gu, L. Z., Sun, H., and Chen, J. H. (2017b). Histone deacetylases 3 deletion restrains $PM_{2.5}$-induced mice lung injury by regulating NF-κB and TGF-β/Smad2/3 signaling pathways. *Biomed. Pharmacother.* 85, 756–762. doi: 10.1016/j.biopha.2016.11.094

Han, B., Li, S., Lv, Y., Yang, D., Li, J., Yang, Q., et al. (2019). Dietary melatonin attenuates chromium-induced lung injury via activating the Sirt1/Pgc-1α/Nrf2 pathway. *Food Funct.* 10, 5555–5565. doi: 10.1039/C9FO01152H

He, M., Ichinose, T., Yoshida, Y., Arashidani, K., Yoshida, S., Takano, H., et al. (2017). Urban $PM_{2.5}$ exacerbates allergic inflammation in the murine lung via a TLR2/TLR4/MyD88-signaling pathway. *Sci. Rep.* 7:11027. doi: 10.1038/srep46914

Heck, T. G., Scomazzon, S. P., Nunes, P. R., Schöler, C. M., Da Silva, G. S., Bittencourt, A., et al. (2017). Acute exercise boosts cell proliferation and the heat shock response in lymphocytes: correlation with cytokine production and extracellular-to-intracellular HSP70 ratio. *Cell Stress Chaperones* 22, 271–291. doi: 10.1007/s12192-017-0771-3

Hu, W., Hu, M., Hu, W., Jimenez, J. L., Yuan, B., Chen, W., et al. (2016). Chemical composition, sources and aging process of sub-micron aerosols in Beijing: contrast between summer and winter. *J. Geophys. Res. Atmos.* 121, 1955–1977. doi: 10.1002/2015JD024020

Kampa, M., and Castanas, E. (2008). Human health effects of air pollution. *Environ. Pollut.* 151, 362–367. doi: 10.1016/j.envpol.2007.06.012

Lakens, D. (2013). Calculating and reporting effect sizes to facilitate cumulative science: a practical primer for *t*-tests and ANOVAs. *Front. Psychol.* 4:863. doi: 10.3389/fpsyg.2013.00863

Lee, Y. Y., Yang, Y. P., Huang, P. I., Li, W. C., Huang, M. C., Kao, C. L., et al. (2015). Exercise suppresses COX-2 pro-inflammatory pathway in vestibular migraine. *Brain Res. Bull.* 116, 98–105. doi: 10.1016/j.brainresbull.2015.06.005

Leite, A. B., Lima, H. N., Flores, C. O., Oliveira, C. A., Cunha, L. E. C., Neves, J. L., et al. (2021). High-intensity interval training is more effective than continuous training to reduce inflammation markers in female rats with cisplatin nephrotoxicity. *Life Sci.* 266:118880. doi: 10.1016/j.lfs.2020.118880

Li, D., Zhang, R., Cui, L., Chu, C., Zhang, H., Sun, H., et al. (2019). Multiple organ injury in male C57BL/6J mice exposed to ambient particulate matter in a real-ambient PM exposure system in Shijiazhuang, China. *Environ. Pollut.* 248, 874–887. doi: 10.1016/j.envpol.2019.02.097

Li, J., Zhou, Q., Liang, Y., Pan, W., Bei, Y., Zhang, Y., et al. (2018). miR-486 inhibits $PM_{2.5}$-induced apoptosis and oxidative stress in human lung alveolar epithelial A549 cells. *Ann. Transl. Med.* 6:209. doi: 10.21037/atm.2018.06.09

Loxham, M., Davies, D. E., and Holgate, S. T. (2019). The health effects of fine particulate air pollution. *BMJ* 367:l6609. doi: 10.1136/bmj.l6609

Ma, M., Li, S., Jin, H., Zhang, Y., Xu, J., Chen, D., et al. (2015). Characteristics and oxidative stress on rats and traffic policemen of ambient fine particulate matter from Shenyang. *Sci. Total Environ.* 526, 110–115. doi: 10.1016/j.scitotenv.2015.04.075

Ma, H., Wang, H., Zhang, H., Guo, H., Zhang, W., Hu, F., et al. (2020). Effects of phenanthrene on oxidative stress and inflammation in lung and liver of female rats. *Environ. Toxicol.* 35, 37–46. doi: 10.1002/tox.22840

MacInnis, M. J., and Gibala, M. J. (2017). Physiological adaptations to interval training and the role of exercise intensity. *J. Physiol.* 595, 2915–2930. doi: 10.1113/JP273196

Maret, W. (2012). New perspectives of zinc coordination environments in proteins. *J. Inorg. Biochem.* 111, 110–116. doi: 10.1016/j.jinorgbio.2011.11.018

Matute-Bello, G., Downey, G., Moore, B. B., Groshong, S. D., Matthay, M. A., Slutsky, A. S., et al. (2011). An official American Thoracic Society workshop report: features and measurements of experimental acute lung injury in animals. *Am. J. Respir. Cell Mol. Biol.* 44, 725–738. doi: 10.1165/rcmb.2009-0210ST

Menon, G. K., Lee, S. E., and Lee, S. H. (2018). An overview of epidermal lamellar bodies: novel roles in biological adaptations and secondary barriers. *J. Dermatol. Sci.* 92, 10–17. doi: 10.1016/j.jdermsci.2018.03.005

Metsios, G. S., Moe, R. H., and Kitas, G. D. (2020). Exercise and inflammation. *Best Pract. Res. Clin. Rheumatol.* 34:101504. doi: 10.1016/j.berh.2020.101504

Molmen-Hansen, H. E., Stolen, T., Tjonna, A. E., Aamot, I. L., Ekeberg, I. S., Tyldum, G. A., et al. (2012). Aerobic interval training reduces blood pressure and improves myocardial function in hypertensive patients. *Eur. J. Prev. Cardiol.* 19, 151–160. doi: 10.1177/1741826711400512

Mulugeta, S., Gray, J. M., Notarfrancesco, K. L., Gonzales, L. W., Koval, M., Feinstein, S. I., et al. (2002). Identification of LBM180, a lamellar body limiting membrane protein of alveolar type II cells, as the ABC transporter protein ABCA3. *J. Biol. Chem.* 277, 22147–22155. doi: 10.1074/jbc.M201812200

Nieman, D. C., and Pence, B. D. (2020). Exercise immunology: future directions. *J. Sport Health Sci.* 9, 432–445. doi: 10.1016/j.jshs.2019.12.003

Ning, J., Li, P., Zhang, B., Han, B., Su, X., Wang, Q., et al. (2019). miRNAs deregulation in serum of mice is associated with lung cancer related pathway deregulation induced by $PM_{2.5}$. *Environ. Pollut.* 254:112875. doi: 10.1016/j.envpol.2019.07.043

Pedersen, B. K., and Hoffman-Goetz, L. (2000). Exercise and the immune system: regulation, integration, and adaptation. *Physiol. Rev.* 80, 1055–1081. doi: 10.1152/physrev.2000.80.3.1055

Peng, J., Zhang, L., Meng, Q., Zhang, F., Mao, X., Liu, J., et al. (2019). Adverse impact of ambient $PM_{2.5}$ on expression and trafficking of surfactant protein A through reactive oxygen species damage to lamellar bodies. *Toxicol. Lett.* 315, 47–54. doi: 10.1016/j.toxlet.2019.08.015

Qin, F., Cui, S., Dong, Y., Xu, M., Wang, Z., Qu, C., et al. (2021). Aerobic exercise ameliorates particulate matter-induced lung injury in aging rats. *Environ. Pollut.* 280:116889. doi: 10.1016/j.envpol.2021.116889

Qin, F., Dong, Y., Wang, S., Xu, M., Wang, Z., Qu, C., et al. (2020a). Maximum oxygen consumption and quantification of exercise intensity in untrained male Wistar rats. *Sci. Rep.* 10:11520. doi: 10.1038/s41598-020-68455-8

Qin, F., Xu, M. X., Wang, Z. W., Han, Z. N., Dong, Y. N., and Zhao, J. X. (2020b). Effect of aerobic exercise and different levels of fine particulate matter ($PM_{(2.5)}$) on pulmonary response in Wistar rats. *Life Sci.* 254:117355. doi: 10.1016/j.lfs.2020.117355

Qiu, H., Yu, I. T., Tian, L., Wang, X., Tse, L. A., Tam, W., et al. (2012). Effects of coarse particulate matter on emergency hospital admissions for respiratory diseases: a time-series analysis in Hong Kong. *Environ. Health Perspect.* 120, 572–576. doi: 10.1289/ehp.1104002

Rietberg, M. B., Veerbeek, J. M., Gosselink, R., Kwakkel, G., and Van Wegen, E. E. (2017). Respiratory muscle training for multiple sclerosis. *Cochrane Database Syst. Rev.* 12:Cd009424. doi: 10.1002/14651858.CD009424.pub2

Schmitz, G., and Müller, G. (1991). Structure and function of lamellar bodies, lipid-protein complexes involved in storage and secretion of cellular lipids. *J. Lipid Res.* 32, 1539–1570. doi: 10.1016/S0022-2275(20)41642-6

Song, C., Liu, L., Chen, J., Hu, Y., Li, J., Wang, B., et al. (2020). Evidence for the critical role of the PI3K signaling pathway in particulate matter-induced dysregulation of the inflammatory mediators COX-2/PGE(2) and the associated epithelial barrier protein Filaggrin in the bronchial epithelium. *Cell Biol. Toxicol.* 36, 301–313. doi: 10.1007/s10565-019-09508-1

Su, X., Tian, J., Li, B., Zhou, L., Kang, H., Pei, Z., et al. (2020). Ambient $PM_{2.5}$ caused cardiac dysfunction through FoxO1-targeted cardiac hypertrophy and macrophage-activated fibrosis in mice. *Chemosphere* 247:125881. doi: 10.1016/j.chemosphere.2020.125881

Sur, R., Babad, J. M., Garay, M., Liebel, F. T., and Southall, M. D. (2008). Anti-inflammatory activity of sertaconazole nitrate is mediated via activation of a p38-COX-2-PGE2 pathway. *J. Invest. Dermatol.* 128, 336–344. doi: 10.1038/sj.jid.5700972

Tala, W., and Chantara, S. (2019). Use of spent coffee ground biochar as ambient PAHs sorbent and novel extraction method for GC-MS analysis. *Environ. Sci. Pollut. Res. Int.* 26, 13025–13040. doi: 10.1007/s11356-019-04473-y

Tian, Y., Liu, H., Wu, Y., Si, Y., Li, M., Wu, Y., et al. (2019). Ambient particulate matter pollution and adult hospital admissions for pneumonia in urban China: a national time series analysis for 2014 through 2017. *PLoS Med.* 16:e1003010. doi: 10.1371/journal.pmed.1003010

Toledo, A. C., Magalhaes, R. M., Hizume, D. C., Vieira, R. P., Biselli, P. J., Moriya, H. T., et al. (2012). Aerobic exercise attenuates pulmonary injury induced by exposure to cigarette smoke. *Eur. Respir. J.* 39, 254–264. doi: 10.1183/09031936.00003411

Tsai, M. H., Hsu, L. F., Lee, C. W., Chiang, Y. C., Lee, M. H., How, J. M., et al. (2017). Resveratrol inhibits urban particulate matter-induced COX-2/PGE(2) release in human fibroblast-like synoviocytes via the inhibition of activation of NADPH oxidase/ROS/NF-κB. *Int. J. Biochem. Cell Biol.* 88, 113–123. doi: 10.1016/j.biocel.2017.05.015

Valavanidis, A., Fiotakis, K., Vlahogianni, T., Bakeas, E. B., Triantafillaki, S., Paraskevopoulou, V., et al. (2006). Characterization of atmospheric particulates, particle-bound transition metals and polycyclic aromatic hydrocarbons of urban air in the Centre of Athens (Greece). *Chemosphere* 65, 760–768. doi: 10.1016/j.chemosphere.2006.03.052

Wang, M., Aaron, C. P., Madrigano, J., Hoffman, E. A., Angelini, E., Yang, J., et al. (2019a). Association between long-term exposure to ambient air pollution and change in quantitatively assessed emphysema and lung function. *JAMA* 322, 546–556. doi: 10.1001/jama.2019.10255

Wang, H., Gao, Z., Ren, J., Liu, Y., Chang, L. T., Cheung, K., et al. (2019b). An urban-rural and sex differences in cancer incidence and mortality and the relationship with $PM_{(2.5)}$ exposure: an ecological study in the southeastern side of Hu line. *Chemosphere* 216, 766–773. doi: 10.1016/j.chemosphere.2018.10.183

Wang, J., Liu, S., Li, G., and Xiao, J. (2020). Exercise regulates the immune system. *Adv. Exp. Med. Biol.* 1228, 395–408. doi: 10.1007/978-981-15-1792-1_27

Wewege, M., Van Den Berg, R., Ward, R. E., and Keech, A. (2017). The effects of high-intensity interval training vs. moderate-intensity continuous training on body composition in overweight and obese adults: a systematic review and meta-analysis. *Obes. Rev.* 18, 635–646. doi: 10.1111/obr.12532

Xu, F., Xu, Z., Zhang, R., Wu, Z., Lim, J. H., Koga, T., et al. (2008). Nontypeable *Haemophilus influenzae* induces COX-2 and PGE2 expression in lung epithelial cells via activation of p38 MAPK and NF-kappa B. *Respir. Res.* 9:16. doi: 10.1186/1465-9921-9-16

Yang, B., Guo, J., and Xiao, C. (2018). Effect of $PM_{2.5}$ environmental pollution on rat lung. *Environ. Sci. Pollut. Res. Int.* 25, 36136–36146. doi: 10.1007/s11356-018-3492-y

Yu, K. R., Lee, J. Y., Kim, H. S., Hong, I. S., Choi, S. W., Seo, Y., et al. (2014). A p38 MAPK-mediated alteration of COX-2/PGE2 regulates immunomodulatory properties in human mesenchymal stem cell aging. *PLoS One* 9:e102426. doi: 10.1371/journal.pone.0115041

Zhang, S., Zhang, W., Zeng, X., Zhao, W., Wang, Z., Dong, X., et al. (2019). Inhibition of Rac1 activity alleviates $PM_{2.5}$-induced pulmonary inflammation via the AKT signaling pathway. *Toxicol. Lett.* 310, 61–69. doi: 10.1016/j.toxlet.2019.04.017

Zhou, M., Wang, H., Zeng, X., Yin, P., Zhu, J., Chen, W., et al. (2019). Mortality, morbidity, and risk factors in China and its provinces, 1990-2017: a systematic analysis for the global burden of disease study 2017. *Lancet* 394, 1145–1158. doi: 10.1016/S0140-6736(19)30427-1

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SYSTEMATIC REVIEW
published: 15 November 2021
doi: 10.3389/fphys.2021.754731

Calorie Restriction With Exercise Intervention Improves Inflammatory Response in Overweight and Obese Adults: A Systematic Review and Meta-Analysis

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OPEN ACCESS

Edited by:
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Reviewed by:
Guido Gembillo, University of Messina, Italy
Marli Maria Knorst, Federal University of Rio Grande do Sul, Brazil

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Specialty section:
This article was submitted to Integrative Physiology, a section of the journal Frontiers in Physiology

Received: 06 August 2021
Accepted: 05 October 2021
Published: 15 November 2021

Citation:
Liu Y, Hong F, Lebaka VR, Mohammed A, Ji L, Zhang Y and Korivi M (2021) Calorie Restriction With Exercise Intervention Improves Inflammatory Response in Overweight and Obese Adults: A Systematic Review and Meta-Analysis. Front. Physiol. 12:754731. doi: 10.3389/fphys.2021.754731

Background/Purpose: In this systematic review and meta-analysis, we assessed the effects of exercise (EX) combined with calorie restriction (CR) intervention on inflammatory biomarkers, and correlations between biomarkers and participants' characteristics were calculated in overweight and obese adults.

Methods: An article search was conducted through PubMed, Web of Science, EMBASE, the Cochrane database, Scopus, and Google Scholar to identify articles published up to April 2021. Studies that examined the effect of EX + CR intervention on inflammatory biomarkers, including C-reactive protein (CRP), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-α), and compared them with a CR trial in overweight and obese adults were included. We calculated the pooled effect by meta-analysis, identified the correlations (between inflammatory biomarkers and participants' characteristics) through meta-regression, and explored the beneficial variable through subgroup analysis. The Cochrane risk of bias tool and Methodological Index for Non-randomized Studies were used to assess the risk of bias for the included trials.

Results: A total of 23 trials, including 1196 overweight and obese adults, were included in the meta-analysis. The pooled effect showed that EX + CR intervention significantly decreased CRP levels ($P = 0.02$), but had no effect on IL-6 ($P = 0.62$) and TNF-α ($P = 0.11$). Meta-regression analysis showed that the effect of EX + CR on CRP, IL-6, and TNF-α changes was correlated with lifestyle behavior of adults (Coef. = −0.380, $P = 0.018$; Coef. = −0.359, $P = 0.031$; Coef. = −0.424, $P = 0.041$, respectively), but not with age and BMI. The subgroup analysis results revealed that participants with sedentary lifestyle behavior did not respond to EX + CR intervention, as we found no changes in CRP, IL-6, and TNF-α concentrations ($P = 0.84$, $P = 0.16$, $P = 0.92$, respectively). However, EX + CR intervention significantly decreased CRP (P = 0.0003; SMD = −0.39; 95%CI: −0.60 to −0.18), IL-6 (P = 0.04; SMD = −0.21; 95%CI: −0.40 to −0.01) and TNF-α (P = 0.006; SMD = −0.40, 95%CI: −0.68 to −0.12) in adults without a sedentary lifestyle or with a normal lifestyle. Furthermore, the values between sedentary and normal lifestyle subgroups were statistically significant for CRP, IL-6, and TNF-α.

Conclusion: Our findings showed that combination EX + CR intervention effectively decreased CRP, IL-6, and TNF-α in overweight and obese adults with active lifestyles, but not with sedentary lifestyle behavior. We suggest that 'lifestyle behavior' is a considerable factor when designing new intervention programs for overweight or obese adults to improve their inflammatory response.

Keywords: physical exercise, diet, C-reacting protein, meta-regression, inflammation, obesity

INTRODUCTION

According to the latest reports from the World Health Organization (WHO), worldwide prevalence of obesity has tripled since 1975. More than 1.9 billion adults (18 years or older) were overweight (39%) in 2016. Of these, more than 650 million adults (13%) were obese (WHO, 2021). The energy imbalance between calories consumption and calories expenditure is the fundamental cause of being overweight and obese. Increased intake of energy-dense foods (fat and sugar) over a period of time, and increased physical inactivity due to increased sedentary behavior are the primary contributors of being overweight and obese (Blüher, 2019; WHO, 2021). Weight gain has been associated with subclinical inflammation, which is mainly attributed to the secretion of various pro-inflammatory biomarkers (Saltiel and Olefsky, 2017). The inflammation caused by overnutrition or obesity is characterized by the activation of various immune cells, which release pro-inflammatory cytokines, such as tumor necrosis-alpha (TNF-α), interleukin-6 (IL-6), and C-reactive protein (CRP). Studies have shown that increased production of these inflammatory biomarkers is closely associated with the prevalence of chronic diseases, such as type 2 diabetes (T2D), cardiovascular diseases (CVDs), chronic kidney disease, and cancer (Coussens and Werb, 2002; Hotamisligil, 2006). It is further stated that more than 50% of all deaths worldwide are directly or indirectly linked with the progression of inflammatory-related diseases (Furman et al., 2019).

Recent evidence identified a strong association between obesity and severity of the coronavirus disease-19 (COVID-19), the ongoing global pandemic (Albashir, 2020). The severity of COVID-19 is represented by an excessive production of IL-6 (Mehta et al., 2020), TNF-α (Mortaz et al., 2021), and CRP (Chen et al., 2020). The need of intensive care for obese COVID-19 patients was reportedly higher compared to patients with a normal weight (Lighter et al., 2020), and disease severity increased with body mass index (BMI) and existence of inflammatory diseases (Simonnet et al., 2020). A study demonstrated that CRP and BMI were significantly higher in critical COVID-19 patients. Among the non-survivors, 88.24% of patients reportedly had a higher BMI (>25 kg/m^2) (Peng et al., 2020). Therefore, controlling the release of inflammatory biomarkers is crucial for prevention of chronic diseases and to enhance the treatment efficiently in COVID-19 patients. Since excessive calorie intake and adiposity cause systemic inflammation, calorie restriction (CR) without malnutrition is a potential strategy to treat the inflammation and inflammatory diseases (Kökten et al., 2021). On the other hand, any form of physical activity or exercise has been documented to promote weight loss and decrease chronic low-grade inflammation, which together can attenuate several complications caused by inflammation (Nimmo et al., 2013). There is an increasing interest on investigating the combined effect of exercise (EX) and CR on an inflammatory system (Tolkien et al., 2019;Zheng et al., 2019). CR intervention (4-week) has been shown to decrease bodyweight and fasting blood glucose and insulin levels of obese women. This beneficial effect of CR was accompanied by decreased systemic inflammation in obese women (Ott et al., 2017). A previous meta-analysis concluded that exercise can improve the inflammatory response in overweight and obese adults, in which aerobic exercise had a greater beneficial effect (Yu et al., 2017). Another recent meta-analysis on older adults demonstrated that exercise intervention decreased IL-6 and CRP levels, but not TNF-α. However, this study was unable to establish a dose-related response to exercise and chronic inflammation (Monteiro-Junior et al., 2018).

Owing to the independent positive effects of exercise and CR interventions on inflammatory biomarkers, researchers wondered whether a combination of EX plus CR would amplify the beneficial effects compared to CR alone. To address this, Fisher at al. studied the independent effect of energy restriction alone and also in combination with exercise on changes in inflammatory mediators in overweight premenopausal women. The results showed that weight loss was associated with a reduction of inflammatory mediators; however, combining exercise and energy restriction did not alter the response (Fisher et al., 2011). Contrarily, another study reported a greater reduction of subcutaneous fat (not intra-abdominal fat) with a combination of EX plus CR compared to CR alone in postmenopausal women (van Gemert et al., 2019). Another study on obese sedentary adults reported no significant additional

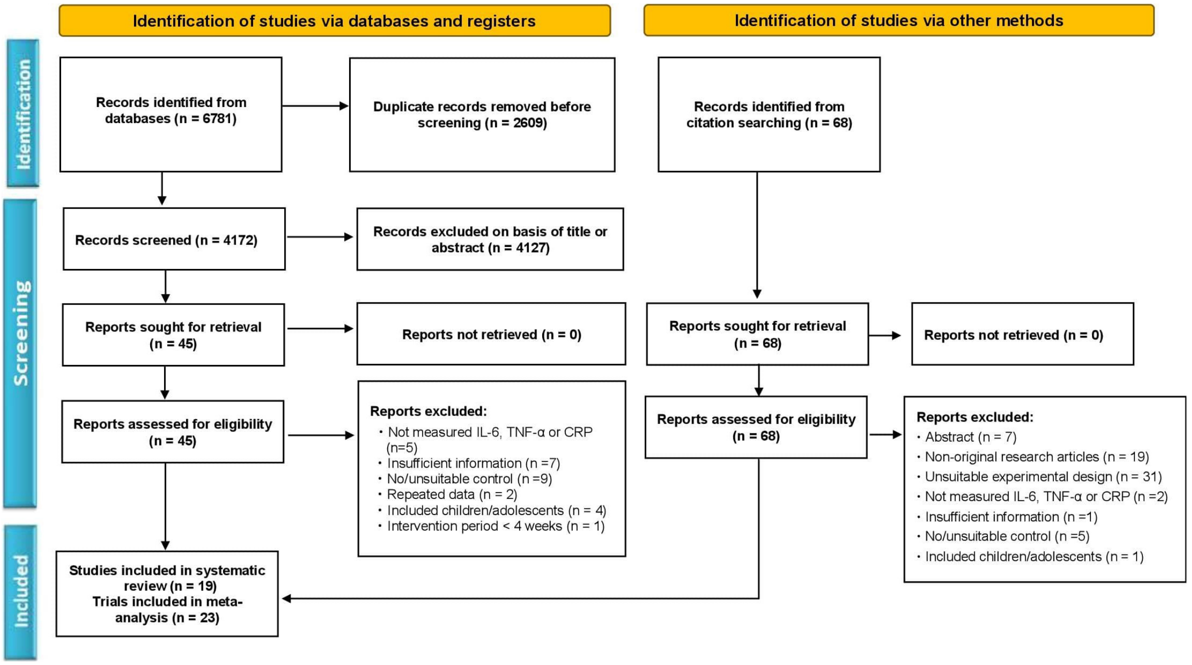


FIGURE 1 | Flowchart of the study selection according to the Preferred Reporting Items for the Systematic Reviews and Meta-Analysis (PRISMA) method.

benefit of exercise on inflammatory response in a CR plus exercise intervention group (Trussardi Fayh et al., 2013). Compared with CR alone, combination of EX plus CR intervention exerts only a minor influence on adipocytokines and inflammatory cytokines in postmenopausal women (Giannopoulou et al., 2005). A recent meta-analysis concluded that a combination of EX plus CR largely decreased the inflammatory cytokines and CRP than CR alone in overweight and obese adults (Khalafi et al., 2021).

These controversial results of research studies and meta-analyses may be due to the differences in participants' characteristics and/or intervention protocol. From the perspective of participants' characteristics, no meta-analysis has yet analyzed the effect of EX plus CR intervention on inflammatory biomarkers in overweight and obese adults. It is therefore necessary to pool the data from EX plus CR interventions and examine the influence of participant's characteristics on improving the inflammatory cytokines. In this study, we conducted a systematic review and meta-analysis of trials and explored the effect of EX plus CR on the alterations of CRP, IL-6, and TNF-α in overweight and obese adults. We further examined the association between characteristics of participants (age, BMI, and lifestyle behavior) and inflammatory biomarkers to identify the influential and effective variable that decreases inflammation.

METHODS

Search Strategy

A thorough article search was conducted primarily using PubMed, Web of Science, EMBASE, the Cochrane database, Scopus, and Google Scholar. Specific keywords, including "exercise" OR "physical activity" OR "training" OR "aerobic exercise" OR "resistance exercise" OR "strength training" AND "caloric restriction" OR "restricted diet" OR "low calorie diet" OR "weight loss" were independently used with "inflammation" OR "interleukin-6" OR "tumor necrosis factor-α" OR "C-reactive protein" to search for articles. Furthermore, a manual search from the reference list of the included articles was conducted to identify relevant articles. Studies published until April 2021 were systematically searched by a team of authors (YL, FH, and YZ), and the influence of calorie restriction with exercise on inflammatory biomarkers was analyzed in overweight and obese adults with different lifestyle behavior.

Inclusion and Exclusion Criteria

The article review and selection process was strictly followed according to the updated guidelines of Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) as described before (Moher et al., 2009; Page et al., 2021a,b). The detailed selection process with the number of articles in each step is depicted as a flowchart in **Figure 1**.

According to the selection criteria, a team of authors (YL, FH, and YZ) independently reviewed and assessed the relevant articles. Any differences in opinion among the authors on inclusion or exclusion of articles were solved by discussion with the corresponding author (MK). The inclusion criteria were: (1) All participants in the trials were overweight and obese adults; (2) the intervention group adopted calorie restriction with any type of therapeutic exercise (aerobic, resistance, mobility exercises, etc.), whilst the control group only underwent calorie restriction; (3) trials compared the effect of exercise and/or calorie restriction with respective controls; (4) trials reported inflammatory biomarkers (IL-6, TNF-α, or CRP) data as outcome measures after intervention, and (5) all studies were published in English as full-text articles. The exclusion criteria were: (1) Studies involving minors (<18 years) or animals; (2) articles with repeated data; (3) non-original research articles, such as protocols, case reports, meta-analyses, and systemic reviews, and (4) articles with inadequate information about the characteristics of the participants or intervention.

Outcome Measures and Data Extraction

Two review authors, YL and FH, independently extracted the study characteristics, and the same was verified by another review author, MK. Three authors (VRL, AM, and LJ) provided in-depth analyses of the results and coordinated data extraction and interpretation. The information extracted from each study included values of inflammatory biomarkers (CRP, IL-6, and TNF-α), characteristics of participants (number, sex, baseline age, and baseline BMI), baseline lifestyle (sedentary or normal lifestyle), and details of intervention protocol (type of exercise, intensity, frequency, duration). Publication details, including name of authors, year of publication, and study conducted region were also extracted, and are described in **Table 1**.

Quality Assessment

The quality of the included randomized controlled trials (RCTs) was assessed using the Cochrane risk of bias tool (Higgins et al., 2011). The Cochrane risk of bias assessment tool consists of seven domains, namely random sequence generation/allocation concealment (selection bias), blinding of participants/personnel (performance bias), blinding of outcome assessment (detection bias), incomplete outcome data (attrition bias), selective reporting (reporting bias), and other biases. The quality of each domain was rated as "low risk," "high risk," or "unclear," and were indicated with green (+), red (−), and yellow (?) colors and symbols, respectively. The quality of trials was assessed by two authors (YL and FH), and discrepancies were resolved through discussion with a third reviewer (MK) to reach a consensus. The quality of the non-randomized controlled trials (non-RCTs) was assessed with the Methodological Index for Non-randomized Studies (MINORS) entry, and non-RCTs with MINORS scores > 12 were included in the study (Slim et al., 2003).

Statistical Analysis

In this study, Cochrane Collaboration's Review Manager (RevMan 5.3., Copenhagen, Denmark) was employed to analyze the effect of intervention (EX + CR) and participants characteristics on inflammatory biomarkers (IL-6, TNF-α, and CRP) of overweight and obese adults. The outcome measures were summarized through standardized mean difference (SMD) and 95% confidence intervals (95% CI). Inter-study heterogeneity was tested using I^2 statistic, when $I^2 \geq 50\%$, this means strong heterogeneity. Then meta-regression analysis followed by subgroup analysis were performed to assess the sources of heterogeneity, and to explore the effective characteristics that promote the benefits of intervention. We used STATA version 12 (StataCorp., College Station, TX, United States) to run the meta-regression analysis, and examined the relationships between effect size estimates and the following covariates: (1) baseline age, (2) baseline BMI, and (3) baseline lifestyle behavior. We found baseline lifestyle behavior (sedentary or normal lifestyle) was correlated with the changes of inflammatory mediators. Sedentary behavior refers to any walking behavior that is characterized by energy expenditure ≤1.5 metabolic equivalents (METs), while in a sitting, reclining, or lying posture (Tremblay et al., 2017; Thivel et al., 2018). The included trials were subgrouped based on the lifestyle behavior (sedentary or normal) of adults, and the effective lifestyle behavior in response to intervention was determined. We then assessed the differences between subgroups using the test for heterogeneity in Review Manager version 5.3. The Egger's test was conducted to examine potential publication bias. The level of statistical significance was set at $P < 0.05$.

RESULTS

Search Results and Selection of Studies

We initially obtained a total of 6,849 articles, including 6781 from the electronic databases (PubMed, Web of Science, Scopus, EMBASE, SportDiscus, ScienceDirect, and Google Scholar) search, and 68 from the manual search. After removing duplicates (2,677), the remaining 4,172 articles were carefully screened by title and abstract, and a further 4,116 were excluded because of unsuitability. Then the full text of the 56 articles was thoroughly reviewed, and 37 of them were excluded due to the following reasons; not measuring IL-6, TNF-α, or CRP levels, insufficient information, no/unsuitable control, repeated data, included children or adolescents, and intervention period less than 4 weeks. Finally, 19 articles consisting of 23 trials met the inclusion criteria, and were included in the systematic review and meta-analysis. The informative flowchart (PRISMA) of article search, screening, and selection is presented in **Figure 1**.

Summary of the Included Studies

According to the inclusion and exclusion criteria, a total of 19 articles published between 2004 and 2019 were included for the systematic review, meta-analysis, and meta-regression analysis. These studies were from various parts of the world, most were from the United States (10), with one each from Australia, Canada, Denmark, Iran, Japan, Korea, Netherlands, Spain, and Tunisia. The total sample size was 1196 participants, consisting of 688 adults from the intervention group and 508 adults from the control group. The mean age of participants in the trials ranged from 18 to 75 years. The types of exercise intervention included aerobic (You et al., 2004; Giannopoulou et al., 2005; Silverman et al., 2009; Christiansen et al., 2010; Straznicky et al., 2010; Fisher et al., 2011; Snel et al., 2011; Lakhdar et al., 2013; Oh et al., 2014; Ryan et al., 2014; Lam et al., 2015; Weiss et al., 2016; Galedari et al., 2017; Rejeski et al., 2019), resistance (Brochu et al., 2009; Fisher et al., 2011; García-Unciti et al., 2012; Galedari et al., 2017; Rejeski et al., 2019), a combination of both aerobic

TABLE 1 | Characteristics of the included studies, presented in chronological order.

First author	Country	Sex	Sample EX + CR/CR	Participants characteristics			Exercise type/intensity	Frequency (t/wk)	Duration	Outcomes	MINORS
				Age (years)	BMI (kg/m2)	Life-style					
Cho et al., 2019	South Korea	M/F	9/8	EX + CR: 34.5 ± 5.7 CR: 33.5 ± 5.0	EX + CR: 28.0 ± 2.6 CR: 27.8 ± 3.4	NP	AE + RE: NR	≥3	8 weeks	CRP	–
Rejeski et al., 2019	United States	M/F	AE + CR: 79 RE + CR: 75 CR:68	66.9 ± 4.7	33.5 ± 3.5	NP	AE: 12–14RPE RE: 40–75%1RM	4	72 weeks	CRP, IL-6	–
Galedari et al., 2017	Iran	M	AE + CR: 12 RE + CR: 10 HT + CR: 10 CR: 8	AE + CR: 28.8 ± 6.1 RE + CR: 31.7 ± 7.7 HT + CR: 30.8 ± 7.6 CR: 32.6 ± 6.8	AE + CR: 28.9 ± 1.32 RE + CR: 29.0 ± 2.9 HT + CR: 29.6 ± 1.5 CR: 29.2 ± 2.4	NP	AE: 65–70%HR_{max} RE: 60–80%1RM HT: 90–95%HR_{max}	3	12 weeks	TNF-α	–
Weiss et al., 2016	United States	M/F	19/17	EX + CR: 57 ± 7 CR: 57 ± 5	EX + CR: 28.3 ± 1.8 CR: 27.7 ± 1.7	SP	AE: moderate- and high-intensity	7	12–14 weeks	CRP	–
Lam et al., 2015	United States	M/F	8/8	EX + CR: 37.9 ± 1.8 CR: 39.0 ± 2.1	EX + CR: 27.9 ± 0.6 CR: 27.7 ± 0.5	NP	AE: NR	5	24 weeks	CRP, IL-6, TNF-α	–
Oh et al., 2014	Japan	M	52/20	EX + CR: 49.1 ± 1.3 CR: 53.2 ± 2.1	EX + CR: 29.2 ± 0.4 CR: 28.5 ± 0.8	NP	AE: >40%HR_{max}	3	12 weeks	CRP, IL-6, TNF-α	18
Bouchonville et al., 2014	United States	M/F	28/26	EX + CR: 70 ± 4 CR: 70 ± 4	EX + CR: 37.2 ± 5.4 CR: 37.2 ± 4.5	SP	AE + RE AE: 65–85%HR_{max} RE: 65–85%1RM	3	48 weeks	CRP	–
Ryan et al., 2014	United States	F	37/40	EX + CR: 60 ± 1 CR: 61 ± 1	EX + CR: 32 ± 1 CR: 33 ± 1	SP	AE: 50–85%HRR	3	24 weeks	CRP	16
Lakhdar et al., 2013	Tunisia	F	10/10	EX + CR: 38.90 ± 4.37 CR: 38.90 ± 3.94	EX + CR: 32.98 ± 2.17 CR: 33.02 ± 1.89	NP	AE: 55–80%HR_{max}	3	24 weeks	IL-6, TNF-α	–
García-Unciti et al., 2012	Spain	F	13/12	EX + CR: 48.6 ± 6.4 CR: 51.4 ± 5.5	EX + CR: 35 ± 3.1 CR: 34.6 ± 3.4	SP	RE: 50–80%1RM	2	16 weeks	IL-6	–
Fisher et al., 2011	United States	F	AE + CR: 43 RE + CR: 54 CR: 29	20–41	AE + CR: 28 ± 1 RE + CR: 28 ± 1 CR: 28 ± 1	SP	AE: 65–80%HR_{max} RE: 60–80%1RM	3	Until a BMI < 25kg/m^2	CRP, IL-6, TNF-α	–
Snel et al., 2011	Netherlands	M/F	14/13	EX + CR: 56 ± 2 CR: 59 ± 2	EX + CR: 36.4 ± 1.1 CR: 37.9 ± 1.4	NP	AE: 70%VO_{2max}	5	16 weeks	CRP, IL-6, TNF-α	18
Christiansen et al., 2010	Denmark	M/F	21/19	EX + CR: 37.5 ± 8 CR: 35.6 ± 7	EX + CR: 34.2 ± 3 CR: 35.3 ± 4	NP	AE: NR	3	12 weeks	IL-6	–
Straznicky et al., 2010	Australia	M	20/20	EX + CR: 54 ± 1 CR: 55 ± 1	EX + CR: 31.8 ± 0.8 CR: 32.2 ± 0.9	SP	AE: 65%HR_{max}	3	12 weeks	CRP	–
Silverman et al., 2009	United States	F	46/40	EX + CR: 60 ± 5 CR: 58 ± 5	EX + CR: 32.1 ± 4.2 CR: 32.6 ± 4.6	SP	AE: 50–75%HRR	3	24 weeks	IL-6, TNF-α	19
Brochu et al., 2009	Canada	F	36/71	EX + CR: 57.2 ± 5.0 CR: 58.0 ± 4.7	EX + CR: 32.6 ± 4.9 CR: 32.2 ± 4.6	SP	RE: 65–80%1RM	3	24 weeks	CRP	–
Giannopoulou et al., 2005	United States	F	11/11	EX + CR: 57.4 ± 1.7 CR: 58.5 ± 1.7	EX + CR: 33.7 ± 1.9 CR: 34.3 ± 1.9	NP	AE: 65–70%VO_{2max}	3–4	14 weeks	CRP, IL-6, TNF-α	–
Nicklas et al., 2004	United States	M/F	64/71	EX + CR: 68 ± 7 CR: 68 ± 5	EX + CR: 33.9 ± 5.6 CR: 34.4 ± 4.9	SP	AE + RE AE: 50–75%HRR RE: NR	3	72 weeks	CRP, IL-6, TNF-α	–
You et al., 2004	United States	F	17/17	EX + CR: 59 ± 1 CR: 57 ± 1	25–40	SP	AE: 50–70%HRR	3	24 weeks	CRP	–

M, male; F, female; EX, exercise; CR, calorie restriction; AE, aerobic exercise; RE, resistance exercise; HT, high-intensity interval training; AE + RE, combination of aerobic and resistance exercise; SP, sedentary people; NP, normal people; NR, not reported; %HR_{max}, percentage of maximal heart rate; %VO_{2max}, percentage of maximal oxygen uptake; %HRR, percentage of heart rate reserve; %1RM, percentage of one-repetition maximum; RPE, ratings of perceived exertion (6–20); t/wk, times/week; MINORS, methodological index for non-randomized studies.

and resistance (Nicklas et al., 2004; Bouchonville et al., 2014; Cho et al., 2019), and high-intensity interval training (Galedari et al., 2017). The exercise intervention duration ranged from 8 to 72 weeks with a frequency of 3 to 7 times per week. The characteristics of patients (sex, age, BMI, and life style behavior) and exercise intervention (type, intensity, frequency, and duration) are presented in **Table 1**.

Exercise Plus Calorie Restriction Decreases C-Reactive Protein Levels in Overweight and Obese Adults

Among the included trials (*n* = 23), 16 trials (981 participants) reported the effect of exercise plus CR intervention on alterations in CRP levels and compared the changes with a CR trial alone. The pooled outcome of meta-analysis revealed that exercise plus CR intervention significantly ($P = 0.02$) decreased CRP in overweight and obese adults. The standardized mean difference (SMD) of decreased CRP with EX + CR intervention was −0.16 with 95% CI: −0.29 to −0.03 (**Figure 2** and **Supplementary Figure 1**). These findings revealed that overweight or obese adults required a combination of exercise and CR to decrease their CRP levels.

Exercise Plus Calorie Restriction Does Not Affect IL-6 and TNF-α in Overweight and Obese Adults

The changes in IL-6 concentrations with EX + CR intervention were reported in 13 trials, and the differences were compared with CR trials. Results of the pooled outcome showed that an EX + CR intervention for a period of 12–72 weeks did not influence IL-6 concentrations in overweight and obese adults ($P = 0.62$; SMD = −0.04; 95% CI: −0.18 to 0.11) (**Figure 3** and **Supplementary Figure 2**). Besides, the effect of exercise plus CR on TNF-α concentration was evaluated in 12 trials of 560 overweight and obese adults. Similar to the IL-6 response, EX + CR-mediated changes in TNF-α were also not statistically significant ($P = 0.11$; SMD = −0.14; 95% CI: −0.31 to 0.03). Although TNF-α tended to decrease with EX + CR, such a change was not favorable to experimental intervention (**Figure 4** and **Supplementary Figure 3**).

Meta-Regression Analysis: Correlations Between Participants' Characteristics and Inflammatory Biomarkers

Meta-regression analysis was conducted to identify the correlations between characteristics of participants (age, BMI, and lifestyle behavior) and inflammatory biomarkers (CRP, IL-6, and TNF-α). One of the key findings in our analysis is that 'lifestyle behavior' of participants was significantly correlated with the changes of CRP (Coef. = −0.380; $P = 0.018$), IL-6 (Coef. = −0.359; $P = 0.031$), and TNF-α (Coef. = −0.424; $P = 0.041$) after exercise plus CR intervention. In contrast, age and BMI of participants were not correlated with the changes of any inflammatory biomarkers (**Table 2**). These results indicate that lifestyle behavior, either sedentary or normal, is the key variable that is involved in improving the inflammatory mediators following EX + CR intervention.

Subgroup Analysis Results

Normal Lifestyle With EX + CR Intervention Decreases C-Reactive Protein, Not Sedentary Lifestyle

Subgroup analysis was performed to identify the effective lifestyle (sedentary or normal) behavior of adults that could influence the beneficial effects of EX + CR intervention on inflammatory mediators. As shown in **Figure 2**, participants (213 from 7 trials) without sedentary behavior (normal lifestyle) showed greater beneficial effects of EX + CR intervention. This was evidenced by a substantial decrease of CRP levels ($P = 0.0003$; SMD = −0.39; 95%CI: −0.60 to −0.18) in normal-lifestyle adults after EX + CR intervention. However, CRP levels in participants with sedentary lifestyle behavior did not respond to EX + CR intervention. The heterogeneity of the subgroup analysis explained that the intervention effect of EX + CR on CRP levels was influenced by lifestyle behavior of adults ($I^2_{\text{in-subgroup}} < 50\%$; $I^2_{\text{between-subgroups}} = 87\%$) (**Figure 2**).

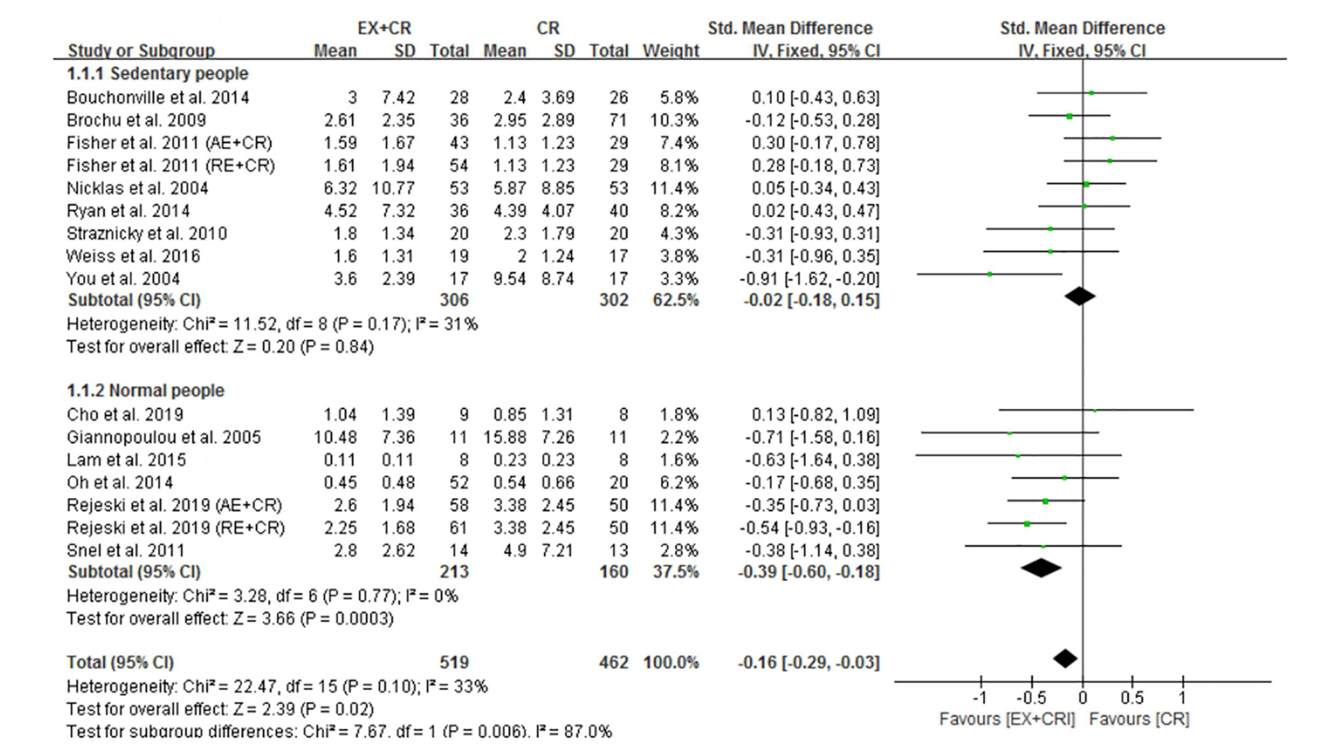
EX + CR Intervention Decreases IL-6 and TNF-α in Adults Without Sedentary Lifestyle

It is worth pointing out that EX + CR intervention had no effect on IL-6 in overweight and obese adults. Nevertheless, when trials separated participants based on lifestyle behavior, the IL-6 concentrations were significantly decreased in normal-lifestyle participants (237 from 8 trials) after EX + CR intervention ($P = 0.04$, SMD = −0.21; 95%CI: −0.40 to −0.01). Contrary to this, participants (209 from 5 trials) with sedentary lifestyle behavior did not show any positive response to EX + CR intervention (**Figure 3**). The heterogeneity ($I^2 = 83.1\%$) between the subgroups was strong, while the heterogeneity ($I^2 = 0\%$) within the subgroups was negligible, which reveals that the results of this study were relatively reliable (**Figure 3**).

Next, we found that a combination of exercise and CR intervention remarkably decreased TNF-α concentrations in participants with normal lifestyle behavior ($P = 0.006$; SMD = −0.40, 95%CI: −0.68 to −0.12), but not in adults with sedentary lifestyle (**Figure 4**). We further identified that there was no significant heterogeneity within the subgroups ($I^2 = 0\%$), but there was a significant heterogeneity between the subgroups ($I^2 = 80.6\%$), indicating the influential role of lifestyle behavior of participants on improving TNF-α (**Figure 4**).

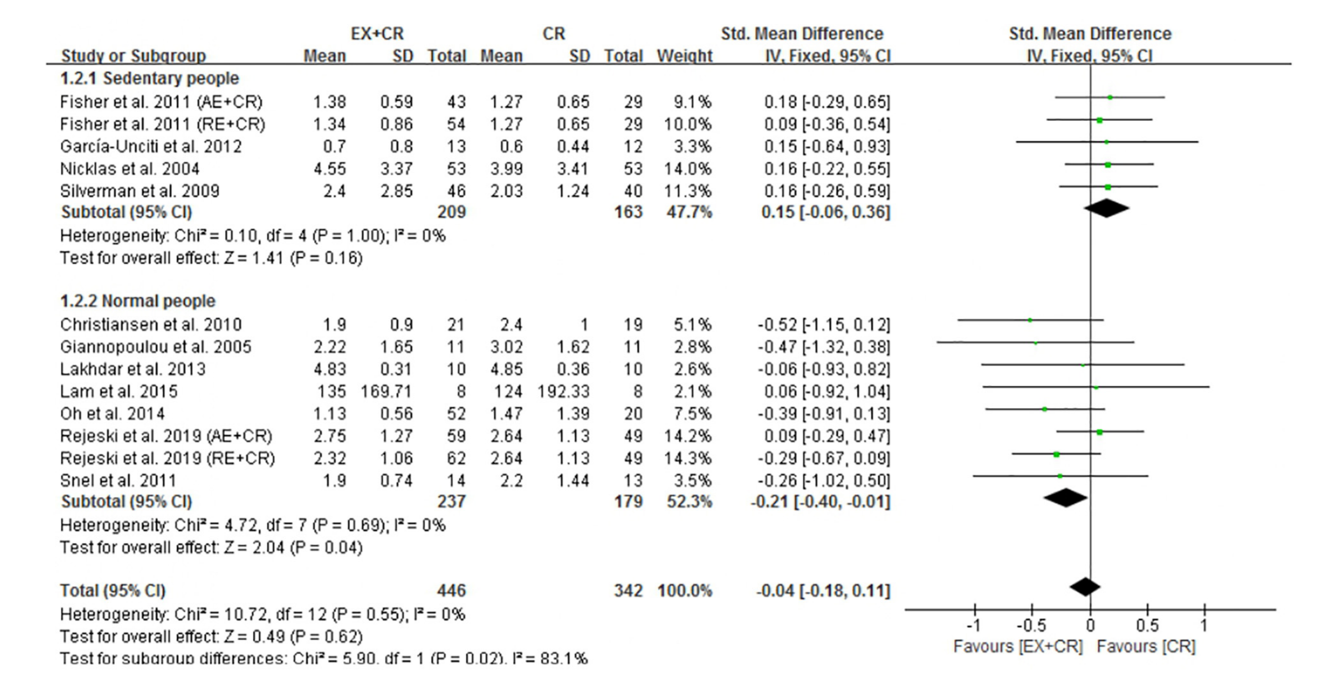
Summary of Risk of Bias

Risk of bias in the included studies (19) was assessed according to the Cochrane risk of bias tool, and the judgment is presented in **Figure 5**. For the selection bias, more studies (13 trials) reported a low risk of random sequence generation, and 12 trials were judged to have a low risk of allocation concealment. Physical exercise is the main intervention among all the trials, and therefore it may not be feasible to adopt the blind method. As a result, several studies were judged as having a high risk of performance bias (16 trials). However, reporting of such a high risk of performance bias does not compromise the quality of the



Study or Subgroup	EX+CR Mean	EX+CR SD	EX+CR Total	CR Mean	CR SD	CR Total	Weight	Std. Mean Difference IV, Fixed, 95% CI
1.1.1 Sedentary people								
Bouchonville et al. 2014	3	7.42	28	2.4	3.69	26	5.8%	0.10 [-0.43, 0.63]
Brochu et al. 2009	2.61	2.35	36	2.95	2.89	71	10.3%	-0.12 [-0.53, 0.28]
Fisher et al. 2011 (AE+CR)	1.59	1.67	43	1.13	1.23	29	7.4%	0.30 [-0.17, 0.78]
Fisher et al. 2011 (RE+CR)	1.61	1.94	54	1.13	1.23	29	8.1%	0.28 [-0.18, 0.73]
Nicklas et al. 2004	6.32	10.77	53	5.87	8.85	53	11.4%	0.05 [-0.34, 0.43]
Ryan et al. 2014	4.52	7.32	36	4.39	4.07	40	8.2%	0.02 [-0.43, 0.47]
Straznicky et al. 2010	1.8	1.34	20	2.3	1.79	20	4.3%	-0.31 [-0.93, 0.31]
Weiss et al. 2016	1.6	1.31	19	2	1.24	17	3.8%	-0.31 [-0.96, 0.35]
You et al. 2004	3.6	2.39	17	9.54	8.74	17	3.3%	-0.91 [-1.62, -0.20]
Subtotal (95% CI)			**306**			**302**	**62.5%**	**-0.02 [-0.18, 0.15]**
Heterogeneity: Chi² = 11.52, df = 8 (P = 0.17); I² = 31%								
Test for overall effect: Z = 0.20 (P = 0.84)								
1.1.2 Normal people								
Cho et al. 2019	1.04	1.39	9	0.85	1.31	8	1.8%	0.13 [-0.82, 1.09]
Giannopoulou et al. 2005	10.48	7.36	11	15.88	7.26	11	2.2%	-0.71 [-1.58, 0.16]
Lam et al. 2015	0.11	0.11	8	0.23	0.23	8	1.6%	-0.63 [-1.64, 0.38]
Oh et al. 2014	0.45	0.48	52	0.54	0.66	20	6.2%	-0.17 [-0.68, 0.35]
Rejeski et al. 2019 (AE+CR)	2.6	1.94	58	3.38	2.45	50	11.4%	-0.35 [-0.73, 0.03]
Rejeski et al. 2019 (RE+CR)	2.25	1.68	61	3.38	2.45	50	11.4%	-0.54 [-0.93, -0.16]
Snel et al. 2011	2.8	2.62	14	4.9	7.21	13	2.8%	-0.38 [-1.14, 0.38]
Subtotal (95% CI)			**213**			**160**	**37.5%**	**-0.39 [-0.60, -0.18]**
Heterogeneity: Chi² = 3.28, df = 6 (P = 0.77); I² = 0%								
Test for overall effect: Z = 3.66 (P = 0.0003)								
Total (95% CI)			**519**			**462**	**100.0%**	**-0.16 [-0.29, -0.03]**
Heterogeneity: Chi² = 22.47, df = 15 (P = 0.10); I² = 33%								
Test for overall effect: Z = 2.39 (P = 0.02)								
Test for subgroup differences: Chi² = 7.67, df = 1 (P = 0.006), I² = 87.0%								

FIGURE 2 | Forest plot of the effects of exercise plus calorie restriction (EX + CR) intervention on C-reactive protein (CRP) changes in overweight and obese adults with different (subgroup analysis) lifestyle behaviors.



Study or Subgroup	EX+CR Mean	EX+CR SD	EX+CR Total	CR Mean	CR SD	CR Total	Weight	Std. Mean Difference IV, Fixed, 95% CI
1.2.1 Sedentary people								
Fisher et al. 2011 (AE+CR)	1.38	0.59	43	1.27	0.65	29	9.1%	0.18 [-0.29, 0.65]
Fisher et al. 2011 (RE+CR)	1.34	0.86	54	1.27	0.65	29	10.0%	0.09 [-0.36, 0.54]
García-Unciti et al. 2012	0.7	0.8	13	0.6	0.44	12	3.3%	0.15 [-0.64, 0.93]
Nicklas et al. 2004	4.55	3.37	53	3.99	3.41	53	14.0%	0.16 [-0.22, 0.55]
Silverman et al. 2009	2.4	2.85	46	2.03	1.24	40	11.3%	0.16 [-0.26, 0.59]
Subtotal (95% CI)			**209**			**163**	**47.7%**	**0.15 [-0.06, 0.36]**
Heterogeneity: Chi² = 0.10, df = 4 (P = 1.00); I² = 0%								
Test for overall effect: Z = 1.41 (P = 0.16)								
1.2.2 Normal people								
Christiansen et al. 2010	1.9	0.9	21	2.4	1	19	5.1%	-0.52 [-1.15, 0.12]
Giannopoulou et al. 2005	2.22	1.65	11	3.02	1.62	11	2.8%	-0.47 [-1.32, 0.38]
Lakhdar et al. 2013	4.83	0.31	10	4.85	0.36	10	2.6%	-0.06 [-0.93, 0.82]
Lam et al. 2015	135	169.71	8	124	192.33	8	2.1%	0.06 [-0.92, 1.04]
Oh et al. 2014	1.13	0.56	52	1.47	1.39	20	7.5%	-0.39 [-0.91, 0.13]
Rejeski et al. 2019 (AE+CR)	2.75	1.27	59	2.64	1.13	49	14.2%	0.09 [-0.29, 0.47]
Rejeski et al. 2019 (RE+CR)	2.32	1.06	62	2.64	1.13	49	14.3%	-0.29 [-0.67, 0.09]
Snel et al. 2011	1.9	0.74	14	2.2	1.44	13	3.5%	-0.26 [-1.02, 0.50]
Subtotal (95% CI)			**237**			**179**	**52.3%**	**-0.21 [-0.40, -0.01]**
Heterogeneity: Chi² = 4.72, df = 7 (P = 0.69); I² = 0%								
Test for overall effect: Z = 2.04 (P = 0.04)								
Total (95% CI)			**446**			**342**	**100.0%**	**-0.04 [-0.18, 0.11]**
Heterogeneity: Chi² = 10.72, df = 12 (P = 0.55); I² = 0%								
Test for overall effect: Z = 0.49 (P = 0.62)								
Test for subgroup differences: Chi² = 5.90, df = 1 (P = 0.02), I² = 83.1%								

FIGURE 3 | Forest plot of the effects of exercise plus calorie restriction (EX + CR) intervention on IL-6 changes in overweight and obese adults with different (subgroup analysis) lifestyle behaviors.

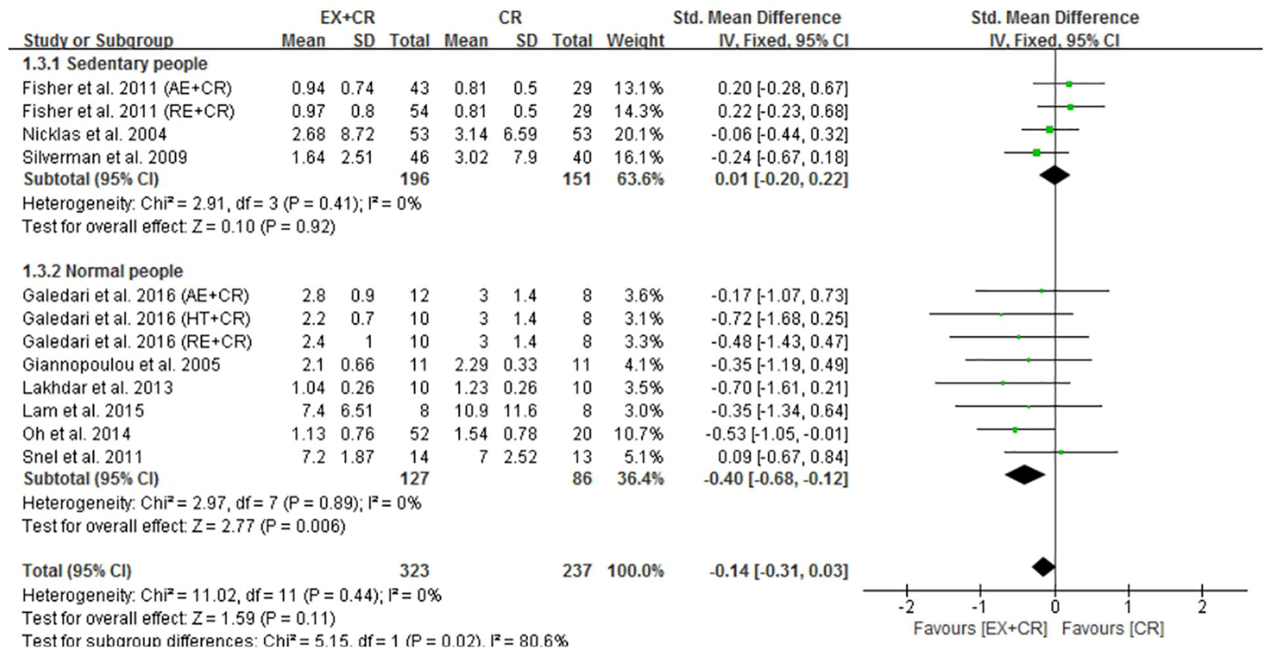


FIGURE 4 | Forest plot of the effects of exercise plus calorie restriction (EX + CR) intervention on TNF-α changes in overweight and obese adults with different (subgroup analysis) lifestyle behaviors.

TABLE 2 | Meta-regression analysis for participants' characteristics.

Inflammatory biomarkers	Characteristics of participants	Coefficient	Standard error	*T*-value	*P*-value
	Age	0.0026002	0.0099456	0.26	0.798
CRP	BMI	−0.0339752	0.0279858	−1.21	0.246
	Lifestyle	−0.3804664	0.142546	−2.67	0.018*
	Age	0.0104658	0.0082666	1.27	0.237
IL-6	BMI	−0.0206236	0.0304715	−0.68	0.513
	Lifestyle	−0.3592889	0.1457418	−2.47	0.031*
	Age	0.0125738	0.007944	1.58	0.152
TNF-α	BMI	−0.0121833	0.0379839	−0.32	0.755
	Lifestyle	−0.4249011	0.1811258	−2.35	0.041*

**Represents a significant correlation between the inflammatory biomarkers and characteristics of population.*

study (Hong et al., 2019; Liu et al., 2019). No studies were judged to have a detection bias. We identified one trial with an attrition bias, three trials with reporting bias, and two trials with other bias. The quality of the four non-RCT studies was evaluated using the MINORS evaluation criteria. One trial scored 19 points, two trials scored 18 points, and one trial scored 16 points.

DISCUSSION

To the best of our knowledge, this is the first systematic review and meta-analysis to compare the combination effect of exercise and CR with CR alone on inflammatory biomarkers in obese and overweight adults. We included 23 trials, which reported the changes in CRP, IL-6, and TNF-α concentrations with response to EX + CR and CR alone treatments. The pooled outcome results showed that combination of EX + CR intervention significantly decreased CRP levels in overweight and obese adults, while IL-6 and TNF-α did not respond to EX + CR intervention. Meta-regression analyses revealed that the changes in CRP, IL-6, and TNF-α were correlated with lifestyle behavior, but not correlated with age and BMI of participants. Subgroup analysis results further explored that normal lifestyle with EX + CR intervention effectively decreased CRP, IL-6, and TNF-α concentrations. However, baseline sedentary lifestyle behavior with EX + CR intervention could not decrease the inflammatory mediators in overweight and obese adults. These findings imply that participants' lifestyle behavior, especially active lifestyle is important to achieve the beneficial effects of EX + CR intervention on improving the inflammatory biomarkers in overweight and obese adults.

IL-6 and TNF-α are the two important pro-inflammatory cytokines that mediate the process of inflammation and healing. However, excessive production of IL-6 and TNF-α over a period of time causes systemic inflammation, which is eventually involved in developing metabolic diseases, including CVDs, diabetes, various cancer types, chronic kidney disease, non-alcoholic fatty liver disease (NAFLD), and neurodegenerative conditions (Furman et al., 2019). Both cytokines, IL-6 and TNF-α, are responsible for the production and release of CRP, an acute phase protein that induces the inflammatory stage (Popko et al., 2010). Elevated levels of IL-6 and CRP are strongly associated with development of type 2 diabetes in healthy middle-aged women (Pradhan et al., 2001). Several lifestyle factors, including

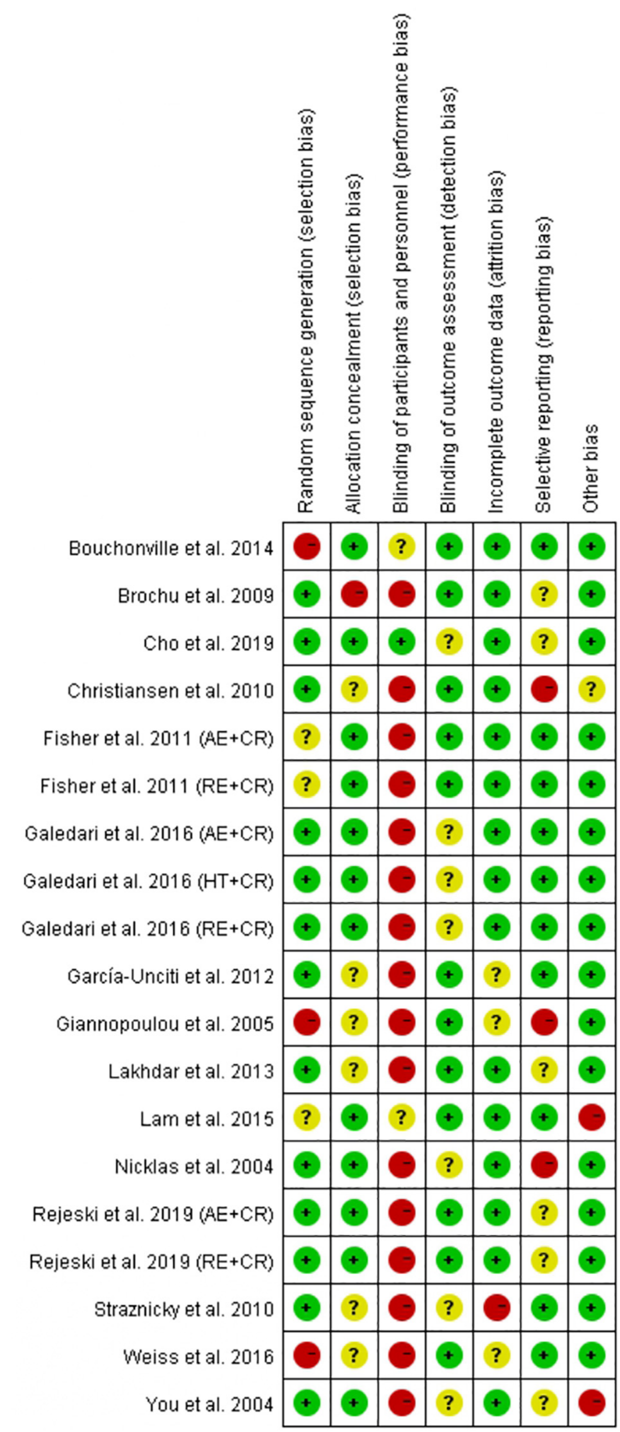


FIGURE 5 | Summary of the risk of bias for the trials included in this meta-analysis.

physical inactivity, poor diet, nighttime blue light exposure, smoking, and psychological stress are reported to promote systemic inflammation. Therefore, lifestyle modifications may reduce inflammatory proteins and improve metabolic health (Furman et al., 2019; Kökten et al., 2021). Studies have targeted these modifiable risk factors to decrease the inflammatory response, however, the corresponding reduction of inflammatory biomarkers is limited due to various reasons. Here, we addressed the influential role of lifestyle behavior on improving CRP, IL-6, and TNF-α with exercise plus CR intervention.

Exercise and dietary intake are important lifestyle interventions that are reported to orchestrate inflammatory biomarkers independently or together. A study with a very low calorie diet (CR) for 4 weeks has been shown to reduce inflammatory biomarkers in obese women, and this beneficial effect of CR was accompanied by a significant weight loss (Ott et al., 2017). In a randomized clinical trial, a modified alternate-day fasting diet was reported to be more effective than CR on weight loss and reduction of CRP levels in patients with metabolic syndrome. Despite this, the changes in IL-6 and TNF-α were not statistically different between the diets (Razavi et al., 2021). On the other hand, a meta-analysis reported positive effects of aerobic exercise intervention on reduction of CRP, IL-6, and TNF-α in middle-aged and older adults, but not on IL-4 (Zheng et al., 2019). The anti-inflammatory effect of exercise may be attributed to a decrease of adipose tissue; however, its independent effect on inflammation has not yet been elucidated. Evidence suggests that exercise can directly affect the immune cells by regulating systemic inflammatory mediators without relying on the loss of bodyweight (Gleeson et al., 2011). The anti-inflammatory effect of exercise is independent of weight loss and it can inhibit pro-inflammatory mediators, stimulate anti-inflammatory pathways, and thereby regulate insulin sensitivity. Nevertheless, it is not clear whether a combination of exercise and CR has a greater beneficial effect than that of CR alone on inflammatory cytokines in obese adults.

One of the key findings of our meta-analysis is that exercise plus CR has greater beneficial effects than CR alone in decreasing inflammatory mediators when participants are subgrouped based on their lifestyle behavior. Lifestyle modification with the Mediterranean diet (CR) and exercise intervention for 2 years induced significant weight loss and reduced CRP, IL-6, and TNF-α in healthy obese women. Nevertheless, the deteriorated adipokine profile was not improved with combination of CR and exercise intervention (Gomez-Huelgas et al., 2019). In a recent meta-analysis, exercise plus CR intervention significantly decreased IL-6 and TNF-α levels and marginally decreased CRP in overweight and obese adults (Khalafi et al., 2021). In contrast to our findings, subgroup analysis for BMI showed a significant decrease of CRP and TNF-α with higher BMI values, but not IL-6 (Khalafi et al., 2021). We performed meta-regression analysis for participants' characteristics (age, BMI, and lifestyle), and found that lifestyle behavior is significantly correlated with decreased CRP, IL-6, and TNF-α after exercise plus CR intervention. BMI and age variables were not correlated with decreased inflammatory biomarkers in overweight and obese adults. Here, our findings emphasize the importance of lifestyle behavior in enhancing the beneficial effects of exercise plus CR intervention. The differences with previous findings may be due to the differences in population characteristics. Our study included only overweight or obese adults, and children below 18 years were excluded. Therefore, our conclusions are convincing to construct an intervention program for overweight or obese adults to improve their inflammatory response.

The mechanism of improving inflammatory markers by exercise intervention is not completely clear, which may be achieved by improving the hypoxia state of adipose tissue (Dumitriu et al., 2007), promoting the phenotype transformation of macrophages in adipose tissue (Kawanishi et al., 2010), and regulating the function of peripheral blood cells (Yeh et al., 2009). Although changes in body weight and composition are not necessarily factors that affect inflammatory markers, changes in body composition (muscle and fat content) caused by exercise may indirectly affect the inflammatory response of an individual (Beasley et al., 2009). A meta-analysis compared the effects of exercise and CR on body weight and composition, and found that CR has a larger impact on total body weight loss, but exercise has a superior effect in reducing visceral adipose tissue (VAT) (Verheggen et al., 2016), which is an important source of pro-inflammatory factors, such as IL-6 and TNF-α. In addition, skeletal muscle is the key source of inflammatory mediators involved in systemic inflammation. A meta-analysis of 13 articles showed that resistance exercise-induced increased muscle mass contributes to control of inflammatory biomarkers in older adults (Sardeli et al., 2018). The positive effect of exercise on muscle mass is not limited to resistance exercise or high-intensity exercise. In a study, overweight to obese adults were randomly assigned into diet-induced weight loss alone or diet-induced weight loss combined with exercise intervention groups for 4 months. The results showed that diet-induced weight loss (intentional CR) significantly decreased skeletal muscle mass in overweight to obese adults. Interestingly, moderate aerobic exercise combined with an intentional weight loss trial attenuated the loss of skeletal muscle mass (Chomentowski et al., 2009).

Although exercise and CR are important strategies to curb the level of inflammation, paying attention to the different characteristics of participants is the primary concern to achieve the goal. This study emphasizes that individual lifestyle behavior (sedentary or non-sedentary) is the key factor to achieve an intervention effect. Sedentary behavior refers to any walking behavior characterized by energy expenditure $\leq$ 1.5 metabolic equivalents (METs), while in a sitting, reclining, or lying posture (Tremblay et al., 2017; Thivel et al., 2018). To the best of our knowledge, this is the first meta-analysis and subgroup analysis to evaluate the combination effect of EX + CR on inflammatory mediators in overweight and obese adults with sedentary behavior. We found that exercise plus CR intervention could not improve the CRP, IL-6, and TNF-α response in overweight and obese adults with long-term sedentary behavior. An existing meta-analysis mainly revealed the combination effect of EX + CR on inflammatory mediators from the aspects of intervention duration, exercise type, and BMI, but did not disclose the influence of sedentary behavior of participants on the effectiveness of the intervention (Khalafi et al., 2021).

It is well known that sedentary lifestyle behavior is the main cause of obesity, and both obesity and sedentary behavior are strongly associated with developing inflammatory-related diseases (Park et al., 2020). Apart from the fact that obesity can cause chronic inflammation, sedentary behavior itself also contributes to chronic inflammation and inflammatory diseases in the course of life (Burini et al., 2020). Adults who have sedentary behavior for a long time may have impaired metabolic health and cannot benefit from exercise or other interventions. Previous studies have reported that long-term sedentary behavior can lead to inflammation of subcutaneous adipose tissue, and negative health consequences, including type 2 diabetes, obesity, hypertension, and CVDs (Grøntved and Hu, 2011; Biswas et al., 2015). A multi-ethnic cross-sectional study with a large sample has shown that sedentary behavior is associated with high levels of TNF-α and leptin and low adiponectin-to-leptin ratios. The degree of these associations does not vary with ethnic groups, and is independent of related co-variables (including moderate to high intensity exercise) (Allison et al., 2012). Moreover, exercise combined with nutrient intake or new exercise methods seems to improve the inflammatory response. A study on sedentary men showed that high intensity exercise plus honey intake significantly decreased IL-6 concentrations, but had no effect on other inflammatory mediators (Bakhtyar et al., 2018). Another study on sedentary middle-aged men reported that cycling training decreased only CRP levels, while small-sided games decreased both CRP and IL-6 concentrations and increased muscle mass (Mendham et al., 2014). Therefore, it is suggested that we should further investigate the effect of exercise combined with nutrient intake or a new exercise protocol on inflammatory factors.

Limitations

The intervention duration of the included trials ranged between 8 and 72 weeks. Although the duration range appears to be wide, this may not influence the final outcome of our study. Meta-regression analysis results also showed no significant correlation between intervention duration and inflammatory mediators. In addition, our study did not provide the statistical evidence to demonstrate the influence of exercise type, intensity, or frequency on inflammatory biomarkers. We included trials that performed any type of exercise, and therefore it is inconclusive which exercise type has greater beneficial effects in combination with CR. Further studies with meta-regression analysis are necessary to identify the influential role of exercise variables on changes in inflammatory mediators in overweight and obese adults.

CONCLUSION

Our findings demonstrated that a combination of exercise with calorie restriction could improve the CRP, IL-6, and TNF-α response in overweight and obese adults with normal lifestyle behavior, but not in adults with sedentary behavior. Therefore, lifestyle behavior is the key variable that influences the beneficial effects of exercise plus CR intervention on inflammatory biomarkers. Whenever designing interventional programs for obese or overweight adults, ‘lifestyle behavior’ should be considered as an important characteristic of adults to achieve the goal of intervention on the inflammatory system.

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

YL, FH, and MK designed the study. YL, FH, and YZ performed the article search and screening. YL and FH performed statistical analyses and drafted the manuscript. VL, AM, and LJ assisted in interpretation of data and provided additional suggestions. YL, FH, YZ, and MK reviewed the full-text articles and extracted the data.YZ and MK revised and finalized the manuscript. All authors read and approved the submission.

FUNDING

This study was partially supported by a grant (no. jykf20069) from the Open Research Fund of College of Teacher Education, Zhejiang Normal University, Zhejiang, China.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2021.754731/full#supplementary-material

REFERENCES

Albashir, A. A. D. (2020). The potential impacts of obesity on COVID-19. *Clin. Med. (Lond)* 20, e109–e113. doi: 10.7861/clinmed.2020-0239

Allison, M. A., Jensky, N. E., Marshall, S. J., Bertoni, A. G., and Cushman, M. (2012). Sedentary behavior and adiposity-associated inflammation: the multi-ethnic study of atherosclerosis. *Am. J. Prev. Med.* 42, 8–13. doi: 10.1016/j.amepre.2011.09.023

Bakhtyar, T., Naser, A., and Khayat, S. M. A. (2018). IL-6 and IL-12 levels in response to honey supplementation combined with HIIT training in sedentary individuals. *Iran. J. Allergy Asthma Immunol.* 17:232.

Beasley, L. E., Koster, A., Newman, A. B., Javaid, M. K., Ferrucci, L., Kritchevsky, S. B., et al. (2009). Inflammation and race and gender differences in computerized tomography-measured adipose depots. *Obesity (Silver Spring)* 17, 1062–1069. doi: 10.1038/oby.2008.627

Biswas, A., Oh, P. I., Faulkner, G. E., Bajaj, R. R., Silver, M. A., Mitchell, M. S., et al. (2015). Sedentary time and its association with risk for disease incidence, mortality, and hospitalization in adults. *Ann. Intern. Med.* 162, 123–132. doi: 10.7326/M14-1651

Blüher, M. (2019). Obesity: global epidemiology and pathogenesis. *Nat. Rev. Endocrinol.* 15, 288–298. doi: 10.1038/s41574-019-0176-8

Bouchonville, M., Armamento-Villareal, R., Shah, K., Napoli, N., Sinacore, D. R., Qualls, C., et al. (2014). Weight loss, exercise or both and cardiometabolic risk factors in obese older adults: results of a randomized controlled trial. *Int. J. Obes. (Lond)* 38, 423–431. doi: 10.1038/ijo.2013.122

Brochu, M., Malita, M. F., Messier, V., Doucet, E., Strychar, I., Lavoie, J., et al. (2009). Resistance training does not contribute to improving the metabolic profile after a 6-month weight loss program in overweight and obese postmenopausal women. *J. Clin. Endocrinol. Metab.* 94, 3226–3233. doi: 10.1210/jc.2008-2706

Burini, R. C., Anderson, E., Durstine, J. L., and Carson, J. A. (2020). Inflammation, physical activity, and chronic disease: an evolutionary perspective. *Sports Med. Health Sci.* 2, 1–6. doi: 10.1016/j.smhs.2020.03.004

Chen, W., Zheng, K. I., Liu, S., Yan, Z., Xu, C., and Qiao, Z. (2020). Plasma CRP level is positively associated with the severity of COVID-19. *Ann. Clin. Microbiol. Antimicrob.* 19:18. doi: 10.1186/s12941-020-00362-2

Cho, A. R., Moon, J.-Y., Kim, S., An, K.-Y., Oh, M., Jeon, J. Y., et al. (2019). Effects of alternate day fasting and exercise on cholesterol metabolism in overweight or obese adults: a pilot randomized controlled trial. *Metabolism* 93, 52–60. doi: 10.1016/j.metabol.2019.01.002

Chomentowski, P., Dubé, J. J., Amati, F., Stefanovic-Racic, M., Zhu, S., Toledo, F. G. S., et al. (2009). Moderate exercise attenuates the loss of skeletal muscle mass that occurs with intentional caloric restriction-induced weight loss in older, overweight to obese adults. *J. Gerontol. A Biol. Sci. Med. Sci.* 64, 575–580. doi: 10.1093/gerona/glp007

Christiansen, T., Paulsen, S. K., Bruun, J. M., Pedersen, S. B., and Richelsen, B. (2010). Exercise training versus diet-induced weight-loss on metabolic risk factors and inflammatory markers in obese subjects: a 12-week randomized intervention study. *Am. J. Physiol. Endocrinol. Metab.* 298, E824–E831. doi: 10.1152/ajpendo.00574.2009

Coussens, L. M., and Werb, Z. (2002). Inflammation and cancer. *Nature* 420, 860–867. doi: 10.1038/nature01322

Dumitriu, I. E., Bianchi, M. E., Bacci, M., Manfredi, A. A., and Rovere-Querini, P. (2007). The secretion of HMGB1 is required for the migration of maturing dendritic cells. *J. Leukoc. Biol.* 81, 84–91. doi: 10.1189/jlb.0306171

Fisher, G., Hyatt, T. C., Hunter, G. R., Oster, R. A., Desmond, R. A., and Gower, B. A. (2011). Effect of diet with and without exercise training on markers of inflammation and fat distribution in overweight women. *Obesity (Silver Spring)* 19, 1131–1136. doi: 10.1038/oby.2010.310

Furman, D., Campisi, J., Verdin, E., Carrera-Bastos, P., Targ, S., Franceschi, C., et al. (2019). Chronic inflammation in the etiology of disease across the life span. *Nat. Med.* 25, 1822–1832. doi: 10.1038/s41591-019-0675-0

Galedari, M., Azarbayjani, M. A., and Peeri, M. (2017). Effects of type of exercise along with caloric restriction on plasma apelin 36 and HOMA-IR in overweight men. *Sci. Sport* 32, e137–e145. doi: 10.1016/j.scispo.2016.12.002

García-Unciti, M., Izquierdo, M., Idoate, F., Gorostiaga, E., Grijalba, A., Ortega-Delgado, F., et al. (2012). Weight-loss diet alone or combined with progressive resistance training induces changes in association between the cardiometabolic risk profile and abdominal fat depots. *Ann. Nutr. Metab.* 61, 296–304. doi: 10.1159/000342467

Giannopoulou, I., Fernhall, B., Carhart, R., Weinstock, R. S., Baynard, T., Figueroa, A., et al. (2005). Effects of diet and/or exercise on the adipocytokine and inflammatory cytokine levels of postmenopausal women with type 2 diabetes. *Metabolism* 54, 866–875. doi: 10.1016/j.metabol.2005.01.033

Gleeson, M., Bishop, N. C., Stensel, D. J., Lindley, M. R., Mastana, S. S., and Nimmo, M. A. (2011). The anti-inflammatory effects of exercise: mechanisms and implications for the prevention and treatment of disease. *Nat. Rev. Immunol.* 11, 607–615. doi: 10.1038/nri3041

Gomez-Huelgas, R., Ruiz-Nava, J., Santamaria-Fernandez, S., Vargas-Candela, A., Alarcon-Martin, A. V., Tinahones, F. J., et al. (2019). Impact of intensive lifestyle modification on levels of adipokines and inflammatory biomarkers in metabolically healthy obese women. *Mediators Inflamm.* 2019:4165260. doi: 10.1155/2019/4165260

Grøntved, A., and Hu, F. B. (2011). Television viewing and risk of type 2 diabetes, cardiovascular disease, and all-cause mortality: a meta-analysis. *JAMA* 305, 2448–2455. doi: 10.1001/jama.2011.812

Higgins, J. P. T., Altman, D. G., Gøtzsche, P. C., Jüni, P., Moher, D., Oxman, A. D., et al. (2011). The cochrane Collaboration's tool for assessing risk of bias in randomised trials. *BMJ* 343:d5928. doi: 10.1136/bmj.d5928

Hong, F., Ye, W., Kuo, C. H., Zhang, Y., Qian, Y., and Korivi, M. (2019). Exercise intervention improves clinical outcomes, but the "time of session" is crucial for better quality of life in breast cancer survivors: a systematic review and meta-analysis. *Cancers (Basel)* 11:706. doi: 10.3390/cancers11050706

Hotamisligil, G. S. (2006). Inflammation and metabolic disorders. *Nature* 444, 860–867. doi: 10.1038/nature05485

Kawanishi, N., Yano, H., Yokogawa, Y., and Suzuki, K. (2010). Exercise training inhibits inflammation in adipose tissue via both suppression of macrophage infiltration and acceleration of phenotypic switching from M1 to M2 macrophages in high-fat-diet-induced obese mice. *Exerc. Immunol. Rev.* 16, 105–118.

Khalafi, M., Symonds, M. E., and Akbari, A. (2021). The impact of exercise training versus caloric restriction on inflammation markers: a systemic review and meta-analysis. *Crit. Rev. Food Sci. Nutr.* 1–16. doi: 10.1080/10408398.2021.1873732

Kökten, T., Hansmannel, F., Ndiaye, N. C., Heba, A., Quilliot, D., Dreumont, N., et al. (2021). Calorie restriction as a new treatment of inflammatory diseases. *Adv. Nutr.* 12, 1558–1570. doi: 10.1093/advances/nmaa179

Lakhdar, N., Denguezli, M., Zaouali, M., Zbidi, A., Tabka, Z., and Bouassida, A. (2013). Diet and diet combined with chronic aerobic exercise decreases body fat mass and alters plasma and adipose tissue inflammatory markers in obese women. *Inflammation* 36, 1239–1247. doi: 10.1007/s10753-013-9661-8

Lam, Y. Y., Ghosh, S., Civitarese, A. E., and Ravussin, E. (2015). Six-month calorie restriction in overweight individuals elicits transcriptomic response in subcutaneous adipose tissue that is distinct from effects of energy deficit. *J. Gerontol. A Biol. Sci. Med. Sci.* 71, 1258–1265. doi: 10.1093/gerona/glv194

Lighter, J., Phillips, M., Hochman, S., Sterling, S., Johnson, D., Francois, F., et al. (2020). Obesity in patients younger than 60 years is a risk factor for COVID-19 hospital admission. *Clin. Infect. Dis.* 71, 896–897. doi: 10.1093/cid/ciaa415

Liu, Y., Ye, W., Chen, Q., Zhang, Y., Kuo, C. H., and Korivi, M. (2019). Resistance exercise intensity is correlated with attenuation of HbA1c and insulin in patients with type 2 diabetes: a systematic review and meta-analysis. *Int. J. Environ. Res. Public Health* 16:140. doi: 10.3390/ijerph16010140

Mehta, P., Mcauley, D. F., Brown, M., Sanchez, E., Tattersall, R. S., Manson, J. J., et al. (2020). COVID-19: consider cytokine storm syndromes and immunosuppression. *Lancet* 395, 1033–1034. doi: 10.1016/S0140-6736(20)30628-0

Mendham, A. E., Duffield, R., Marino, F., and Coutts, A. J. (2014). Small-sided games training reduces CRP, IL-6 and leptin in sedentary, middle-aged men. *Eur. J. Appl. Physiol.* 114, 2289–2297. doi: 10.1007/s00421-014-2953-3

Moher, D., Liberati, A., Tetzlaff, J., and Altman, D. G. (2009). Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *PLoS Med.* 6:e1000097. doi: 10.1371/journal.pmed.1000097

Monteiro-Junior, R. S., De Tarso Maciel-Pinheiro, P., Da Matta Mello Portugal, E., Da Silva, Figueiredo, L. F., Terra, R., et al. (2018). Effect of exercise on inflammatory profile of older persons: systematic review and meta-analyses. *J. Phys. Act. Health* 15, 64–71. doi: 10.1123/jpah.2016-0735

Mortaz, E., Tabarsi, P., Jamaati, H., Dalil Roofchayee, N., Dezfuli, N. K., Hashemian, S. M., et al. (2021). Increased serum levels of soluble TNF-α receptor is associated with ICU mortality in COVID-19 patients. *Front. Immunol.* 12:592727. doi: 10.3389/fimmu.2021.592727

Nicklas, B. J., Ambrosius, W., Messier, S. P., Miller, G. D., Penninx, B. W. J. H., Loeser, R. F., et al. (2004). Diet-induced weight loss, exercise, and chronic inflammation in older, obese adults: a randomized controlled clinical trial. *Am. J. Clin. Nutr.* 79, 544–551. doi: 10.1093/ajcn/79.4.544

Nimmo, M. A., Leggate, M., Viana, J. L., and King, J. A. (2013). The effect of physical activity on mediators of inflammation. *Diabetes Obes. Metab.* 15(Suppl. 3), 51–60. doi: 10.1111/dom.12156

Oh, S., Tanaka, K., Tsujimoto, T., So, R., Shida, T., and Shoda, J. (2014). Regular exercise coupled to diet regimen accelerates reduction of hepatic steatosis and associated pathological conditions in nonalcoholic fatty liver disease. *Metab. Syndr. Relat. Disord.* 12, 290–298. doi: 10.1089/met.2013.0143

Ott, B., Skurk, T., Hastreiter, L., Lagkouvardos, I., Fischer, S., Büttner, J., et al. (2017). Effect of caloric restriction on gut permeability, inflammation markers, and fecal microbiota in obese women. *Sci. Rep.* 7:11955. doi: 10.1038/s41598-017-12109-9

Page, M. J., Mckenzie, J. E., Bossuyt, P. M., Boutron, I., Hoffmann, T. C., Mulrow, C. D., et al. (2021a). The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *J. Clin. Epidemiol.* 134, 178–189.

Page, M. J., Mckenzie, J. E., Bossuyt, P. M., Boutron, I., Hoffmann, T. C., Mulrow, C. D., et al. (2021b). Updating guidance for reporting systematic reviews: development of the PRISMA 2020 statement. *J. Clin. Epidemiol.* 134, 103–112. doi: 10.31222/osf.io/jb4dx

Park, J. H., Moon, J. H., Kim, H. J., Kong, M. H., and Oh, Y. H. (2020). Sedentary Lifestyle: overview of Updated Evidence of Potential Health Risks. *Korean J. Fam. Med.* 41, 365–373. doi: 10.4082/kjfm.20.0165

Peng, Y. D., Meng, K., Guan, H. Q., Leng, L., Zhu, R. R., Wang, B. Y., et al. (2020). [Clinical characteristics and outcomes of 112 cardiovascular disease patients infected by 2019-nCoV]. *Zhonghua Xin Xue Guan Bing Za Zhi* 48, 450–455.

Popko, K., Gorska, E., Stelmaszczyk-Emmel, A., Plywaczewski, R., Stoklosa, A., Gorecka, D., et al. (2010). Proinflammatory cytokines Il-6 and TNF-α and the development of inflammation in obese subjects. *Eur. J. Med. Res.* 15(Suppl. 2), 120–122. doi: 10.1186/2047-783X-15-S2-120

Pradhan, A. D., Manson, J. E., Rifai, N., Buring, J. E., and Ridker, P. M. (2001). C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. *JAMA* 286, 327–334. doi: 10.1001/jama.286.3.327

Razavi, R., Parvaresh, A., Abbasi, B., Yaghoobloo, K., Hassanzadeh, A., Mohammadifard, N., et al. (2021). The alternate-day fasting diet is a more effective approach than a calorie restriction diet on weight loss and hs-CRP levels. *Int. J. Vitam. Nutr. Res.* 91, 242–250. doi: 10.1024/0300-9831/a000623

Rejeski, W. J., Marsh, A. P., Fanning, J., Ambrosius, W. T., Walkup, M. P., and Nicklas, B. J. (2019). Dietary weight loss, exercise, and inflammation in older adults with overweight or obesity and cardiometabolic disease. *Obesity (Silver Spring)* 27, 1805–1811. doi: 10.1002/oby.22600

Ryan, A. S., Ge, S., Blumenthal, J. B., Serra, M. C., Prior, S. J., and Goldberg, A. P. (2014). Aerobic exercise and weight loss reduce vascular markers of inflammation and improve insulin sensitivity in obese women. *J. Am. Geriatr. Soc.* 62, 607–614. doi: 10.1111/jgs.12749

Saltiel, A. R., and Olefsky, J. M. (2017). Inflammatory mechanisms linking obesity and metabolic disease. *J. Clin. Invest.* 127, 1–4. doi: 10.1172/JCI92035

Sardeli, A. V., Tomeleri, C. M., Cyrino, E. S., Fernhall, B., Cavaglieri, C. R., and Chacon-Mikahil, M. P. T. (2018). Effect of resistance training on inflammatory markers of older adults: a meta-analysis. *Exp. Gerontol.* 111, 188–196. doi: 10.1016/j.exger.2018.07.021

Silverman, N. E., Nicklas, B. J., and Ryan, A. S. (2009). Addition of aerobic exercise to a weight loss program increases BMD, with an associated reduction in inflammation in overweight postmenopausal women. *Calcif. Tissue Int.* 84, 257–265. doi: 10.1007/s00223-009-9232-z

Simonnet, A., Chetboun, M., Poissy, J., Raverdy, V., Noulette, J., Duhamel, A., et al. (2020). High prevalence of obesity in severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) requiring invasive mechanical ventilation. *Obesity (Silver Spring)* 28, 1195–1199. doi: 10.1002/oby.22831

Slim, K., Nini, E., Forestier, D., Kwiatkowski, F., Panis, Y., and Chipponi, J. (2003). Methodological index for non-randomized studies (minors): development and validation of a new instrument. *ANZ J. Surg.* 73, 712–716. doi: 10.1046/j.1445-2197.2003.02748.x

Snel, M., Van Diepen, J. A., Stijnen, T., Pijl, H., Romijn, J. A., Meinders, A. E., et al. (2011). Immediate and long-term effects of addition of exercise to a 16-week very low calorie diet on low-grade inflammation in obese, insulin-dependent type 2 diabetic patients. *Food Chem. Toxicol.* 49, 3104–3111. doi: 10.1016/j.fct.2011.09.032

Straznicky, N. E., Lambert, E. A., Nestel, P. J., Mcgrane, M. T., Dawood, T., Schlaich, M. P., et al. (2010). Sympathetic neural adaptation to hypocaloric diet with or without exercise training in obese metabolic syndrome subjects. *Diabetes* 59, 71–79. doi: 10.2337/db09-0934

Thivel, D., Tremblay, A., Genin, P. M., Panahi, S., Rivière, D., and Duclos, M. (2018). Physical activity, inactivity, and sedentary behaviors: definitions and implications in occupational health. *Front. Public Health* 6:288. doi: 10.3389/fpubh.2018.00288

Tolkien, K., Bradburn, S., and Murgatroyd, C. (2019). An anti-inflammatory diet as a potential intervention for depressive disorders: a systematic review and meta-analysis. *Clin. Nutr.* 38, 2045–2052. doi: 10.1016/j.clnu.2018.11.007

Tremblay, M. S., Aubert, S., Barnes, J. D., Saunders, T. J., Carson, V., Latimer-Cheung, A. E., et al. (2017). Sedentary behavior research network (SBRN) – terminology consensus project process and outcome. *Int. J. Behav. Nutr. Phys. Act.* 14:75. doi: 10.1186/s12966-017-0525-8

Trussardi Fayh, A. P., Lopes, A. L., Fernandes, P. R., Reischak-Oliveira, A., and Friedman, R. (2013). Impact of weight loss with or without exercise on abdominal fat and insulin resistance in obese individuals: a randomised clinical trial. *Br. J. Nutr.* 110, 486–492. doi: 10.1017/S0007114512005442

van Gemert, W. A., Peeters, P. H., May, A. M., Doornbos, A. J. H., Elias, S. G., Van Der Palen, J., et al. (2019). Effect of diet with or without exercise on abdominal fat in postmenopausal women – a randomised trial. *BMC Public Health* 19:174. doi: 10.1186/s12889-019-6510-1

Verheggen, R. J. H. M., Maessen, M. F. H., Green, D. J., Hermus, A. R. M. M., Hopman, M. T. E., and Thijssen, D. H. T. (2016). A systematic review and meta-analysis on the effects of exercise training versus hypocaloric diet: distinct effects on body weight and visceral adipose tissue. *Obes. Rev.* 17, 664–690. doi: 10.1111/obr.12406

Weiss, E. P., Albert, S. G., Reeds, D. N., Kress, K. S., Mcdaniel, J. L., Klein, S., et al. (2016). Effects of matched weight loss from calorie restriction, exercise, or both on cardiovascular disease risk factors: a randomized intervention trial. *Am. J. Clin. Nutr.* 104, 576–586. doi: 10.3945/ajcn.116.131391

WHO (2021). *Fact sheet: Obesity and Overweight. 9 June*, 2021 Edn. Geneva: Wordl Health Organization.

Yeh, S. H., Chuang, H., Lin, L. W., Hsiao, C. Y., Wang, P. W., Liu, R. T., et al. (2009). Regular Tai Chi Chuan exercise improves T cell helper function of patients with type 2 diabetes mellitus with an increase in T-bet transcription factor and IL-12 production. *Br. J. Sports Med.* 43, 845–850. doi: 10.1136/bjsm.2007.043562

You, T., Berman, D. M., Ryan, A. S., and Nicklas, B. J. (2004). Effects of hypocaloric diet and exercise training on inflammation and adipocyte lipolysis in obese postmenopausal women. *J. Clin. Endocrinol. Metab.* 89, 1739–1746. doi: 10.1210/jc.2003-031310

Yu, N., Ruan, Y., Gao, X., and Sun, J. (2017). Systematic review and meta-analysis of randomized, controlled trials on the effect of exercise on serum leptin and adiponectin in overweight and obese individuals. *Horm. Metab. Res.* 49, 164–173. doi: 10.1055/s-0042-121605

Zheng, G., Qiu, P., Xia, R., Lin, H., Ye, B., Tao, J., et al. (2019). Effect of aerobic exercise on inflammatory markers in healthy middle-aged and older adults: a systematic review and meta-analysis of randomized controlled trials. *Front. Aging Neurosci.* 11:98. doi: 10.3389/fnagi.2019.00098

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