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Nutritional and physical activity strategies to boost immunity, antioxidant status and health, volume II

Edited by
Mallikarjuna Korivi, Lebaka Veeranjaneya Reddy and Arifullah Mohammed

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Nutritional and physical activity strategies to boost immunity, antioxidant status and health, volume II

Topic editors

Mallikarjuna Korivi — Zhejiang Normal University, China

Lebaka Veeranjaneya Reddy — Yogi Vemana University, India

Arifullah Mohammed — Universiti Malaysia Kelantan, Malaysia

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Geoffrey A. Head,
Baker Heart and Diabetes Institute, Australia

*CORRESPONDENCE
Weibing Ye,
ywbls@zjnu.cn
Veeranjaneya Reddy Lebaka,
lvereddy@gmail.com

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Editorial: Nutritional and physical activity strategies to boost immunity, antioxidant status and health, Volume II

Mallikarjuna Korivi[1], Arifullah Mohammed[2], Weibing Ye[1]* and Veeranjaneya Reddy Lebaka[3]*

[1]Exercise and Metabolism Research Center, College of Physical Education and Health Sciences, Zhejiang Normal University, Jinhua, China, [2]Department of Agriculture, Faculty of Agro-Based Industry, Universiti Malaysia Kelantan, Jeli, Kelantan, Malaysia, [3]Department of Microbiology, Yogi Vemana University, Kadapa, India

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Editorial on the Research Topic
Nutritional and physical activity strategies to boost immunity, antioxidant status and health, Volume II

In addition to the existing knowledge, here we have emphasized the recent developments on the role of nutritional supplements and physical activity in improving immunity, inflammatory response, redox signaling, and health status. This Research Topic includes research articles, reviews, and meta-analyses, which demonstrate the beneficial effects of physical activity with or without a combination of nutritional interventions among healthy individuals and patients. A comprehensive review by Shao et al. highlights that the intake of proper nutrients together with physical activity can improve the energy balance, immune system, and functional ability of various systems. This study enlightens that the incorporation of various fruits and vegetables, whole grains, proteins, and probiotics are vital for sustaining overall health. In addition to physical activity, supplementation with certain probiotics, plant-derived compounds, and functional foods may improve the immune system and prevent diseases (Shao et al.). Plant-based natural products are widely used in the treatment of several inflammatory diseases, including rheumatoid arthritis (RA). This chronic inflammatory and autoimmune disease is represented by impaired inflammatory response, pain, peripheral joint swelling, and articular cartilage damage. A study by Majeed et al. demonstrates that extracts of the *Boswellia serrata* plant reduce clinical signs of joint swelling and preserve the matrix proteins by inhibiting the hydrolyzing enzymes. This is further accompanied by decreased oxidative stress and pro-inflammatory mediators after *B. serrata* treatment (Majeed et al.). In line with this, irisin is a novel molecule that has been investigated for the regulation of metabolic syndrome-induced male infertility. A

review by Sengupta et al. states that irisin can reverse the adversities of metabolic syndrome-induced male fertility disruptions and ameliorates spermatogenesis and steroidogenesis. This could be possibly due to its direct and/or indirect beneficial effects of altering insulin resistance, oxidative stress, inflammation, and testicular functions (Sengupta et al.).

Regardless of nutritional patterns or exercise behavior, negative lifestyle behavior (i.e. the consumption of alcohol) has been associated with the occurrence of various diseases, including alcoholic fatty liver. Excessive alcohol consumption is frequently associated with anxiety and depression and neuropeptide-Y (NPY) is associated with the positive or negative emotions of a healthy adult (Chen et al.). A meta-analysis was conducted to evaluate the role of neuropeptide-Y (NPY) in the development of alcoholism. This large-scale meta-analysis concludes that NPY rs16139 polymorphism is not associated with alcoholism (Chen et al.). On the other hand, COVID-19 patients after the early phase of discharge may also experience some negative emotions, such as anxiety or depression. Such mental health in patients further leads to a decrease in the quality of life, which was particularly seen among female patients (Hu et al.). Therefore, the implementation of gender-specific rehabilitation programs and other interventions is necessary to improve the psychological status, quality of life, and physical function among COVID-19 discharged patients (Hu et al.).

The beneficial effects of exercise and nutritional interventions could be influenced by various factors, including characteristics of participants (age, gender, health status, and body weight) and exercise variables (frequency, intensity, type, and duration). To address this issue, a systematic review and meta-analysis are conducted on patients with non-alcoholic fatty liver disease (NAFLD) and concludes that exercise intervention can decrease serum transaminases in patients. However, the beneficial effects are associated with patients' age, not with gender, body mass index, and exercise variables. To be specific, young NAFLD patients are more highly responsive to the exercise intervention than older patients (Hong et al.). It is well-known that carbohydrate is the primary energy source for endurance exercise, and supplementation of carbohydrate with or without protein could influence performance and post-exercise recovery. A study by Tan and others Tan et al. demonstrates that consumption of a pre-exercise carbohydrate drink containing natural soy or whey protein had no immediate benefit on performance improvement and antioxidant status. However, fatigue recovery appears to be improved in endurance performance on day 2 after supplementation (Tan et al.), which indicates the importance of dietary supplements during post-exercise recovery.

Practicing traditional exercise programs, such as yoga, Tai-chi, Qigong and square dance has long been claimed to promote or maintain psychological and physical health. In a comparative study, older adults with either a Tai-chi or square dance practice show better immune function, physical health, and life satisfaction than the sedentary control adults. However, the Tai-chi practitioners show better physical health and immune function outcomes than the square dance practitioners. These findings suggest that Tai-chi exercise could be a practical strategy to promote overall health in Chinese older adults (Su and Zhao.). Local government attention and reforms on exercise participation and nutrition are beneficial in improving public health. A study from China validates the influential role of government attention on the Chinese population's nutrition, health, and exercise (Zhang et al.). The findings reveal that increased implementation of government policies related to nutrition, exercise, and health has led to promoting the level of nutrition, exercise, and health of Chinese nationals. Furthermore, the total production of various food types, dietary structure, number of medical institutions, number of sports venues, and average life expectancy of the population has been constantly increasing since the reforms implemented by the Chinese government (Zhang et al.).

It is indicated that dietary habits and exercise interventions are playing a key role in improving immune function, inflammatory response, antioxidant status, and psychological well-being among adults. Nevertheless, to achieve the maximum benefits without adverse effects, the intervention programs should be tailored according to the physical status, age, and health condition of an individual.

Author contributions

MK and VL drafted, edited, and finalized the editorial. AM and WY organized the order of articles and assessed the key findings from the articles. All authors contributed to the article and approved the submitted version.

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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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Irisin, Energy Homeostasis and Male Reproduction

Pallav Sengupta[1†], Sulagna Dutta[2†], Ivan Rolland Karkada[1†], Roland Eghoghosoa Akhigbe[3,4] and Suresh V. Chinni[5]*

[1] Physiology Unit, Faculty of Medicine, Bioscience and Nursing, MAHSA University, Kuala Lumpur, Malaysia, [2] Department of Oral Biology and Biomedical Sciences, Faculty of Dentistry, MAHSA University, Kuala Lumpur, Malaysia, [3] Department of Physiology, Ladoke Akintola University of Technology, Ogbomoso, Nigeria, [4] Reproductive Biology and Toxicology Research Laboratories, Oasis of Grace Hospital, Osogbo, Nigeria, [5] Department of Biotechnology, Faculty of Applied Sciences, AIMST University, Bedong, Malaysia

Irisin is a novel skeletal muscle- and adipose tissue-secreted peptide. It is conventionally regarded as an adipomyokine and is a cleaved fragment of Fibronectin type III domain-containing protein 5 (FNDC5). It is involved in the browning of white adipose tissue, glucose tolerance, and reversing of metabolic disruptions. Fertility is closely linked to energy metabolism and the endocrine function of the adipose tissue. Moreover, there is established association between obesity and male infertility. Irisin bears strong therapeutic promise in obesity and its associated disorders, as well as shown to improve male reproductive functions. Thus, irisin is a molecule of great interest in exploring the amelioration of metabolic syndrome or obesity-induced male infertility. In this review we aim to enumerate the most significant aspects of irisin actions and discuss its involvement in energy homeostasis and male reproduction. Though current and future research on irisin is very promiscuous, a number of clarifications are still needed to reveal its full potential as a significant medicinal target in several human diseases including male infertility.

Keywords: energy metabolism, irisin, male infertility, reproduction, metabolic syndrome

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Arifullah Mohammed, Universiti Malaysia Kelantan, Malaysia

Reviewed by:
Sirajudeen KNS, International Islamic University Malaysia, Malaysia
Mukhtar Ahmed, King Saud University, Saudi Arabia
Yavuz Erden, Bartin University, Turkey

****Correspondence:***
Suresh V. Chinni
v_suresh@aimst.edu.my;
cvsureshgupta@gmail.com

† These authors have contributed equally to this work

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INTRODUCTION

Metabolic syndrome and energy dyshomeostasis are among the major disruptors of male reproductive health (Morrison and Brannigan, 2015). Obesity links with male infertility by multitudinous mechanisms (Katib, 2015; Bhattacharya et al., 2020). Men with metabolic disorders often have disturbed levels of reproductive hormones, most prominently with low testosterone and high estradiol (Pauli et al., 2008). These hormonal imbalance reportedly corresponds to the severity of metabolic syndrome (Rosenblatt et al., 2015). Insulin Resistance (IR), inflammation and Oxidative Stress (OS) also are underlying players in the mechanism how excess body fat disrupts reproductive functions (Morrison and Brannigan, 2015).

Various classical and non-classical hormones and factors have been discussed in bridging the knowledge gap among metabolic disorders, energy dyshomeostasis and male infertility (Alahmar et al., 2019; Bhattacharya et al., 2019; Dutta et al., 2019a,b,c; İrez et al., 2019; Sengupta et al., 2019a,b). Irisin, discovered by Boström et al. (2012), is a novel myokine/adipokine secreted by skeletal muscle as well as adipose tissues. Irisin is a remarkable molecule which is mainly induced via exercise, and in the adipose tissues, it converts white adipocytes into metabolically

active brown adipocytes, thereby holding promise as a therapeutic in obesity (Zhang et al., 2014, 2016b). Irisin has been shown to improve insulin sensitivity, enhancing cognitive capacities, thereby reversing metabolic imbalances and associated disorders (Perakakis et al., 2017). Despite its essential contribution in energy homeostasis, its detailed physiological actions on reproduction are yet to be explored. Few studies on animals indicate positive impact of irisin upon male fertility (Nanees and Reham, 2018; Tekin et al., 2019; Luo et al., 2021). Thus, it may be assumed that irisin, via reversing obesity, also ameliorates the obesity-induced disruptions in male fertility. The present article thus reviews the available literature pertaining to the versatile roles of irisin in metabolism, energy homeostasis and male reproduction and discusses the possible involved mechanisms.

IRISIN: AN ADIPO-MYOKINE AND ITS RECEPTORS

The precursor of irisin is a transmembrane glycoprotein, Fibronectin type III domain-containing protein 5 (FNDC5) which was detected for the first time in 2002 (Ferrer-Martínez et al., 2002; Teufel et al., 2002), and is also called FRCP2 and Pep. The FNDC5 proteolytic cleavage produces irisin in response to Peroxisome proliferator-activated receptor gamma coactivator 1 alpha (PGC-1 α) activation (Boström et al., 2012). Irisin bears a molecular weight of 12 kDa with 112 amino acid residues (Boström et al., 2012), while the structure of irisin is yet to fully revealed.

Irisin was discovered through a study in search of factors secreted in response to PGC-1α by the skeletal muscles (Schumacher et al., 2013). PGC-1α mediates the physiological benefits of exercise such as white-to-brown fat conversion (Handschin and Spiegelman, 2008), improvement of insulin sensitivity and signaling (Wenz et al., 2009). Studies have also demonstrated that irisin is primarily released in response to exercise (Boström et al., 2012; Huh et al., 2012).

Barely 6 years after the discovery of irisin, Kim et al. (2018) clearly demonstrated that the physiological actions of irisin are mediated via αV integrins located in osteocytes, myocytes, and adipose tissues. They showed that irisin therapy ameliorated hydrogen peroxide-induced apoptosis in MLO-Y4 (osteocyte-like) cell-line, demonstrating that irisin confers protection against apoptosis and induces bone resorption by upregulating sclerostin (Kim et al., 2018). In addition, they demonstrated that FNDC5-knockout mice had significantly lower expression of receptor activator of nuclear factor kappa-B ligand (RANKL) mRNA (Kim et al., 2018). Quantitative proteomics analyses in MLO-Y4 osteocytes identified five cell surface proteins as possible receptor candidates for irisin. Among them, only integrin β1 which binds with α-integrins to form obligate heterodimers, is known to trigger downstream signaling; phosphorylation of focal adhesion kinase (FAK), protein kinase B (AKT), and cyclic AMP (cAMP) response element-binding protein (CREB) (Schaller et al., 1994; Giancotti and Ruoslahti, 1999; D'Amico et al., 2000). Irisin-treated MLO-Y4 cells showed phosphorylation of FAK, AKT, CREB, and Zyxin (Kim et al., 2018). This infers that irisin activates a pathway of integrin-like signaling. The αV/β5 integrin had the highest binding affinity while other integrin complexes showed weak binding to irisin. Their quantitative proteomics with mass spectrometry revealed that αV is the most abundant integrin protein in MLO-Y4 cells followed by integrin β1, integrin α5, integrin β5, integrin β3, integrin β6, and integrin β8. Furthermore, it was reported that HEK293T cells with forced expression of integrin αV/β5 but not αV/β3 showed enhanced FAK phosphorylation with irisin treatment. Inhibition of αV/β5 absolutely blocked the observed irisin-driven phosphorylation of FAK, CREB, and Zyxin. This finding highlights the role of integrin αV/β5 in irisin-mediated functions (Kim et al., 2018).

Although the physiological roles of irisin are still evolving, recent studies have shown that irisin secretion is not limited to the osteocyte, myocytes and adipose tissue as it has been found in a variety of tissues. In central nervous system (CNS), irisin expression has been detected in the Purkinje cells of the cerebellum (Dun et al., 2013), spinal cord and cerebral cortex (Huh et al., 2012). In peripheral tissues, it is reported in liver, kidney (Aydin, 2014), salivary glands (Aydin et al., 2013), cardiac muscles (Aydin et al., 2017), skin and testis (Aydin et al., 2014). Among the male reproductive tissues, testis bears the highest irisin expressions followed by prostate gland, while within testis, irisin is mostly expressed in the developing germ cells, peritubular cells and Leydig cells (The Human Protein Atlas, 2021; **Figure 1**).

IRISIN IN ENERGY HOMEOSTASIS AND METABOLIC SYNDROME

Irisin partially bridges the knowledge gap on the interactions of working tissues with other tissues to mediate energy homeostasis. Although irisin is primarily known as a myokine, it is also released from adipose tissue (Moreno-Navarrete et al., 2013; Roca-Rivada et al., 2013), earning its name as an adipokine. It mediates the beneficial metabolic effects of exercise (Grygiel-Górniak and Puszczewicz, 2017). Irisin induces the expression of mitochondrial uncoupling protein 1 (UCP1) and conversion of white to brown adipose tissue, resulting in raised energy expenditure by increased thermogenesis (Zhang et al., 2017). Thus, irisin holds promise to be a therapeutic molecule in mitigation of metabolic syndrome and related disorders.

Irisin aids skeletal muscle glucose uptake, facilitate glucose and lipid metabolism in liver, serving as insulin sensitizing hormone and reversing conditions of hyperlipidemia and hyperglycemia in metabolic disorders (Chen et al., 2016). Recent studies depicted that irisin stimulates glucose uptake in muscle cells via the calcium/ROS and P38/AMP activated protein kinase (AMPK) mediated pathway (Mu et al., 2001; Zhang et al., 2014; **Figure 2**). Irisin can ameliorate insulin resistance (IR) by its influence on the functions of the tissues, mainly liver and pancreas, that are involved in the etiology of type 2 diabetes (Moreno-Navarrete et al., 2013; Chen et al., 2016). Moreover, thyroid hormones play significant roles in metabolism,

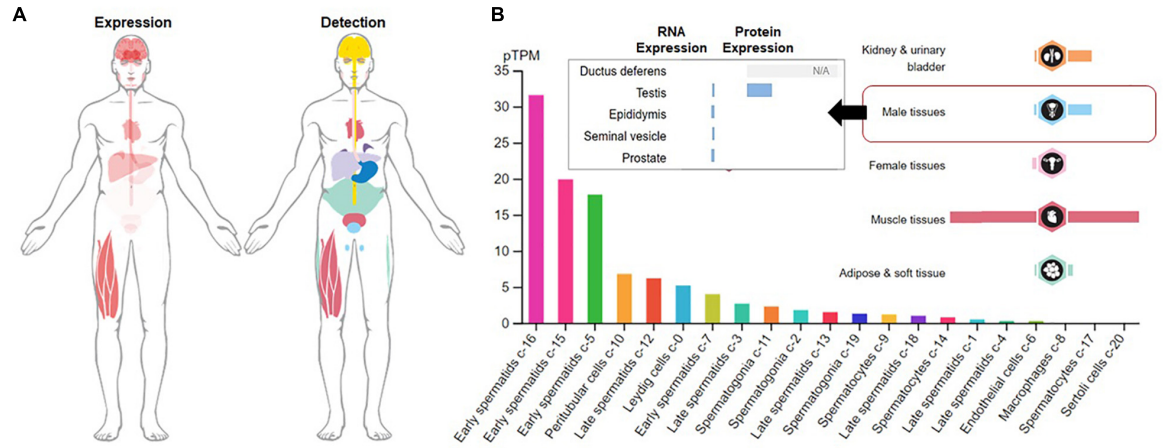

FIGURE 1 | Expression and detection of Fibronectin type III domain containing 5 (FNDC5). **(A)** Global expression and detection of FNDC5 RNA/protein; **(B)** FNDC5 RNA (normalized expression) and protein expression in overall male reproductive tissues and specific testicular cells.

and it is reported that triiodothyronine (T3) can increase the levels of adiponectin and leptin as well as improve insulin sensitivity, while irisin has least impact on adipokines levels, but it plays role in prevention of obesity or body weight regulation owing to its effects upon lipid profile (De Oliveira et al., 2020). Despite, the fact that central irisin administration inhibits the hypothalamic-pituitary-thyroid axis, it appears to be a key regulator of food intake and energy metabolism (Tekin et al., 2018). As irisin impacts on hypothalamic-pituitary-thyroid axis, it indirectly may impact on reproductive functions (Sengupta and Dutta, 2018).

In mature adipocytes, irisin excites white-to-brown fat conversion by elevating UCP1 and PR/SET Domain 16 (PRDM16) via upregulation of p38 mitogen-activated protein kinase (MAPK) and extracellular signal-regulated kinase (ERK) signaling (Boström et al., 2012; Raschke et al., 2013; Huh et al., 2014; Zhang et al., 2014, 2016a). Several studies examined the link between circulating irisin, adiposity, and obesity in humans but with inconsistent results, which may be explained by the fact that irisin may be involved in compensatory mechanism for altered metabolism in obesity and thereby different metabolic status of the specific obese individual determines its levels. Some studies reported a positive correlation between serum irisin levels, body mass index (BMI) and adiposity (Stengel et al., 2013; Crujeiras et al., 2014b), whereas others found inverse association among the circulating irisin levels, BMI and the amount of fat tissue or could not detect any significant change in circulating irisin levels (Huh et al., 2012; Gouni-Berthold et al., 2013). Positive relation of irisin with fat mass, waist circumference, waist-to-hip ratio and leptin levels have also been evidenced in obese subjects (Stengel et al., 2013; Crujeiras et al., 2014b) while a negative association was shown between irisin and adiponectin (Blüher et al., 2014). Furthermore, irisin levels were significantly reduced following weight loss due to bariatric surgery, an effect attributed to a lower fat-free mass and decreased FNDC5 mRNA expression in skeletal muscle (Huh et al., 2012). On the other hand, the reduction in irisin levels was reversed in patients who regained their original weight (Crujeiras et al., 2014a). This suggests that elevated irisin levels could be a compensatory mechanism for the abnormal metabolism and insulin sensitivity characteristic of obese individuals (Huh et al., 2012). Obesity is characterized by systemic inflammation (Bhattacharya et al., 2020), significant imbalance in cytokine secretion that is a strong predictor of developing IR and type-2 diabetes (Fantuzzi, 2005). In addition to cytokines, the activated toll-like receptor 4 (TLR4) is also strongly associated with IR as it increases TNF-α expression, that in turn affects insulin signaling pathway in muscle and adipose tissue (Könner and Brüning, 2011). Interestingly, irisin treatment suppressed expression of pro-inflammatory cytokines, nuclear factor-kappa B (NF-κB), TNF-α, and IL-6 in a concentration dependent manner. Irisin reduced MCP-1 expression in the cultured adipocytes which subsequently attenuated migration of macrophages in the presence of irisin. Moreover, irisin induced the phenotypic switching of adipose tissue macrophages from M1 (pro-inflammatory) to M2 (anti-inflammatory) state (Dong et al., 2016). Therefore, FNDC5/irisin expression is associated with some anti-inflammatory markers (Moreno-Navarrete et al., 2013).

IRISIN ENERGY HOMEOSTASIS AND MALE REPRODUCTIVE FUNCTIONS

Report by Kim et al. (2018) upended most of the conflicting data on irisin receptor, but it also raises questions and opens up studies in other fields. Are αV/β5 integrin receptors expressed in the male reproductive tract? If yes, what are their specific functions? Would inhibition of irisin (or FNDC5) or its binding to αV/β5 affect male fertility adversely? (Kim et al., 2018). Albeit available data establishing a link between irisin and male reproductive function is scarce, its secretion in the seminal vesicle, penis, and testis (Huh et al., 2012;

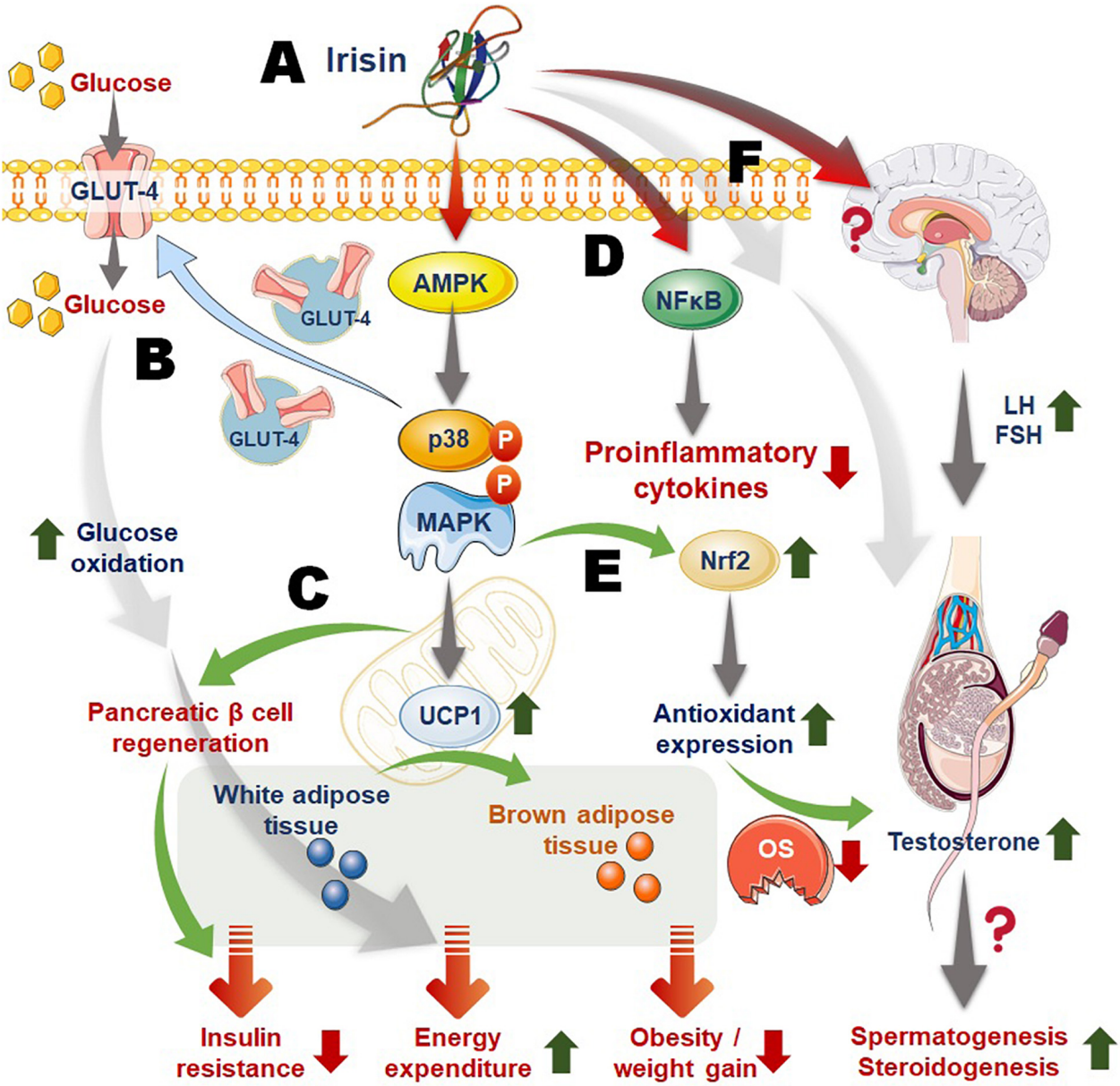

FIGURE 2 | Mechanism of irisin actions linking energy homeostasis, obesity, inflammation, and male reproduction. **(A)** Irisin acts via activation of the AMP activated protein kinase (AMPK), P38, MAPK (mitogen activated protein kinase) pathway; **(B)** irisin-activated pathway upregulates glucose transporter 4 (GLUT4) expression and transportation to membrane, aiding cellular glucose uptake that follows increased glucose metabolism and energy expenditure; **(C)** irisin also induces the expression of mitochondrial uncoupling protein 1 (UCP1) that aids conversion of white adipose tissue to brown adipose tissue, resulting in raised total body energy expenditure, as well as facilitates pancreatic β-cell regeneration that contribute to irisin-mediated reversing of insulin resistance; **(D)** irisin downregulates nuclear factor kappa-B (NF-kB) thereby playing role in suppressing inflammatory responses; **(E)** irisin-induced activation of Nrf2 (nuclear factor erythroid-2 related factor) may increase production of antioxidant enzymes thereby curbing excess reactive oxygen species (ROS) and oxidative stress (OS); **(F)** irisin may act on the HPG (hypothalamic-pituitary-gonadal) axis or directly upon the testicular cells to regulate male reproductive functions. Moreover, irisin actions to improve metabolic balance as well as to reverse obesity, inflammation and OS, may confer ameliorative impact upon obesity/inflammation/OS-mediated male infertility.

Aydin, 2014) might infer that it exerts some autocrine and paracrine effects on the male reproductive function. In addition, since energy balance has been established to play key roles in maintaining optimal reproductive function, irisin-driven energy homeostasis may be beneficial to the male reproductive function (**Figure 2**).

IRISIN AND STEROIDOGENESIS

The hypothalamic-pituitary-gonadal (HPG) axis is the main endocrine regulator of the male reproductive functions (Akhigbe et al., 2020). The hypothalamic signal to the pituitary gland is via the gonadotropin-releasing hormone (GnRH)

(Dhillo et al., 2005; Corradi et al., 2016). The pituitary gland sends signal to the testis by releasing follicle stimulating hormone (FSH) and luteinizing hormone (LH) from the pituitary gonadotrophs. FSH and LH exert their effects on the testis by binding to FSH-R that is predominantly expressed in the Sertoli cells within the seminiferous tubules and LH-R that is expressed in the interstitial Leydig cells, respectively (Ramaswamy and Weinbauer, 2014). In response to LH signaling, the conversion of cholesterol into testosterone through series of biochemical events (Akhigbe et al., 2020). The gonadotropins establish the adult population of Sertoli, Leydig and stem germ cells and their functions, thus maintaining normal spermatogenesis (Ramaswamy and Weinbauer, 2014). Energy dyshomeostasis has been associated with upregulation of estrogen receptor expression in the male hypothalamus (Chimento et al., 2014). In turn, this triggers a negative feedback mechanism and inhibits the pulsatile release of GnRH, resulting in decline in FSH and LH release and impaired testosterone production. Energy dyshomeostasis also increases the level of aromatase, which raises the conversion of testosterone to estrogen, thus inhibiting testicular function and suppressing circulatory androgen (Hammoud et al., 2006).

Irisin (and FNDC5), possibly via elevation of expression of mitochondrial UCP1, activates thermogenesis and lipolysis with resultant maintenance of energy balance. This might downregulate the expression of estrogen receptor in the hypothalamus, ensuring optimal pulsatile GnRH release and consequent FSH and LH release into the circulatory, resulting in Leydig cell-dependent testosterone production. Re-establishment of energy balance by irisin may also inhibit the conversion of testosterone to estrogen via repression of aromatase activity. Irisin-led elevation of UCP1 and PR/SET Domain 16 (PRDM16) via upregulation of p38 MAPK and ERK signaling (Boström et al., 2012; Huh et al., 2014; Zhang et al., 2014, 2016a) may not only cause white-to-brown fat conversion, but also blunt estrogen-induced cytokine-mediated inflammation.

It is possible that irisin-induced upregulation of p38 MAPK and ERK signaling activates nuclear factor erythroid-2 related factor (Nrf2) (Zhao et al., 2014), resulting in increased expression and activities of antioxidants and protection against ROS attack, oxidative stress and inflammation of the testis (Copple et al., 2017; Askari et al., 2018). Thus, it preserves testicular integrity and function, and promoting testosterone production by mitigating effects of oxidative stress (OS), which has been reported to induce inflammation, and vice versa (Akhigbe et al., 2020), with consequent apoptosis of the testicular tissue and testicular dysfunction.

Irisin may also exert regulatory role on the HPG axis. Kisspeptins, a family of peptides encoded by the KISS1 gene, has been reported to be expressed in the hypothalamus and testis among other tissues (West et al., 1998; Pinilla et al., 2012). The KISS1/GPR54 system plays a central role in the initiation of HPG axis, testosterone production, pubarche, and fertility maintenance (Navarro and Tena-Sempere, 2011). Kisspeptin system thus governs the HPG axis. Impaired expression of the kiss1 gene results in metabolic dysfunction and hypogonadism (Castellano et al., 2006). Reports suggest significant role of kisspeptin neuronal network in connecting energy homeostasis to the reproductive axis (Brown et al., 2008; Hameed et al., 2011). However, the exact mechanism of kisspeptin signaling is unclear. Moreover, kisspeptin signaling has also has a regulatory role in adipose tissue metabolism and it has been found to trigger irisin release (Shamas et al., 2019). Reports also showed that administration of irisin and kisspeptin increased neuropeptide Y (NPY) levels (Ferrante et al., 2016; Orlando et al., 2018) depicting the role of NPY in linking the kisspeptin and irisin pathways. Increase in irisin levels following kisspeptin administration also validate kisspeptin-mediated irisin release via direct irisin neurons stimulation in the hypothalamus or by the active skeletal muscles. It is also being suggested that the interactions between irisin and kisspeptin neurons are involved in regulation of reproductive functions (Tekin et al., 2019). The available reports suggest that irisin, when administered alone trigger reproductive hormones (Jiang et al., 2017), but mediate reverse effects when combined with other factors (such as GnRH and insulin) (Poretsky et al., 2017). Current studies are insufficient to elucidate the effects of irisin on the HPG axis and thus on reproductive functions. Current research is thus not enough to elucidate the impacts of irisin upon the HPG axis with reports claiming irisin to be inhibitory (Poretsky et al., 2017; Tekin et al., 2019), activator (Jiang et al., 2017), or ineffectual (Huh et al., 2014) on reproductive endocrine axis.

IRISIN, SPERMATOGENESIS, AND SPERM QUALITY

Energy dyshomeostasis-led androgen suppression adversely affects spermatogenesis via suppression of testosterone (Bieniek et al., 2016). This results in oligozoospermia and azoospermia (Sermondade et al., 2013). Irisin-mediated upregulation of the expression of Elov13, Cox7a, and Otop1 genes and increased energy expenditure maintain energy homeostasis (Boström et al., 2012). It has been shown that irisin administration in obese male rats could downregulate IR, decrease BMI, enhance the serum levels of FSH and LH, increase testosterone levels thereby resulting in improved spermatogenesis and increased sperm parameters, namely sperm count and motility (Nanees and Reham, 2018). Moreover, *in vitro* study demonstrated the possible role of irisin in spermatogenesis owing to increased irisin expressions in Sertoli cells and undifferentiated spermatogonia transcripts in organotypic primate testicular tissue culture (Wahab et al., 2020).

Several studies have linked obesity with adverse male fertility profile (Jensen et al., 2004; Sermondade et al., 2013). The obesogenic environment stimulates various adipose tissue-derived hormones, among which leptin is widely studied and rise in leptin following energy imbalance leads to increased circulatory estrogen levels, resulting in increased conversion of androgen to estrogen, thereby reducing testosterone levels (Fantuzzi, 2005; Sengupta et al., 2019a). It also reduces sex hormone-binding globulin production (Tsai et al., 2004) thereby restricting the availability of free testosterone. Obesity also mediates increase in pancreatic insulin production and peripheral tissue insulin resistance (Farooqi et al., 2003). Reports

claim that obesity-induced altered testosterone production, spermatogenesis and semen quality may be carried out via some common mechanisms that involve OS (Sengupta et al., 2019a). The membranes of the sperm cells are rich in polyunsaturated fatty acid that predisposes them to reactive oxygen species (ROS) attack and oxidative damage including damaged sperm DNA integrity (Selvam et al., 2020). It is credible to suggest that irisin-induced activation of Nrf2 via upregulation of p38 MAPK and ERK signaling (Zhao et al., 2014) may confer protection against ROS attack and oxidative damage to the testis and the sperm cells, thereby enhancing spermatogenesis and sperm quality (**Figure 2**). Thus, the rise in energy consumption and thermogenesis along with the Nrf2 signaling induced by irisin (Zhang et al., 2014; Askari et al., 2018) would likely cause a decline in energy dyshomeostasis-driven rise in obesity-led oxidative damage. However, studies are needed to validate the most likely assumption that the above mentioned irisin signaling pathway may result in improved insulin sensitivity and sex hormone-binding globulin production, restore testosterone production and functions, spermatogenesis as well as semen quality.

CONCLUSION

Irisin is an important novel molecule to be investigated for regulation of metabolic syndrome-induced male infertility. This article describes the major elements of irisin functions and discusses the relevance of irisin in energy homeostasis and male reproduction. Irisin can reverse the adversities of metabolic syndrome-mediated disruptions of male fertility and ameliorates spermatogenesis and steroidogenesis, possibly via its direct and/or indirect beneficial impact of amending insulin resistance, inflammation, OS, imbalanced HPG axis and testicular functions. It thus may provide new approach to treat male reproductive disorders by addressing the root causes of infertility. In-depth investigations are needed to reveal the detailed irisin signaling pathways in regulation of male reproductive functions. While irisin holds high promise in bridging the knowledge gap between energy homeostasis and male fertility various facets await to be explored to show its full potential as a key molecule in reverting metabolic syndrome-induced male reproductive dyshomeostasis.

AUTHOR CONTRIBUTIONS

SD, PS, and IK contributed to design the review and conceived the study. SD, PS, IK, RA, and SC drafted, edited, and reviewed the manuscript. SC procured the grant for the publication. All the authors have given their consent for submission.

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Boswellia serrata Extract Containing 30% 3-Acetyl-11-Keto-Boswellic Acid Attenuates Inflammatory Mediators and Preserves Extracellular Matrix in Collagen-Induced Arthritis

*Muhammed Majeed[1,2], Kalyanam Nagabhushanam[2], Lincy Lawrence[1], Rameshprabu Nallathambi[1], Varadharajan Thiyagarajan[1] and Lakshmi Mundkur[1]**

[1]Sami-Sabinsa Group Limited, Bangalore, India, [2]Sabinsa Corporation, East Windsor, NJ, United States

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Mallikarjuna Korivi, Zhejiang Normal University, China

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Luca Mario Giacomelli, University of Genoa, Italy
Sadiq Umar, University of Illinois at Chicago, United States

***Correspondence:**
Lakshmi Mundkur
lakshmi@sami-sabinsagroup.com

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Boswellia serrata extracts have been traditionally employed for the treatment of inflammatory diseases. In the present study, we have evaluated the mechanism of activity of Boswellin Super® FJ (BSE), a standardized extract of *B. serrata* containing not less than 30% 3-acetyl-11-keto-β-boswellic acid along with other β-boswellic acids. The *in vitro* anti-inflammatory activities were carried out in RAW 264.7 macrophages or human peripheral blood mononuclear cells stimulated with bacterial lipopolysaccharides (LPS) and treated with 1.25-5 μg/ml BSE. The anti-arthritic activity of the extract was evaluated in a rat model of collagen-induced arthritis. BSE at 40 and 80 mg/kg and celecoxib 10 mg/kg were orally dosed for 21 days. BSE showed significant ($p < 0.05$) inhibition of inflammation (TNF-α, IL-6, nitric oxide, and COX-2 secretion) and downregulates the mRNA levels of TNF-α, IL-6, IL1-β, and inducible nitric oxide synthase in macrophages. BSE treatment reduced the levels of phosphorylated-NF-κB (P65), suggesting an anti-inflammatory activity mediated by blocking this key signal transduction pathway. In addition, BSE showed inhibition ($p < 0.05$) of collagenase, elastase, hyaluronidase enzymes, and a reduction in reactive oxygen species and matrix-degrading proteins in RAW 264.7 macrophages stimulated with LPS. BSE treatment significantly ($p < 0.05$) reduced the arthritic index, paw volume, and joint inflammation comparable to celecoxib in collagen-induced arthritis (CIA) in rats. The circulating anti-collagen antibodies were reduced in BSE and celecoxib-treated animals as compared to the CIA. In confirmation with *in vitro* data, BSE showed a significant ($p < 0.05$) dose-dependent effect on C-reactive protein, prostaglandin E2, and erythrocyte sedimentation rate, which is widely used as a blood marker of inflammation. Further, BSE treatment suppressed the cartilage oligomeric matrix protein and significantly enhanced the hyaluronan levels in synovial fluid. As observed by collagen staining in joints, the loss of matrix proteins was lower in BSE-treated animals, suggesting that BSE could preserve the extracellular matrix in RA. The extract showed inhibition of collagenase enzyme activity *in vitro*, further strengthening this hypothesis. BSE treatment was found

to be safe, and rats displayed no abnormal behavior or activities. The results suggest that Boswellin Super® mediates its activity by preserving matrix proteins, reducing pro-inflammatory mediators, and oxidative stress.

Keywords: inflammation, autoimmunity, matrix proteins, collagen-induced arthritis, acetyl-11-keto-β-boswellic acid, *Boswellia serrata*

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic, inflammatory, and autoimmune disease, with a global prevalence estimated to range from 0.24–1% in different countries (Safiri et al., 2019). It is characterized by inflammation and swelling of the peripheral joints, pain, and articular cartilage damage. Synovial hyperplasia and tissue inflammation are the characteristic features of the disease. The synovium is also the source of various proteins and proteases that degrade articular cartilage and activate osteoclasts, leading to bone erosions, typically at the junction of bone and cartilage (Kyburz and Finckh, 2013).

During synovial hyperplasia, monocytes recruited into the synovium secrete cytokines and chemokines, inducing synovial fibroblast proliferation, pro-inflammatory cytokine production, matrix-degrading enzymes secretion, and sustained synovial hypertrophy (Bartok and Firestein, 2010). The cytokines, such as the receptor activator of nuclear factor-kappa-β ligand and granulocyte-macrophage colony-stimulating factor, control the osteoclast differentiation. Other inflammatory cytokines present in the synovium are tumor necrosis factor-alpha (TNF-α), interleukin (IL)-1β, and IL-6, contributing to bone erosion. Nitric oxide (NO), reactive oxygen species (ROS), prostaglandins, leukotrienes, platelet-activating factor, and enzymes [lipoxygenases, cyclooxygenases (COX-1 and COX-2), and phospholipases] are other inflammatory markers in RA. Abnormalities in the immune system leading to the presence of autoantibodies known as rheumatoid factors and immigration of T and B cells into the synovium are characteristic features of RA (Scherer et al., 2020).

RA is clinically managed using nonsteroidal anti-inflammatory drugs, corticosteroids, disease-modifying anti-rheumatic drugs (DMARDs), and biological response modifiers associated with several adverse effects (Schaffer et al., 2006). Conventional DMARDs, including methotrexate, hydroxychloroquine, and sulfasalazine, are widely used in RA therapy. Biologics are engineered proteins that target cytokines, or inflammatory cells or pathways related to tissue damage. The most common biologics are TNF-α antagonist like etanercept (Enbrel®) infliximab (Remicade®) adalimumab (Humira®), certolizumab pegol (Cimzia®), and golimumab (Simponi®). Other biologics include IL-1 inhibitor anakinra (Kineret®) and T-cell co-stimulation blocker abatacept (Orencia®). The patient response to DMARD is slow in many cases, and biologics are associated with adverse effects like susceptibility to infection and malignancies (Curtis and Singh, 2011).

Natural products and supplements could reduce the pain and morbidity associated with RA (Yang et al., 2016). The extracts of *B. serrata* (Family: Burseraceae) are widely used in traditional medicine due to their anti-oxidative, anti-inflammatory, and anti-arthritic properties (Ahmed et al., 2013; Mannino et al., 2016; Majeed et al., 2020a). Boswellic acids (BAs) are triterpenes present in the oleo gum resins of *Boswellia* species. Around 12 different pentacyclic triterpenes (BAs) have been identified (Buchele and Simmet, 2003). Among them, β-boswellic acid (BBA) and 3-acetyl-11-keto-beta-boswellic acid (AKBBA) have received significant pharmacological interest (Ammon, 2016). BAs have been reported to reduce the inflammation and associated markers in both RA and OA in clinical studies. AKBBA is the most potent inhibitor of 5 lipoxygenases (5-LO) and the leukotriene-mediated inflammatory pathways (Schweizer et al., 2000). Apart from leukotriene inhibition, AKBBA inhibits the COX-1 activity in human platelets, suppresses NF-κB activation and pro-inflammatory cytokines.

Although the *Boswellia* extracts with different BAs have been extensively studied, comprehensive evaluation of standardized extracts is scarce. Consistency of the chemical constituents of the extract is ensured by standardization. It is essential to study the activity of the standardized extract to confirm their safety and efficacy as they differ from the total plant extract which is used in traditional medicine. In the present study, we evaluated the mechanism of activity of Boswellin Super® FJ (BSE), a standardized extract of *Boswellia serrata* containing not less than 30% 3-acetyl-11-keto-β-boswellic acid along with other β-boswellic acids, *in vivo* in a collagen-induced arthritis model. The safety of this composition has been established earlier (Majeed et al., 2020b).

MATERIALS AND METHODS

Reagents

Freund's complete adjuvant (AR001), collagen type II, and calcium ionophore A23187 (C7522) were procured from Sigma-Aldrich (St. Louis, MO, United States). Dulbecco's modified minimal essential medium (DMEM) from Life Technologies (CA, United States) and fetal bovine serum (FBS) from GIBCO/Invitrogen (Carlsbad, CA, United States). DuoSet and Quantikine ELISA kits were from R&D Systems (Minneapolis, Minnesota, United States).

Boswellia Extract

Boswellin® Super (BSE) FJ is a standardized extract of *B. serrata*.

Cell Culture

Mouse macrophage cell line (RAW 264.7) was purchased from National Centre for Cell Science (Pune, India) and maintained as a monolayer culture in DMEM, supplemented with 10% (v/v) FBS at 37°C in a humidified 5% CO_2 incubator. Cytotoxicity/

cell viability of both PBMC and RAW264.7 was measured after 24 h of exposure to BSE by MTT assay (Liu et al., 2018).

Isolation of PBMC

The human blood sample (5 ml) was collected from one of the manuscript authors as a volunteer with informed consent in EDTA vacutainer tubes. The sample was diluted with an equal volume of RPMI media and overlayed on Histopaque. The tubes were centrifuged at 400 x g for 30 min at room temperature. The peripheral blood mononuclear cells (PBMCs) were aspirated from the buffy coat in the Ficoll-plasma interface, washed with PBS, and resuspended in Hanks balanced salt solution. Cell viability and count were determined using the trypan blue dye exclusion method (Grievink et al., 2016).

Antioxidant Assay

RAW 264.7 ($5 \times l0^4$ cells/well) macrophage cells were seeded in 96 well black microplates and allowed to grow overnight. Cells were induced with H_2O_2 (25 mm) and treated with different concentrations of BSE in DMEM 1% FBS for 4 hours. Freshly prepared DCFH-DA reagent (10 µg/ml) was added to all the wells. The fluorescence was documented at a wavelength of 485:520 (Ex: Em) nm, after 30 min incubation at 37°C using the BMG FluoStar Optima microplate reader. The ROS scavenging percentage was calculated with respect to the fluorescence intensity of H_2O_2-treated control cells.

Anti-inflammatory Assay

RAW 264.7 ($8 \times l0^5$ cells/well) macrophage cells were seeded in six well plates and allowed to grow overnight. Cells were induced with LPS (500 ng/ml) and or without different BSE concentrations for 24 h. The cell supernatant was collected, and the cells were lysed using RIPA buffer (Hi-Media, India). Protein concentration in cell lysates was estimated by the Bradford method (Sigma, United States). The culture supernatants were used for nitrite estimation using the Griess reaction. The cell lysates were used to analyze the presence of IL1-β using a DuoSet ELISA kit, according to the manufacturers' instructions (R&D Systems, Minneapolis, Minnesota, United States).

Alternately, human PBMC purified from healthy adults (5×10^5 cells/well) were seeded in 96 well plates and stimulated with LPS (500 ng/ml) in the presence of BSE for 24 h. The supernatants were used for TNF-α and IL6 estimation using ELISA kits as per the manufacturer's instruction (R&D Systems, Minneapolis, Minnesota, United States).

Prostaglandin Estimation

Prostaglandin was estimated using a commercial kit (Quantikine ELISA kit, R&D Systems, Minneapolis, Minnesota, United States), using the 24 h LPS and sample-treated PBMC cells (5×10^5 cells/well) supernatant. The concentration of prostaglandin was calculated based on the standard provided in the kit.

LTB4 Estimation

PBMC (5×10^5 cells/well) were treated with sample along with Calimycin (10 µm) and incubated for 30 min at 37°C. The incubation was stopped by rapid cooling in an ice bath followed by centrifugation at 250 g for 10 min at 4°C. The concentrations of LTB4 in cell supernatants were measured by ELISA (R&D Systems, Minneapolis, Minnesota, United States) as per the manufacturer's instructions. The cell supernatants were stored at −20°C until assay. The percentage inhibition of LTB4 was calculated to Calimycin-treated control (Kovács et al., 2014).

Nitrite Determination

The nitrite accumulated in the culture medium was measured as an indicator of NO production based on the Griess reaction. Briefly, 50 µl of cell culture medium was mixed with 50 µl of Griess reagent I [1% (w/v) sulfanilamide in 5% (v/v) phosphoric acid] and 50 µl Griess reagent II [0.1% (w/v) N-(1-Naphthyl) ethylenediamine dihydrochloride], incubated at room temperature for 10 min, and then, the absorbance was taken at 540 nm.

Collagenase Inhibition

Inhibition of collagenase enzyme was estimated by using the Enzchek® gelatinase/collagenase assay kit, Invitrogen, United States. Type IV collagenase enzyme from *Clostridium histolyticum* with DQ™ gelatin was used as the substrate. Different concentrations of BSE (80 µl) were preincubated with 20 µl of gelatin substrate (12.5 µg/ml). A volume of 100 µl of the collagenase enzyme solution was added (final concentration of 0.4 U/ml), and the fluorescence intensity was measured at 485 nm and 520 nm after 30 min. Enzyme activity of control (buffer) was recorded as a negative control. The extent of inhibition was calculated by using the equation, $[(B\text{-}BC) - (T\text{-}C)/(B\text{-}BC) \times 100]$, where B is the fluorescence in the presence of the enzyme, BC is the fluorescence in the absence of the enzyme, T is the fluorescence of enzyme activity in the presence of BSE, and TC is the fluorescence of the BSE alone.

Hyaluronidase Inhibition

The assay was performed following the method as described by Tung et al. (1994). A volume of 50 µl hyaluronidase (20 Uml^{-1}) in enzyme diluent (20 mm sodium phosphate with 77 mm sodium chloride and 0.01% (w/v) Bovine serum albumin, pH 7.0 at 37°C) was mixed with 50 µl of different concentrations of BSE diluted with enzyme diluent followed by incubation at 37°C for 10 min. The reaction was then initiated by the addition of 100 µl of hyaluronic acid (0.03%) prepared in 300 mM sodium phosphate buffer, pH 5.35 at 37°C as substrate solution and incubated at 37°C for 45 min. Cetylpyridinium chloride (1 ml) was used to precipitate the undigested hyaluronic acid. After incubation at room temperature for 10 min, 200 µl of each sample was transferred to 96 well microplates, and the absorbance of the reaction mixture was measured at 600 nm using a microplate reader

(Sunrise, TW, Tecan). All solutions were prepared freshly. The absorbance in the absence of enzyme was used as a control value for maximum inhibition. The inhibition percentage was calculated using the formula (Absorbance of Control-Absorbance of Sample/Absorbance of Control) × 100.

Elastase Inhibition

RAW 264.7 cells at a density of 6×10^5 were seeded in a six well plate and incubated overnight and stimulated with 50 ng/ml LPS along with different concentrations of BSE. The culture medium was collected after 16 h to check the elastase enzyme activity in the supernatant. A volume of 50 µl of assay buffer was preincubated with 50 µl of elastin substrate (25 µg/ml) in a 96 well black microtiter plate. 100 µl of the culture supernatant was added fluorescence intensity was measured at 485 nm and 520 nm after 30 min. The percentage inhibition of enzyme activity was calculated compared to the fluorescence intensity of LPS-treated control cells.

MMP Activity (Zymography)

RAW 264.7 cells (6×10^5) in a six well plate and incubated overnight for cell adhesion. The cells were stimulated with 50 ng/ml LPS along with different concentrations of BSE. Culture medium or cell lysate was collected after 16 h for Matrix metalloproteinase (MMP) gelatin zymography. Samples were mixed in Laemmli buffer lacking 2-mercaptoethanol and incubated at room temperature for 15 min for gelatin zymography. Equal amounts of protein were loaded and separated on 8% SDS-PAGE containing 0.1% gelatin from bovine skin, type B (Sigma-Aldrich). Gels were washed for 20 min, thrice in zymogram wash buffer (2.5% Triton X-100, 50 mm Tris-HCl, pH 7.5, 5 mm $CaCl_2$, and 1 µm $ZnCl_2$) and incubated overnight at 37°C in zymogram developing buffer (2.5% Triton X-100, 50 mm Tris-HCl, pH 7.5, 5 mm $CaCl_2$, 1 µm $ZnCl_2$, and 150 mm NaCl). Gels were then stained with 0.5% Coomassie Brilliant Blue R-250 (Bio-Rad) for 1 h and destained twice for 30 min in 10% acetic acid and 40% methanol solution.

Gene Expression by qRT-PCR

RAW 264.7 cells were treated with LPS as described in the earlier sections (6×10^5 in a six well plate and stimulated with 50 ng/ml LPS along with different concentrations of BSE). After 16 h of treatment, total cellular RNA was isolated using Trizol reagent® (Ambion, Life Technologies), followed by RNase-free DNase I treatment (Thermo Fisher Scientific) to remove any genomic DNA. One microgram of total RNA was reverse transcribed into cDNA using the revert-aid first strand cDNA synthesis kit (Thermo Fisher Scientific), and quantitative real-time PCR (qRT-PCR) was performed using the SYBR Green qPCR master mix (Thermo Scientific) using Light cycler 96 (Roche Life Science). β-actin gene expression was used as housekeeping control. The following primers were used for the analysis.

Name	Primer sequence (Forward)	Primer sequence (Reverse)
m iNos2	CAGCTGGGCTGTACAAACCTT	CATTGGAAGTGAAGCGGTTCG
m TNF α	CACAGAAAGCATGATCCGCGACGT	CGGCAGAGAGGAGGTTGACTTTCT
m IL-1β	CCAGCTTCAAATCTCACAGCAG	CTTCTTTGGGTATTGCTTGGGATC
m IL- 6	TCCTACCCCAACTTCCAATGCTC	TTGGATGGTCTTGGTCCTTAGCC
m β-actin	GCAGGAGTACGATGAGTCCG	ACGCAGCTCAGTAACAGTCC

Immunoblotting

Cellular protein (75 µg) was loaded per lane in denatured 10% polyacrylamide gel (SDS-PAGE), transferred to a polyvinylidene difluoride membrane (Invitrolon™ PVDF, Thermo Fisher Scientific, United States). Membranes were incubated with anti-mouse primary antibodies against total and p-NFκB and β-actin (Cell Signaling Technology, Europe) for 18 h at 4°C and horseradish peroxidase-conjugated secondary antibody (G-Biosciences, United States) for 2 h at 37°C. ECL (Pierce ECL plus, Thermo Scientific, United States) was used to detect the immunoreactive proteins. The blots were quantified using ImageJ software (version 1.52a, National Institute of Health, United States).

Animals and Experimental Design

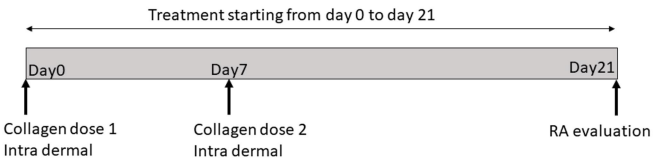
Wistar rats were housed under standard air-conditioned laboratory conditions. The temperature was maintained at a maximum: 24°C and a minimum of 23°C and relative humidity at a maximum: 63% and a minimum of 48% with 12 h light and 12 h dark cycle. The maximum and minimum temperature and relative humidity in the experimental room were recorded once daily. The male Wistar rats (8 weeks weighing 220–240 g) were randomized into five groups of six animals, as shown in the experimental design. CIA was induced in Groups 2–5, and treatments were given by oral gavage. The experiment was carried out in accordance with the guidelines for animal experimentation of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India, with approval from the Institutional Animal Ethics Committee (approval no. 1165/PO/RcBiBt-S/NRc-L/08/CPCSEA).

Collagen-Induced Arthritis

Chicken type II collagen (CII, 2 mg/ml in 0.1 M acetic acid, dissolved overnight at 4°C) was emulsified in an equal volume of complete Freund's adjuvant (CFA) prepared by dissolving heat-inactivated Bacillus Calmette-Guerin in IFA to a final concentration of 0.5 mg/ml. Rats were immunized with 0.2 ml of CII emulsion at the base of the tail by the intradermal route. On day 7, a booster dose was administered (Rosloniec et al., 2001). Immunized rats received BSE (40 and 80 mg/kg per day or celecoxib 10 mg/kg by oral gavage) or vehicle, starting from day 0 to 21. The test formulations were suspended in 0.5% sodium carboxymethylcellulose and dosed at the volume of 10 ml/kg body weight. At the end of the study, rats were euthanized by decapitation by cervical dislocation as per the American Veterinary Medical Association guidelines by a trained

veterinary physician (Leary et al., 2020). Blood (1 ml) and synovial fluid (0.8–1 ml) were collected from all the animals for biochemical analysis.

Experimental Design



Groups	Treatment
1	Control
2	Collagen induced arthritis(CIA)
3	CIA+ BSE 40mg/kg
4	CIA+BSE 80mg/kg
5	CIA+ Celecoxib 10mg/kg

Evaluation Parameters

Arthritic Index Score

A standardized method of arthritis scoring was used to evaluate the degree of swelling and erythema of all four paws. No sign as 0; – Redness without edema as 1, Redness with mild edema as 2, Redness with severe edema as 3, and Redness, severe edema, and stiffness in movement (Yu et al., 2008).

Motility Test Score

The motility of the rats was observed for 5 min and scored as 0 for walking easily, 1 when the rats walked with little difficulty, toe touching the floor, and 2, when the rats walked with difficulty, avoiding touching the toes of the inflamed paw to the floor (Amdekar et al., 2012).

Stair-Climbing Ability

Overnight fasted rats were trained to climb a staircase with steps at 5, 10, and 15 cm for 1 week, having water at the second and food at the third step. The climbing ability of the rats was scored as 0 if the rats did not climb. A score of 1 when the rats climbed to the first step, 2, when they climbed into steps 1 and 2, and 3, if the rats climbed all the three steps (Amdekar et al., 2012).

Dorsiflexion Pain

The left hind paw of the rats was gently flexed five times at 5 s intervals, resulting in a squeak or withdrawal of the leg. The pain was scored as 0 – when rat showed no squeaking and no withdrawal of leg was scored as 0, either squeaking or withdrawal of leg as 1, and both squeaking and withdrawal of leg.

Edema Measurement

Digital vernier calipers measured the edema in the paw and ankle joints.

Histopathological Assessments

For histological analysis, rats' hind paws and knee joints were removed and fixed in 4% paraformaldehyde for 24 h and decalcified in phosphate-buffered saline containing 10% EDTA. Joints were processed and embedded in paraffin, and 4 μm thick sections were taken. The sections were deparaffinized using xylene and prepared for staining by rehydration in a graded concentration of ethanol in water and finally in water (Zhuqian et al., 2019). The slides containing sections were stained by hematoxylin and Eosin for general histology and using Picrosirius red (0.1% Sirius red in saturated picric acid solution) for 60 min, followed by washing with 0.5% acetic acid to visualize collagen (Schmitz et al., 2010). Sections were examined at 10x and 40x magnification in a research microscope using brightfield mode (Nikon Eclipse). The collagen area fraction was calculated by dividing the collagen area by the total tissue area.

Estimation of Hyaluronan in Synovial Fluid

Hyaluronan was estimated using a commercial kit (Quantikine Hyaluronan Immunoassay Kit, R&D Systems). The synovial fluid was diluted 300 times to get the hyaluronan concentration within the standard curve. The concentration of hyaluronan was calculated based on the standard provided in the kit. The results were expressed as hyaluronan concentration/mg protein of synovial fluid.

ESR and CRP

ESR was measured using ESR analyzer (HumaSRate 24PT. Medsource, Ozone Biomedicals, United States), and CRP was estimated using a commercial kit (Sigma-Aldrich, United States).

Serum Collagen Antibody Estimation

Collagen antibody estimation was done by following the standard estimation methods. The diluted serum samples were incubated in collagen-coated plates for 90 min at 37°C; the plates were washed with PBST (0.05% tween) and then subjected to differential washing. Anti-CII antibodies were detected using horseradish peroxidase-conjugated anti-rat-IgG (Sigma Chemical Co., St. Louis, Mo.) diluted 1:1000 and incubated for 90 min at 37° C. The reaction was revealed with a substrate solution consisting of TMB substrate. The reaction was stopped after incubation for 20 min at room temperature with 2 N H_2SO_4 and absorbance read at 450 nm (Yasmin et al., 2014).

Estimation of Comp Levels in Synovial Fluid

COMP was estimated using a commercial kit (ELISA kit, NOVUS Biologicals, Colorado, United States). The synovial fluid was diluted three times to get the COMP concentration within the standard curve. The concentration of COMP was calculated based on the standard provided in the kit. The results were expressed as pg/mg protein of synovial fluid.

Statistical Analysis

All the data are presented as mean ± SD. The *in vitro* experiments were repeated thrice in duplicates while animal

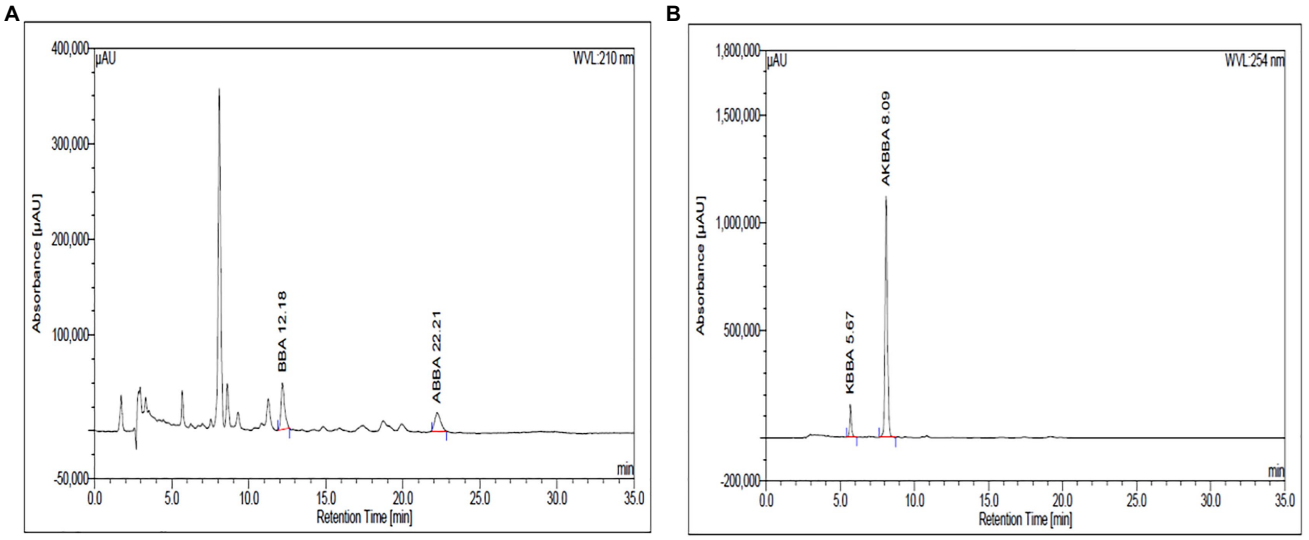


FIGURE 1 | The β-Boswellic acids in BSE were analyzed by HPLC using a C18 column and a mobile phase of acetonitrile and water in the ratio of 90:10 with 0.05 ml of glacial acetic acid. BBA and ABBA are detected at 210 nm **(A)** and KBBA and AKBBA at 254 nm **(B)**.

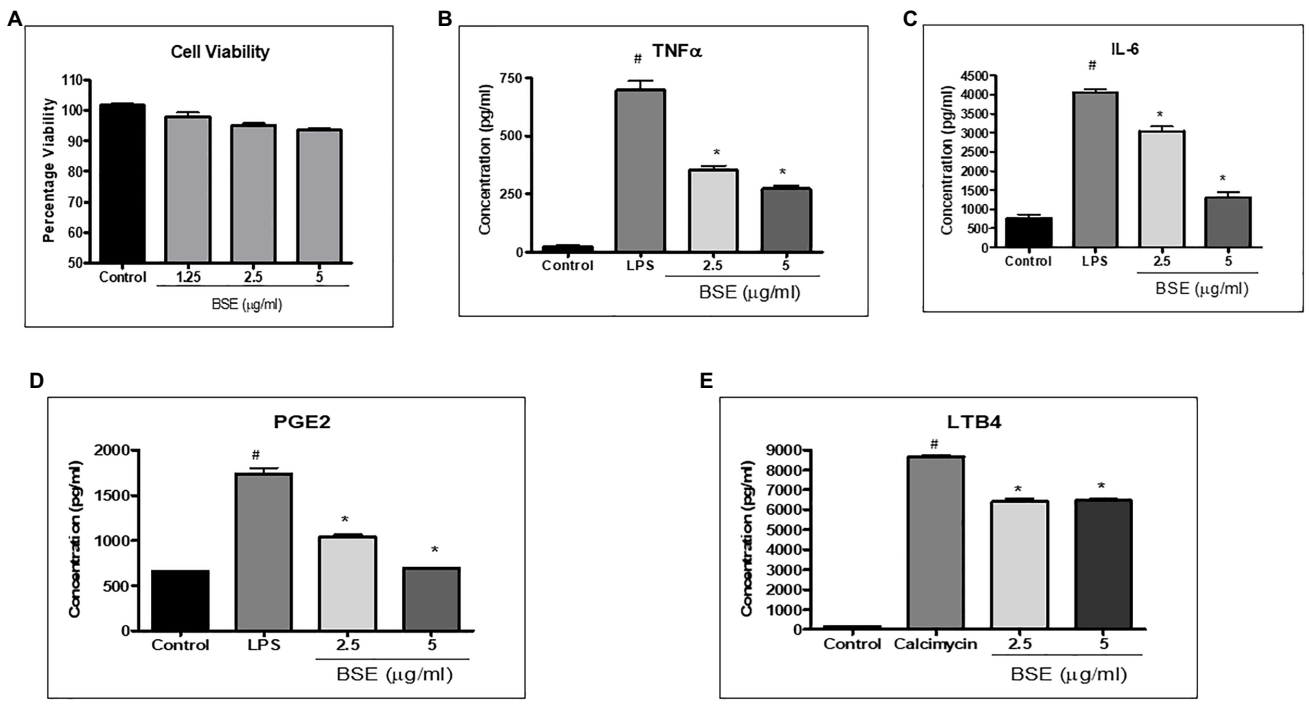


FIGURE 2 | (A) Viability of PMBC cells in the presence of different concentrations of BSE effect of BSE on **(B)** TNF-α, **(C)** IL-6, and **(D)** PGE2 in the supernatant of peripheral blood mononuclear cells (PBMC) activated with bacterial lipopolysaccharides, and **(E)** LTB4 in the supernatant of peripheral blood mononuclear cells (PBMC) induced with Calimycin. $^{\#}p < 0.05$ compared to control; $^{*}p < 0.05$ compared to the negative control (LPS for TNF-α, IL-6, and PGE2 and Calimycin for LTB4).

experiments were carried out with N = 6 animals per group. The data were analyzed using the one-way ANOVA followed by the Turkey multiple comparisons test. A value of $p < 0.05$ was considered statistically significant in comparison with disease control.

RESULTS

B. serrata Extract

Boswellin® Super (BSE) is a standardized extract of *B. serrata*. BSE contains at least 30% 3-O-Acetyl-11-Keto- beta-boswellic acid,

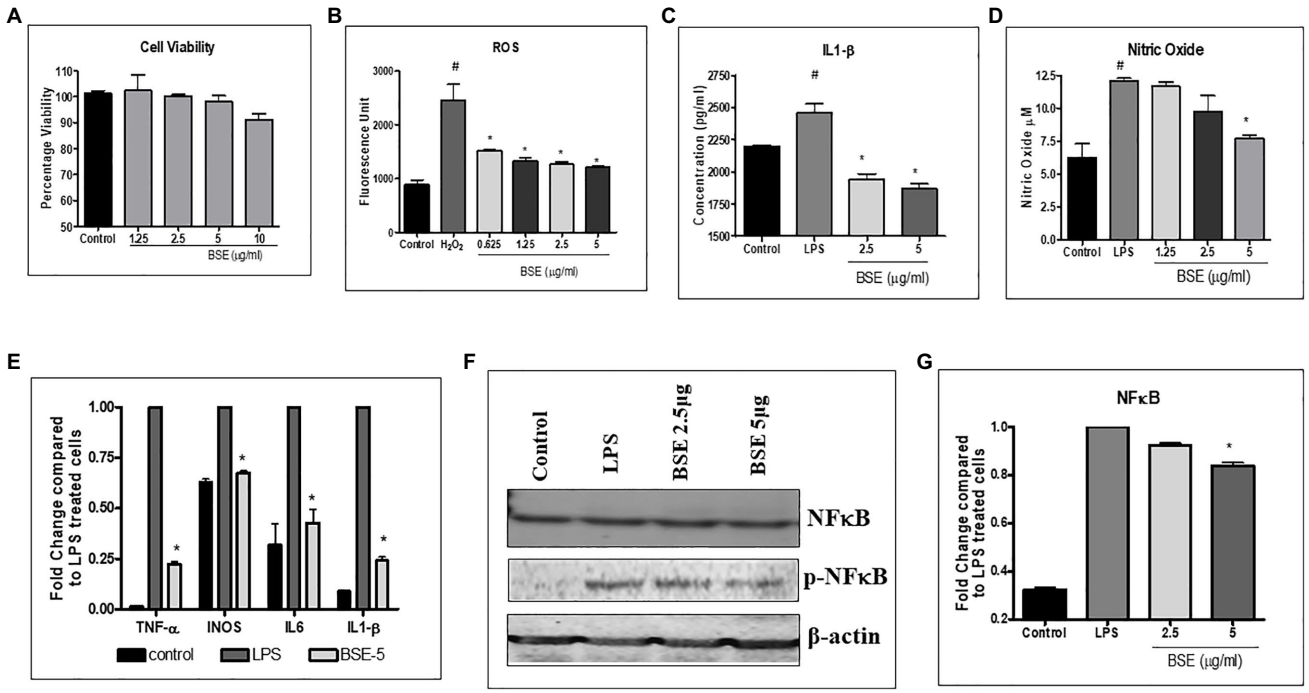


FIGURE 3 | (A) Viability of RAW macrophage cells in the presence of different concentrations of BSE **(B)** effect of BSE on intracellular reactive oxygen species (ROS) in RAW 264.7 macrophage cells induced with 25 mM hydrogen peroxide (H_2O_2). **(C)** IL-1β and **(D)** nitric oxide (NO) in the supernatant of RAW 264.7 macrophage cells induced with LPS (500 ng/ml). **(E)** Effect of BSE on relative gene expression of inflammatory biomarkers iNOS, TNF-α, IL-6, and IL-1β in LPS (50 ng/ml) induced RAW 264.7 macrophage cells. **(F)** and **(G)** effect of BSE on relative protein expression of inflammatory biomarker p-NFκB in LPS (50 ng/ml) induced RAW 264.7 macrophage cells. $^{\#}p < 0.05$ compared to control; $^{*}p < 0.05$ compared to negative control H_2O_2 for ROS and LPS for IL-1β and NO.

7.5% of beta-boswellic acid, 3.5% of 3-O-Acetyl-beta-boswellic acid (ABBA), and 1.5% 11-Keto-beta-boswellic acid (KBBA) as analyzed by HPLC. The content of the total identified beta-boswellic acids was between 50 and 55% in the extract. The HPLC chromatograms are shown in **Figures 1A,B**, wherein BBA and ABBA are detected at 210 nm and KBBA and AKBBA at 254 nm.

Effect of BSE on Antioxidant and Anti-inflammatory Markers *in vitro*

The cell viability of PBMC and RAW 264.7 was not affected at concentrations up to 5 µg/ml (**Figures 2A, 3A**); hence, all the cell-based assays were carried out at a maximum concentration of 5 µg/ml of BSE. The secretion of inflammatory cytokines TNF-α and IL-6 from LPS stimulated human PBMC was inhibited in a dose-dependent manner by BSE treatment. At the 5 µg/ml concentration, BSE showed 61.1% inhibition of TNF-α and 67.6% inhibition of IL-6 induced by LPS (**Figures 2B,C**). Significant inhibition was observed in the production of PGE2 (40%) induced by LPS and LTB4 (25.9%) induced by Calimycin in PBMC (**Figures 2D,E**).

As shown in **Figure 3B**, treatment with BSE caused a dose-related inhibition in the production of intracellular ROS induced by H_2O_2 in RAW 264.7 macrophage cells. The levels of IL-1β (26.5%) and nitric oxide production (38%) were reduced by BSE treatment in RAW 264.7 macrophages stimulated with LPS (**Figures 3C,D**). Concurrent with these results, the mRNA levels of iNOS TNF-α IL-6 and IL1-β were downregulated in LPS stimulated cells treated with 5 µg/ml BSE (**Figure 3E**). We did not observe any change in IL-8 expression in BSE-treated cells. The protein levels of phosphorylated-NF-κBp65 were also lowered by BSE in the same cells (**Figures 3F,G**), suggesting an anti-inflammatory activity *via* inhibiting the p-NF-κB signaling cascade.

Effect of BSE on Extracellular Matrix Protein and Degrading Proteases

The proteins, such as collagen, hyaluronan, and elastin present in the extracellular matrix, provide lubrication, absorb, and distribute the compressive load, and withstand shear stress during joint movement. To understand the effect of BSE on matrix-degrading enzymes, we evaluated the inhibition of collagenase, hyaluronidase, and elastase enzymes by BSE, *in vitro*. Further, its effect on matrix metalloprotease (MMP)-9, the protease that degrades gelatin and collagen, was assessed by zymography.

BSE showed dose-dependent inhibition of collagenase enzyme activity with 45% inhibition at a concentration of 500 µg/ml (**Figure 4A**). It showed potent inhibition of hyaluronidase activity, with an IC_{50} of 13.07 µg/ml and moderate inhibition of elastase (**Figures 4B,C**). The enhanced expression of MMP-9 induced by LPS (50 ng/ml) was reduced by 5 and 2.5 µg/ml of BSE extract (**Figures 4D,E**).

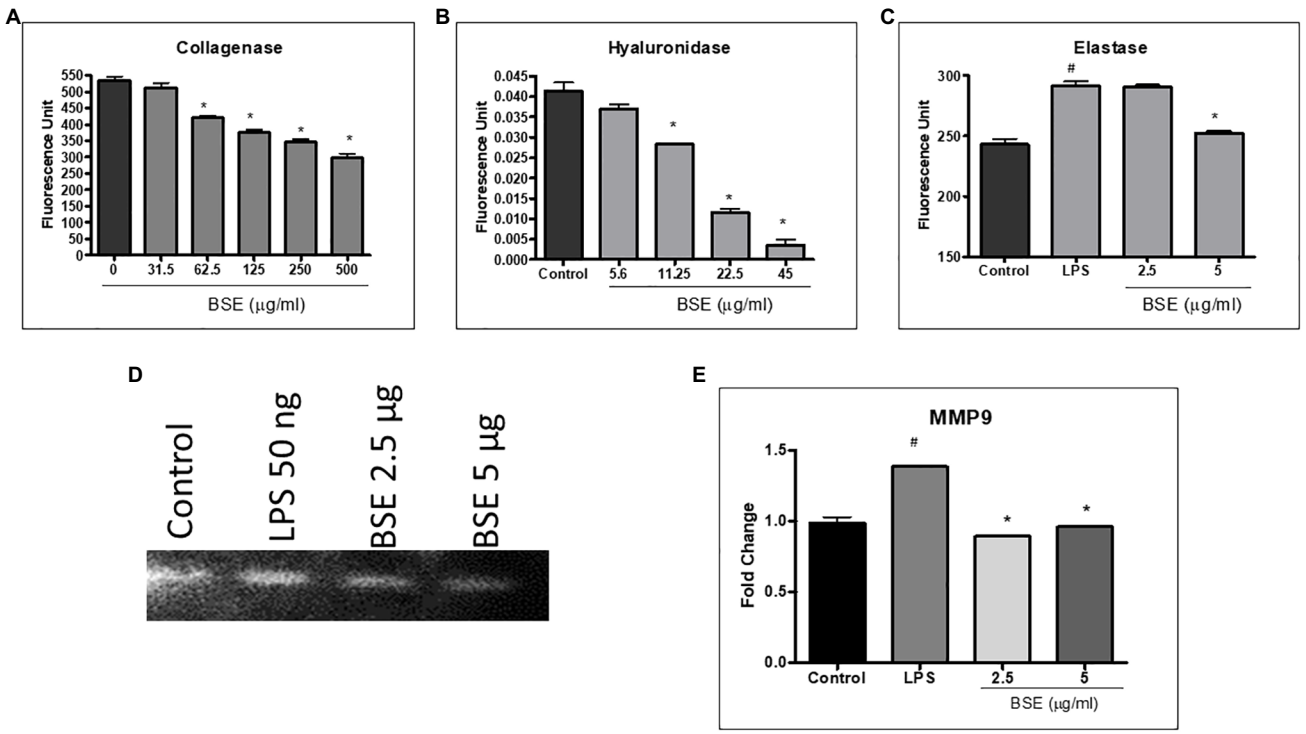


FIGURE 4 | Effect of BSE on **(A)** collagenase enzyme inhibition, **(B)** hyaluronidase enzyme inhibition, and **(C)** elastase enzyme inhibition. **(D)** and **(E)** MMP-9 in cell lysates of RAW 264.7 macrophage cells induced with LPS detected by zymography. $^{\#}p<0.05$ compared to control; $^{\#\#\#}p<0.005$ compared to control; $^{*}p<0.05$ compared to the negative control (vehicle control for collagenase and hyaluronidase enzyme inhibition and LPS for elastase enzyme inhibition and MMP9).

Effect of BSE on Collagen-Induced Arthritis in Rats

The collagen immunized animals showed a significant increase in the arthritic index compared to control animals. The arthritic index showed a significant decrease in animals treated with BSE (49.9 and 59.9% at 40 and 80 mg/kg) and celecoxib (74.8%; **Figure 5A**). The arthritic rats showed a significant increase in paw inflammation, measured in terms of increased volume. BSE reduced the paw volume by 36.6 and 37.5% at 40 and 80 mg/kg, which was comparable to celecoxib (40%, $p<0.05$) for both treatments (**Figure 5B**). The joint thickness almost doubled in RA animals compared to control animals, which was significantly reduced by 28.2 and 37.8% ($p<0.05$) at 40 and 80 mg/kg by BSE in comparison with the positive control drug, celecoxib (**Figure 5C**). The dorsiflexion pain test showed that all the animals in the RA group had severe pain and were unable to bear the flexing of the paw. Treatment with BSE significantly reduced pain by 50.0 and 58.3% ($p<0.05$) at 40 and 80 mg/kg (**Figure 5D**), whereas celecoxib showed a 66.5% reduction.

Due to inflamed paws, the motility of the arthritic animals was severely impaired, and the untreated RA-induced animals walked with difficulty. BSE eased motility by 40.1 and 59.8% ($p<0.05$) at 40 and 80 mg/kg. BSE at 80 mg/kg was equivalent to celecoxib (59.8%) treatment (**Figure 5E**).

In the stair-climbing test, animals with RA were unable to climb stairs due to inflamed paws, while the control animals easily climbed all three steps. BSE improved the climbing ability by 38.9 and 50.0% at 40 and 80 mg/kg, compared to celecoxib (66.67%; **Figure 5F**).

Histological Evaluation

Histological examination of the ankle joints of rats from different groups further supported the therapeutic effect of BSE on CIA. As shown in **Figure 6**, the ankle joint of the control group showed intact bone architecture and articular cartilage, open joint space, and absence of inflammatory cell infiltration. The RA model showed pathological changes with bone erosion, inflammatory cell infiltration, and narrow joint space. BSE and celecoxib treatments restored the morphology of bone and cartilage structure. Further, an alleviation of inflammatory cell infiltration and synovial hyperplasia was observed in the treated animals. BSE downregulated the total pathological score, which was comparable to celecoxib (**Table 1**).

Effect of BSE on Preserving Collagen in the Joints

The extracellular matrix of cartilage is composed primarily of type 2 collagen, proteoglycans containing hyaluronic acid, chondroitin sulfate (CS), fibers, fibronectin, and laminin. We evaluated the presence of total collagen in the joints by staining with picrosirius red using a light microscope. Picrosirius red staining is widely used to visualize the distribution of

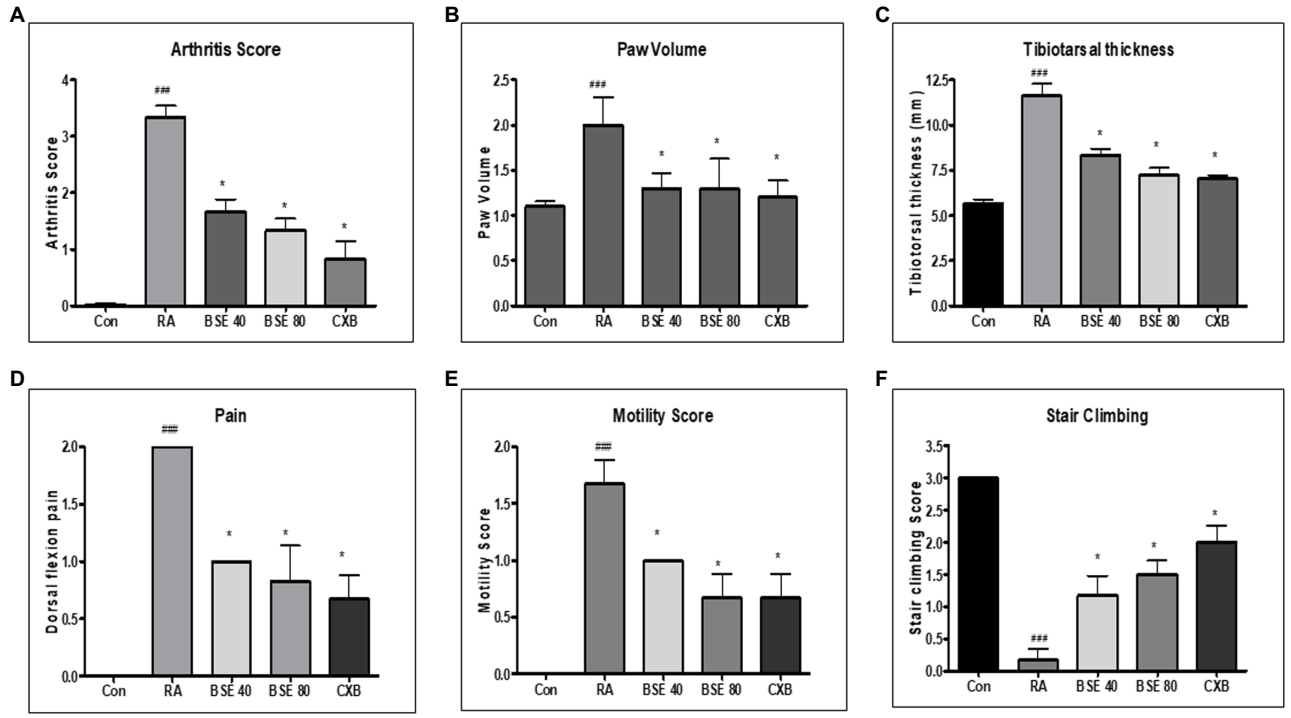


FIGURE 5 | (A) Effect of BSE on arthritis index, **(B)** paw volume in collagen-induced arthritis. **(C)** tibiotarsal thickness, **(D)** dorsiflexion pain, **(E)** motility score, and **(F)** stair climbing in collagen-induced arthritis in rats. A standardized method of arthritis scoring was used to evaluate the degree of swelling and erythema of all four paws. The motility pattern, stair climbing, and pain were scored as described in the methods section. $^{###}p < 0.005$ compared to control; $^{*}p < 0.05$ compared to RA animal, BSE 40 and BSE 80, Boswellin Super Extract 40 and 80 mg/kg; CXB, celecoxib 10 mg/kg; and RA, CIA control.

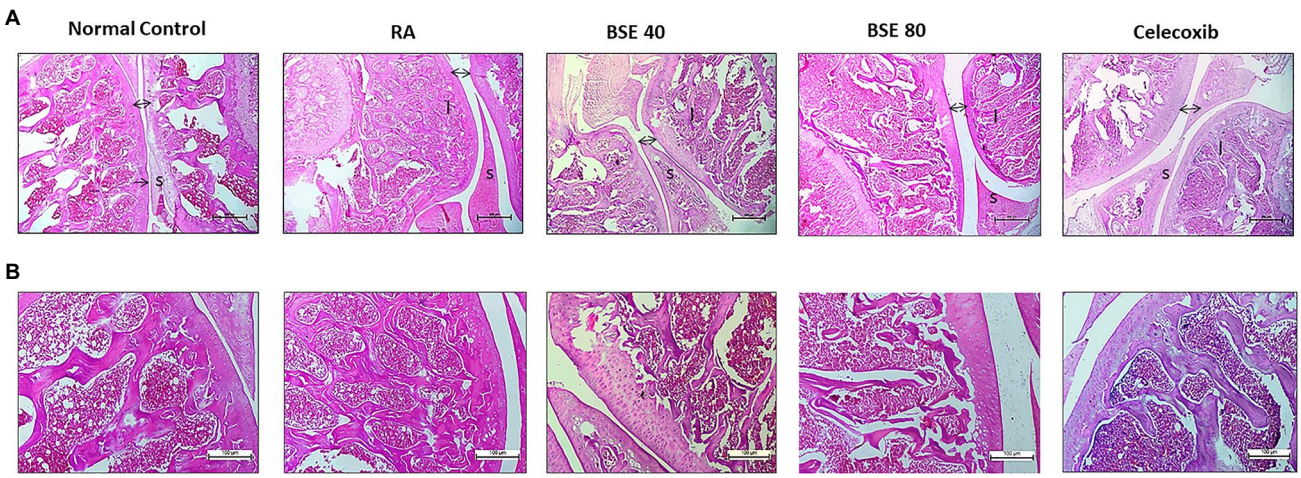


FIGURE 6 | (A) H- and E-stained histopathological images of a typical ankle joint of each group. A, 4X and **(B)** 10X resolution. S, synoviocytes and synovial hyperplasia in diseased control. A double arrow indicates space between cartilage bone of joints, and increased space between bone cartilage was found in diseased control; "I" indicates inflammation in the bone marrow. BSE 40 and BSE 80, Boswellin Super Extract 40 and 80 mg/kg; CXB, celecoxib 10 mg/kg; and RA, rheumatoid arthritis.

collagen in tissue sections by brightfield or polarization microscopy (Vogel et al., 2015). The loss of collagen in BSE-treated rats was significantly lower compared to CIA control rats, as observed by a higher intensity of picrosirius staining (**Figures 7A,B**). These results could be correlated with the results of cartilage and bone erosion observed in the H&E-stained sections. Hyaluronan was significantly reduced in arthritic animals, which was restored in BSE-treated rats. The significance

TABLE 1 | Histopathological scores of ankle issues.

Group	Synovial tissue hyperplasia	Inflammatory cell infiltration	Articular cartilage erosion
Control	0	0	0
RA	9.33 ± 0.33	9.17 ± 0.31	9.17 ± 0.31
Celecoxib 10	6.17 ± 0.48*	4.83 ± 0.31*	5 ± 0.37*
BSE40	6.0 ± 0.26*	6.67 ± 0.33*	7.5 ± 0.43
BSE80	1.50 ± 0.22*	5 ± 0.37*	5.67 ± 0.56*

*BSE40 and BSE80, Boswellin® Super 40 and 80 mg/kg; CXB, celecoxib 10 mg/kg; and RA, rheumatoid arthritis. *$p < 0.05$ compared to RA animal.*

was not achieved probably due to variability between the concentrations in the animals (**Figure 8A**).

The cartilage oligomeric matrix protein (COMP) concentration increased in the synovial fluid of untreated arthritic animals. COMP levels significantly decreased in animals treated with BSE at 80 mg/kg (**Figure 8B**).

Effect of BSE on Inflammatory and Matrix-Degrading Markers in Rats

Collagen-induced arthritis elicits anti-collagen antibodies in rats. ELISA was used to evaluate the presence of anti-collagen antibodies in serum. Animals with RA had very high levels of antibodies to collagen. BSE treatment reduced the collagen antibodies in the serum. The effect of BSE at 80 mg/kg was better than celecoxib (**Figure 8C**).

C-reactive protein concentration in serum and erythrocyte sedimentation ratio increased in the untreated arthritic animals, which were reduced in animals treated with BSE (**Figure 8D**).

DISCUSSION

The present study demonstrates that BSE, a standardized extract of *Boswellia serrata* containing 30% 3-acetyl-11-keto-β-boswellic acid, reduces inflammation, oxidative stress and, most importantly, preserves the matrix proteins by inhibiting the enzymes which hydrolyze these proteins.

BSE showed a reduction in clinical signs of joint swelling, which could be correlated with its anti-inflammatory activity. RA is an inflammatory disease characterized by chronic and persistent inflammation of synovial membranes, causing cartilage destruction and bone erosion (Zhao et al., 2015). Although recent medications have largely improved the course of the disease, they are associated with notable side effects. Hence alternative and complementary medicine is sought to relieve the symptoms and reduce the side effects of drugs. Extracts from *Boswellia serrata* gum resin, with different compositions of boswellic acids, have been used to treat a variety of inflammatory conditions like arthritis and inflammatory bowel disease (Ammon, 2016). While joint pain is a common symptom of OA and RA, the etiology of the two diseases is highly different. OA is a degenerative disease, while RA is an immune disease with the involvement of a self-destructive inflammatory immune response.

Inflammatory mediators, such as TNF-α, IL-6, and PGE-2, play a pivotal role in synovial hyperplasia observed in RA (Takayanagi, 2007). Downregulation of these inflammatory components, consequently, reduces the severity and progression of the disease. The stimulation of the production of TNF-α and NO could be linked to the activation of the NF-κB. Suppressing the NF-κB transcriptional activity in the macrophages is beneficial to suppress the expression of iNOS, COX-2, 5-LOX, and other inflammatory pathways (Peng et al., 2011). BSE markedly inhibited the phosphorylated-NF-κB (p65), suggesting that the anti-inflammatory activity is mediated by suppressing the key signal-transducing protein. NO is synthesized in excess from the synovium of inflamed joints and exacerbates joint damage and causes T-cell dysfunction contributing to bone loss in patients with RA (Lever, 2001). BSE significantly inhibited NO levels in cell supernatant and gene expression of iNOS in a dose-dependent manner. Apart from the anti-inflammatory activity, BSE could also quench the intracellular ROS in macrophages. Oxidative stress and ROS can activate various signaling pathways having a vital importance in the pathophysiology of RA (Phull et al., 2018). Excessive ROS can cause direct or indirect damage to the cartilage, matrix proteins, and DNA.

Collagen-induced arthritis, which shares the immunological and pathological features of human RA, is the most widely used animal model for the evaluation of novel therapeutic strategies for RA. BSE showed a reduction in clinical features of RA in the mice model comparable to the effect of the nonsteroidal inflammatory drug celecoxib. The main pathological characteristics of RA are inflammatory cell infiltration and pannus formation, causing joint inflammation, deformity, and the loss of motility and activity. BSE treatment reduced systemic inflammation and the swelling in joints essentially by reducing inflammation. The efficacy of BSE at 80 mg/kg was comparable to celecoxib for most of the parameters studied.

The immune system is involved in the progression and maintenance of inflammation in RA. Collagen antibodies reacting to type II collagen in the cartilage were observed to be associated with disease severity, higher levels of inflammatory cytokines in serum and erythrocyte sedimentation ratio (Cook et al., 1996; Bevaart et al., 2010). BSE could reduce the serum levels of anti-collagen antibodies, which could be correlated with the significant reduction in inflammation and arthritis symptoms.

Cartilage degradation is an important pathology of RA. The synovial membrane can be divided into two layers, the continuous surface layer of cells (intima), consisting of fibroblast-like synoviocytes and macrophages, and the underlying subintimal issue having resident fibroblasts and infiltrating cells in a collagenous extracellular matrix (Smith, 2011; Smith and Wechalekar, 2015). The synovial cells secrete hyaluronic acid for lubrication in a normal joint and maintain homeostasis in matrix synthesis and degrading enzymes. Hyperplasia of intimal cells results in increased production of matrix metalloproteinases (MMP) which degrade the ECM of cartilage and synovial membrane (Siebuhr et al., 2012).

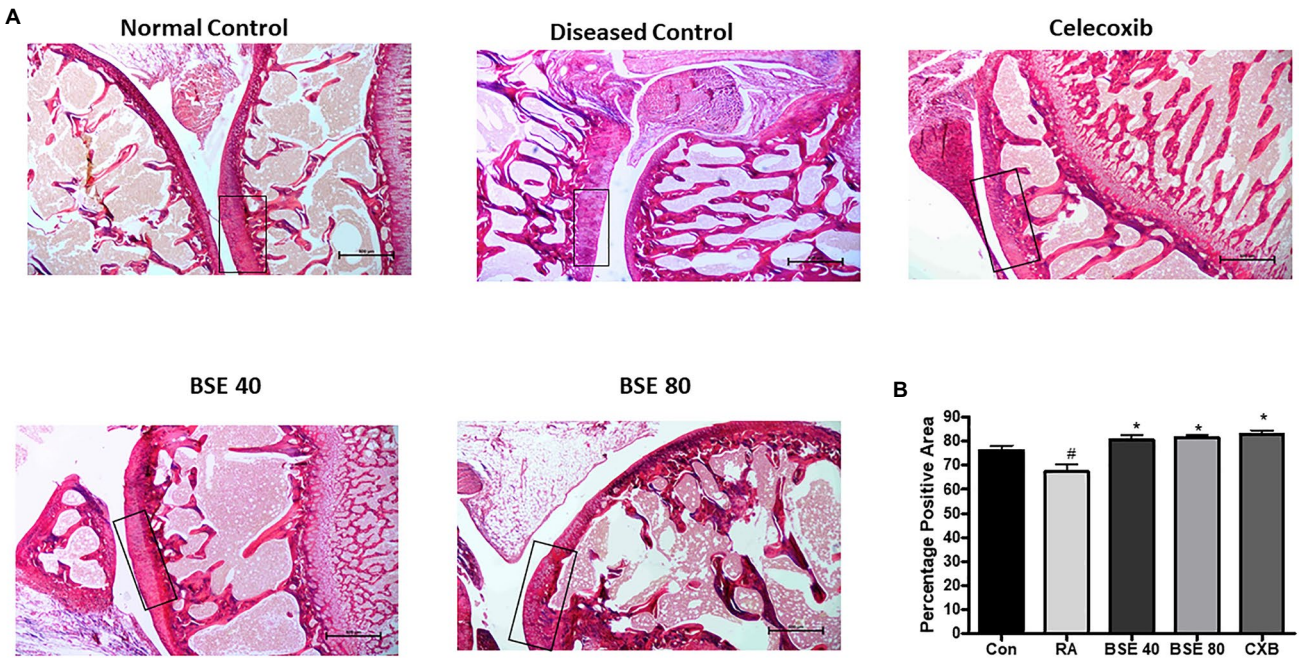


FIGURE 7 | (A) Picrosirius stained images of ankle joint. **(B)** Percentage positive area out of total tissue area. The box represents the intact collagen, which is degraded in the CIA control. BSE 40 and BSE 80, Boswellin. Super Extract 40 and 80 mg/kg; CXB, celecoxib 10 mg/kg; and RA, CIA control. $^{*}p < 0.05$ compared to RA animal; $^{\#}p < 0.005$ compared to control.

The inflammatory cytokines from hyperplasic synovium also perpetuate joint damage (Guo et al., 2018). The MMP mediated degradation of extracellular matrix proteins release COMP, and therefore, it is suggested to be a marker of disease progression with prognostic value (Kullich et al., 2006). Reduction of COMP in BSE-treated rats could be beneficial in minimizing joint destruction, which will be highly beneficial for RA patients. Apart from these markers, hyaluronan plays a significant role in protecting the articular cartilage by blocking the loss of proteoglycans and helps in nutrient transport. In RA, hyaluronan additionally acts as an anti-inflammatory molecule by inhibiting the adherence of immune complexes to neutrophils and protects the synovial tissues from the attachment of inflammatory mediators (Tamer, 2013). Enhanced hyaluronan levels in synovial fluid further support the role of BSE in protecting the cartilage in RA rats. Interestingly, the increase in hyaluronan levels by BSE was superior in celecoxib, although it was not statistically significant. In substantiation with these results, BSE inhibited the activity of hyaluronidase and MMP9 enzyme activity *in vitro*, which could be the mechanism of action of BSE in increasing hyaluronan in the synovial fluid. Further, BSE treatment significantly inhibited collagenase and elastase enzyme activities *in vitro*, suggesting that BSE could reduce the ECM degradation and preserve the extracellular matrix. Further, the total collagen content was higher in BSE-treated animals, suggesting ECM integrity.

The histopathological observations of the joints further supported the results observed in the alterations in the biomarkers. Increased infiltration of inflammatory cells, the congestion and hyperplasia of synovium, the damage of cartilage, and bone erosion were observed in the CIA rats, while BSE treatment significantly reduced these changes.

In summary, these results suggest that the anti-arthritic effect of BSE is mediated by regulating pro-inflammatory cytokines, nitric oxide, and oxidative stress along with preserving matrix proteins and reducing joint damage, which are the key markers for RA. We have earlier demonstrated the efficacy of BSE (standardized extract with 30% AKBBA) in mitigating clinical symptoms in osteoarthritic patients (Majeed et al., 2019). These results suggest that BSE could be a potential supplement to reduce the pain and inflammation associated with rheumatoid arthritis. In RA, recurrence of symptoms (flares) while tapering DMARDs is a common occurrence. The clinical trial with BSE as an adjunct therapy would initially be aimed at prolonging remission and stabilizing the disease. Larger trials can be planned based on the preliminary results of this trial.

CONCLUSION

Based on the results, it can be concluded, BSE has a significant effect on RA in a collagen-induced autoimmune arthritis model. The anti-arthritic effect was comparable to the standard anti-inflammatory drug celecoxib at higher concentrations. Thus, BSE may be considered as a promising supplement for managing RA in a clinical setting. Future clinical trials are warranted to translate the preclinical results to human use.

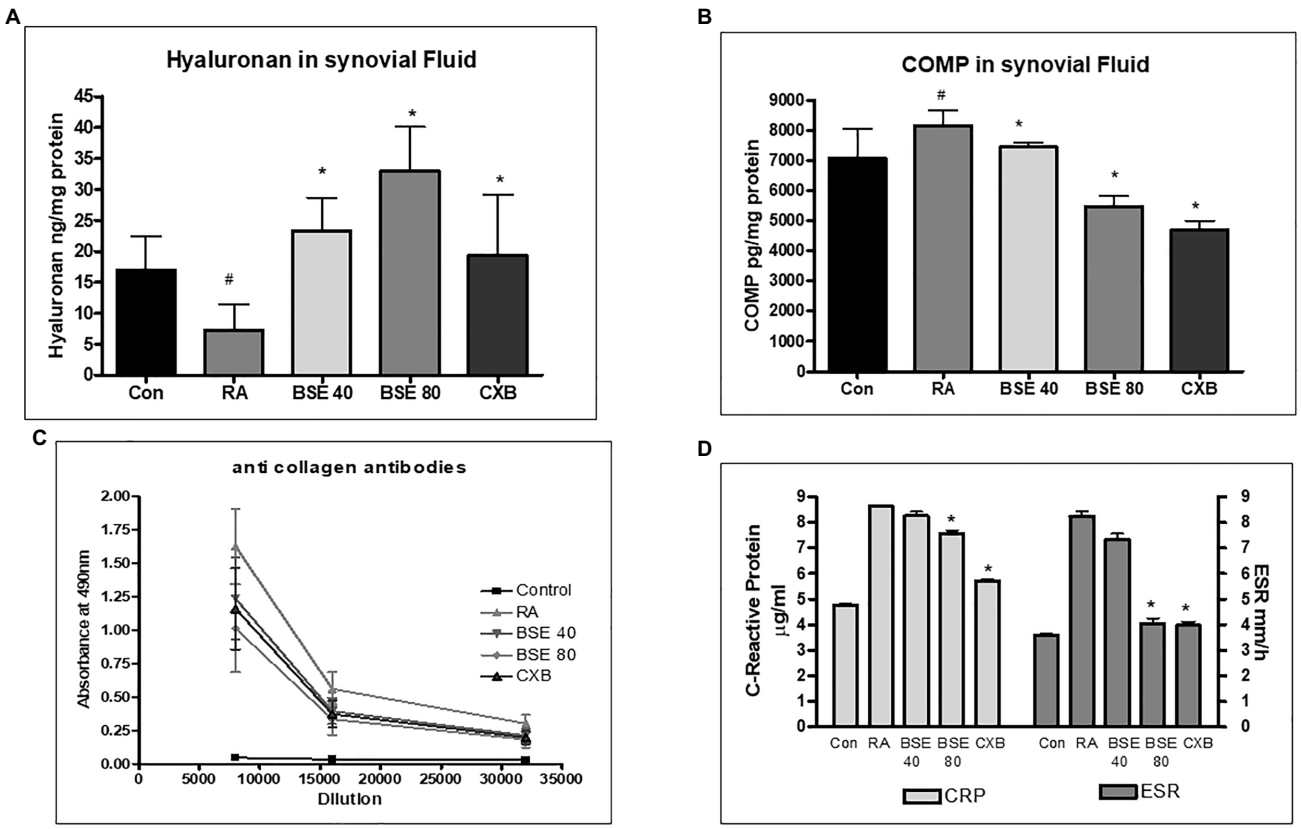


FIGURE 8 | Effect of BSE on **(A)** hyaluronan in synovial fluid and **(B)** cartilage oligomeric matrix protein (COMP) in synovial fluid, **(C)** collagen antibodies in serum, and **(D)** serum C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) levels in collagen-induced arthritis in rats. $^{\#}p < 0.05$ compared to control; $^{*}p < 0.05$ compared to RA animal; BSE40 and BSE80, Boswellin® Super 40 and 80 mg/kg; CXB, celecoxib 10 mg/kg; and RA, rheumatoid arthritis.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The animal study was reviewed and approved by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India, with the approval from the Institutional Animal Ethics Committee (approval no. 1165/PO/RcBiBt-S/NRc-L/08/CPCSEA).

AUTHOR CONTRIBUTIONS

MM and KN: conceptualization. LM and KN: methodology. LL, RN, and VT: formal analysis, investigation, and writing—original draft preparation. MM: resources. LM and RN: data curation. LM, KN, and MM: writing—review and editing. LM: supervision. All authors have read and agreed to the published version of the manuscript.

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Conflict of Interest: All authors are employees of the Sami-Sabinsa Group Limited and Sabinsa Corporation. Boswellin® Super is marketed by the Sami-Sabinsa Group Limited and Sabinsa Corporation.

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Comparative Study of the Effects of Tai Chi and Square Dance on Immune Function, Physical Health, and Life Satisfaction in Urban Empty-Nest Older Adults

*ZhongJun Su[1] and JieXiu Zhao[2]**

[1] College of Physical Education and Health, Wenzhou University, Wenzhou, China, [2] China Institute of Sport Science, Beijing, China

Objective: To compare the effects of Tai Chi and Square dance on immune function, physical health, and life satisfaction in urban, empty-nest older adults.

Methods: This cross-sectional study included 249 older adults (60–69 years) who were categorized into Tai Chi (n = 81), Square dance (n = 90), and control groups (n = 78). We evaluated immunoglobulin G (IgG) and interleukin-2 (IL-2) levels by enzyme-linked immunosorbent assay (ELISA), natural killer (NK) cell cytotoxicity by MTT assay, physical health indices by physical fitness levels, and life satisfaction by Life Satisfaction Index A (LSIA) scores.

Results: Immune function, physical health, and life satisfaction in older adults in the Tai Chi and Square dance groups were significantly better than those in the control group ($P < 0.05$). Regarding immune function and physical health, the Tai Chi group exhibited significantly higher levels of IgG (15.41 ± 0.26 g/L vs. 11.99 ± 0.35 g/L, $P < 0.05$), IL-2 (4.60 ± 0.20 ng/mL vs. 4.45 ± 0.21 ng/mL, $P < 0.05$), and NK cell cytotoxicity (0.28 ± 0.02 vs. 0.22 ± 0.02, $P < 0.05$) than the square dance group, significantly lower waist-to-hip ratio (0.87 ± 0.02 vs. 0.89 ± 0.02, $P < 0.05$), resting pulse (78.4 ± 4.6 beats/min vs. 81.0 ± 3.1 beats/min, $P < 0.05$), systolic blood pressure (132.0 ± 5.2 mmHg vs. 136.2 ± 3.2 mmHg, $P < 0.05$), diastolic blood pressure (80.0 ± 2.6 mmHg vs. 83.0 ± 2.7 mmHg, $P < 0.05$), and significantly higher vital capacity (2978.0 ± 263.0 mL vs. 2628.3 ± 262.8 mL, $P < 0.05$) and duration of one-leg standing with eyes closed (16.2 ± 1.9 s vs. 12.0 ± 1.7 s). However, there was no significant difference in LSIA scores between the Tai Chi and Square dance groups (12.05 ± 1.96 vs. 13.07 ± 1.51, $P > 0.05$). Further, there was a significant correlation between LSIA scores and immune function (r = 0.50, P = 0.00) and physical health (r = 0.64, P = 0.00).

Conclusion: (1) Both Tai Chi and square dance practitioners had better health outcomes, compared with sedentary individuals; (2) Tai Chi practitioners had better physical health and immune function than Square dance practitioners. (3) Tai Chi

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Edited by:
Mallikarjuna Korivi,
Zhejiang Normal University, China

Reviewed by:
Siew Cheok Ng,
University of Malaya, Malaysia
Yuewei Liu,
Sun Yat-sen University, China

****Correspondence:***
JieXiu Zhao
zhaojiexiu@ciss.cn

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and Square dance exercises had similar effects on life satisfaction among urban empty-nest older adults.

Suggestions: For urban empty-nest older adults who want to have better physical health and immune function, long-term Tai Chi exercise may be a better choice; however, those who are concerned about life satisfaction can choose either Tai Chi or Square dance exercise.

Keywords: Tai Chi, Square dance, immune function, physical health, life satisfaction, empty nest elderly

INTRODUCTION

Due to market reform, economic restructuring, the miniaturization of the family structure, and population aging in China, approximately 50% of the older adults in China are currently empty nesters (Zhen, 2016); it is estimated that by 2030, the proportion will reach 90% (Wang G. et al., 2017). Compared with regular older adults, empty nesters constitute a special group of older adults who are prone to suffering from "empty nest syndrome," which is characterized by a series of psychological disorders, such as feelings of loneliness, emptiness, and depression, which adversely affect their mental health (Guo and Sun, 2018). Mental health not only contributes considerably to life satisfaction but also to immune function. For example, physical and mental health potentially influence life satisfaction in older adults (Pinto et al., 2016; Lombardo et al., 2018), and psychoneuroimmunology studies have indicated that thoughts, emotional patterns, and psychological dynamics are strongly interrelated with immune response (Vasile, 2020). Another research has demonstrated a positive correlation of increased job satisfaction with natural killer (NK) cell number and plasma immunoglobulin G (IgG) concentration (Nakata et al., 2013) as well as a significant relationship between mental resilience, perceived immune function, and health (Van Schrojenstein Lantman et al., 2017).

Hence, life satisfaction is an important psychological factor reflecting the mental health and quality of life of older empty nesters (Zou and Yang, 2017), and it is an abstract and synthetic concept, which involves spiritual, physical, and social factors of individuals in daily life (Holmes and Dickerson, 1987). Since feelings of loneliness, depression, and emptiness, among others, are common in older empty nesters and are associated with adverse health consequences from both mental and immune health perspectives, an intensified focus on introducing more effective intervention strategies targeted at mitigating these feelings, is imperative. It is also important to improve their mental health, immune function, and life satisfaction.

Currently, non-pharmacological strategies, such as exercise, are becoming more popular because of their multifunctional effects and the uncertain efficacy and possible side effects of pharmacological strategies. To date, several kinds of fitness programs, such as Tai Chi and Square dance exercises, have been adopted by the older population in China. Tai Chi exercise is a traditional Chinese physical exercise characterized by meditation and low-to-moderate intensity activity, and it is practiced worldwide by older adults. In addition to improving muscle strength (Manson et al., 2013b; Wehner et al., 2021), balance (Wehner et al., 2021), body mass index (Manson et al., 2013a,b), and systolic blood pressure (SBP) (Manson et al., 2013a), research has also found Tai Chi exercise to have favorable effects on immunity (Yeh et al., 2006; Ho et al., 2013)as well as physical and mental health in older adults (Holly and Helen, 2012; Zheng et al., 2017). Square dance is considered an expansion of line dancing and was introduced in 2004, to China (Li, 2011). Public places where dance sessions are usually conducted consist of music, companions, and leader(s); further, because it is easy to learn and it produces a cheerful atmosphere, Square dance is significantly popular among middle-aged and older Chinese adults, especially among older women. Research has revealed the positive effects of Square dance on depressive symptoms and quality of life-related mental well-being (Wang et al., 2020), physical health and psychological mood (Sun and Wang, 2020), and immunity (Pei et al., 2013) in older adults. To the best of our knowledge, only a few studies have investigated the effectiveness of Square dance, and no study has comparatively evaluated the effects of Tai Chi and Square dance on mental health and immune function in the older population. Research on the differences in effect on mental health and immune function between Tai Chi and Square dance may offer positive guidance to older adults in selecting an appropriate exercise program.

The purpose of this study was to compare the effects of Tai Chi and Square dance exercises on immunity and life satisfaction in empty-nest older adults. We also aimed to evaluate the effects of these exercise on other physical health indicators, including waist-to-hip ratio, SBP, diastolic blood pressure (DBP), vital capacity, resting pulse, and balance. We hypothesized that (1) both Tai Chi and Square dance exercises can have better effect on immunity, physical health, and life satisfaction in empty-nest older adults, (2) Tai Chi exercise has more better effect than Square dance on all the aforementioned indicators in empty-nest older adults, and (3) a significant correlation exists between these indicators.

MATERIALS AND METHODS

Study Population

In this cross-sectional study, 249 empty-nest older adults aged 60–69 years were recruited and categorized into Tai Chi (n = 81, female/male [F/M] = 61/20), Square dance (n = 90, F/M = 65/25), and control group (n = 78, F/M = 60/18). In the Tai Chi and Square dance groups, empty-nest older adults were recruited

by cluster sampling and those in the control group with the help of communities. The inclusion criteria for empty-nest older adults in the Tai Chi and Square dance groups were as follows: (1) empty-nest older adults: those without offspring or whose offspring lived in other places; (2) aged 60–69 years; (3) unlimited by gender; (4) engagement in regular exercise for at least 2 years, no less than 120 min/week, and more than 3 times/week; (5) no obvious diseases, such as neurological, cardiovascular, psychiatric, and/or metabolic disease prior to the exercise. The control participants are sedentary due to our choice, because the subjects in Tai Chi or Square dance group only do regular Tai Chi or Square dance exercise, so subjects are sedentary in control group is one of our inclusion criteria.

A sedentary lifestyle was defined as not having participated in exercise for more than once per week for the last year (Audette et al., 2006). All the eligible literate participants provided written informed consent, and for the illiterate ones, the consent statement was read out and signed by the researcher after obtaining their permission. The study's protocol was approved by Ethics Committee of Wenzhou University (WZU-083).

Exercise

Tai Chi and Square dance sessions are in the form of a self-organized clubs. Each club has a chief organizer who is responsible for its leadership. Music is being played during exercise. Tai Chi exercises are conducted in the morning (6:00–7:10 a.m.) and Square dance in the evening (7:00–8:10 p.m.), with the exercise venue being a park or square.

Measures

Physical Health

Waist-to-Hip Ratio

Waist-to-hip ratio (WHR) is the ratio of the circumference of the waist to that of the hips. We employed measurement methods used by previous researchers (Yang et al., 2017). Waist circumference was measured at a level midway between the lowest rib and the iliac crest using a measuring tape, and hip circumference was measured using the same tape at the widest position of the buttocks, with the tape along a plane parallel to the floor and not compressing the skin, after inhalation and exhalation. Waist and hip circumferences were measured three times for each participant and were accurate to the nearest 0.1 cm, with the average of the three measurements being used for further data analysis.

Blood Pressure and Resting Heart Rate

According to the American Heart Association's standardized protocol (Perloff et al., 1993), we measured SBP, DBP, and resting heart rate (RHR) three times for each participant using an electronic sphygmomanometer (Omron HEM-7071A, Japan), after having them sit for at least 5 min. In cases where there was a difference of more than 5 mmHg or 5 beats/min, the two closest values were adopted (Wang P. et al., 2017). We encouraged participants to avoid alcohol, cigarette smoking, coffee, tea, and excessive exercise for at least 30 min prior to measuring their blood pressure and pulse rate (Wang et al., 2012).

Vital Capacity

Vital capacity (VC) is the maximum volume of air exhaled slowly and completely after trying to inhale, that is, VC (mL) = tidal volume + expiratory reserve volume + inspiratory reserve volume (Liu et al., 2017).

We measured VC using previously described methods (Huang et al., 2019). Briefly, VC was measured using a spirometer (Jianmin, GMCS-III type A, Xinheng Oriental Technology Development Co., Ltd, Beijing, China) according to the National Physical Health Test standard guidelines of China as per the following protocol: (1) in a standing position, take 1–2 deep breaths; (2) hold the Venturi handle (the pressure hose is above the Venturi); (3) shift the head slightly backward; (4) attempt to inhale deeply until one can no longer breathe in; and (5) subsequently exhale steadily into the mouthpiece for as long as possible until there is no air left. The maximum value was recorded after three acceptable maneuver attempts. The average of the three measurements was used for further data analysis.

One-Leg Standing With Eyes Closed

We used the method described in the National Physical Health Test standard guidelines of China. Briefly, upon the assessor's command, participants were asked to lift the non-dominant leg off the ground and keep their dominant leg vertical; in this position, participants were asked to stand for as long as possible with the time measured to the nearest to 0.01 s using a stopwatch (JinQue, JD-3B, Shanghai Automation Instrument Co., Ltd.). Before the test measurement was conducted, participants practiced 3–5 trials in the same position as that used in the official measurement. The test was stopped when participants were no longer able to maintain the requirements of the test position.

Immune Function

Overnight fasting peripheral venous blood (2 mL) was collected by qualified nurses from all participants at approximately the same time (7:30 a.m.) in a vacuum tube (Cangzhou Yongkang Pharmaceutical Products Co., Ltd., China) for measurement of IgG, Interleukin-2 (IL-2) and NK cell cytotoxicity levels, and forbid exersing, drinking and coffee the night before last.

Immunoglobulin G and Interleukin-2

We measured IgG and IL-2 levels using previously reported methods (Meng et al., 2019). The blood samples were centrifuged at 10,000 r/min for 10 min; thereafter, we collected the serum to measure the concentrations of IgG and IL-2 using the commercial enzyme-linked immunosorbent assay (ELISA) kit according to the manufacturer's protocol. Absorbance was measured using an ELISA reader (Bio-Rad, California, United States).

Natural Killer Cell Cytotoxicity

We used peripheral blood mononuclear cells (PBMCs) to assess NK cell cytotoxicity. PBMCs were isolated by density gradient centrifugation using Ficoll-Hypaque (Tianjin Haoyang Biological Manufacture Co., Ltd., China) according to the manufacturer's operation manual. We performed the proliferation and cytotoxicity assays using freshly isolated PBMCs.

Natural Killer Cell Isolation and Purification

PBMCs in the middle cloud layer were extracted using density gradient centrifugation and washed twice using phosphate buffer solution (Wuhan Boster Biological Technology Co., Ltd.); To every 10^7 cells, 70 μL buffer was added for resuspension, followed by 20 μL CD56 magnetic bead antibody, and subsequently incubated at 2–8°C for 15 min; 1 mL buffer was added for uniform mixing, centrifuged at 300 r/min for 5 min, and subsequently resuspended in 500 μL buffer. The MS separation column was placed in a MiniMACS^{TM} separation magnetic field (Miltenyi Biotec, German), and the column wall was wetted with 500 μL buffer before use; the collecting tube was set in place and the resuspended cells placed on the column, and the column was subsequently washed with 3 × 500 μL buffer. Finally, the separation column was separated from the magnetic field, placed on a new collection tube, and 1 mL buffer was promptly injected to flush down NK cells. NK cells were collected and cultured. A small number of NK cells were labeled with CD56-FITC to verify if the purity exceeded 95%.

Natural Killer Cell Culture

The NK cells' density was adjusted to 2 × 10^5/mL. Inoculation was performed in 96-well plates in the RPMI 1640 culture system containing 10% inactivated fetal bovine serum and IL-2 (100 U/mL) and cultured in a 5% CO_2 incubator at 37°C.

Natural Killer Cytotoxicity Measurement

NK cells cultured for 48 h were effector cells (E), and K562 cells in the logarithmic growth phase were target cells (T), with E:T = 20:1. Simultaneously, three parallel multiple pores, namely, the target cell pore, effector cell pore, and medium blank control pore, were set up and cultured in a 5% CO_2 incubator at 37°C for 12 h. CCK-8 (10 μL; Dojindo, Japan) was added to each well and recultured for 4 h. Absorbance (OD value) was determined using a microplate reader at 450 nm wavelength as previously described (Mehla et al., 2010), and the average value was used for further analysis. Cytotoxicity = (1– [OD value of effector pore of target cell—OD value of effector pore]/OD value of target cell) × 100%.

Life Satisfaction Index A

This scale includes 20 items, and each item has three options, namely, "agree," "disagree," and "uncertain." The total score was the sum of each item, with a score range of 0–20 points; a higher score indicated a higher life satisfaction (Neugarten Bernice et al., 1961).

Data Analysis

Descriptive statistics were used to present the demographic characteristics of the total population in the three groups. Shapiro-Wilk and Levene's tests were used for normal distribution and homogeneity test of variance for the continuous data respectively. Continuous variables are presented as mean ± SD and categorical data was expressed as a percentage. One way ANOVA was used to evaluate the differences in immune function, physical health, and life satisfaction data among the three groups, and the Bonferroni *post hoc* test was used to determine significant differences between groups if there is significant differences overall. The chi-squared test was performed to examine the demographic homogeneity among three groups; canonical correlations tests was conducted to evaluate the correlation between the life satisfaction index A (LSIA) and immunity and physical health, of which LSIA was set as aggregation 1; IgG, NK cytotoxicity, and IL-2 were set as aggregation 2; and physical health was set as aggregation 3. Results were considered statistically significant or very significant if their two-tailed p-value was < 0.05 or 0.01, respectively. IBM SPSS Statistics software for Windows (version 25; IBM Corporation, Somers, NY, United States) was used for statistical analysis.

RESULTS

We had conducted a *post hoc* power analysis according to our sample size for statistics power by G*power 3.0.10. The parameter is set as follows: α is 0.05, the effect size is 0.3, and the total sample size is 249, then the calculated power (1-βerr prob) for F-test, X^2-test and Correlation-test is 0.99, 0.96–0.99 and 0.99, respectively, belongs to large effect size.

Demographic Characteristics

There were 81, 90, and 78 participants in the Tai Chi, Square dance, and control groups, respectively, with average ages of 64.4 ± 2.2, 64.6 ± 2.3, and 64.5 ± 2.3 years, respectively. No significant differences were observed in demographic data across the three group, almost three quarters of the participants in the three groups were women. The majority of the participants in the Tai Chi and Square dance groups reported that they had not participated in other forms of regular exercise, except for occasional walking; the same was reported in the control group. No statistically significant differences in demographic variables were observed across the three groups or between Tai Chi and Square dance group (**Table 1**).

Immunity of the Participants

Compared with the control group, Tai Chi and Square dance significantly improved IgG, IL-2, and NK cytotoxicity levels ($p < 0.05$). Further, the effects of Tai Chi were significantly superior to those of Square dance ($p < 0.05$; **Table 2**).

Physical Health of the Participants

Compared with control group, Tai Chi had better effects on all the indicators ($p < 0.05$); Square dance had better effects on all the indicators, except for WHR ($p < 0.05$). Moreover, the effects of Tai Chi were significantly superior to those of Square dance ($p < 0.05$; **Table 3**).

Life Satisfaction of the Participants

In both the Tai Chi and Square dance groups, the LSIA index was significantly higher than that in the control group ($p < 0.05$), and no significant differences were noted between the Tai Chi and Square dance groups (**Table 4**).

TABLE 1 | Demographics of the participants in the three groups (n = 249).

Variables	Group			X^2/F	P
	Tai chi (n = 81)	Square dance (n = 90)	Control (n = 78)		
Age (Mean ± SD)	64.4 ± 2.2	64.6 ± 2.3	64.5 ± 2.3	0.16	0.86
Gender					
Female	61 (75.3%)	65 (72.2%)	60 (76.9%)	0.51	0.77
Male	20 (24.7%)	25 (27.8%)	18 (23.1%)		
Marital status					
Married	49 (60.5%)	61 (67.8%)	46 (59.0%)	1.62	0.44
Single or widowed	32 (39.5%)	29 (22.2%)	32 (41.0%)		
Education					
None	17 (21.0%)	15 (16.7%)	16 (20.5%)	1.04	0.98
Elementary	47 (58.0%)	55 (61.1%)	46 (59.0%)		
High school	6 (7.4%)	9 (10.0%)	7 (9.0%)		
Special school and above	11 (13.6%)	11 (12.2%)	9 (11.5%)		
Frequency of exercise/week					
3–5 times/week	71 (87.7%)	82 (91.1%)	–	0.54	0.46*
≥ 6 times/week	10 (12.3%)	8 (8.9%)	–		
Duration of exercise/time	47.8 ± 5.1	45.2 ± 3.1		0.21	0.52*
Total years of exercise					
2–4 years	49 (60.5%)	58 (64.4%)	–	0.70	0.40*
≥ 5 years	32 (39.5%)	32 (35.6%)	–		

**Indicates the significant difference between Tai Chi and Square dance group.*
SD standard deviation.

Correlation Between Life Satisfaction Index A, Immunity, and Physical Health

Significant correlation was observed between LSIA, immunity and physical health (p = 0.00; **Table 5**).

DISCUSSION

The purpose of this study was to compare the effects of Tai Chi and Square dance exercises on empty-nest older adults by mainly measuring the following three indicators: immune function, physical health, and life satisfaction. To the best of our knowledge, this study is the first to compare the effects of long-term practice of Tai Chi and Square dance on immunity, physical health, and life satisfaction in empty-nest older adults.

Our study demonstrated that Tai Chi had a significantly greater effect on immunity and physical health indicators in older adults ($p < 0.05$) compared with the other two groups; however, no such effect was observed with regard to life satisfaction when compared with Square dance ($p > 0.05$). Further, there were significantly different effects on immunity and physical health indicators as well as life satisfaction ($p < 0.05$) between the Square dance and control groups in older adults except for WHR ($p > 0.05$).

TABLE 2 | Immunity of the participants in the three groups (n = 249).

Variables	Tai chi (n = 81)	Square dance (n = 90)	Control (n = 78)
IgG (g/L)	15.41 ± 0.26#*	11.99 ± 0.35*	9.92 ± 0.22
IL-2 (ng/mL)	4.60 ± 0.20#*	4.45 ± 0.21*	4.09 ± 0.19
NK cell cytotoxicity	0.28 ± 0.02#*	0.22 ± 0.02*	0.20 ± 0.02

*# and * indicate significant differences when compared with Square dance and Control groups, respectively. IgG immunoglobulin G; IL-2 interleukin-2; NK natural killer.*

The reason underlying the different effects of the Tai Chi and Square dance exercises may be their unique characteristics. Tai Chi exercise integrates physical, psychosocial, spiritual, and behavioral components to promote mind-body interactions (Wang, 2011). It is a moderate-intensity exercise, as no more than 55% maximal oxygen intake is required (Wang et al., 2004), and it should be practiced in harmony with the Tai Chi philosophy by utilizing and manipulating Qi via Tai Chi exercise (Zheng et al., 2017). Qi is a very important concept in Chinese classical philosophy and medicine. It is not a body organ which can be anatomically identified by its location like the chakras of yoga (Cho et al., 2019). In Tai Chi exercise, it is emphasized that "Qi sinks into Dantian," "Qi runs all over the body," and "middle Qi passes through the top," all of which is important to eliminate diseases and improve human function. Smooth flow of Qi makes the body comfortable, and stagnation makes the body sick. Although a deep understanding of the essence of Qi is still lacking, one can feel the existence of Qi while practicing Tai Chi exercise, as smooth flow of Qi improves fingertip numbness, distension, etc.

Square dance, which integrates Chinese style dancing and music with energetic and similar rhythms (Zhou, 2014), introduced in China around 2004 and considered an expansion of line dancing (Li, 2011), is just a medium-intensity exercise. Research shows that Tai Chi exercise has significantly better effect on cognitive function and emotion in older people than Square dance (Zhang et al., 2014), as well as better effect on enhancing lower extremity strength, balance, and flexibility than brisk walking (Audette et al., 2006). Davidson et al. (2003)

TABLE 3 | Physical health of the participants in the three groups (n = 249).

Variables	Tai Chi (n = 81)	Square dance (n = 90)	Control (n = 78)
WHR	0.87 ± 0.02#*	0.89 ± 0.02	0.89 ± 0.02
SBP (mmHg)	132.0 ± 5.2#*	136.3 ± 3.2*	140.0 ± 3.0
DBP (mmHg)	80.0 ± 2.6#*	83.0 ± 2.7*	89.4 ± 2.7
Vital capacity (mL)	2978.0 ± 263.0#*	2628.3 ± 262.8*	2279.1 ± 240.8
RHR (beat/min)	78.4 ± 4.6#*	81.0 ± 3.1*	83.6 ± 3.4
OSEC (s)	16.2 ± 1.9#*	12.0 ± 1.7*	7.0 ± 1.7

*# and * indicate significant differences when compared with Square dance and Control groups, respectively. WHR waist-to-hip ratio; SBP systolic blood pressure; DBP diastolic blood pressure; RHR resting heart rate; OSEC one-leg standing with eyes closed.*

TABLE 4 | Life satisfaction of the participants in the three groups (n = 249).

Variables	Tai Chi (n = 81)	Square dance (n = 90)	Control (n = 78)
Life satisfaction	12.05 ± 1.96*	13.07 ± 1.51*	9.04 ± 1.54

**Indicates significant difference when compared with the Control group.*

TABLE 5 | Correlation between LSIA, immunity and physical health (n = 249).

Canonical correlation	Aggregation 1		
	R	F	P
Aggregation 2	0.50	27.28	0.00
Aggregation 3	0.64	28.59	0.00

Aggregation 1, Life satisfaction index; Aggregation 2, IgG (g/L) + NK cell cytotoxicity + IL-2 (ng/mL); Aggregation 3, Systolic blood pressure (mmHg) + diastolic blood pressure (mmHg) + resting heart rate (beat/min) + one-leg standing with eyes closed (s) + waist-to-hip ratio + Vital capacity (mL).

directly found that an 8-week clinical training program in mindfulness meditation significantly increases the left-sided anterior activation and immune function, and that activated left-sided anterior of the brain was associated with enhanced NK-cell activity (Kang et al., 1991; Davidson et al., 1999). Irwin et al. (2003) confirmed that Tai Chi potentially increases varicella-zoster virus specific cell-mediated immunity in older adults and potentially improves T-helper cell function (Yeh et al., 2009). Tai Chi exercise, which focuses on producing inner calmness, would have both physical and psychological therapeutic value (Docker, 2006). Therefore, we have reason to believe that the comprehensive nature of Tai Chi exercise rendered it significantly superior to Square dance in improving physical health and immune function.

The mechanism underlying Tai Chi exercise's ability to significantly increase immune function compared with Square dance may be related to a more pronounced increase in antibody titer, telomerase activity, reverse gene expression, reduced DNA damage, etc. Jacobs et al. (2011) found that meditation training could suppress immune cell aging because meditation can significantly increase immunocyte telomerase activity in normal people. Moreover, Goon et al. (2008) found that practicing Tai Chi exercise for 7 years provided a significantly effective DNA repair mechanism, reduced DNA damage, and increased lymphocyte apoptosis and proliferation in older adults; the upregulated lymphocyte apoptosis and proliferation with Tai Chi exercise may also be beneficial in preventing replicative senescence during aging. Our study also found Tai Chi exercise to be significantly effective in improving WHR ($p < 0.05$), which was in accordance with the results of Siu et al. (2021), who found that Tai Chi exercise can significantly decrease waist circumference. A previous study Minuzzi et al. (2018) has indicated that high levels of aerobic fitness may help prevent the accumulation of senescent T-cells during the natural aging process. Davidson et al. (2003) proved that more antibodies against the influenza-virus vaccine were formed in the meditation training group. Buric et al. (2017) found that mind-body interventions can downregulate the nuclear factor kappa B pathway, which antagonizes the effects of chronic stress on gene expression.

Chronic diseases and objective losses in functionality are considered to be strongly related to low levels of life satisfaction in older adults. Pinto et al. (2016) found self-rated health to be a mediator variable between physical and mental health and life satisfaction in older adults. In our study, some participants claimed that after practicing Tai Chi, they seldom caught a cold, even when the weather changed. The body's innate immune function declines with aging (Solana et al., 2012). Research has shown life satisfaction to be related to physical and mental health (Puvill et al., 2016; Lombardo et al., 2018), and life satisfaction is an important psychological factor reflecting the mental health and quality of life of empty-nest older adults (Zou and Yang, 2017). Hence, physical health, immune function, and life satisfaction significantly correlated (**Table 5**). Our results are consistent with those of other researchers; for example, Teixeira Vaz et al. (2019) found a positive relationship between physical activity and life satisfaction in older adults.

Compared to Tai Chi exercise, Square dance is a group activity that is more relaxing, less serious, and does not involve Kung Fu. Xie et al. (2021) found the group camaraderie in Square dance to be positively associated with the subjective well-being of middle-aged and empty-nest older women. Therefore, we consider Tai Chi and Square dance to be instrumental in improving empty-nest older adults' life satisfaction through different mechanisms, that is, through Tai Chi's movement characteristics and Square dance's sense of camaraderie; however, no significant difference was observed.

Our study has several limitations. First, the cross-sectional design of this study is not very rigorous because we did not measure the data before and after the experiment, and this has a certain impact on the accuracy of the experimental results; however, we made efforts to control the accuracy by measuring the physical activities of participants. A more precise study design should be a randomized controlled trial, so this cross-sectional design because of the time and financial constraints is the limitation of our research method. Second, the disadvantage of cluster sampling is that the sampling error caused by the large differences between groups is often greater than that of simple random sampling. Third, there are some differences in exercise frequency, time, duration, and standardization of the samples obtained by cluster sampling. Finally, the members in a self-organized club may be those who have similar personalities and a common language and feel happy, which influences the effect of exercise on life satisfaction.

Therefore, these limitations should be considered when interpreting the results of this study.

CONCLUSION AND SUGGESTIONS

Overall, our study demonstrated that both Tai Chi and Square dance exercises promote better physical health, immune function, and life satisfaction in empty-nest older adults; Tai Chi exercise was significantly superior to Square dance in physical health and immune function. Hence, we recommend Tai Chi as the preferred choice of exercise for empty-nest older adults who desire to have better physical fitness and immune function and either Tai Chi or square dance as an equally suitable choice for those desiring to have better life satisfaction.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee of Wenzhou University (WZU-083). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

JZ was responsible for the overall experimental design, index selection, financial support, manuscript polishing, and data analysis. ZS was responsible for the experiment implementation, index test, and first draft writing. Both authors contributed to the article and approved the submitted version.

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REVIEW
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Physical Activity and Nutritional Influence on Immune Function: An Important Strategy to Improve Immunity and Health Status

Tianyi Shao[1†], Henu Kumar Verma[2†], Babita Pande[3], Vincenzo Costanzo[4], Weibing Ye[5], Yuyan Cai[6] and L. V. K. S. Bhaskar[7*]*

[1]College of Teacher Education, Zhejiang Normal University, Jinhua, China, [2]Department of Immunopathology, Institute of lungs Biology and Disease, Comprehensive Pneumology Center, Helmholtz Zentrum, Munich, Germany, [3]Department of Physiology, All India Institute of Medical Science, Raipur, India, [4]Department of Experimental, Diagnostic and Specialty Medicine, University of Bologna, Bologna, Italy, [5]College of Physical Education and Health Sciences, Zhejiang Normal University, Jinhua, China, [6]Department of Physical Education, Guangdong University of Technology, Guangzhou, China, [7]Department of Zoology, Guru Ghasidas Vishwavidyalaya, Bilaspur, India

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***Correspondence:**
Yuyan Cai
caiyyan@gdut.edu.cn
L. V. K. S. Bhaskar
lvksbhaskar@gmail.com
orcid.org/0000-0003-2977-6454

†These authors have contributed equally to this work

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Physical activity (PA) and nutrition are the essential components of a healthy lifestyle, as they can influence energy balance, promote functional ability of various systems and improve immunity. Infections and their associated symptoms are the common and frequent challenges to human health that are causing severe economic and social consequences around the world. During aging, human immune system undergoes dramatic aging-related changes/dysfunctions known as immunosenescence. Clinically, immunosenescence refers to the gradual deterioration of immune system that increases exposure to infections, and reduces vaccine efficacy. Such phenomenon is linked to impaired immune responses that lead to dysfunction of multiple organs, while lack of physical activity, progressive loss of muscle mass, and concomitant decline in muscle strength facilitate immunosenescence and inflammation. In the present review, we have discussed the role of nutrition and PA, which can boost the immune system alone and synergistically. Evidence suggests that long-term PA is beneficial in improving immune system and preventing various infections. We have further discussed several nutritional strategies for improving the immune system. Unfortunately, the available evidence shows conflicting results. In terms of interaction with food intake, PA does not tend to increase energy intake during a short time course. However, overcoming nutritional deficiencies appears to be the most practical recommendation. Through the balanced nutritious diet intake one can fulfill the bodily requirement of optimal nutrition that significantly impacts the immune system. Supplementation of a single nutrient as food is generally not advisable. Rather incorporating various fruits and vegetables, whole grains, proteins and probiotics may ensure adequate nutrient intake. Therefore, multi-nutrient supplements may benefit people having deficiency in spite of sufficient diet. Along with PA, supplementation of probiotics, bovine colostrum, plant-derived products and functional foods may provide additional benefits in improving the immune system.

Keywords: nutrition, physical activity, immune fuction, exercise, aging

INTRODUCTION

It is well established that physical activity (PA) and healthy nutrition are vital lifestyle factors that influence lifelong health by improving body composition, musculoskeletal health, physical and cognitive performance. Optimal physical activity and proper nutrition are also important to prevent metabolic diseases such as obesity, diabetes mellitus, and cardiovascular disease. The WHO has identified lack of physical activity as a significant risk factor for global mortality. They recommended that some amount of physical activity is better than none and more physical activity is better for optimal health outcomes (Reardon et al., 2019; Bull et al., 2020). While the health benefits of nutrition and physical activity are frequently studied separately, it is now becoming increasingly clear that combining nutrition and physical activity can produce more significant positive health consequences and boost the immune system when compared to strategies that focus solely on one or the other. Regular moderate exercise has been shown to reduce the risk of infection compared to a sedentary lifestyle (Silişteanu and Covaşă, 2015). Similarly, a large systematic analysis from 195 countries shows that a poor diet lacking optimal nutrition is linked to adverse health outcomes. While association among many lifestyle (physical activity and diet)-related benefits, such as improved fitness, overall health/well-being, and perceived quality of life, are well documented (GBD 2017 Diet Collaborators, 2019).

The potential benefits to immunity and infection susceptibility are significant but widely ignored. Infection, including respiratory tract infection and its associated symptoms, is the most common and frequent presentation, and it can have severe economic and social consequences (Varricchio et al., 2020). It has also been noticed that prolonged periods of exercise and intense training are linked to an increased risk of infection. Acute bouts of strenuous exercise cause a temporary dwindle in the immune function that can last up to 24 h after the workout (Walsh et al., 2011). During an individual lifespan, the PA or the nutritional requirement alters during different development stages and naturally decreasing with aging. Age-related quantitative and qualitative changes occur in the immune system known as immunosenescence (Longo et al., 2015).

Clinically, immunosenescence refers to the gradual deterioration of immune system mostly observed with aging of an individual that increased the exposure to infections, increased viral reactivation, and reduced vaccine efficacy and is linked to impaired immune responses leading to pathologies (Weyand and Goronzy, 2016). Therefore majority of older adults are more prone to death by suffering from multiple chronic diseases, and they are living lives in poor health and with disabilities due to the burden of chronic diseases. This is expected to put a strain on healthcare and social costs. Lack of physical activity, progressive loss of muscle mass, and the concomitant decline in muscle strength facilitate immunosenescence and inflammation (Fulop et al., 2020). The early history of immunological changes with physical activity and importance of nutrients is poorly documented. Some recent findings on this context are reported in timeline **Figure 1**. Although research into the effects of physical activity and exercise on the immune system is ongoing, evidence suggests that long-term PA activity is beneficial for immune enhancement and infection prevention. In this review, we have discussed the role of PA and nutrition in boosting the immune system. We have also discussed PA and exercise, and their relationship to various nutritional strategies for improving the immune system.

OVERVIEW OF THE IMMUNE SYSTEM

The immune system is a complex system, and it is influenced by an ideal environment without nutrient deficiencies that are critical in immune cell triggering, interaction, differentiation, or functional expression (Zheng et al., 2020). There are two types of the immune response. Innate immunity is the first immune response that comes into play in response to invading pathogens/foreign bodies and is quick but not specific. The components of the innate immune responses are phagocytes like macrophages and monocytes, neutrophils, mast cells, eosinophils, etc. The more specific and long-term immune response is the adaptive immune response. T and B cells are the specialized cells for long-term immunity. These cells recognize the antigen elicit specific immune responses rapidly against particular pathogens. The cytotoxic T cells with CD8 receptor kill the infected cells or tumor cells, and the T helper cells with CD4 receptor help other immune cells. B cells produce different antibody or immunoglobulin (Ig) classes that target antigens and destroy the pathogens or any foreign antigens. The severity of infection varies by gender and age, and it is linked with comorbidities; as a result, the immune system develops. Several factors influence the immune system and its competence, including nutrition (Maggini et al., 2018).

RELATIONSHIP BETWEEN PHYSICAL ACTIVITY AND IMMUNE SYSTEM

The lack of sufficient daily PA is an underappreciated primary cause of the majority of chronic conditions. Overwhelming evidence supports the notion that decreases in daily PA are the primary causes of chronic diseases and rehabilitative treatment for inactivity-caused dysfunctions (Booth et al., 2017). Although the terms "physical activity" and "exercise" are frequently used interchangeably, it is essential to understand the differences. According to the Center for disease control (CDC), PA is any bodily movement produced by the contraction of skeletal muscle that increases energy expenditure at baseline level. Exercise is a type of physical activity that is planned and carried out with the goal of improving physical fitness (Seibert et al., 2019).

Majority of research conducted over the past century has investigated how physical activity affects the immune system (Campbell and Turner, 2018; Chastin et al., 2021). Recently

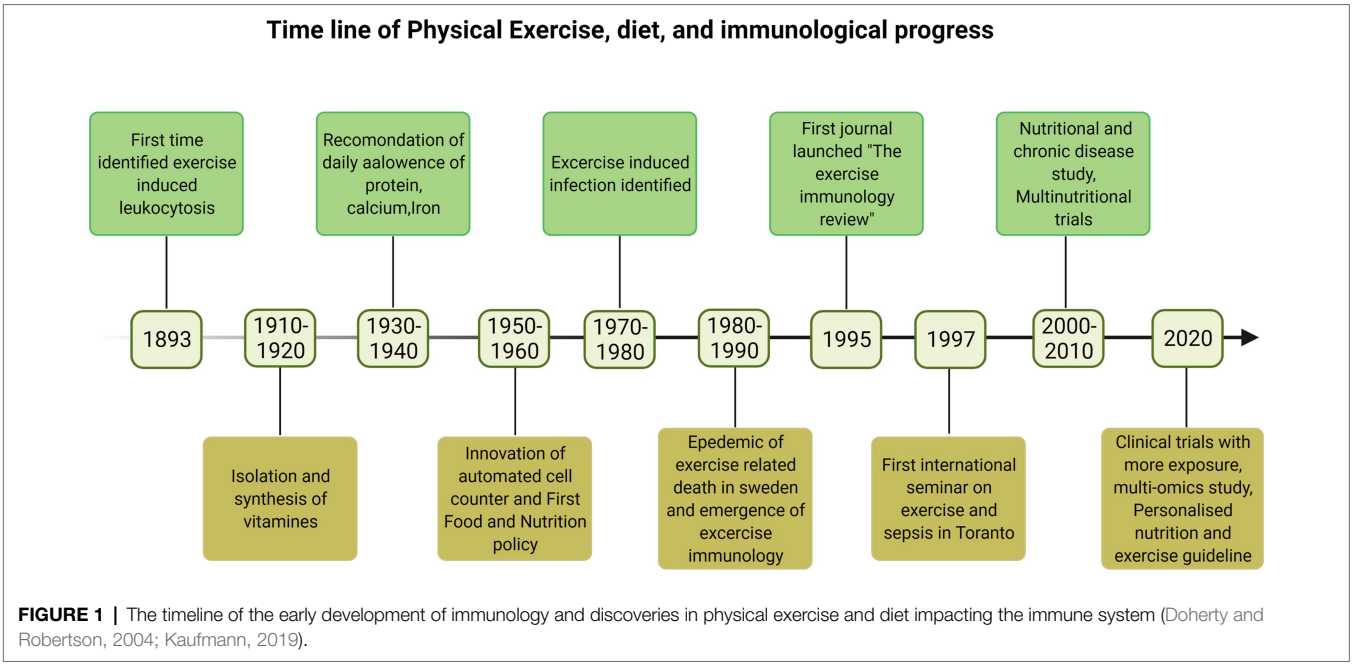

FIGURE 1 | The timeline of the early development of immunology and discoveries in physical exercise and diet impacting the immune system (Doherty and Robertson, 2004; Kaufmann, 2019).

two meta-analyses have shown that physical activity prevents the risk of upper respiratory tract infections and provides immunity, nevertheless supportive data are minimal and the results are inconclusive due to a small number of samples and unavailability of published studies (Rocco et al., 2018; Grande et al., 2020). According to the meta-analyses, regular physical activity is associated with 31% lower risk of infectious disease and 37% lower risk of infectious disease-related mortality.

Conversely, evidence is mounting that physical inactivity and its consequences, such as adipose tissue accumulation and muscle dysfunction, have a negative impact on both innate and adaptive immunity. Further, it is now widely accepted that intensive physical activity improves immune function and that once the recovery process is complete, beneficial adaptations occur. Physical activity interventions of 3–5 times per week for an average of 30 min resulted in higher CD4 T cells and salivary immunoglobulin IgA and lower levels of neutrophils. Neutrophils are the most abundant white blood cells, the primary effectors of pathogen clearance, and the first white blood cells recruited during infection.

Furthermore, IgA has several other functions; the most crucial role is to act as an anti-inflammatory response and strengthen the mucosal barrier to pathogens and the body's first line of defense (Hansen et al., 2019). They not only enhance the response and memory of other immune cells, but they are also involved in direct effector mediating pathogen clearance. The increased concentrations of CD4 T cells found in these analyses suggest that regular physical activity strengthens the immune system and results in a faster response (Swain et al., 2012; Laidlaw et al., 2016). Jung et al. (2018) found that physical inactivity and metabolic abnormalities are associated with reduced NK cell activity.

Neutrophils, in particular, play a significant role in chronic inflammation, and an elevated neutrophil count is frequently considered as a marker of chronic inflammation. A 10 years follow-up study by Hamer et al. (2012) demonstrated that physically active participants at baseline contain lower C-reactive protein and interleukin-6 levels. Thus PA may be necessary for preventing the proinflammatory state with aging. Recently Fedewa et al. (2017) found that extensive exercise training is associated with decreased CRP levels.

This mounting evidence suggests that moderate to vigorous physical activity on a regular basis may play an essential role in immune system and response effectiveness, providing enhanced protective immunity against infections. Furthermore, more research in relation to stress and recovery responses and exercise is needed to understand the beneficial effects of exercise on immune functions. This implies that people should be encouraged to engage in daily PA in order to strengthen their immune system and reduce the risk of infectious disease and infectious disease-related mortality.

AGING AND THE IMMUNE SYSTEM

The immune system represents a defense mechanism to protect the human body against foreign hosts, including bacteria and viruses, and alter intrinsic matter, such as cancer cells (Nicholson, 2016). The bone marrow is the main actor involved in the immune response. It contains hematopoietic cells responsible for the used production of immune cells, which migrate to peripheral lymphoid organs to complete their maturation and obtain the capacity to recognize non-self-components (Morsink et al., 2020).

Innate immunity refers to a non-specific and fast mechanism that can detect and destroy common microbial components. However, it is limited in the number of receptors to recognize specific pathogens. The innate response mainly includes the

anatomical and physiological barriers, such as skin and low gastric pH, and the phagocytosis mechanism, mediated by a wide range of cells, including macrophages and natural killers (NK). Conversely, adaptive immunity constitutes a more complex response, typically mediated by T and B cells, which express a multitude of receptors to permit antigen identification. This type of immunity is highly specific and requires a migration of immune cells to infection sites to enter in contact with exogenous pathogens (Zhang et al., 2014).

After naive B and T cells are formed in the bone marrow and thymus, they migrate to secondary lymphoid tissues such as the spleen. This process is robust in children to generate a diverse immune repertoire and fill peripheral lymphoid compartments. Although aging decreases the number of naive B and T cells migrating from primary to secondary lymphoid organs, B and T cell development does not stop completely, even in the elderly, some functional thymic tissue exists (Nikolich-Zugich et al., 2012; Montecino-Rodriguez et al., 2013). As we all know, an increased number of memory T cells is a well-known feature of aging. When these cells are re-exposed to the specific invading antigen, they quickly divide and express molecules such as cytokine proteins supporting the fight against infection. When the pathogens are eliminated, most effector cells die, but a small pool of protective memory cells remains, prepared to respond rapidly if reinfection occurs (Aiello et al., 2019). This phenomenon is a significant factor contributing to the accumulation of $CD8^+$ memory cells in people aged 60 years and older (Kaech and Cui, 2012).

Similarly, aging has a significant impact on B cell functions, as many other B-cell biomarkers of aging have been identified. Natural aging is accompanied by progressive biological changes in the immune system (**Figure 2**). They are linked to low recombination and somatic hypermutation of immunoglobulin genes, resulting in decreased antibody production and decreased vaccination or infection. These mechanisms are responsible for changes that occur with age in both innate and adaptive immunity. They may be responsible for autoimmune disorders, cancer, and an inadequate response to vaccination (Blomberg and Frasca, 2013).

Macrophages are among the first cells to react when exogenous materials infringe the primary human protective barriers. They accomplish many crucial functions to destroy the microbes, including triggering inflammation and phagocytosis. Furthermore, macrophages are also involved in tissue homeostasis and wound healing through the recruitment of fibrogenic factors and the elimination of cellular debris (Zhang et al., 2014). It is widely accepted that aged macrophages exhibit a lower expression of major histocompatibility complex II (MHC-II) and toll-like receptors (TLR; Handunnetthi et al., 2010), which may impair the antigen presentation mechanism and reduceds the production of interleukin (IL)-7 (Mittal and Roche, 2015). In addition, old macrophages are associated with reduced phagocytosis and chemotaxis events as well as increased levels of proinflammatory cytokines, including tumor necrosis factor α (TNFα), IL-6 and IL-1β (Turner et al., 2014; Kany et al., 2019).

Indeed, an imbalanced cytokine expression represents a hallmark of the aging process, as confirmed by an overall increase in c-reactive protein (CRP), impairment of transforming growth factor-beta (TGF β) pathway and other cytokines, such as IL-4 and IL-10 (Ferrucci and Fabbri, 2018; Kong et al., 2020). This contributes to a significant age-related reduction in inflammation, leading to a higher risk of mortality in the elderly population (Batista et al., 2020). Aging caused a decrease the production of IL-12, a fundamental interleukin required for T-cell immune response, decreases, resulting in an impairment of the antigen presentation process mediated by accessory cells such as dendritic and langerhans cells (Mbongue et al., 2014).

Another example of aging-related immune system impairment is the altered function of neutrophils, which are the main characters in the fight against microbial infection. However, there is no reduction in their number during chemotaxis, phagocytosis, and superoxide production, which leads to increased susceptibility to cell death by apoptosis (Ponnappan and Ponnappan, 2011). Mathur et al. (2008) showed some age-related functional differences by carrying out *in vitro* experiments on eosinophils collected by peripheral blood of young and old patients. Remarkably, eosinophilic cells of aged people exhibited a substantial decline in the degranulation process and a slight reduction in ROS production, which suggest an alteration of cytotoxic functions.

NK cells are leucocytes designed to destroy tumor or virus-infected cells by releasing lytic proteins such as granzymes and perforin. Older people have higher NK cells, as evidenced by more elevated specific markers, particularly CD56, compared to the young (Paul and Lal, 2017).

People regularly involved in physical activity show lower signs of immunosenescence. Recent studies highlighted a higher frequency of naïve T-cells and B regulatory cells as well as increased levels of thymoprotective cytokines in elder people regularly involved in cycling activity during their lives compared to untrained people (Duggal et al., 2018; Tylutka et al., 2021). Moreover, influenza vaccine responses are improved in elder trained population, as demonstrated by higher antibody titers against H1N1 and H3N2 strains of influenza A following 10 months of aerobic physical exercise (Simpson et al., 2015). In addition, old trained people exhibit a lower extent of inflammatory markers compared to inactive people. This could be at least partially due to the action of muscle mass, which have immunoprotective and anti-inflammatory effects by releasing protective factors called "myokines" (Pedersen and Febbraio, 2012).

On the other hand, immune system is also affected by dietary lifestyle. There is evidence that lack or deficiencies of minerals and vitamins as well as an excess of saturated fatty acids negatively affect immune responses. An important difference in terms of immunity changes can be made among healthy and undernourished elder people. Pae et al. (2012) show that old people with protein dietary deficiency exhibit an alteration of most cell-mediated immunity parameters, such as lymphocytes proliferation and cytokines synthesis, as well as reduced macrophage function and phagocytosis.

Diminished nutritional status, including an abnormal value in serum albumin, zinc or folate levels, is associated with significant change in T-cell subset and in T-cell function (Raiten et al., 2015). Moreover, a study proved that supplementation with vitamins and micronutrients, such as zinc and selenium,

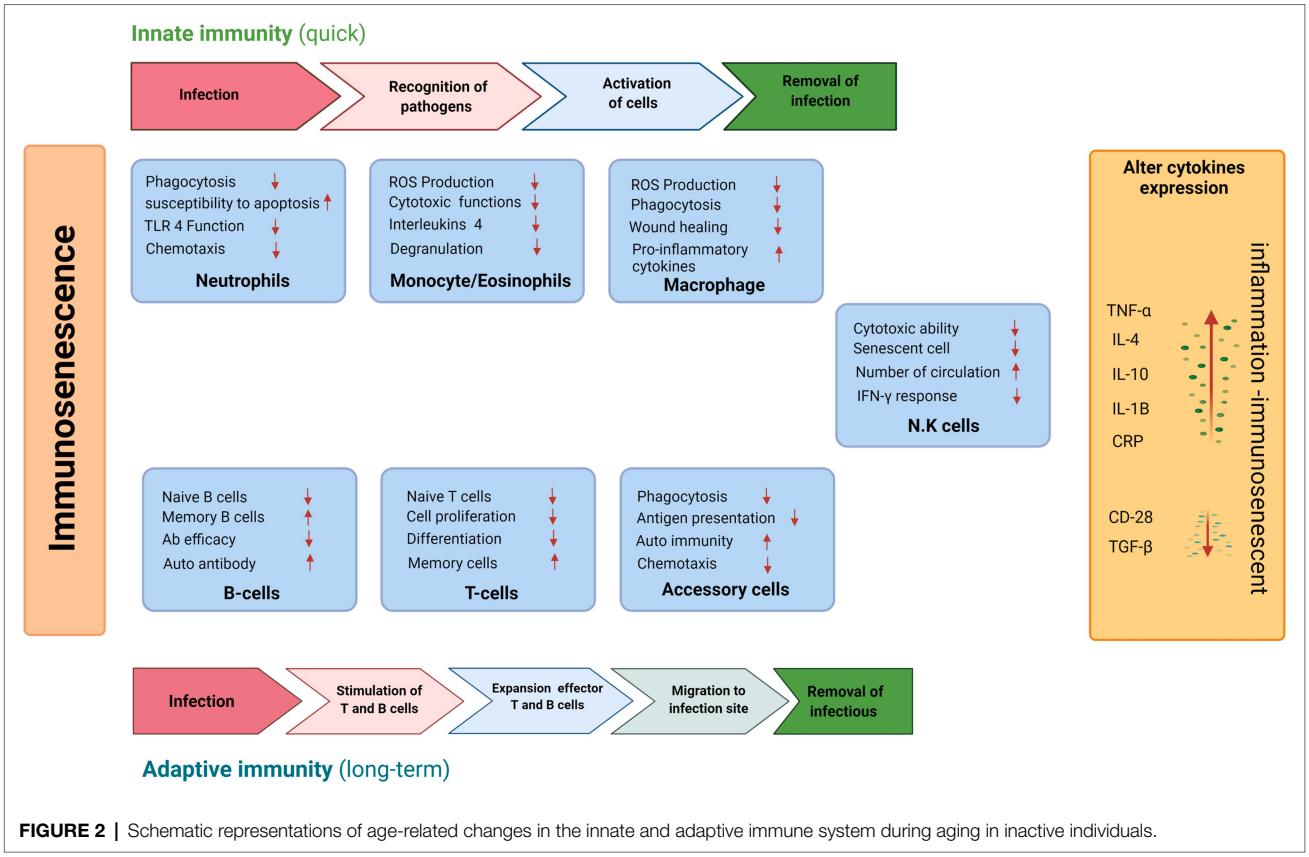

FIGURE 2 | Schematic representations of age-related changes in the innate and adaptive immune system during aging in inactive individuals.

improved immune response in old people, as justified by raise in $CD4^+$ T-cells subset, NK cells, and allowed a better antibody response against influenza vaccines (Schmoranzer et al., 2009).

THE ROLE OF NUTRITION IN THE IMMUNE SYSTEM

Nutritional interventions have been a part of some cultures or traditions like the Indian and the Chinese for a long time. Thus, nutrition intervention could be an essential therapeutic way to manage many diseases in hospitals, clinics, and homes (Reber et al., 2019). Nutrition supplementations reduce not only chronic diseases but also immune-mediated side effects. Rather than looking at energy boost, adequate and proper nutrition provides much more by enhancing the immune functions and providing a healthier life.

The immune system is the body's defense system that ensures overall health and survival; it protects the body from invading pathogens or microflora residing in the body (gut microbiome) and regulating its inner system. To combat the infection, the immune system remains in active mode all the time. There is more energy expenditure for adequate clearance of the pathogens; thus, the body's immune system needs a continuous energy supply. At this time, sufficient nutrition only can provide enough power to immune cells during the infection.

The invading pathogens lead to loss of appetite, malabsorption, increased nutrient demand, and loss of critical endogenous nutrients. The risk and severity of infection differ according to immune competence, development, maturation, and the immune system's decline. Therefore, nutrition is one of the significant factors impacting the body's immune system or defense mechanism.

Thus nutrition, primarily adequate and appropriate nutrition, is an essential determinant of optimal immune functions such as trigger of immune cells, interaction, differentiation, or functional expression of these cells. Malnutrition deteriorates the immune system and increases the vulnerability to infectious pathogens (Zheng et al., 2020). Nutrition can play a role in effective responses against pathogens and modulates the immune functions to combat stress, disease, and injury.

Nutrition can come from either an exogenous or endogenous source. Exogenous sources are diets that provide vitamins or micronutrients. Some micronutrients and dietary components play specific immune roles, such as activating immune functions. Endogenous sources, such as the stored form of energy in body cells or tissues, become instantaneous sources that can be derived through various mechanisms, such as when required, in the absence or insufficiency of exogenous nutrients.

CARBOHYDRATE

The primary energy source is carbohydrate in the human body, and it can also be found in immune cell components. It has been reported that carbohydrates can help boost the immune

system. It has been proven that carbohydrates can modulate the immune system properties as studied in exercise or athlete performance (Gunzer et al., 2012; Kerksick et al., 2018). Individuals with a poor carbohydrate diet are more prone to immune dysfunction during exercise; however, it is also helpful during exercise-induced adaption like mitochondrial biogenesis and lipolysis (Mata et al., 2019).

After exercise, a balanced and simple carbohydrate diet, such as 30–60 g/h, has immunomodulatory effects as observed in athletes. These effects are decreased systemic IL-6 release neutrophils, monocytes, lymphocytes, less suppression of $CD4^+$ and $CD8^+$ T cells, which causes leukocyte distribution and immune responses such as neutrophil degranulation and oxidative stress, lymphocyte proliferation and functions (Maydych et al., 2017; Peake et al., 2017).

However, the impact of carbohydrate supplementation to recover from upper respiratory tract infections (URTI) during strenuous exercise or games in athletes has been least explored (Nieman and Mitmesser, 2017). In this context, conflicting reports showed the ineffectiveness of carbohydrate supplementation to minimize URTI risk (Jones and Davison, 2019). While animal models have shown that a carbohydrate-rich diet reduces the risk of herpes simplex virus 1 [HSV-1] infection and mortality (Harper et al., 2021), it has also been shown that carbohydrate intake after exercise debilitates human T cells' declining migration towards human rhinovirus–infected airway epithelial cells, as seen *in vitro* (Peake et al., 2017). Therefore, more research regarding the effectiveness of carbohydrate supplementation with clinical relevance in a case like URTI is required.

Further, it has been shown that the activity of NK cells increased by taking the carbohydrate-rich diet compared with the fat-rich diet during training (Gunzer et al., 2012; Clark and Mach, 2016). These researchers have documented that a fat-rich diet is harmful to the immune system compared to a carbohydrate-rich diet, though the reason is not clear.

Carbohydrate Supplementation Before and During Exercise

Long-term carbohydrate supplementation during strenuous exercise has been shown to reduce exercise-induced circulating cytokines and support the redistribution of immune cells such as neutrophils, monocytes, NK cells, and lymphocytes (Nieman and Wentz, 2019). The immune-modulatory role of carbohydrates is more effective when there are optimal blood glucose concentrations and reduce stress hormones such as catecholamines and glucocorticoid secretion during and after exercise (Nieman and Pence, 2020). The carbohydrate intake during exercise did not change the muscle glycogen; however, the muscle glycogen has decreased in response to systemic release of IL-6 during exercise, reducing carbohydrate supplementation (Alghannam et al., 2018).

According to a study, carbohydrate consumption reduces antigen-stimulated proliferative lymphocyte responses on the second day before exercise while an increasing trend was observed in lymphocyte proliferative responses to mitogen stimulation after exercise (Bishop et al., 2005). Thus, the carbohydrate supplementation as a function of time (day) of pre and post-exercise has a crucial role in adaptive immune response and is helpful to enhance immune functions during long-term exercise or do two consecutive exercises with short recovery periods.

MICRONUTRIENTS

In the present scenario, micronutrients and immune function studies have much interest. Vitamin C (ascorbic acid or antioxidant vitamin), vitamin D, and zinc are all essential contributors to immune response. Immune dysfunction can also be caused by a lack of certain micronutrients, such as iron, zinc, and vitamin A.

It has been assumed that immune dysfunction caused by any micronutrient deficiency can be restored by consuming that individual micronutrient and adjusting to the optimal or normal level in the body (Gombart et al., 2020). The benefits and effects of micronutrients on immunity have been widely studied (Maggini et al., 2018). The deficiency of micronutrient zinc that acts as a cofactor in enzymes leads to immune dysfunction related to adaptive and innate immune response (Pecora et al., 2020). The role of zinc specifically has been found to improve patients' immunity with sepsis (Alker and Haase, 2018). Evidence also shows that additional intake of certain micronutrients (more than current RDAs) improves the immune system and reduces susceptibility to infections (Berger et al., 2021).

Antioxidant Vitamins

Vitamins E and C are the antioxidants that occur naturally in fruits and vegetables which protect the body from free oxygen radicals formed during inflammatory reaction or activation of phagocytosis of invading microorganisms (Kurutas, 2016). The recommended quantity of vitamin E is 15 mg for adult females and males, and that of vitamin C is 75 mg and 90 mg for females and males, respectively; so there could be possible health hazards if the dose exceeds. (Ristow et al., 2009) reported that intake of a higher dose of vitamins C and E in combination after 4 weeks of intense exercise prevents ROS defense-related enzymes in human skeletal muscle. Therefore, reports regarding the benefits of vitamins E and C administration alone or in combination on muscle mass and strength is also varying. Intense exercise generates free radicals that are the primary reason for muscle injury, fatigue, and poor performance (Apostolopoulos et al., 2015). This also indicates that athletes who are generally engaged in exercise seem to experience greater efficacy of intake of vitamins E and C and improvement in athletic performance when taken in adequate amount.

It has also been examined that vitamin E supplements are insufficient to improve the performances at altitude, specifically they are not effective for quick and short-term improvement in an athlete's performance. Enhanced antioxidant capacity decreases the systemic inflammatory biomarkers in elite endurance athletes at altitude (Koivisto et al., 2019). The

instances cited above signify that antioxidant supplements like vitamins E and C are more likely to hinder the anabolic signaling pathways that deteriorate the adaptations to resistance training (Dutra et al., 2019). Thus it is suggested that athletes should be aware while consuming antioxidants like vitamins E and C directly. Fruits and vegetables are good sources of vitamin E and C. These nutrients are not in concentrated form but present along with other minerals and bioactive compounds that meet the required amount of vitamins E and C.

As a water-soluble vitamin, vitamin C is considered safe and non-toxic with a few side effects, so many used to take a high dose of vitamin C supplements (Hemila and Chalker, 2013). The routine and regular consumption of vitamin C is very well known to reduce the chances of common cold and, if contracted, reduce the duration of common cold (Hemila and Chalker, 2013). Vitamin C also showed an inverse relationship with phagocytosis (Fantacone et al., 2020). There are cases where the increased antioxidant is beneficial; for example, high doses of antioxidant vitamins (10× RDA) have affectively minimized the chances of infection in ultra-endurance athletes (Salehi et al., 2018). In football players, reduction in oxidative stress has been documented when vitamins C and E were consumed in combination (De Oliveira et al., 2019). Administration of vitamin E alone before exercise under the hypoxic condition at high altitude restores the interleukin (IL)-6, TNF-α, IL-1ra and IL-10 to normal level (Santos et al., 2016). It has been suggested that it might be due to antioxidant like vitamin C mediated decreased level of stress hormone and cytokine production.

A high antioxidant content in body attenuated IL-6 responses to a stressor (exercise) through reducing IL-6 production in healthy individuals (Walsh, 2019). Reports also purported about the side effects of intake of high dose single type of antioxidant. A high doses of vitamin E potentially acts as modulator proinflammatory agents, oxidative stress and NF-κB pathway having role in neuroprotection and cancer (Ungurianu et al., 2021). However, a high dose of antioxidant seems to be favorable to individuals at high risk of URTI to enhance the immunity to reduce URTI risk (Davison et al., 2014). It has been alleged that the high concentration of these antioxidants interferes with the normal physiological functions and decreases benefits of physical activity or the normal training response such as decrease in muscle strength (Paulsen et al., 2014).

Vitamin D

Vitamin D is an essential nutritional supplement that strengthens the bones and is vital for calcium and bone homeostasis. Substantial evidence supports the role of low vitamin D content with compromised immunity and increased risk of illness. The role of vitamin D has been explored in immune function like deficiency of this vitamin has been linked to increased susceptibility to infection. An association between vitamin D content and Acute Respiratory Tract Infection risk has also been observed (Pham et al., 2019).

Vitamin D is a secosteroid hormone known to interact with more than 200 genes *via* nuclear receptors expressed on various tissues of the body (Nurminen et al., 2019), including the immune cells. Thus vitamin D facilitates the innate and adaptive immune responses and modulates a wide range of physiological functions. The immune cells such as B cells, T cells, and antigen-presenting cells expressed the receptor for the vitamin D and can synthesize active vitamin D metabolite. Vitamin D receptors are important to activate the cathelicidin antimicrobial peptide (CAMP) in activated monocytes or macrophages. Vitamin D participates in innate antimicrobial response through activation of Toll-like receptors (TLR) that increased the expression of both 1-α-hydroxylase and vitamin D receptors (VDR; Di Rosa et al., 2011). It inhibits B cell proliferation and blocks B cell differentiation and immunoglobulin secretion (Medrano et al., 2018). In addition, vitamin D acts in an autocrine manner to elicit an immune response. Deficiency in vitamin D has been shown to augment autoimmunity besides increasing the risk of infection. Vitamin D supplementation is thus helpful to individuals suffering from vitamin D deficiency mediated autoimmune diseases.

Vitamin D also has an inverse relationship with the reactive oxygen species production (Berridge, 2016). The majority of individuals from different countries, primarily from developed countries, suffer from vitamin D deficiency (Sizar et al., 2021). Though there are dietary sources for vitamin D, it is mainly synthesized beneath the skin when exposed to solar ultraviolet B radiation from sunlight. Thus seasonal availability of direct sunlight, for example, lack of solar light in winter, affects vitamin D synthesis, especially in temperate countries.

Accordingly, vitamin D deficiency can be adjusted with intake of vitamin D rich diet or exposure to sunlight. Once the normal level is achieved, there may be no further benefits from further vitamin D consumption. Studies to date are mostly cross-sectional or observational. Only the beneficial effect of vitamin D supplementation and deficiency surfaced the respective vitamin as an index of compromised immune function. Indeed vitamin D intervention based on more research could evaluate the actual impact of vitamin D deficiency or overdose.

Multivitamin and Mineral Supplement

Studies show that a multivitamin and mineral supplement (MVM) improves individuals' immune functions and immune status, as documented in older people with deficiency in minerals and vitamins. There is a natural decline in immune functions during aging, such as decrease in neutrophil function that amplifies by diet deficit in multiple vitamin and mineral lacks (Zhang et al., 2015a; Maggini et al., 2018).

A MVM supplement contains at least three vitamins and at least one mineral; therefore, combinations of nutrients (multivitamins and mineral supplements) could be a more effective means to recover from a deficiency than a single nutrient, which may imbalance the nutrient content in the body (Bird et al., 2017). Experimentally, a good supplement of MVM containing a high amount of many redox-active and immunomodulatory micronutrients such as Redoxon® Vita Immune (VI) MVM supplements has been studied. This product intake for at least 12 weeks reduces the incidence of infection

in older adults. It boosts serum vitamin C and zinc content compared to older adults without MVM supplements who were more susceptible to infections (Fantacone et al., 2020). However, the respective authors did not observe any significant changes in neutrophil function compared to a placebo group; this may account for the participants' optimal blood and zinc content.

Additionally, the deficiency indicates physiological demand for normal immune functions or vitamins/minerals for enhancing athletic/training performance. Furthermore, nutrients obtained from food rather than from supplements could provide a natural blend of nutrients, accomplishing the goal of a multi-nutrient supplement. It may also provide nutrients that work synergistically and are more beneficial than single nutrients.

Supporting this, it has been observed that pregnant women with both fruits and vegetables in their diet showed a moderate reduction in upper respiratory tract infection (URTI) during pregnancy compared to women without or with fruit or vegetables food consumption (Li and Werler, 2010). It would seem that a potentially beneficial strategy is to eat more fruits and vegetables as part of a healthy and balanced diet.

Micronutrients are critical for the proper function of the immune system and play a vital role in promoting health and nutritional wellbeing when micronutrients are taken from a nutritionally balanced and diverse diet like fruits and vegetables and animal source foods. As per various epidemiological studies, the Mediterranean diet is a dietary type that is believed to be rich in balanced micronutrients and considered as one of the healthiest nutritional guidelines (Guasch-Ferre et al., 2017; Carlos et al., 2018).

Dairy-Derived Supplements or Functional Foods

Bovine Colostrum

Bovine colostrum, also known as "early milk," is the initial milk and is abundant in bioactive components produced by cows during the first 48 or 72 h after birth. It is enriched in nutrients, both in terms of macronutrients and micronutrients compared to milk. The colostrum plays a vital role in improving body composition by building muscle mass and strength (Blair et al., 2020). Natural supplements help to lose body fat and muscle healing after strenuous exercise in athletes (Yoon and Kim, 2020). It has been shown that uptake of bovine colostrum (60 g/day) for 8 weeks with resistance training increases the leg press strength and decreases the bone resorption in older adults, male and female, compared to older adults taking whey protein supplements (Willoughby et al., 2018). A similar finding has been reported in physically active young males and females with bovine colostrum supplements (20 g/day) for 8 weeks (Główka et al., 2020). The decrease in body mass while taking colostrum could be attributed to the burning of fat. Additionally, colostrum is rich in calcium, and calcium is an essential factor in regulating lipid metabolism by reducing adipocyte lipid deposition and increasing lipolysis (Zhang et al., 2019).

Colostrum is abundant in immunoglobulins like IgG, IgA, and IgM that confer immunity (McGrath et al., 2016). In addition, bovine colostrum are a good source of other bioactive components such as growth factors like epidermal and platelet-derived growth factors, cytokines, vitamins like A, B, C, D, E, K, and antimicrobial factors antioxidant enzymes (McGrath et al., 2016). In recent years bovine colostrum has been studied in relation to nutrition and immunology for its role to confer immunity and good health. Recently, Davison (2021) in his review elaborated in detail about the immune properties of colostrum such as boosting immune functions or activating the immune cell by increasing neutrophil, oxidative burst and degranulation, as well as cytokine production by neutrophils and peripheral blood mononuclear cells. In addition, the low molecular weight fractions of bovine colostrum consumption supplement the phagocytic activity of monocyte and polymorphonuclear, along with momentary changes in the number of NK cells in gut mucosa (Jensen et al., 2012).

It is hypothesized that consumption of low molecular weight components of bovine colostrum elicits transient accumulation and release of new NK cells. Thus, the benefits of micro fractions of bovine colostrum in humans that are easily digested or pass through gut lining could be envisioned. However, such a hypothesis needs intensive investigation. Supplementation of bovine colostrum of 20 g/day in male subjects for 4 weeks acts as an immune booster in exercise-induced stress through significant increase in the neutrophil function and salivary lysozyme while lack of such effect has been observed after 12 weeks of regimen (Jones et al., 2019). Of course, reduced salivary bacteria amount and incidence of upper respiratory illness during winter while doing intensive exercise have been documented. Even a low dose (3.2 g/day) of bovine colostrum for 3 days has been found to be effective in minimizing exercise-induced muscle damage (EIMD) without deteriorating the performance in soccer players (Kotsis et al., 2018).

Some studies also showed that after colostrum supplementation, secretary IgA concentration remained unchanged compared to the control group observed in IgA-deficient children through decreased infection severity or in athletes (Patiroglu and Kondolot, 2013; Shing et al., 2013). It was observed that individuals taking vaccination of oral *Salmonella typhi* Ty21a along with bovine colostrum for 1 week showed an increasing pattern of IgA compared to controls though non-significant (He et al., 2001). Thus, as mentioned earlier, the results reflect and produce evidence favoring bovine colostrum beneficial effects by boosting immunity and rendering resistance to infection to normal healthy subjects or athletes doing intensive exercise.

In summary, the available evidence to date suggests that the benefits of consuming bovine colostrum are manifold. It benefits not only immune-compromised old adults or exercise-induced stressful athletes but also active humans. Research reports show the bovine colostrum supplementation manifests its beneficial effects just after 1 h of consumption to 12 weeks regularly. However, it seems that the duration of supplementation impacts the different types of immune functions. Being easily digestible and nutrient-rich has multiple functions, such as eliciting a systemic immune response in the gastrointestinal part and reducing upper respiratory tract infections. The duration and dose of the bovine colostrum need to be standardized to increase its efficacy for overall health benefits.

Probiotics

Probiotics are living microorganisms favorable to humans and are present in certain food items or provided as food supplements that reach the intestine in sufficient numbers to benefit the host's health. It is well known that around 100 trillion microorganisms harboring in the human gut that coevolved prevent pathogenic bacteria from transmitting through the gut, or else the imbalance cause diseases that are known during the time of Hippocrates (460–370 EC), who alleged that "all diseases begin in the gut" (Terpou et al., 2019).

The role of diet and other environmental factors modulating the density and metabolic activity of the human gut microbiota is recognized. Many beneficial bacteria are transported to gut *via* fermented foods such as fermented milk or dairy products (curd). Therefore the microorganisms, which are helpful for human health, have been commercialized in the form of probiotics. Initially, it was considered that probiotics only improve gut health; nonetheless, ample evidence supports the role of probiotics in conferring many aspects of immunity, specifically adaptive immunity (Azad et al., 2018). An adequate amount of probiotics positively modulates immunity by enhancing the intestinal mucosa and epithelial health's pathogen blocking or barrier functions (Monteagudo-Mera et al., 2019). Specifically, probiotics proved helpful to people with poor immune systems suffering from frequent gastrointestinal infections caused by enteric bacteria, *Staphylococcus* and antibiotic-associated imbalance in gut microflora (Kothari et al., 2019; Stavropoulou and Bezirtzoglou, 2020). It has been reported that the gut microbial flora interacts with intestinal mucosal epithelial cells and activates the T17 helper cells (T_H17) and regulatory T cells (T_{reg}) that are involved in inflammatory response against helminths (Pandiyan et al., 2019).

The commercial probiotics also contain other strains of beneficial bacteria such as *lactobacillus*, *Bifidobacterium*, *Propionibacterium*, *Faecalibacterium*, and specific yeast species (Fijan, 2014). However, only certain strains are efficient and valuable to provide immunity and effectively combat infection when appropriately consumed. In addition, the immunity varies depending upon an individual's age, gender, genetic constitution, and health status. Therefore the standard for the formulation, dose, and microbial strain type of probiotics should be considered as stated prior to treatment.

Several reports documented a negative impact on health as an outcome of the interaction between probiotics and host gut microbes, specifically in immune-compromised risk populations. The various adverse effects of probiotics are seen in risk groups such as people suffering from bacterial or fungal sepsis, systemic and localized infection, allergies, transfer of antibiotic resistance genes and bowel ischemia (Sanders et al., 2010; Zhang et al., 2015b). Majority of the adverse effects referred are associated with the *Lactobacillus*, *Bifidobacterium*, and *Endocarditis* species in probiotics. Besides, probiotic strains can also display antagonistic effects towards the host gut microbes like nutrient competition, co-aggregation with pathogens, and elicit response (De Melo Pereira et al., 2018).

Probiotics are also helpful in reducing upper respiratory tract infection (URTI) incidence and duration, though the reports are meager. A double-blinded, randomized controlled study has shown that if probiotics with specific bacterial composition are administered for 12 weeks, it significantly reduces the URTI and cold symptoms in the probiotic group compared to placebo with a significant increase in immune components such as IFN-γ and sIgA in serum (Zhang et al., 2018). A decrease in duration of RTI symptoms and severity of symptoms with an increase in immune cells like T helper cells ($CD4^+$) and T suppressor cells ($CD8^+$) has also been noted after probiotics administration (Kim et al., 2018).

Probiotics have been observed to augment the serum cytokines, the efficiency of influenza vaccine and minimizing occurrence and duration of respiratory infections in COVID patients, so probiotics as an adjuvant to treat the COVID patients have been advocated (Darbandi et al., 2021). The reports regarding the effects of probiotics on the occurrence, duration, and severity of viral respiratory infections are inconsistent. In healthy adults, consuming a probiotic of specific strains followed by influenza virus vaccination resulted in a significant decrease in the frequency of antigen-specific IgA and T-helper type 1 response (Vitetta et al., 2017). The research reports regarding the response of probiotics to the vaccination are mostly in healthy adults or different probiotic strains. Therefore, randomized and placebo-controlled adjusted for age, vaccination, and composition of probiotics and administration duration may come out with overall benefits of probiotics (Maidens et al., 2013; Lehtoranta et al., 2014).

The side effect of antibiotics can also be reduced through probiotics following treatment. A large meta-analysis on 2,972 patients in intensive care units suffering from ventilator-associated pneumonia (VAP) showed a decrease in infection rate and incidence of VAP, suggesting that probiotics treatment is a promising way to treat patients (Manzanares et al., 2016). Nonetheless, more trials are recommended with different groups of patients to avoid side effects and increase the efficacy of probiotics. The reports from studies in animal models infected with influenza virus and then oral or mucosal administration of different strains of probiotics bacteria *Lactobacillus* also support and confirm earlier findings of using probiotics as a nutritional supplement or immune booster (Aponte et al., 2020).

Recent evidence and ongoing research suggest that the intestinal microbiota have a bidirectional effect on mood and psychiatric disorders. A meta-analysis of existing research reports showed administration of probiotics reduces depression, and it depends upon the dose and microbiota strain of the probiotics (Ng et al., 2018). Probiotics also treat inflammatory bowel disease and enhance cognitive functions through the gut-brain axis (Wasilewski et al., 2015; Bagga et al., 2018; Bermudez-Humaran et al., 2019; Zagorska et al., 2020).

It has been observed that the physically active people like athletes' guts are rich in microbial population compared to those of sedentary people evaluated through fecal content (Clarke et al., 2014). Probiotics have been considered as important ergonomic supplements for improving the physical performance in athletes on short- or long-term consumption that is 2 weeks to 3 months besides, significant difference has been observed in the different diet and physical activity level between physically

active and sedentary people (Marttinen et al., 2020). The gut microbiota influence an individual's physical performance by modulating digestion and energy harvest during strenuous physical activity (Nay et al., 2019). A study in animals and humans found that probiotic microbe supplementation increased muscle mass, energy generation, and improved physical performance (such as Lactobacillus plantarum TWK10), or were able to modulate systemic and airway immune functions, such as serum TNF-level reduction immediately after marathon (like Lactobacillus casei Shirota; Chen et al., 2016; Vaisberg et al., 2019).

Prebiotics

The other way to modulate the gut microflora is through prebiotics; these are the undigestibale and unabsorbed food ingredients (mostly the oligosaccharides) that help to harbour useful bacteria population like Bacteroidetes and Firmicutes, and their activity for the host gut health (Vieira et al., 2013). Intake of fiberous food acts as prebiotics in the gut and modulates the mucosal immune functions and reduces the risk of enteric inflammation and like by increasing anti-inflammatory cytokines and reducing proinflammatory cytokines and the systemic immune response (Shokryazdan et al., 2017). The undigested food can also concert to short-chain fatty acids like butyrate that is absorbed by the gut and helps to reduce inflammatory disorders through increasing of T-regulatory cells (Treg) and reducing IFN-γ.

PLANT-DERIVED AND HERBAL SUPPLEMENTS

Echinacea

Echinacea is the common name for a genus of plant species native to North America, also known as coneflower. *Echinacea purpurea* (purple coneflower) is most commonly used as herbal medicine to treat respiratory infections and in immune intervention studies. Echinacea has been observed to minimize the common cold incidence by around 58% and lessen the duration of infection by about 1.4 days (Shah et al., 2007). The administration of commercial-grade of *Echinacea purpurea* supplement for 28 days has been observed to improve the immune functions in the mucosal area after strenuous exercise in three consecutive 30 s Wingate cycling tests by reducing the magnitude of exercise-induced decrease in salivary IgA concentration and release (Hall et al., 2007).

Hall et al. (2007) suggested that the possible immune function mechanism could increase cytokine production like IL-6 or 1, tumor necrosis factor, and activation of phagocytes and lymphocytes. *In vitro* cultures also reported that fresh *Echinacea purpurea* juice activates macrophages to produce cytokine (Lim, 2014). Several bioactive components are obtained from different parts of Echinacea, such as glycoproteins, caffeic acid phenolic compounds, flavanoids responsible for conferring both innate and adaptive immunity. A recent review elaborated on Echinacea's immunomodulatory functions, such as inhibiting $CD4^+$ and $CD8^+$ T lymphocytes and improving macrophage phagocytosis by increasing lysosomal activity and nitric oxide production, re-establishing splenic NK cell activity, activating Th1 and Th2 cytokines for antibody production (Nagoor Meeran et al., 2021). However, a meta-analysis by Karsch-Völk et al. (2014) found that many Echinacea products slightly reduce the risk of catching a cold by 10–20% in healthy individuals.

The effectiveness depends upon the species, plant parts and bioactive content. In this line, Wang and Frueh, also did not notice any adequate treatment of common cold using Echinacea extract (Wang and Frueh, 2015). Echinacea has been shown in numerous studies to modulate both systemic and local immunity. The mechanisms immunomodulatory role of Echinacea supplements is not known currently. Nevertheless, it seems that the benefits come from bioactive compounds such as caffeic acids and derivatives, phenolic compounds, flavonoids, and alkalines (Sellami et al., 2018).

POLYPHENOLS AND OTHER PLANT-DERIVED SUBSTANCES

Polyphenols are the compound that act as antioxidants and are present in medicinal plants as secondary metabolites, fruits, nuts, seed, vegetables, spices, cereals, and beverages. Polyphenol protects from harmful ultraviolet radiation and invading pathogens (Tungmunnithum et al., 2018). Flavonoids having various types (flavonols, flavones, and flavanones) show similar biological activities. Flavones include apigenin, baicalein, luteolin, and rutin. Quercetin and kaempferol are examples of flavonols. Hesperidin and naringin are two flavanones with growth-inhibitory effects in cancers such as colon, prostate, liver, stomach, cervix, pancreas, breast, and leukemia (Kumar and Pandey, 2013; Zhou et al., 2016).

Polyphenol consumption in diets is also reported to prevent the risk of developing a chronic and fatal disease like cardiovascular disorder, cancer, atherosclerosis, diabetes, osteoporosis and neurodegenerative diseases (Kozłowska and Szostak-Węgierek, 2017; Behl et al., 2020). Polyphenols like flavanoids or quercetin effectively cure cardiovascular disorders due to their antioxidant, anti-platelet, anti-inflammatory properties, augmenting endothelial or blocking expression of metalloproteinase 1 (MMP1) functions or chronic diseases cancer (Li et al., 2016). Polyphenols with immunomodulatory functions, such as aglycones epicatechin and catechin extracted from almond, demonstrated antimicrobial and antiviral activities against *S. aureus* and reduced herpes simplex virus titer *in vitro* (Musarra-Pizzo et al., 2020). Recently the oral administration of polyphenols to patients suffering from Nickel-Mediated Allergic Contact Dermatitis showed altered decease inflammatory biomarkers like interferon-γ, IL-4, IL-17, pentraxin three and NO. At the same time, the placebo group did not exhibit any change (Magrone et al., 2019).

The importance of polyphenols in URTI has also been studied. The incidence of exercise-induced URTI in cyclists has been minimized within 2 weeks by taking 1,000 mg of quercetin daily for 3 weeks before and 2 weeks after 3 days

(Myburgh, 2014). The daily consumption of nonalcoholic beer containing polyphenol in a fix quantity 1–1.5 L/day for 3 wk. before and 2 weeks after a marathon decreases the URTI incidence and IL-6 and blood leucocytes (Scherr et al., 2012). On the other hand, food items like dark chocolate having polyphenol eaten for 2 weeks did not exhibit any significant immune effect measured through serum cytokine and cortisol despite a decrease in some oxidative stress markers (Stellingwerff et al., 2014). In conclusion, reports substantiated the benefits of polyphenols in some cases of providing immunity against the risk of URTI and combating infection. Nonetheless, the mechanisms behind this immunomodulatory role are still unclear, and further research could elucidate the mechanisms and pathways of the role of polyphenols in immune functions.

In summary, evidence is emerging for a potential beneficial effect of polyphenols on URTI risk and infection clearance. However, the mechanisms remain to be elucidated and more research is required to determine whether polyphenols should be recommended for immune support.

EFFECTS OF EXERCISE AND DIET/NUTRITION ON IMMUNITY

Evidence shows that some benefits, particularly physical activity and a proper diet, can reinforce the immune mechanisms and prevent the inflammaging process (Weyh et al., 2020). It has been demonstrated that active exercise ameliorates the overall immune response in regularly trained people, conferring a wide range of advantages. As shown by the pioneering work of Nieman and Wentz (2019) women recruited for their regular practical exercises exhibited higher NK and T lymphocyte function compared to sedentary women of the same age who underwent 12 weeks of exercise.

Among the beneficial effects of physical activity, lowering of $CD14^+$ and $CD16^+$ monocytes can also be counted, representing vital proinflammatory cells involved in the onset of immune diseases, such as rheumatoid arthritis and cardiovascular disorders (Ma et al., 2019; Mannucci et al., 2021). The role of exercise in regulating the expression of TLR (full form) was examined in individuals who underwent intense physical activity. TLRs are highly expressed on the cell surface of immune cells such as monocytes and macrophages. These receptors play an essential role in innate immunity and adaptive immunity, stimulating antimicrobial activity by activating the nuclear factor-kappa B (NFkB) pathway and releasing proinflammatory cytokines (Vijay, 2018).

As shown by Oliveira and Gleeson (2010) sustained cycling activity was able to reduce the TLR4 expression significantly by 45% after 1 h of intense training in comparison with the control group. However, this effect was not permanent. The downregulation of TLR after prolonged exercise is not fully understood yet. Still, it may be caused by high levels of IL-6 released after training, which may compete with the physiological NFkB pathway of TLR, as discussed in several studies (Favere et al., 2021). Moreover, IL-6 secretion exhibits proinflammatory properties by stimulating the release of immunity regulators, including IL-10 and IL-1 receptor agonist (Dinarello, 2018).

According to the study of Spielmann et al. (2011) aerobic activity influences the frequency of T cells, leading to a decrease in senescent $CD4^+$, $CD8^+$, and CD28-CD57 T cells. In contrast, it causes a rise in the number of naïve $CD8^+$ T cells. The reduced amount of senescent T cells can be explained through the mechanism of cell death, such as apoptosis, induced by intense physical activity (Minuzzi et al., 2018).

Although moderately exercising people experience less mortality and lower risk of infections than sedentary individuals, evidence also shows a higher tendency to suffer from upper respiratory tract infections (URTI) during intense or prolonged exercise in athletes. This may be due to changes in inflammatory pathways following low physical activity, resulting in higher susceptibility to pathogens (Jones and Davison, 2019).

Another interesting approach to strengthening the immune system is provided by nutritional strategies, which should work in synergy with exercise to maintain immune response efficiency. Proper nutrition must respond to pathogen stimuli, such as during fever, and avoid chronic inflammation. Conversely, malnutrition negatively affects the immune functions with variable effects according to patient age and extent of nutritional deficiency.

A plethora of micronutrients have been studied for their multiple properties in the maintenance of the immune system. The role of zinc as a cofactor for many proteins has been widely studied. As indicated by Andreini et al. (2006) more than 2000 human proteins are zinc-bonded to exert their physiological functions. The importance of zinc as a modulator of the immune system is evidenced by the impairment of innate and adapted immunity in zinc deficiency. In detail, decreased amount of zinc causes lower cytotoxicity of NK cells, reduced phagocytosis capacity of neutrophils, auto-reactivity of T cells and higher apoptosis rate of B cells (Maywald et al., 2017).

Glutamine is a pivotal modulator of the immune system, as observed through its ability to enhance the phagocytosis activity in macrophages, lymphocyte proliferation and cytotoxic activity in neutrophils. Glutamine is rapidly consumed by immune cells, especially during diseases, reaching a similar metabolic rate as glucose. Therefore, glutamine is generally used in the clinic routine for dietetic supplementations of immune depress patients (Cruzat et al., 2018).

Vitamin D is also thought to play an important role in modulating the immunity system, as confirmed by higher inflammation and increased risk of diabetes and rheumatoid arthritis in Vitamin D-deficient patients. Vitamin D exerts positive effects on innate immunity by increasing autophagy and chemotaxis in immune cells and reinforcing human physical barriers, such as intestinal and corneal tissues (Chirumbolo et al., 2017). Although vitamin D seems to stimulate regulatory T cells' activity in many diseases, there are not enough data in the literature confirming a significant role of vitamin D in the regulation of adaptive immunity.

There is a strong link between gut microbiota and immunity because microbiota trains and stimulates immune system

TABLE 1 | Common nutritional intervention and key food involved to stimulate human immune system.

Nutritional intervention and immunity	
Carbohydrate intervention	• Restore exercise-induced immunodepression (Gunzer et al., 2012; Kerksick et al., 2018), role in endurance-exercise-induced adaption like mitochondrial biogenesis and lipolysis (Mata et al., 2019). • Immunomodulatory effects like decrease in systemic IL-6 release neutrophils, monocytes, lymphocytes, reduce suppression of $CD4^+$ and $CD8^+$ T cells, causes leukocyte distribution, neutrophil degranulation and oxidative stress, lymphocyte proliferation and functions (Maydych et al., 2017; Peake et al., 2017). • Increased-natural killer (NK; Gunzer et al., 2012; Clark and Mach, 2016). • Minimized exercise-induced increased circulating cytokines, redistribution of immune cells like neutrophils, monocytes, NK cells and lymphocytes (Nieman and Wentz, 2019). • Upper respiratory tract infections (URTI) recovery (Nieman and Mitmesser, 2017); reduction in risk of infection and mortality from viral infections like herpes simplex virus 1 [HSV-1] (Harper et al., 2021).
Micronutrients including (antioxidant vitamins-vitamins C and E); vitamin D and multivitamin	• High doses of antioxidant vitamins (i.e., 10× RDA, recommended dietary allowance) effectively minimized the chances of infection in ultra-endurance athletes (Salehi et al., 2018); high dose of antioxidant decreased risk of URTI (Davison et al., 2016). • Vitamins C and E in combination reduce oxidative stress in athletes (De Oliveira et al., 2019) • Vitamin C inversely related to phagocytosis (Fantacone et al., 2020) • Vitamin E before exercise under hypoxic condition at high altitude restores interleukin (IL)-6, TNF-α, IL-1ra, and IL-10 to normal level (Santos et al., 2016); decreases systemic inflammatory biomarkers in elite endurance athletes at altitude (Koivisto et al., 2019); in high dose acts as modulator proinflammatory agents, oxidative stress and NF-κB pathway having role in neuroprotection and cancer (Ungurianu et al., 2021). • An association exists between vitamin D content and Acute Respiratory Tract Infection risk (Pham et al., 2019). • Vitamin D participates in innate antimicrobial response through activation of Toll like receptors (TLR) that increased expression of both the 1-α-hydroxylase and vitamin D receptors (VDR; Chun et al., 2014). • Vitamin D decreases reactive oxygen species production • Neutrophil function decreased substantially in individuals with diet deficit in multiple vitamins and mineral lacks (Maggini et al., 2018). • MVM supplements for at least 12 weeks reduce the incidence of infection, increased vitamin C and zinc content (Fantacone et al., 2020) • Zinc improves immunity of patients with sepsis (Alker and Haase, 2018)
Bovine colostrum	• Improves body composition increase, muscle mass and strength and burn fat (Davison, 2021) • Confer immunity; abundant in immunoglobulins like IgG, IgA and IgM that (McGrath et al., 2016). • Good source of other bioactive components such as growth factors like epidermal and platelet-derived growth factors, cytokines, vitamins like A, B, C, D, E, K and antimicrobial factors like antioxidant enzymes (McGrath et al., 2016). • Boost immune functions or activating the immune cell by increasing neutrophil, oxidative burst and degranulation, as well as cytokine production by neutrophils and peripheral blood mononuclear cells (Davison, 2021).
Probiotics	• Enhances pathogen (enteric bacteria and staphylococcus) blocking or barrier functions of intestinal mucosa, epithelial health; confer adaptive Immunity (Georgieva et al., 2015; Terpou et al., 2019; Stavropoulou and Bezirtzoglou, 2020). • In risk groups leads to bacterial or fungal sepsis, systemic and localized infection, allergies, transfer of antibiotic resistance genes and bowel ischemia (Kothari et al., 2019). • Some strains show antagonistic effects towards the host gut microbes like nutrient competition, co-aggregation with pathogens, and elicit response (De Melo Pereira et al., 2018). • Reduce URTI and cold symptoms with a significant increase in IFN-γ and sIgA in serum (Zhang et al., 2018). • Increase serum cytokines, efficiency of influenza vaccine and minimizing occurrence and duration of respiratory infections in COVID patients (Darbandi et al., 2021). • Reduce infection rate and incidence of ventilator-associated pneumonia (VAP; Manzanares et al., 2016).

(Continued)

TABLE 1 | Continued

Nutritional intervention and immunity	
Plant-derived and herbal supplements (including Echinacea, polyphenols and other plant-derived substances)	• Provide both innate and adaptive immunity through its bioactive components (glycoproteins caffeic acid phenolic compounds, flavanoids; Jain and Pasare, 2017) • Have immunomodulatory functions like inhibiting $CD4^+$ and $CD8^+$ T lymphocytes and enhancing the phagocytosis ability of macrophages by increasing the lysosomal activity and nitric oxide production, re-establish splenic NK cells activity, activating Th1 and Th2 cytokines for antibody production (Abel et al., 2018) • Possess antioxidant and antibacterial properties, growth-inhibitory effects in different cancers like colon, prostate, liver, stomach, cervix, pancreas, breast, and leukemia (Zhou et al., 2016; Sharifi-Rad et al., 2018) • Prevent the risk of disease like cardio-vascular disorder, cancer, arthrosclerosis, diabetes, osteoporosis and neurodegenerative diseases (Kozłowska and Szostak-Węgierek, 2017) • Modulate gut immune system by increasing intraepithelial T cells and mucosal eosinophils, and activating inflammatory response against helminthes parasites (Motran et al., 2018) • Altered decease inflammatory biomarkers like interferon-γ, IL-4, IL-17, pentraxin 3, and NO in patients of Nickel-Mediated Allergic Contact Dermatitis (Magrone et al., 2019)

function, compensating for maintaining a symbiotic relationship with gut hosts. In addition, many studies have found that gut microbiota can help regulate immune homeostasis in innate and adaptive responses (Belkaid and Hand, 2014; Belkaid and Harrison, 2017).

According to the literature, exercise and diet modifications have a strong combined effect on immune system. This effect, however, can vary depending on the type, intensity and duration of the interventions. As shown by Roberts et al. (2006) the combination of daily aerobic exercise and a low-fat, high fiber diet in healthy patients and patients with metabolic syndrome induced a reduction of inflammation, leucocytes adhesion and chemotaxis capacity, and oxygen species production as well as a decrease in metabolic syndrome markers after 3 weeks of treatment. Moreover, the experiments by Hill et al. (2007) suggested combining diet and exercising interventions to enforce the immunity and to prevent obesity, cardiovascular diseases and diabetes mellitus. Indeed, supplementation for 12 weeks with fish-oil and moderate physical activity in overweight and obese volunteers was able to decrease the oxygen species production and maintain the bactericidal activity of neutrophils.

Additionally, Meksawan et al. (2004) demonstrated that different dietary fat intakes, associated with a short and intense exercise, gave variable effects in controlling pro-inflammatory markers after 3 weeks of interventions in untrained healthy patients. Physical activity and proper nutrition or diet habits affect the process of immunosenescence and inflammation (Weyh et al., 2020), and boost the immunity that in turn improve the quality of life (Davison et al., 2016). In older adults, exercise and dietary protein intervention have shown to prevent muscles loss, improved muscle functions and quality of life (Strasser et al., 2018). Kommers et al. (2019) examined a positive association of probiotic supplementation on quality of life of constipated young female, while no impact of intense exercise on quality of life. Though there is meager report on the commercial immune booster supplements and active physical exercise in relation to improve or maintain a healthy lifespan, the nutritional supplements like single or MVM, dairy derived supplements, probiotics; all indeed act as aid to boost immune functions that is augmented by physical performances and in combination, so both in a combined way seem to maintain healthy life (**Table 1**).

NUTRITIONAL INTERVENTIONS IN AGEING AND IMMUNOSENESCENCE

Previous research has shown that a decrease in the number of peripheral blood naive cells, with a relative increase in the frequency of memory cells, is an irreversible age-related immune alteration (Quinn et al., 2018; Li et al., 2019). These two changes, along with inflammaging, are considered as the hallmarks of immunosenescence (decreasing strength of the immune system with age). However, improvements in health quality, immunization, and healthy and nutritious diets have increased the average lifespans of most developed-country citizens (Quilici et al., 2015). Immunosenescence is a complex process that affects the immune system as a whole and impairs the ability to respond adequately to invading pathogens.

The changes to the immune system seen with aging may not be permanent, and there is evidence of nutritional interventions promoting beneficial changes in immune cells (Yaqoob, 2017). Although there is enormous heterogeneity among individuals, mainly due to inherited differences in immune response genes that cannot be modified, certain factors, including lifestyle choices and nutritional habits, are flexible and significantly impact the progression of immunosenescence.

Nutrition has been studied as a modifiable factor in immune health for several decades, and this research has evolved into a field known as nutritional immunology. People in Mediterranean countries who consume many fruits, vegetables, legumes, unrefined cereals, skimmed dairy products, fish, and olive oil have a lower risk of heart disease (Caprara, 2018). Further, it is well established that nutrition directly impacts

immunosenescence because deficiencies in several vitamins and minerals, protein-energy malnutrition, and excessive consumption of saturated fatty acids can hinder immune systems. Many studies have been conducted to investigate the effect of nutritional factors on the immune response. Thus, we discuss the role of macronutrients, multivitamins, minerals, and other nutrient components in the immunosenescence process.

To date, most nutritional interventions have concentrated on the use of dietary lipids; among them, micronutrients like vitamin E, carotenoids, and zinc are necessary for increasing immune responses. Vitamin E is the most important lipid-soluble antioxidant in the innate immune system of the cell. Dietary supplements of vitamin E have been shown to improve cell-mediated and humoral immune responses in many species. Several animal and human studies have found that supplementing with vitamin E increases lymphocyte proliferation, immunoglobulin levels, antibody responses, NK cell activity, interleukin (IL)-2 production and decreased IL-6 production (Mahalingam et al., 2011; Capo et al., 2016; Wang et al., 2017; Dalia et al., 2018). Evidence-based studies and clinical trials strongly suggest that vitamin E is effective in improving immune response. Especially significant improvement in Delayed-Type Hypersensitivity (DTH) and lowering the risk of infection in the people with >60 years, and a daily dose of 200 IU/d is most effective in improving immune function in the older people (Meydani et al., 2018).

Zinc is an essential mineral with its deficiency leading to reduced immune cell proliferation, cytokine production, and specific NK cell and neutrophil function reductions. The overall frequency of zinc deficiency worldwide is estimated to be higher than 20% (Wessells and Brown, 2012). Zinc can also have the ability to modulate the development of T helper cells, thereby reducing the cytokine storm, which is characterized by a sudden increase in circulating levels of different proinflammatory cytokines and chemokines. These are associated with a wide variety of infectious and noninfectious diseases and finally lead to systemic immune response impairment, resulting in acute respiratory distress syndrome (ARDS) or multiple organ failure (Griffith et al., 2014; Sokol and Luster, 2015; Soriani et al., 2018). Many studies indicate that age-related macular degeneration (AMD) is a progressive, degenerative disorder that commonly affects the elderly population and can lead to blindness. Further, it is treatable with zinc supplementation (Vishwanathan et al., 2013; Blasiak et al., 2020).

Every stage of immune response is dependent on the availability of specific micronutrients. A plethora of micronutrients have been studied for their multiple properties in the maintenance of the immune system. The importance of micronutrients in the immune system and infection was first discovered in vitamin C deficiency. In 1753, the first recorded controlled clinical trial was published (Noble et al., 2013). Although the recommended dietary allowances for older people indicate that their energy needs are lower than younger people's, the micronutrient requirements are mostly the same. However, micronutrient deficiencies are more common in older people (Conzade et al., 2017). Lower food intake has been linked to less intake of calcium, iron, zinc, B vitamins, and vitamin E in older people, directly related to micronutrient deficiency (Fostinelli et al., 2020).

Fatty acids are an essential energy source, and they can influence immune cell functions by acting as precursors in the synthesis of lipid compounds. Fatty acid metabolic derivatives, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), are precursors for anti-inflammatory molecules that support monocyte recruitment in sites of infection (Schieffer et al., 2014). Fatty acids can have a dual effect on inflammation regulation. *In vitro* and *in vivo* studies show that the intracellular macromolecular complex Nod-like receptor protein 3 (NLRP3) inflammasome promotes proinflammatory cytokines such as IL-1 and IL-18. Additionally, metabolic derivatives of omega-3 fatty acids have been shown to prevent T cell differentiation into proinflammatory and decrease proinflammatory cytokine secretion (Chiurchiu et al., 2016).

Overall it appears that elderly individuals are more sensitive to the immunologic effects of fatty acid, which is advantageous and some disadvantages have also been observed with higher doses. As the doses used and the study designs differ between studies, more research is required to confirm the effects of fatty acid in this age group.

CONCLUSION AND FUTURE PERSPECTIVES

The evidence suggests insufficient data to draw definite conclusions about which diets can boost the immune system prominently. Although the extent of the effects of resistance exercise on immune function remains unclear, most data show that lifestyle factors such as specific dietary preferences, physical activity, and nutrition influence the inflammatory profile (risk of infection). It is further associated with behavioral intervention to improve immune and overall health outcomes in subjects of all ages cooperatively and independently. On the other hand, it is understood that the global burden of chronic diseases is rising exponentially, necessitating long-term systemic strategies to increase daily physical activity levels. However, we know that exercise is a highly effective strategy for promoting a healthy immune system and lowering the risk of maladaptive immune aging. In this perspective, regular physical activity appears to be the most promising approach to combating cellular immunosenescence and inflamm-aging.

In addition, specific foods and supplements may also boost immunity and consequently reduce the risk of respiratory tract infection. Some emerging evidence suggests that other supplements such as bovine colostrum, probiotics, Echinacea, and polyphenols can also enormously boost the immune system. Beside, this study suggests that adequate intake of all good essential variety of fruits and vegetables can overcome the single nutrient deficiency.

In parallel, healthcare professionals and public health policymakers should encourage physical activity for everyone, but especially for older adults and other at-risk individuals, such as those suffering from chronic diseases related to aging and lifestyle. Thus, more short-term and long-term controlled,

randomized interventional clinical trials using different exercise regimens with nutritional habits are needed to identify additional moderating factors and understand the mechanisms of immune cell mobilization and resistance to infections to guide clinical practice. This strategy would allow for a more personalized approach to treatment, which could help to improve the efficacy and acceptability of currently available therapies.

In summary, both exercise and a well-balanced diet can enhance immunity and resistance to infection in a large number of people. Immunosenescence is a new challenge for an aging population (those over 65 years old), so interventions to boost immunity are incredibly beneficial for them. It has a beneficial effect on several immune functions. PA and following a balanced diet throughout life can help to reduce the adverse impact of age-related immune dysfunction. Although the evidence is still preliminary, we hope that this review will be helpful for researchers, clinicians, and academicians better understand the relationship between physical activity, nutrition, and immunosenescence.

AUTHOR CONTRIBUTIONS

TS, HV, BP, VC, WY, YC, and BL contributed substantially to the paper. TS, HV, YC, and BL contributed to conception and design of manuscript. HV, BP, and VC performed the methodological search on the research topic and helped write the draft manuscript. WY, YC, and BL critically revised the manuscript. All authors contributed to the article and approved the submitted version.

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Does the Attention of the Chinese Government Influence Chinese Nutrition, Exercise, and Health? Based on the Content Analysis of the Central Government Work Reports From 1978 to 2020

Wenpeng Zhang[1,2]*, *Yuomei Zhou*[2], *Jingzhan Li*[2], *Tingting Zeng*[2] and *Jinlian Liao*[2]

[1] Department of Research Institute of Healthy China, University of East China Jiaotong University, Nanchang, China,
[2] Department of Physical Education and Health, University of East China Jiaotong University, Nanchang, China

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National Bureau of Plant Genetic Resources (ICAR), India

***Correspondence:**
Wenpeng Zhang
wenpeng98@126.com

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The attention of the Chinese government on nutrition, exercise, and health refers to the attention degree of the Central Government to the nutrition, exercise, and health of Chinese nationals and reflects whether Central Government attach importance to Chinese nationals' nutrition, exercise and health or not and the distribution of resources, which influence the physical quality and health level of Chinese nationals. Based on the attention theory and attention distribution proposed by Herbert Simon, Dai Kai, et al., this study took 43 Central Government Work Reports from 1978 to 2020 as research samples, used literature reviews, and textual analysis methods, and applied the Nvivo12.0 software to conduct qualitative and quantitative analyses about the contents of the Central Government Work Report concerning the nutrition, exercise, and health of Chinese nationals. This research found the following: (1) There has been a relatively huge overall change in the attention of the Central Government, that is, the level of attention, to the nutrition, exercise, and health of Chinese nationals from 1978 to 2020, and the policies related to nutrition, exercise and health of Chinese nationals issued by the Central Government have been growing faster. (2) The income level of the urban and rural residents, the total production of various types of food, dietary structure, the total number of medical and health institutions, the average life expectancy of the Chinese population, and the number of sports venues have been constantly increasing since the reform and opening up, which has effectively promoted the improvement of the nutrition, exercise and health level of Chinese nationals, and it cannot be achieved without the attention and support of the Central Government. However, the change in the lifestyle of Chinese nationals has led to the growth of the modern "Civilization Disease," which is also an important issue that the Central Government needs to handle urgently.

Keywords: the Central Government of the People's Republic of China, nutrition, exercise, health, attention, the distribution of resources, government work

INTRODUCTION

The Outline and Plan of "Healthy China 2030" issued by the Communist Party of China of Central Committee (hereinafter referred to as CPC Central Committee) and the State Council of the People's Republic of China (hereinafter referred to as State Council) that is the Central Government of the People's Republic of China (hereinafter referred to as Central Government) proposed that "health is an inevitable requirement for promoting human beings' all-round development, and the realization of national health and longevity is an important symbol of the country's prosperity, strength, and national rejuvenation" (1). Does the attention degree of the Central Government to the nutrition, exercise, and health of Chinese people influence the health condition of Chinese people? The Central Government Work Report (hereinafter referred to as Government Work Report) refers to the official policy document of the Central Government that summarized the economic and social development of the previous year and set the expected plans and goals for government work in the coming year based on the actual conditions and needs (2), which reflect the attention of the Central Government to various social affairs including nutrition, exercise, and health. Herbert Simon believed that "attention refers to the process in which managers selectively paying attention to certain information while ignoring other parts"(3). However, attention distribution refers to the attention configuration of the action body (4). Based on this, the attention distribution of the government refers to the attention of the Central Government to nutrition, exercise, and health at the same time.

The scope of this research on the government work report from the perspective of attention distribution was wide: vertically, there were both the central level and local level; horizontally, there were the ecological environment level, urban and rural development level, education level, science and technology level, and so on. The researchers analyzed the changed rules of the regional green development based on the government work reports of Beijing, Tianjin, and Hebei province, and provided suggestions for the optimization of the attention level of the regional green development (5). One researcher took 20 provincial policies of eco-environmental protection as the samples to analyze the level, characteristics, and deficiencies of the allocation of the attention of the local government with regards to environmental governance (6). Some researchers took the central policy documents from 1986 to 2019 as the research samples to explore the changing rules of the attention of the Central Government to urban community governance, which provided a reference for promoting urban community governance (7), others analyzed the "*Key Points of Works*" that was issued by the Ministry of Education of the People's Republic of China from 1987 to 2019 through five dimensions, and uncovered the characteristics of the attention distribution of the Central Government to vocational education, and put forward suggestions to optimize the attention distribution levels of the Central Government to vocational education (8). One researcher took 42 Government Work Reports since the reform and opening up as the research samples and found that the attention of the Central Government to scientific and technological innovation has experienced certain periodic changes, and put forward proposals to increase the attention of the Central Government to the field of scientific and technological innovation (9). From the perspective of attention and government decision-making theory, the researchers explored the allocation level of the attention of the Central Government to the cause of aging based on the content analysis of the Government Work Reports from 1978 to 2018, which promoted the development of the cause of aging (10). Whereas, the Chinese researchers who took the Government Work Report as the research samples for analysis found that applied attention theory to nutrition, exercise, and health was rare. Therefore, the research does not only validate the hypothesis that the attention of the Central Government influences Chinese nutrition, exercise, and health, but also fills the gap in the application of attention theory in this field, and promotes the construction of a healthy China.

MATERIALS AND METHODS

Theoretical Foundation

The term "attention" originated from psychology, which refers to the ability of the action body to point and concentrate on something accompanied by a series of psychological processes (11). In the 1940s, Herbert Simon applied "attention" to the field of management, and believed that "attention refers to the process of managers selectively paying attention to some information while ignoring other parts" (3). He also put forward the theory of limited rational decision-making model, which stated that "attention is a kind of scarce resource, and the process of decision-makers' choice is the process of how to effectively distribute limited attention, that is, the process of attention distribution or transfer" (10). In the 1950s, based on the attention distribution proposed by Simon, James March put forward that "attention distribution will be influenced by decision-makers' subjective and objective factors, such as limited rationality, information overload and decision-making environment" (12). In the 1990s, William Ocasio considered that "the decision-making behavior of an organization is built on its attention distribution and influenced by the decision-making environment" (13). Bryan Jones applied "attention" to the field of government decision-making, and believed that "attention is a selection mechanism, all decisions involve selection, and the changes of government policies are constantly varying with the changes of decision-makers' attention" (14).

Research Methods and Tools

Text Analysis

Text analysis is a content analysis method that combines quantitative and qualitative analysis. It was used primarily in the field of informatics and intelligence at the beginning, and then gradually extended to various fields (15). Language usually reflects the cognitive tendency of people, and the cognitive changes of people and the attention degree to things are often reflected in the changes of the frequency of the use of words (12). Therefore, the literal expressions about nutrition, exercise, and health in the Government Work Reports reflected the

attention degree of the Central Government to them and their changing process.

Use of the Nvivo12.0 Software

In 1999, the American QSR company developed a qualitative analysis software -Nvivo (16), which not only helped researchers organize, analyze, and query unstructured or qualitative data, but also improved the reliability and validity of studies (17). At present, Nvivo12.0 is the latest version. According to the need of the research, we used Nvivo12.0 to code and analyze the text content about nutrition, exercise, and health in the Government Work Reports.

Sample Selection

The reasons why the researchers selected 43 Government Work Reports from 1978 to 2020 as the analysis sample were as follows: The first reason was that the Central Government was one of the main management bodies of various social affairs (18), the attention of the Central Government to the nutrition, exercise, and health of Chinese nationals determined the degree of emphasis of the local government to the nutrition, exercise, and health of Chinese nationals, which influenced their physical quality and health level, and the Government Work Report was a real reflection and behavior imprint of the government in dealing with public affairs (19) that was also the representative of the most authoritative, holistic, and prospective (2). The second reason was during "the Ten-Year Cultural Revolution," the development of various social affairs in China was frustrated, and was gradually recovered and adjusted at the beginning of the reform and opening-up, thus the Government Work Reports since the reform and opening-up were selected to ensure the integrity of the samples.

RESULTS AND DISCUSSION

The Changes in the Attention of the Central Government Toward the Nutrition, Exercise, and Health of Chinese National Since the Reform and Opening-Up

The Government Work Report was not only the declaration and programmatic document of CPC Central Committee and State Council on the development of various social affairs (18), but also the baton for the government at all levels to allocate resources and invest energy (2), which implied the intensity of attention and change in the logic of the Party and government on the various social affairs and various fields in China. The "policy discontinuity equilibrium" theory held that "influenced by policy environment and other factors, the policy will experience a long period of stability and sudden change period" (20). Hence, as an official policy document, the Government Work Report over the years would also go through the period. Since the reform and opening up, the discourse expression on the nutrition, exercise, and health of the nationals in the Government Work Report has changed with the policy environment and also reflected the changes in the attention of the Central Government toward the nutrition, exercise, and health of Chinese nationals. After importing the 43 Government Work Reports into Nvivo12.0 and performing a text search query with "nutrition" as the keyword, the research found that there were only five text expressions with "nutrition" as the keyword in **Table 1**, given that nutrition originated from diet and the sources of diet include agriculture, forestry, animal husbandry, fishery, and so on. Therefore, all the words related to the sources of nutrition in the Government Work Reports over the years were the manifestation of the attention of the Central Government to nutrition. And that exercise and health include physical exercise, sports and fitness, medical care, nutrition, and hygiene (21). Hereby, "nutrition, exercise, and health" was considered as the node, and "nutrition," "diet," "food safety," "agriculture," "sports," "fitness," "medical," and "hygiene" were used as the keywords to carry out the text search query and code. Drawing lessons from the hypothesis of Zhi Xu, et al., the research regarded the ratio of the number of words related to nutrition, exercise, and health in the Government Work Report to the total number of words in the Government Work Report of that year as the intensity of the attention of the Central Government to the nutrition, exercise, and health of Chinese nationals (2). After the calculation, it was presented in the form of the combination of bar chart and line chart in **Figure 1**. The research combined the process and staged the characteristics of the Chinese economic, social, and market reform since the reform and opening up with the practice of the nutrition, exercise, and health of the Chinese nationals, and divided the attention of the Central Government to the nutrition, exercise, and health Chinese nationals into three stages: Recovery reform period (from 1978 to1992); Market reform period (from 1993 to 2012); Deepening reform period (from 2013 to 2020).

TABLE 1 | The year of the literal expression with the keyword "nutrition" in Government Work Reports from 1978 to 2020.

Year	Reference point	Coverage rate
1999	1	0.01%
2012	1	0.01%
2013	1	0.01%
2014	2	0.02%
2018	1	0.01%

Recovery Reform Period (From 1978 to 1992)

Bryan Jones believed that "all kinds of factors in the decision-making situation have an effect on decision-making" (14). In different decision-making situations, the Central Government paid different attention levels to the nutrition, exercise, and health of Chinese nationals. Due to the long-term impact of the "cultural revolution," various fields of Chinese social affairs were at a low ebb with many social problems, such as depressed economic development and low living standards. The convening of the Third Plenary Session of the 11th CPC Central Committee ushered in a new historical period of reform and opening up, and the attention of the Central Government to the nutrition, exercise, and health of Chinese nationals also followed the pace of the reform and opening up into a period of recovery and

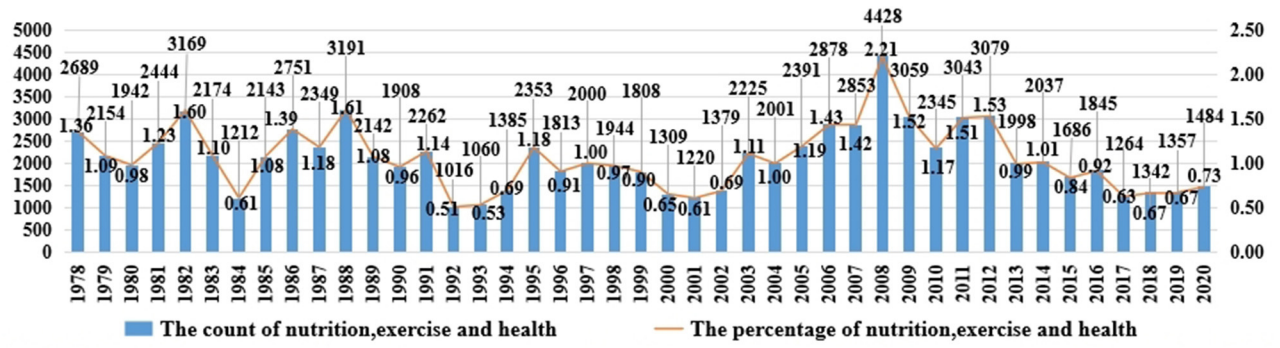

FIGURE 1 | The changes about the counts and percentages of nutrition, exercise and health in Government Work Report from 1978 to 2020.

adjustment. Overall, the intensity of the attention of the Central Government to the nutrition, exercise, and health of Chinese nationals greatly changed during this period. The attention level of the Central Government to the nutrition, exercise, and health of Chinese nationals reached 1.36% in 1978, while the attention level from 1979 to 1981 was lower than that in 1978. The reason was that at the first session of the Fifth National People's Congress, the Government Work Report proposed the general arrangement of "implementing prevention as the main work, extensively launching Patriotic Health Campaigns, and actively carrying out mass sports activities to enhance the physical fitness of Chinese nationals" and the Third Plenary Session of the 11th CPC Central Committee passed the "*Decision on Certain Issues of Accelerating Agricultural Development (draft)"* and "*Regulations on the Work of Rural National Communes (trial draft)"*, which promoted the development of the production undertakings in China, such as agriculture, animal husbandry, forestry, sideline, and fishery. In 1982, the attention level of the Central Government to the nutrition, exercise, and health of Chinese nationals reached 1.6% that because, in January 1982, the CPC Central Committee approved the "*Minutes of the National Rural Work Conference (Released by CPC Central Committee (1982) No.1)"*, and pointed out that "the production team should make plans for the comprehensive development of agriculture, forestry, animal husbandry, sideline, fishery, industry, and commerce in accordance with local conditions, and improve the circulation of rural commodities". In addition, the Central Patriotic Health Campaign Committee and the Ministry of Health of the People's Republic of China jointly issued the "*Notice on Further Development of Patriotic Health Campaigns and the Construction of Socialist Spiritual Civilization."* The attention level of the Central Government to the nutrition, exercise, and health of Chinese nationals was as low as.56% in 1984. The reason for this phenomenon was that the CPC Central Committee issued the "*Some Issues of Current Rural Economic Policy (Released by CPC Central Committee (1983) No.1)"* and "*Notice on Rural Work in 1984 (Released by CPC Central Committee (1984) No.1)"* which have effectively promoted the all-round development of agriculture, forestry, animal husbandry, sideline and fishery in China, and the living standards of the urban and rural residents were further improved. As a result, the number of the literal expressions on nutrition in the Government Work Reports have declined in recent years, and the Central Government has laid its main attention on the system reform and opening up. Compared with 1984, the attention level of the Central Government to the nutrition, exercise, and health of Chinese nationals increased from 1985 to 1991 and reached a peak state of 1.61% in 1988. In October 1984, the CPC Central Committee issued the "*Notice on Further Development of Sports,*" which once again stressed that "sports is closely related to Chinese nationals' health, and we must adhere to the policy of combining popularization and improvement, focusing on school sports". In January 1985, the CPC Central Committee and State Council issued "*Ten Policies on Further Activating the Rural Economy (Released by CPC Central Committee (1985) No.1),*" which was beneficial in improving the tight supply of agricultural products and promoting the rationalization of the rural industrial structure and the diversification of the diet of the urban and rural residents. In April of the same year, the State Council approved the "*Report on Several Issues of Health Work Reform (Released by State Council (1985) NO.62)"* by the Ministry of Health of the People's Republic of China, which unveiled the prolog of health reform and promoted the development of Medical and Health Care in China. In January 1987, the CPC Central Committee issued a notice on "*leading Rural Reform deeper (Released by CPC Central Committee (1987) No.1")* which was an important reason for the peak of the attention of the Central Government in 1998. In January 1989, the State Council approved and forwarded the "*Opinions on Issues Related to the Expansion of Medical and Health Services*" issued by the Ministry of Health, Ministry of Finance, Ministry of Personnel, State Price Bureau, and State Administration of Taxation of the People's Republic of China. However, the attention level of the Central Government to the nutrition, exercise, and health of Chinese nationals fell to 0.51% in 1992, for the reason that: In the 1990s, the State Council approved the *"Regulations on School Sports Work and Regulations on School Health Work*" that was beneficial to improving the physical health level of the youth, so as to enhance the physical quality and health level of Chinese nationals; In October 1991, the State Council issued a "*Notice on Further Invigorating the Circulation of Agricultural Products*", which was beneficial to solving the problem of the lag in the circulation of agricultural

products and laying the foundation for the urban and rural residents to purchase diversified food, obtain the nutrients that their bodies need, and promote good health. Hence, the level of attention of the Central Government was low in 1992.

Market Reform Period (From 1993 to 2012)

The convening of the 14th National Congress of the Communist Party of China marked that the reform and opening up of China has entered a new stage, and the goal of establishing a socialist market economic system has been made. In this context, the attention of the Central Government to the nutrition, exercise, and health of Chinese nationals had also changed. Overall, the attention intensity of the Central Government to the nutrition, exercise, and health of Chinese nationals has changed greatly during this period. In 1993, the attention of the Central Government to the nutrition, exercise, and health of Chinese nationals was as low as.56%. The main reason was that the State Council issued the "*Opinions on Deepening the Reform of the Health and Medical System"* in 1992, which proposed to reform the health management system and the price system of Medical and Health Services. In addition, according to the general goal of establishing a socialist market economy system proposed by the 14th National Congress of the Communist Party of China, the State Council issued the "*Notice on Accelerating the Reform of Grain Circulation System"* to promote the reform of the grain circulation system toward commercialization and market-oriented operation in February 1993. In 1995, the attention of the Central Government to the nutrition, exercise, and health of Chinese nationals increased to 1.18%. The main reasons are as follows: In May 1993, State Council issued the "*Outline for the Reform and Development of China's Food Structure in the 1990s (Released by State Council (1993) NO.40),"* which was the first document on food nutrition that was issued by the country since the founding of the People's Republic of China; the State Council issued the "*Regulations on the Administration of Medical Institutions (Released by State Council (1994) No.194)"*; the CPC Central Committee and State Council issued the "*Opinions on Agricultural and Rural Work in 1994 (Released by CPC Central Committee (1994) No.4)"* and *"Opinions on Doing the Work in Agriculture and Rural Work well in 1995 (Released by CPC Central Committee (1995) No.6)."* From 1996 to 1999, the attention of the Central Government to the nutrition, exercise, and health of Chinese nationals varied little and the trend remained relatively stable. The following reasons could explain this phenomenon: Firstly, in June 1995, the "*Outline for the National Fitness Program (Released by State Council (1995) No.14)"* proposed that "by 2010, the Chinese national physique and health level will be comprehensively improved, and National Fitness system with Chinese characteristics will be basically built", and in August of the same year, the 15th plenary session of the Standing Committee of the 8th National People's Congress adopted the "*Sports Law of the People's Republic of China*", which clearly put forward the requirement that "sports work should adhere to the development of National Fitness Activities as the basis for the development of mass sports activities to improve the physical fitness of the whole nation"; secondly, in January 1997, the CPC Central Committee and State Council promulgated the *"Decision on Health Reform and Development (Released by CPC Central Committee (1997) No.3)"*, which explicitly proposed the goals and guidelines of health work and the principles that need to be followed; thirdly, in October 1998, the "*Resolution on Several Major Issues in Agriculture and Rural Work"* adopted by the Third Plenary Session of the 15th CPC Central Committee proposed that "we must adhere to market-oriented reforms, steadily develop food production, combine agriculture, forestry, animal husbandry and fishery, and ensure the effective supply of agricultural products". From 2000 to 2002, the reasons for the low attention level of the Central Government to the nutrition, exercise, and health of Chinese nationals were: the National Sports Work Conference held in Beijing in 1999, which discussed the *"Outline of Sports Reform and Development from 2001 to 2010"*; In December 2000, the "*Outline of Sports Reform and Development from 2001 to 2010"* mentioned that "the main goals of sports reform and development including an obvious increase in the popularity of mass sports, the full realization of the National Fitness Plan, and the effective enhancement of Chinese nationals' physical quality"; In November 2001, the General Office of State Council issued the "*Outline for the Development of Food and Nutrition in China from 2001 to 2010 (Resealed by State Council Office (2001) No.86),"* which pointed out that "entering the new century, accelerating food development, improving food structure, raising the nutritional level of the whole people and improving people's physical health are the urgent needs for the improvement of the national overall quality"; In January 2002, the CPC Central Committee and State Council issued the *"Opinions on Doing the Agricultural and Rural Work Well in 2002 (Released by CPC Central Committee (2002) No.2)"*, which put forward that "by 2010, the rural health service system and the rural cooperative medical system adapted to the requirements of the socialist market economy system will be basically established in national rural areas". From 2003 to 2012, the attention of the Central Government to the nutrition, exercise, and health of Chinese nationals was high and peaked in 2008 at 2.21%. In July 2002, the CPC Central Committee and State Council issued the *Opinions on Further Strengthening and Improving Sports Work in the New Era* which stated that "carrying out National Fitness Activities and enhancing nationals' physical quality is the fundamental task of sports work". In October of the same year, the CPC Central Committee and State Council issued the *"Decision on Further Strengthening Rural Health (Released by CPC Central Committee (2002) No.13)*." As the key point of Chinese health work, rural health work was linked to the development of the rural productivity and influenced the overall physical fitness and health level of Chinese nationals. In 2003, the severe acute respiratory syndrome (SARS) epidemic situation strengthened the construction of the national public health system and increased the investment of medical and health resources. In January 2005, the CPC Central Committee and State Council issued the "*Opinions on Several Policies to Further Strengthen Rural Work and Improve Comprehensive Agricultural Production Capacity (Released by CPC Central Committee (2005) No.1"*, which put forward new requirements for Chinese agricultural and rural work. The reasons for the peak level of the attention of the Central Government to the

nutrition, exercise, and health of Chinese nationals in 2008 were that: the CPC Central Committee and State Council promulgated the "*Opinions on Developing Modern Agriculture and Solidly Promoting the Construction of a New Rural (Released by CPC Central Committee (2007) No.1)"* and "*Opinions on Effectively Strengthening Agricultural Infrastructure and Further Promoting Agricultural Development and Increasing Farmers' Income (Released by CPC Central Committee (2008) No.1),*" both of which emphasized the issues about agriculture, rural, and farmers (hereinafter referred to as "Three Rural" issues) and the agricultural product safety question; the CPC Central Committee and State Council issued the "*Opinions on Strengthening Youth Sports and Enhancing Youth Physical Fitness (Released by CPC Central Committee (2007) No.7),*" which promoted the healthy growth of the youth and played a role in pushing the development of national health. In March 2009, the "*Opinions on Deepening the Reform of Medical and Health Care System (Released by CPC Central Committee (2009) No.6)"* was issued, which promoted the development of medical and health care and ensured the health of the urban and rural residents in China. In August of the same year, the release of the "*National Fitness Regulations*" not only promoted the development of national fitness activities but also improved the physical quality and health level of Chinese nationals. In January 2010, the CPC Central Committee and State Council issued the "*Several Opinions on Increasing Efforts to Coordinate Urban and Rural Development and Further Strengthening the Foundation of Agricultural Rural Development (Released by CPC Central Committee (2010) No.1),*" which put forward "accelerating the construction of the quality and safety supervision system and inspection and detection system for agricultural products, and actively developing pollution-free agricultural products, green food, organic agricultural products." Therefore, the Central Government has paid high attention to the nutrition, exercise, and health of Chinese nationals since 2003.

Deepening Reform Period (From 2013 to 2020)

The convening of the 18th National Congress of the Communist Party of China marked a new era of socialism with Chinese characteristics (22). The attention of the Central Government to the nutrition, exercise, and health of Chinese nationals has also entered a new stage of deepening reform, following the developmental needs of the new era. From 2013 to 2020, the number of the literal expressions of the nutrition, exercise, and health of Chinese nationals by the Central Government was lower compared with the previous decade, while the number of central-level documents directly issued by the government has been increasing. According to the developmental needs of the new era, the Central Government has made a new deployment arrangement for the relevant work in the field of nutrition for the nationals. In January 2014, the General Office of the State Council issued the "*Outline for the Development of Food and Nutrition in China from 2014 to 2020 (Resealed by State Council Office (2014) No.3)*," proposing that "China's food production is not able to meet the nutritional needs yet, the residents are undernourished and surplus coexist, and the lack of nutritional and health knowledge, which must be given great attention". In October 2016, the CPC Central Committee and State Council issued the "*Outline and Plan of 'Healthy China 2030'."* The outline proposed that "we should formulate and implement a national nutrition plan, deeply carry out the research on the evaluation of the nutritional functions of food, comprehensively popularize dietary nutritional knowledge, issue dietary guidelines suitable for the characteristics of different groups of people, guide residents to form scientific dietary habits, and promote the construction of a healthy diet culture". In July 2017, the General Office of the State Council issued the "*National Nutrition Plan from 2017 to 2030 (Resealed by State Council Office (2017) No.60),*" which put forward that "nutrition is an important material basis for human beings to maintain life, growth, and health, and nationals' nutrition is related to the improvement of nationals' quality and economic and social development." Since the 18th National Congress of the Communist Party of China, the Central Government has also made a series of new deployment arrangements for the work of national fitness. In October 2014, the State Council issued "*Several Opinions on Accelerating the Development of the Sports Industry And Promoting Sports Consumption (Resealed by State Council (2014) No.46")* that put forward "promoting National Fitness as the national strategy of China", which marked a significant leap in the concept and practice of mass sports development in China, recognizing national fitness as the rightful meaning of national health. National fitness is a significant means of national health, it is inevitable to promote the development of national health. In June 2016, the State Council issued the *"National Fitness Plan from 2016 to 2020 (Resealed by State Council (2016) No.37)."* In October of the same year, the *"Outline and Plan of 'Healthy China 2030"'* was issued by the CPC Central Committee and State Council which was a program of action to promote a healthy China, and "Co-construction and sharing, national health" was the strategic theme of building a healthy China. To accelerate the construction of sports power and to vigorously promote the in-depth integration of national fitness and national health, the General Office of the State Council issued the "*Notice on Printing and Distributing the Outline of Building Sports Power (Resealed by State Council Office (2019) No.40"* in September 2019. In September of the same year, for the sake of actively implementing the national fitness initiative and making regular participation in physical exercise a way of life, the General Office of the State Council issued the "*Opinions on Promoting National Fitness and Sports Consumption and Pushing the High-Quality Development of the Sports Industry (Resealed by State Council Office (2019) No.43)."* In October 2020, the General Office of the State Council issued the "*Opinions on Strengthening the Construction of National Fitness Facilities and Develop Mass Sports (Resealed by State Council Office (2020) No.36)"* to boost the construction of fitness facilities, promote the vigorous development of mass sports, and enhance the level of public services for national fitness. The Central Government has also carried out a comprehensive reform in the development of the medical and health services in China. In September 2013, the State Council issued "*Several Opinions on Promoting*

the Development of Health Service Industry (Resealed by State Council (2013) No.40)," which proposed that "accelerating the development of the health service industry is an inevitable requirement for deepening medical reform, improving Chinese nationals' livelihood and enhancing the health quality of the whole Chinese." In March 2015, the General Office of the State Council issued the "*Notice on Printing and Distributing the Outline of the National Medical and Health Service System Planning from 2015 to 2020 (Resealed by State Council Office (2015) No.14)"* to promote the further optimal allocation of the Chinese medical and health resources and build an integrated medical and health service system. In August 2018, the General Office of the State Council issued the "*Notice on Printing and Distributing the Reform Plan for the Division of Financial Affairs Powers and Expenditure Responsibilities between the Central and Local Governments in the Field of Medical and Health (Resealed by State Council Office (2018) No.67)*." In June 2019, the General Office of the State Council issued the "*Notice on Printing and Distributing the Key Work Tasks for Deepening the Reform of the Medical and Health System in 2019 (Resealed by State Council Office (2019) No.28)*," proposing that "putting Chinese national health at the center, implementing prevention as the main work, strengthening disease prevention and health promotion, deepening the linkage reform of medical care, medical insurance and medicine, and firmly promoting the reform of the Medical and Health System effectively implemented and benefiting the Mass". In July 2020, the General Office of the State Council issued the "*Key Work Tasks for Deepening the Reform of the Medical and Health System in the Second Half of 2020 (Resealed by State Council Office (2020) No.25)*," which put forward "strengthening the construction of the public health system and further implement the Healthy China Initiative".

From the above, it can be seen that since the new era, although the Central Government has paid less attention to the nutrition, exercise, and health of Chinese nationals compared with the previous decade, there has been an increasing number of policies at the central level related to nutrition, exercise, and health after entering the new era, most of which were macro-policies and planning programs. On one hand, the promulgation of these policies showed that the CPC Central Committee and the State Council have attached great importance to the nutrition, exercise, and health of Chinese nationals, and to a certain extent promoted the improvement of national nutrition, exercise, and health in China. On the other hand, it indicated the lack of micro and detailed measures on nutrition, exercise, and health. The nutrition policy and action plan of China have promoted the overall nutritional status of Chinese residents to some extent, but there are still some deficiencies that need to be improved. For example, the lack of laws and regulations on nutrition policy protection, lack of long-term mechanism and sustainability, inadequate nutrition policy system, and so on (23). In addition, studies have shown that the elderly sports policy system in China was not sound (24), public sports health policy was still in the stage of continuous development, and the public sports policy of the scientific and standardized aspects still needed to be improved (25), the school sports policy toolbox was not sound and the policy system was not perfect (26), the youth health policy still faced the problems of vague implementation standards and the imperfect linkage mechanism between the executive departments (27).

TABLE 2 | Changes in the per capita net income of rural residents and per capita disposable income of urban residents in China since reform and opening up (Source: Chinese National Bureau of Statistics).

Years	Per capita net income of rural residents	Per capita disposable income of urban residents
1978	134	343
1980	191	478
1990	686	1,510
1992	784	2,027
2000	2,253	6,280
2010	5,951	19,109
2020	17,131	43,834

The Changes in the Nutrition, Exercise, and Health Level of Chinese Nationals Since the Reform and Opening-Up

Since the reform and opening up, the attention of the Central Government to the nutrition, exercise, and health of Chinese nationals has changed significantly. The number of policies related to nutrition, exercise, and health issued by the Central Government has increased, and the level of nutrition, exercise, and health of Chinese nationals has also changed constantly. The per capita income of the Chinese urban and rural residents has been increasing since the reform and opening up. As shown in **Table 2**, the per capita income of rural residents has increased from 134 yuan in 1978 to 17,131 yuan in 2020, and that of urban residents has increased from 343 yuan in 1978 to 43,834 yuan in 2020. Since the reform and opening up, with the improvement of the per capita income of the urban and rural residents in China and the solving of the problem of food and clothing, the demand of the nation for diet has gradually shifted from being full to eating well, and the dietary structure has gradually developed toward a nutritional and scientific direction (28). The dietary structure refers to the amount of all kinds of food in the daily diet of people and their proportion in the diet (29). There are seven main kinds of nutrients in the human diet, namely protein, fat, carbohydrate, vitamin, mineral (including trace element), water, and fiber (30). As shown in **Figure 2A**, the changes in the total production of various types of food in China since the reform and opening up reflected the changes in the overall national diet level, which further reflected the changes in the overall national nutritional level. The total national grain production in China rose from 304.77 million tons in 1978 to 663.843 million tons in 2019. Legumes, which are rich in proteins, fats, carbohydrates, and other nutrients, are part of the daily diet of Chinese residents. The production of legumes in China was low in 1978 with only 7.6108 million tons. After the

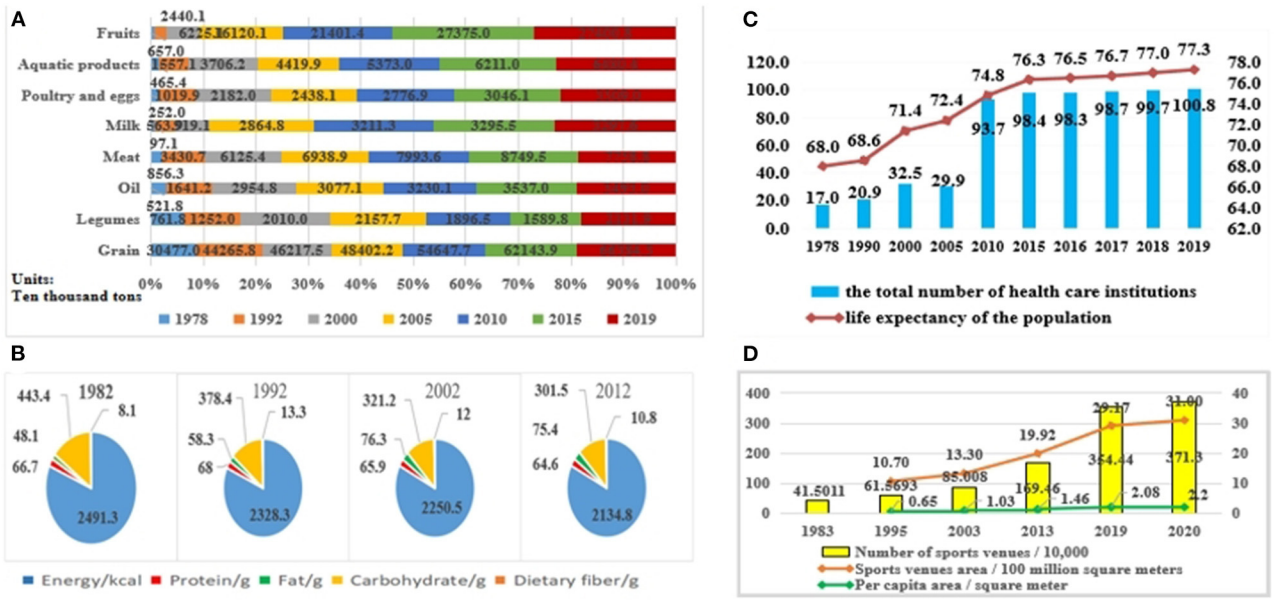

FIGURE 2 | (A) Changes in the total production of various types food in China since reform and opening up (Source: Chinese National Bureau of Statistics). **(B)** The energy and the average intake of major nutrients of Chinese residents from 1982 to 2012 (g/standard person day) (Source: China Health Statistics Yearbook). **(C)** Changes in the Average life expectance of the Chinese population and the total number of Medical and Health institutions since reform and opening up (Source: Chinese National Bureau of statistics). **(D)** Changes in the number, area and per capita area of sports venue in China since reform and opening up (Source: Chinese General Administration of Sport).

establishment of the socialist market economic reform goal, the production of legumes considerably increased, reaching 21.5767 million tons in 2005. The total production of legumes in China has declined due to the global economic crisis. In 2010, the Chinese legumes production fell to 18.7184 million tons. The Chinese legumes production reached 21.139 million tons in 2019. The Chinese oil production rose from 5.218 million tons in 1978 to 34.93 million tons in 2019. The total production of meat, milk, poultry and eggs, aquatic products, and fruits in China has increased relatively. The increase in the total production of these food items was not only due to the changes in the attention level of the Central Government in the area of nutrition, but also the issuance of policy documents related to nutrition, diet, agriculture, etc.

China respectively conducted five national nutrition surveys in 1959, 1982, 1992, 2002, and 2012, and the researchers analyzed the changes in the dietary structure of Chinese residents by using the data of national nutrition surveys comprehensively since the reform and opening up. As seen in **Table 3**; **Figure 2B**, the dietary structure of Chinese residents has undergone major changes. Before the reform and opening up, the dietary structure of Chinese residents was categorized under "high carbohydrate, low protein, low fat, low vitamin pattern". Since the reform and opening up, the dietary structure of Chinese residents has gradually shifted to the "affluent type" pattern of "high energy, high fat, high protein and low dietary fiber" (31). From 1982 to 1992, the average daily intakes of other cereals and tubers decreased significantly, while the average daily intakes of flour and its products, light-colored vegetables, and legumes decreased slightly. On the contrary, the average daily intake of dark-colored vegetables, fish and shrimp, and vegetable oils increased, and the average daily intake of fruits, livestock and poultry meats, rice and its products, eggs and its products, milk and its products, and salt increased slightly. In addition, the average daily intake of protein, fat, and dietary fiber of Chinese residents increased, while the average daily intake of carbohydrates decreased sharply. The average daily intake of energy of Chinese residents also decreased. To effectively avoid the formation of the "affluent type" dietary pattern of "high energy, high fat, high protein, and low dietary fiber", the first policy document on Chinese national dietary structure was issued- "*Outline for the Reform and Development of China's Food Structure in the 1990s (Released by State Council (1993) NO.40).*" From the early 1990s to the beginning of 2000s, the average daily intake of other cereals, fruits, and salt declined slightly, while the average daily intake of tubers, flour and its products, dark-colored vegetables, and light-colored vegetables decreased sharply. The average daily intake of rice and its products, milk and its products, livestock and poultry meats, and vegetable oils increased significantly, while the average daily intake of legumes, eggs and its products, fish, and shrimp, and animal oils increased slightly. In addition, the average daily intake of Chinese residents when it comes to energy, protein, carbohydrates, and dietary fiber declined, while the average daily intake of fat rose. After entering the 21st century, to promote the development of the Chinese national dietary structure into a nutritious and scientific way, the General

TABLE 3 | Changes in the average food intake of Chinese residents from 1982 to 2012 (g/standard person day) (Source: China Health Statistics Yearbook).

Years / Kinds	1982	1992	2002	2012
Other cereals	103.5	34.5	23.6	16.8
Tubers	179.9	86.6	49.1	35.8
Rice and its products	217.0	226.7	238.3	177.7
Flour and its products	189.2	178.7	140.2	142.8
Legumes	13.4	11.2	16.0	10.9
Dark-color vegetables	79.3	102.0	90.8	89.4
Light-color vegetables	236.8	208.3	185.4	180.0
Fruits	37.4	49.2	45.0	40.7
Livestock and poultry meats	34.2	58.9	78.6	89.7
Eggs and its products	7.3	16.0	23.7	24.3
Fish and Shrimp	11.1	27.5	29.6	23.7
Milk and its products	8.1	14.9	26.5	24.7
Vegetable oils	12.9	22.4	32.9	37.3
Animal oils	5.3	7.1	8.7	4.8
Salt	12.7	13.9	12.0	10.5

Office of the State Council issued the "*Notice on Printing and Distributing the Outline for Chinese Food and Nutrition Development from 2001 to 2010 (Resealed by State Council Office (2001) No.86).*" From 2002 to 2012, the average daily intake of other cereals, tubers, rice and its products, and legumes decreased. Meanwhile, the average daily intake of dark-colored vegetables, light-colored vegetables, fruits, fish and shrimp, milk and its products, animal oils, and salt decreased slightly. On the contrary, the average daily intake of flour and its products, eggs and its products, livestock and poultry meats, and vegetable oils all increased. Moreover, the average daily intake of energy, protein, fat, carbohydrates, and dietary fibers of Chinese residents decreased. To address the coexistence of under-nutrition and over-nutrition and to optimize the dietary structure of Chinese residents, in January 2014, the General Office of the State Council issued the "*Outline for the Development of Food and Nutrition in China (2014–2020)(Resealed by State Council Office (2004) No.3).*"

According to the above, we can know that the relationship between the nutrition level and income level of Chinese residents is not always positive. The increase of the per capita income of Chinese residents to a certain extent contributes to the improvement of the nutritional level of the residents, and the diversification of food also contributes to the diversification of the dietary choices of the residents, similarly, promoting the optimization of the dietary structure of the residents, to some extent, may promote the health level of Chinese residents. However, China has a large population base, and there is still a certain gap in the per capita income between the urban and rural residents. Therefore, the eating habits and levels of Chinese nationals are also different. Eating in restaurants, ordering takeout, and other consumer behaviors, bento, fast food, and other eating habits will lower the health level of Chinese people, followed by the modern "Civilization Disease". The modern "Civilization Disease" refers to the disease caused by the bad modern lifestyle, including obesity, hypertension, diabetes, and so on (32). In China, six national health service surveys were conducted in 1993, 1998, 2003, 2008, 2013, and 2018, respectively. The results showed that the number of people suffering from chronic diseases in China increased to 204 million (33, 34). Compared with the results of the first National Health Service survey in 1993, the prevalence of circulatory diseases increased dramatically. In the urban areas, the prevalence of circulatory diseases rose from third to second place, with an increase of nearly half (47.1%), while in the rural areas, the prevalence of such diseases increased by 65.57%, although the ranking remained unchanged. Compared to 1998, the main diseases that increased the prevalence of chronic diseases in the urban areas were hypertension (up to 71%), diabetes (up to 120%), and cerebrovascular diseases (up to 33%). The main diseases that increased the prevalence of chronic diseases in the rural areas were hypertension (up to 134%), gallstones and cholecystitis (up to 38%), and cerebrovascular diseases (up to 47%). In 2008, the prevalence of hypertension diagnosed by doctors increased rapidly by 2.2 times in 15 years, and the prevalence of diabetes increased by 2.8 times in 15 years, and the survey estimated that the total number of chronic disease cases reached 270 million, an increase of 0.7 million from 2003. In 2013, the prevalence of self-reported hypertension was 14.2% among the surveyed population aged 15 years and above which increased by 330.3% in 10 years. The prevalence of self-reported diabetes among those aged 15 years and above was 3.5%, which increased four times in 10 years. The prevalence of overweight and obesity among the population aged 18 years and above accounted for 24.8 and 5.4%, respectively. In 2018, the sixth National Health Service Statistics survey report put forward that the increasing chronic diseases were one of the important problems in the healthy development of China

in the future (35). Therefore, although the increase of the income of Chinese residents will improve their health level to a certain extent, the interaction of various factors such as the improvement of the technology in China, the enhancement of industrialization, the popularization of electronic products, the acceleration of social pace, and the increase of sedentary behavior has resulted in the lack of physical activity of Chinese people and further led to the increasing rates of obesity, diabetes, cardiovascular and other modern "Civilization Diseases". In response to the growth of modern "Civilization Diseases", the General Office of the State Council issued the "*Notice on Printing and Distributing China's Mid-and Long-term Plan for the Prevention and Treatment of Chronic Diseases (Released by State Council Office (2017) No.12)"* in February 2017, which mentioned, "strengthening health education and improving the health quality of the whole people, implementing early diagnosis and treatment to reduce the risk of disease among high-risk groups, promoting the coordination of medical treatment and prevention, realizing the whole process of health management and other measures" (36). In September 2019, Premier Li Keqiang presided over the opening of the China State Council executive meeting, proposing that "the reimbursement rate for over 300 million patients with hypertension and diabetes should be raised to more than 50%" (37). The Customs Tariff Commission of the State Council issued the "*Notice on the adjustment plan of temporary import tariff rate in 2020 (Released by State Council Customs Tariff Commission (2019) No.50)*," which put stated "implementing zero tariff on several new diabetes drugs." (38).

Besides the income of urban and rural residents, dietary structure, and lifestyle, many other factors are affecting the health of Chinese nationals, such as health care, sports, and fitness. Medical and health care are related to the health of hundreds of millions of people. Since the reform and opening up, the development level of medical and health care in China has gradually increased, and the average life expectancy of the Chinese population has also risen. As can be seen in **Figure 2C**, the total number of medical and health institutions in China was 170,000 in 1978. After the establishment of the socialist market economic reform goal, the total number of medical and health institutions in China inflated to around 300,000. Until 2010, the total number of medical and health institutions in China reached 937,000. The main reason for the sharp increase in the total number of medical and health institutions was the *"Opinions on Deepening the Reform of the Medical and Health System (Released by CPC Central Committee (2009) No.6)"* issued by the Central Government and State Council in March 2009, which has not only promoted the development of medical and health undertakings in China but also laid a foundation for the improvement of the whole national health. In addition, the average life expectancy of the Chinese population has increased from 68 years old in 1978 to 77.3 in 2019. The thought of "exercise is a valuable medicine" has existed since ancient times. The leaders of the past generations of the Chinese nation have attached great importance to the physical quality and health level of Chinese nationals and emphasized the importance of exercise and fitness. In June 1952, Chairman Mao Zedong wrote an inscription for the foundation of the All-China Sports Federation which stated to "develop sports and build up the people's physical fitness." In August 1997, Chairman Jiang Zemin wrote an inscription for the national fitness work which stated that "national Fitness benefits the country and Chinese nationals and contributes to the present and future generations". In October 2005, when General Secretary Hu Jintao attended the 10th National Games in Nanjing, he proposed that "carrying out national physical fitness activities to improve the health quality of the whole nation." In August 2013, when meeting with the national mass sports advanced units and advanced individual representatives, General Secretary Xi Jinping emphasized that "national fitness is the foundation and guarantee for all people to enhance their physical fitness and lead a healthy life, national fitness is an important connotation of building moderately prosperous society in all respects, and it's also a vital basis for every person to grow up and live a happy life". It can be seen from **Figure 2D** that since the reform and opening up, the number of sports venues in China has increased from 415,000 in 1983 to 3,713,000 in 2020; the area of sports venues has expanded from 1.07 billion square meters in 1995 to 3.1 billion square meters in 2020; the per capita area of sports has increased from.65 square meters to 2.2 square meters. The increase in the number and the area of sports venues can effectively solve the problem of insufficient venues, thereby promoting the increase of the physical exercise population and further improving the physical fitness of Chinese nationals.

CONCLUSION

In conclusion, there has been a relatively huge overall change in the attention of the Central Government, that is, the level of attention, to the nutrition, exercise, and health of Chinese nationals from 1978 to 2020, and the policies related to the nutrition, exercise, and health of Chinese nationals issued by the Central Government have been growing faster. The income level of the urban and rural residents, the total production of various types of food, dietary structure, the total number of medical and health institutions, the average life expectancy of the Chinese population, and the number of sports venues have been constantly increasing since the reform and opening up, which has effectively promoted the improvement of the nutrition, exercise, and health level of Chinese nationals, and it cannot be achieved without the attention and support of the Central Government. However, the change of the lifestyle of Chinese nationals has led to the growth of the modern "Civilization Disease," which is also an important issue that the Central Government needs to handle urgently. Therefore, the research can not only offer a window into the changes of the attention of the Central Government to the nutrition, exercise, and health of Chinese nationals since the reform and opening up, but also verify the hypothesis that the attention of the government influences the level of the nutrition, exercise, and health of Chinese nationals. Furthermore, the research also summarizes the progress and defects of the development in nutrition, exercise, and health

in China to lay a good foundation for the promotion of a healthy China.

DATA AVAILABILITY STATEMENT

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

AUTHOR CONTRIBUTIONS

TZ and JL: data analysis and review and editing. JL: methodology. YZ: check the data. WZ and YZ: draft the paper. All authors have read and agreed to the published version of the manuscript.

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Meta-Analysis on the Association of Neuropeptide Y rs16139 Variant With the Risk of Alcoholism

Biqing Chen[1†], *Manish Yadav*[2†], *Madhubala Mulkalwar*[3], *Lakkakula Saikrishna*[4], *Henu Verma*[5], *Weibing Ye*[6*] *and L. V. K. S. Bhaskar*[2*]

[1] Department of Sports Operation and Management, Jinhua Polytechnic, Jinhua, China, [2] Department of Zoology, Guru Ghasidas Vishwavidyalaya, Bilaspur, India, [3] Department of Pathology, Shri Shankaracharya Institute of Medical Sciences (SSIMS), Bhilai, India, [4] Department of Public Health, Nellore Municipal Corporation, Nellore, India, [5] Department of Immunopathology, Institute of Lungs Biology and Disease, Comprehensive Pneumology Center, Helmholtz Zentrum, Munich, Germany, [6] Exercise and Metabolism Research Center, College of Physical Education and Health Sciences, Zhejiang Normal University, Jinhua, China

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Andhra University, India

***Correspondence:**
Weibing Ye
ywbls@zjnu.cn
L. V. K. S. Bhaskar
lvksbhaskar@gmail.com

[†]These authors have contributed equally to this work and share first authorships

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Introduction: The neuropeptide-Y (NPY) is involved in the development of alcoholism through NPY receptors. A T>C mutation causes substitution of leucine to proline at codon 7 (L7P; rs16139) in the signal peptide of neuropeptide Y is known to cause a 42% increase in plasma NPY levels. Studies that analyzed the association between *NPY* rs16139 and alcoholism risk did not demonstrate conclusive evidence for this relationship. The present study aims to evaluate the association between *NPY* gene rs16139 variant and alcohol dependence.

Method: An electronic search of databases including PubMed and Google Scholar was performed to retrieve studies investigating the association between *NPY* rs16139 and alcoholism. The pooled odds ratio (OR) with 95% confidence interval (CI) was calculated in allelic and dominant genetic models. Sensitivity analyses and publication bias were assessed in our meta-analysis. The meta-analysis was conducted using the MetaGenyo web tool.

Result: Significant heterogeneity was observed across studies ($p < 0.001$). Our results have shown that there is no significant association between *NPY* rs16139 variant and the risk of alcoholism in allelic (OR = 0.98, 95% CI 0.70–1.38, $p = 0.921$) and dominant models (OR = 0.98, 95% CI 0.69–1.40, $p = 0.919$). Begg's funnel plot and Egger's test have not shown publication bias ($p = 0.332$).

Conclusion: To the best of our knowledge, this is the first meta-analysis that evaluates the relationship between the *NPY* rs16139 polymorphism and the risk of alcoholism. Our large-scale meta-analysis suggests that *NPY* rs16139 polymorphism is not associated with alcoholism. However, further studies are needed to increase our understanding of the relationship between *NPY* variants in alcoholism.

Keywords: NPY, rs16139, alcoholism, meta-analysis, association

INTRODUCTION

Alcohol is one of the most extensively used psychoactive drugs, which has become a part of the culture in many societies. In recent years, alcohol consumption has been rapidly increased worldwide and is responsible for social and medical problems (1). Alcohol use disorder is a chronic, recurrent disease with significant social implications. The family and twin studies suggested a 40 and 60% risk rate related to heredity (2). Genetic predisposition, environmental factors, stress, mental health, age, and gender of the patient are important risk factors for alcoholism (3). Especially, genetic risk factors play a key role in the etiology of alcoholism. Thus, it is essential to understand the genetic basis of alcoholism in order to ascertain an individual's risk of alcohol use disorder and develop effective treatment and prevention programs. Molecular genetic studies to identify the association of genes with alcoholism suggested that many candidate genes such as *ADH1B*, *ALDH2*, *CHRM2*, *DRD2*, *GABRA2*, *OPRM1*, *NPY*, and *SLC10A2* are associated with alcoholism (4–7).

Numerous lines of evidence suggested that the Neuropeptide Y is an angiogenic neurotransmitter whose physiological and behavioral effects are mediated by its receptor subtypes (Y1–Y5) (8, 9). The NPY is highly expressed in the hypothalamus, specifically in the arcuate and paraventricular nuclei, and is involved in energy homeostasis, memory function, and plasticity (10). Neuropeptide Y (NPY) is an evolutionarily conserved neuropeptide that participates in many physiological

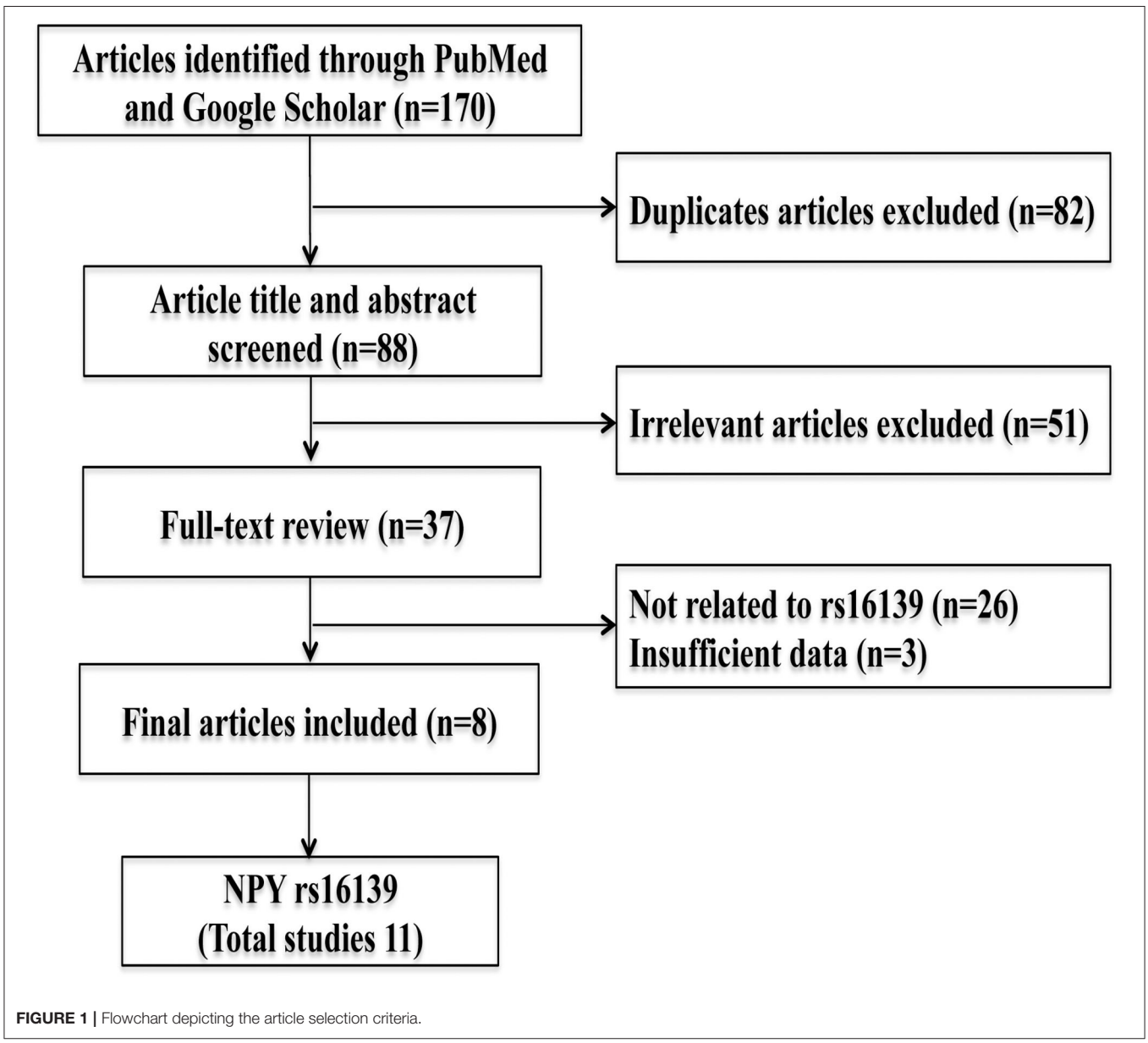

FIGURE 1 | Flowchart depicting the article selection criteria.

TABLE 1 | Summary of characteristics of included studies.

Reference	Country	Alcoholism			Control			HWE
		TT	TC	CC	TT	TC	CC	p value
Ilveskoski et al. (28)	Finland	109	12	0	44	14	0	0.883
Lappalainen et al. (23)_set1	USA	273	34	0	194	8	0	0.900
Lappalainen et al. (23)_set2	USA	144	16	0	194	8	0	0.900
Zhu et al. (24)_ Finnish	Finland	121	14	0	188	24	1	0.900
Zhu et al. (24)_Swedish	Sweden	433	39	0	156	21	0	0.883
Mottagui-Tabar et al. (29)	Sweden	531	50	0	156	21	0	0.883
Kovanen et al. (25)	Finland	459	52	1	439	67	5	0.883
Bhaskar et al. (7)_Kota	India	45	30	5	75	36	4	0.900
Bhaskar et al. (7)_Badaga	India	75	5	0	58	3	0	0.900
Akel et al. (30)	Turkey	75	5	2	75	5	1	0.248
Sengul et al. (26)	Turkey	118	5	0	150	9	0	0.900

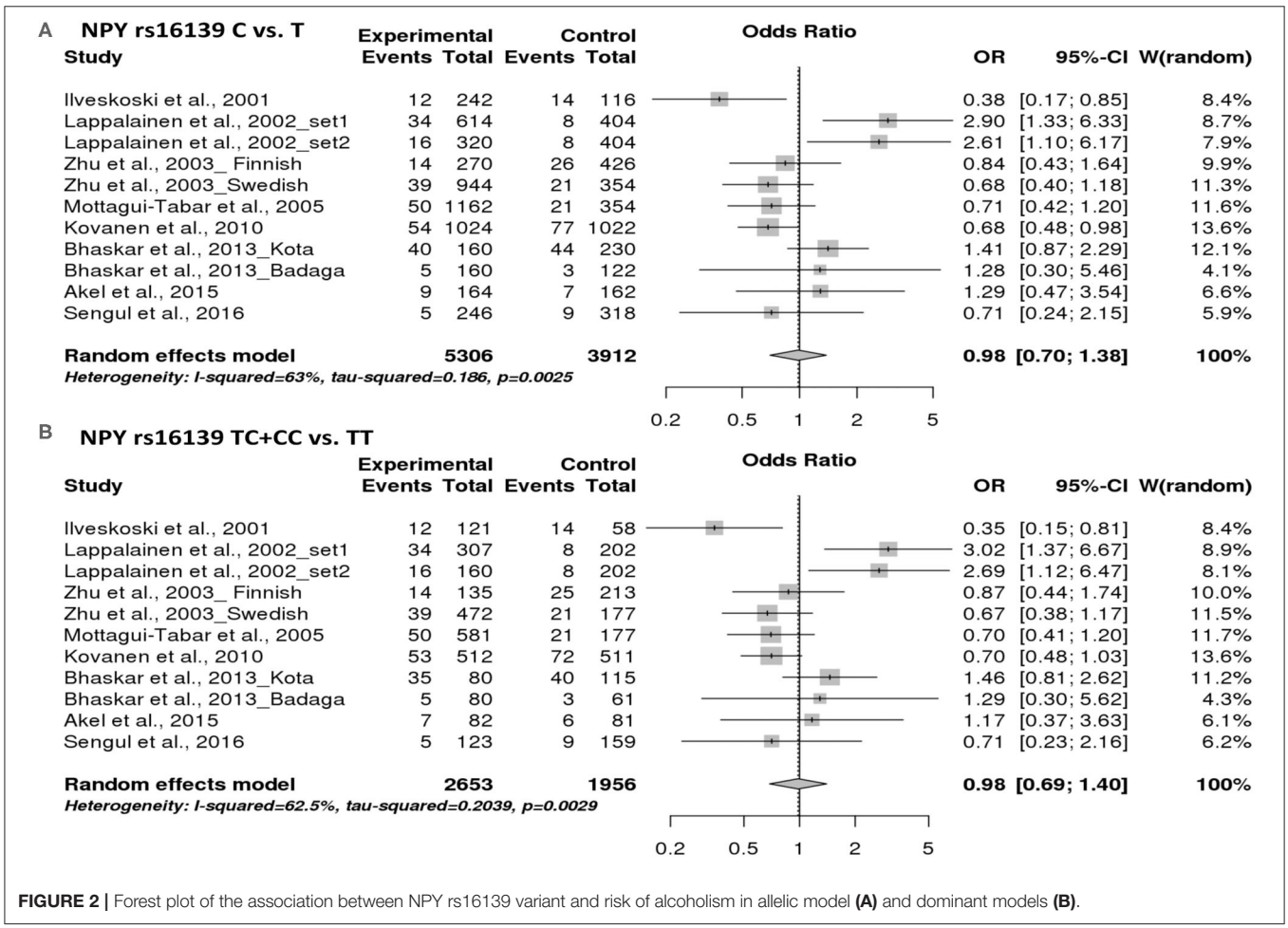

FIGURE 2 | Forest plot of the association between NPY rs16139 variant and risk of alcoholism in allelic model **(A)** and dominant models **(B)**.

functions (11). The link between NPY and alcohol consumption has been studied primarily on animals (12, 13). It is well-known that excessive alcohol consumption is frequently associated with anxiety and depression. The amygdala, a region located deep inside the temporal lobe is known express NPY and is associated with positive and negative emotional effects in healthy subjects (14, 15). The effects of NPY on alcohol-related behaviors have been attributed to their modulation of excitatory and inhibitory transmission in the amygdala and neighboring regions (11). Alcohol consumption was reduced in mice overexpressing NPY,

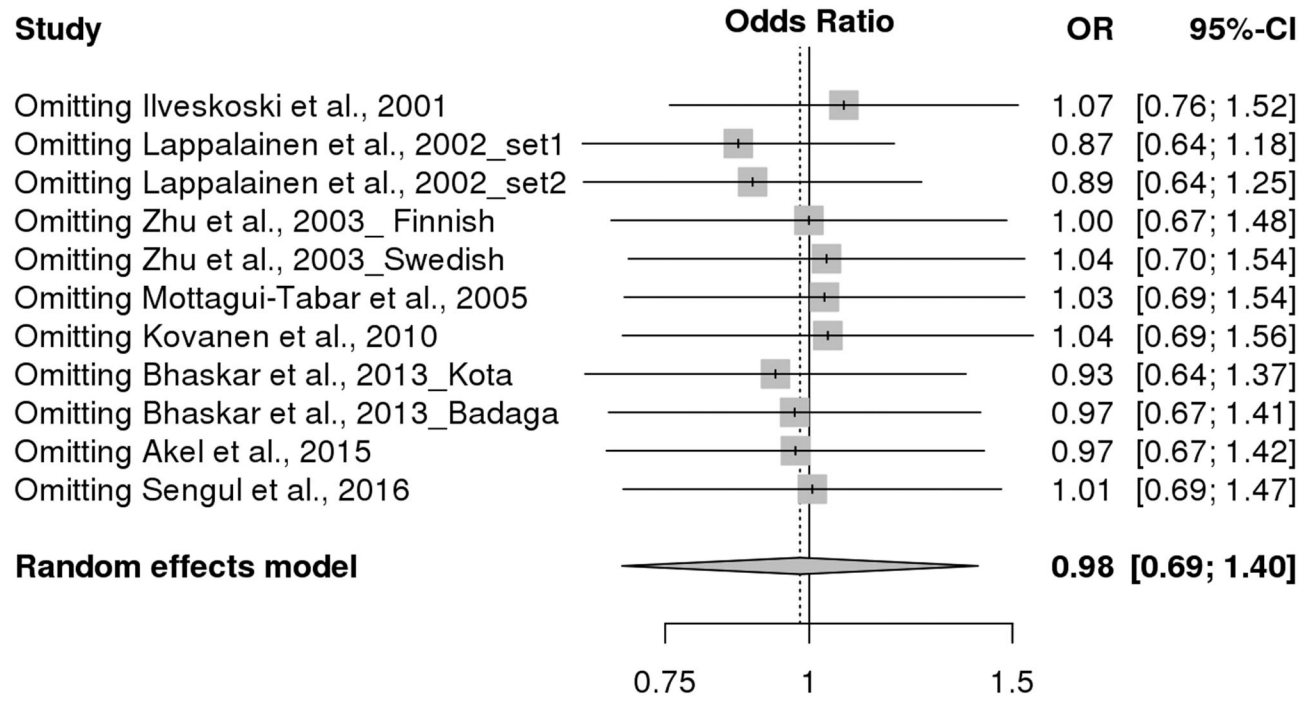

FIGURE 3 | Sensitivity analysis of this meta-analysis.

but increased in mice deficient in NPY (12). In addition, a reduction in ethanol consumption was observed when alcohol-preferring rats were injected with NPY (16). Hence, manipulating the NPY system appears to be a promising target for combating the neural alterations, alcohol use disorder related behaviors, and cognitive deficits caused by many drugs (17).

The gene coding for human NPY is located at the 7p15.1 locus of the 7th chromosome (18). A change in T>C nucleotide at 1,128 is leading to a change in amino acid leucine to proline at codon 7 (L7P) in the signal peptide of neuropeptide Y (19). The substitution of proline for leucine results in a 42% increase in plasma NPY levels (20). The association between *NPY* gene variant and alcoholism has been demonstrated in large cohorts of alcohol users and veterans (21). A promoter SNP, rs16147, alters expression of NPY *in vitro* and seems to contribute for >50% of the variation in expression *in vivo* (22). Further, the link between the NPY rs16139 polymorphism and the risk of alcoholism has been studied in many populations. However, the results are not conclusive (23–26). To date, no meta-analysis has been conducted to investigate the relationship between the NPY gene rs16139 polymorphism and the risk of alcohol use disorders. However, in the present study, we conducted a meta-analysis to evaluate the strength of association between NPY rs16139 variant and the risk of alcoholism.

MATERIALS AND METHODS

Search Strategy and Selection Criteria

According to the PRISMA guidelines (**Figure 1**), all studies examining the association of NPY gene rs16139 with alcoholism have been identified and summarized (27). PubMed, Web of Science and Google Scholar databases were searched using a combination of keywords like Neuropeptide Y (NPY), Leu7Pro, T1128C, rs16139, alcoholism, and alcohol dependence. The studies were eligible for inclusion if they met the following criteria: (1) case-control studies assessing *NPY* rs16139 polymorphisms and alcoholism risk (2) Studies having rs16139 genotypes for estimating the odds ratio. Studies with no specific control group and no detailed genotyping data for calculating odds ratios and 95% confidence intervals have been excluded. From each paper, first author, year of publication, country, genotypes from both alcoholism and control groups were extracted and tabulated (**Table 1**). From all articles, the control group's genotype frequencies were examined for deviations from Hardy-Weinberg equilibrium proportions.

Statistical Analysis

The Cochran's Q test and Higgins and Thompson inconsistency I-squared statistics were used to determine heterogeneity. The association between rs16139 polymorphism and alcoholism was assessed by determining the odds ratios (OR) and 95% confidence intervals (CI) limits. As the homozygous mutant allele is rare and not present in all studies, only the allelic and dominant effects were analyzed. Overall Pooled effects and 95% confidence intervals were estimated and presented as a forest plot. To know each study's influence on the overall effect size, sensitivity analysis was conducted using a "leave-one-out" meta-analysis. It estimated the ORs for the remaining studies. A Begg's funnel

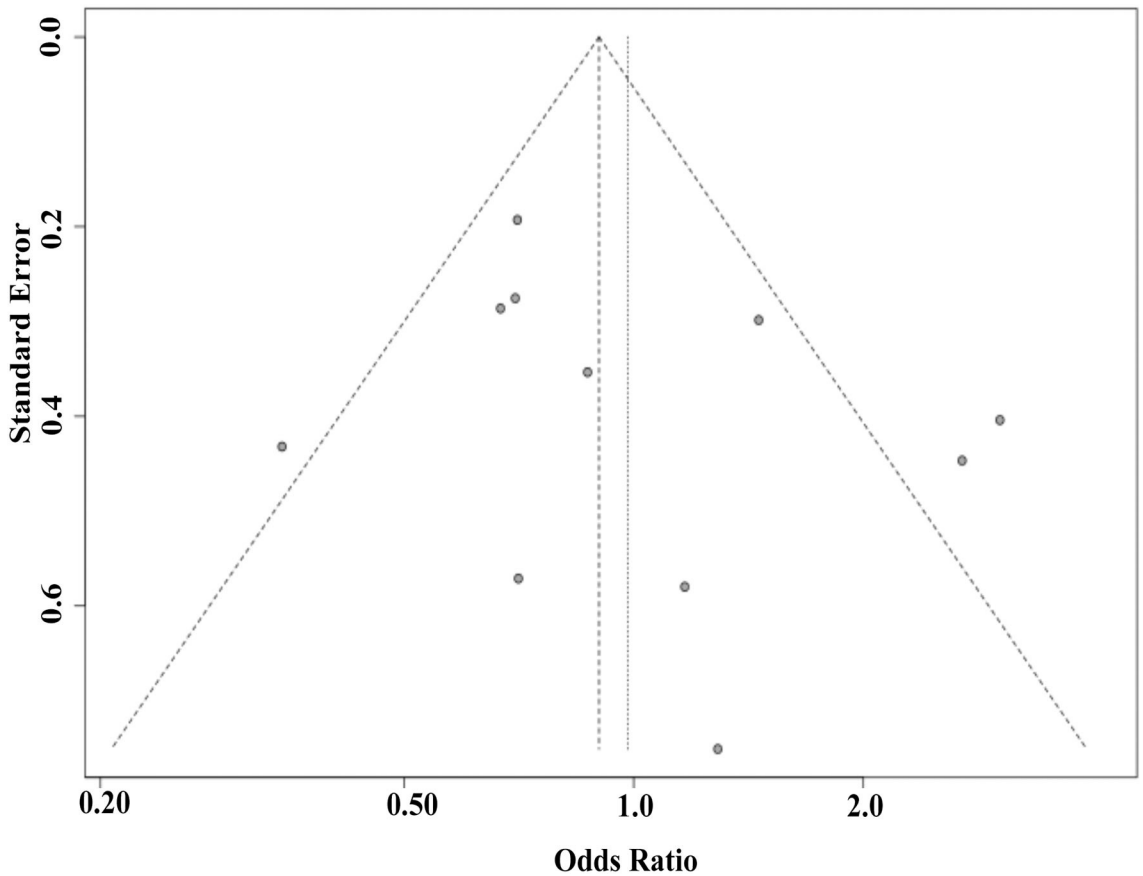

FIGURE 4 | Begg's funnel plot estimating the publication bias.

plot and an Egger's regression test were used to assessing potential publication bias. For conducting the meta-analysis and constructing all plots, MetaGenyo web tool software was used (31).

RESULTS

Characteristics of Included Studies

The search criteria and study selection process is depicted in **Figure 1**. Searching databases identified a total of 172 articles. After excluding 82 duplicate studies, 88 articles were used for further evaluation. After reading the titles and abstracts, 51 irrelevant articles were excluded, and 37 full papers were chosen for further review. Finally, eight case-control studies that met inclusion criteria were selected for data extraction (7, 23–26, 28–30). Three papers included 2 sets of data each (7, 23, 24). The genotype distributions in both alcoholism and control groups are summarized in **Table 1**. In total, 5,306 cases and 3,912 controls were included in the present meta-analysis. Significant between-study heterogeneity was found in both allelic and dominant genetic models (allele model: $P_{heterogeneity=}$ 0.003, I^2 = 63%; dominant model: $P_{Heterogeneity}$ = 0.003, I^2 = 62.5%). Hence, a random-effects model was used for the pooled analysis.

Pooled Analyses for *NPY* RS16139 Polymorphism

In order to find the association between *NPY* rs16139 variant and the risk of alcoholism, 11 studies were included in the pooled analysis. Our results have shown that there is no significant association between the risk of alcoholism and *NPY* rs16139 variant in allelic (OR = 0.98, 95% CI 0.70–1.38, p = 0.921) and dominant models (OR = 0.98, 95% CI 0.69–1.40, $p = 0.919$) (**Figure 2**).

Sensitivity Analysis and Publication Bias

Sensitivity analysis was conducted to identify the effect of each study on pooled estimates. Sensitivity analysis did not reveal any qualitative changes in pooled ORs, indicating that the results of this meta-analysis are robust (**Figure 3**). For *NPY* rs16139 variant, the shape of Begg's funnel plot did not reveal any evidence of publication bias (**Figure 4**). In addition, Egger's test revealed that there was no publication bias ($p = 0.332$).

DISCUSSION

The present study found no significant relation between NPY rs16139 variant and alcoholism in either allelic or dominant models. Although there is significant heterogeneity across studies, sensitivity analysis showed that the results of this meta-analysis are robust. Furthermore, there is no evidence of publication bias.

The relationship between NPY and alcohol consumption has primarily been studied in animals. Based on animal studies, the Pro7 allele has been linked to increased alcohol consumption in humans. A large body of research suggests that the NPY system, including NPY receptors, is involved in developing alcohol and drug use disorder, stress management, and anxiolysis (11). The "Pro7" allele of the rs16139 polymorphism in the NPY gene has been linked to increased mature NPY processing and higher NPY levels in cerebrospinal fluid (32). The "Leu7" allele has been shown to be rare in a depression population and to play a protective role against depression (33, 34). The first evidence for the role of the *NPY* Pro7 variant in regulating human alcohol consumption has been found in Eastern Finland, where individuals with the Pro7 variant showed >34% mean alcohol intake compared to the controls (35). Subsequent studies in Americans and European people also stated that the *NPY* Pro7 allele is more common in alcohol-dependent individuals (23). Further, the low frequency of the pro7 allele had a protective role against alcohol dependence in Finnish patients diagnosed with alcohol use disorder (28). In contrast, some other studies reported no association between *NPY* pro7 allele and alcohol dependence (24) or alcohol withdrawal symptoms (36). Analysis of three promoter polymorphisms and rs16139 could not detect positive correlations with alcohol dependence in the German population (37). The *NPY* Pro7 allele has been extremely rare and exhibits restricted distribution (38, 39). However, one of our previous studies shows the presence of Pro7 allele in many Indian populations (40).

Although the current meta-analysis's findings do not match physiological predictions, decreased expression of NPY has been observed in the alcohol-dependent individuals and during Alcohol withdrawal syndrome (AWS) (41, 42). Later, a systematic analysis of the NPY gene and its receptor revealed that polymorphisms in the NPY gene are not linked to alcoholism or AWS (43). There is significant heterogeneity across studies included in this meta-analysis. In some studies, the controls were social drinkers; in some others, controls were derived from the general population. There is also likely to be heterogeneity in the diagnosis of phenotypes across studies. The *NPY* Pro7 allele shows the discontinuous distribution in mixed ethnicities due to geographically variable selection; the present meta-analysis results have limited applicability.

CONCLUSION

Despite considering some limitations, Accumulating evidence of the NPY system may offer an attractive target for developing novel therapies for alcohol dependence. However, the present meta-analysis suggests that NPY rs16139 polymorphism is not associated with alcoholism. To the best of our knowledge, this is the first meta-analysis to examine the link between the NPY rs16139 and the risk of alcoholism. For the precise results, further studies with large-scale animal and human models are needed to increase our understanding of the relationship between NPY variants in alcoholism.

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

BC, MY, WY, and LB contributed to conception and design of manuscript. MY and MM searched, screened the articles and extracted data. MY and LS performed the data-analyses. BC, HV, and LB provided additional suggestions and assisted in the interpretation of data. BC and MY drafted the manuscript. WY and LB critically revised the manuscript. All authors contributed to the article and approved the submitted version.

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Pre-exercise Carbohydrate Drink Adding Protein Improves Post-exercise Fatigue Recovery

Albert Yi-Wey Tan[1†], Sareena-Hanim Hamzah[1†], Chih-Yang Huang[2,3,4,5,6‡] and Chia-Hua Kuo[7‡]*

[1] Centre for Sport and Exercise Sciences, University of Malaya, Kuala Lumpur, Malaysia, [2] Cardiovascular and Mitochondrial Related Disease Research Center, Hualien Tzu Chi Hospital, Buddhist Tzu Chi Since Medical Foundation, Hualien, Taiwan, [3] Graduate Institute of Biomedical Sciences, China Medical University, Taichung, Taiwan, [4] Department of Medical Research, China Medical University Hospital, China Medical University, Taichung, Taiwan, [5] Department of Biotechnology, Asia University, Taichung, Taiwan, [6] Center of General Education, Buddhist Tzu Chi Medical Foundation, Tzu Chi University of Science and Technology, Hualien, Taiwan, [7] Laboratory of Exercise Biochemistry, University of Taipei, Taipei, Taiwan

Purpose: This study aimed to assess the requirement of protein in pre-exercise carbohydrate drinks for optimal endurance performance at high intensity and post-exercise fatigue recovery.

Methods: Endurance performance at 85% $\dot{V}O_{2peak}$ of young men (age 20 ± 0.9 years, $\dot{V}_{2peak}$ 49.3 ± 0.3 L/min) was measured for two consecutive days using cycling time to exhaustion and total work exerted 2 h after three isocaloric supplementations: RICE (50 g, protein: 1.8 g), $n = 7$; SOY + RICE (50 g, protein: 4.8 g), $n = 7$; and WHEY + RICE (50 g, protein: 9.2 g), $n = 7$.

Results: Endurance performance was similar for the three supplemented conditions. Nevertheless, maximal cycling time and total exerted work from Day 1 to Day 2 were improved in the WHEY + RICE (+21%, $p = 0.05$) and SOY-RICE (+16%, $p = 0.10$) supplemented conditions, not the RICE supplemented condition. Increases in plasma interleukin-6 (IL-6) were observed 1 h after exercise regardless of supplemented conditions. Plasma creatine kinase remained unchanged after exercise for all three supplemented conditions. Increases in ferric reducing antioxidant power (FRAP) after exercise were small and similar for the three supplemented conditions.

Conclusion: Adding protein into carbohydrate drinks provides no immediate benefit in endurance performance and antioxidant capacity yet enhances fatigue recovery for the next day. Soy-containing carbohydrate drink, despite 50% less protein content, shows similar fatigue recovery efficacy to the whey protein-containing carbohydrate drink. These results suggest the importance of dietary nitrogen sources in fatigue recovery after exercise.

Keywords: free radical scavenging capacity, plant-based protein, reduced-to-oxidized glutathione, GSH, GSSG, soy, ferric reducing antioxidant power, FRAP

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Mallikarjuna Korivi, Zhejiang Normal University, China

Reviewed by:
Michael Chia, Nanyang Technological University, Singapore
Fenghua Sun, The Education University of Hong Kong, Hong Kong SAR, China
Sang Ki Lee, Chungnam National University, South Korea

****Correspondence:***
Chia-Hua Kuo
kch@utaipei.edu.tw; kuochiahua@gmail.com

[†] These authors have contributed equally to this work and share first authorship

[‡] These authors have contributed equally to this work and share last authorship

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INTRODUCTION

Protein is not the primary energy substrate supporting the high-intensity exercise. However, protein supplementation is known to accelerate healing during inflammation (Thomas, 1997). Inflammation is the innate immune mechanism responsible for recovering skeletal muscle from damage (Tidball, 2017). During high-intensity exercise, muscle damage is inevitably occurring which immediately triggers cell regeneration in contracting muscle (Wu et al., 2019a; Lee et al., 2021). Nitrogen from protein and amino acids is essential for DNA synthesis and cell regeneration during recovery after intensive exercise (Yang et al., 2018; Tryfidou et al., 2020).

Carbohydrate is considered the most important energy source for ATP synthesis contributing to prolonged high-intensity endurance exercise (Coyle et al., 1986; Hawley and Leckey, 2015). Post-exercise carbohydrate supplementation with a small amount of protein has been shown to accelerate recovery in endurance performance 4 h following cycling relative to carbohydrate supplementation without protein (Saunders, 2007; Hall et al., 2013). Low-protein supplementation delays the resolution of inflammation after muscle-damaging exercise (Yang et al., 2018). However, the benefit of protein addition on endurance performance is abolished when antioxidants are included in the supplement (Romano-Ely et al., 2006), suggesting that free radicals originated from inflammation (phagocytosis) mediate the fatigue recovery. Amino acid is known to activate phagocytosis with increased free radical production *in vitro* (Zhenyukh et al., 2017). Free radicals are found essential for training adaptation against aerobic exercise (Gomez-Cabrera et al., 2008). Soy is a plant-based protein source containing antioxidants (Box et al., 2005). Nevertheless, soy supplementation does not seem to affect pro-inflammatory interleukin-6 (IL-6) levels in randomized clinical trials (Beavers et al., 2009). It remains unknown whether adding natural soy into pre-exercise carbohydrate drinks can influence free radicals, endurance performance, and post-exercise fatigue recovery.

This study aimed to address the question of whether we should include protein sources (whey protein or natural soy) into pre-exercise carbohydrate drinks to optimize endurance performance and post-exercise fatigue recovery. We also examined the association between biomarkers of circulating inflammation/free radicals and endurance performance during fatigue recovery. Timing of pre-exercise supplementation seems to be important for endurance performance. For example, cycling and running times to exhaustion at moderate-to-high intensity [70% maximum oxygen consumption ($\dot{V}_{2max}$)] improve when carbohydrate was supplemented 2–3 h before the exercise challenge (Schabort et al., 1999; Chryssanthopoulos et al., 2002; Chen et al., 2009). However, studies examining endurance performance following carbohydrate supplementation within 1 h before continuous (Hargreaves et al., 1987; Febbraio et al., 2000) and intermittent exercise (Pritchett et al., 2008) show mixed results. In this study, a protein-containing carbohydrate beverage was orally given 2 h before high-intensity exercise at 85% $\dot{V}_{2max}$. We hypothesized (1) enhanced endurance performance after consumption of a protein-containing (whey protein or soy) carbohydrate beverage compared with an isocaloric carbohydrate alone drink, (2) attenuated performance enhancement after pre-exercise soy-containing carbohydrate beverage associated with higher free radical scavenging capacity, and (3) improved post-exercise fatigue recovery, assessed by the same endurance performance test on Day 2 after consumption of a protein-containing carbohydrate beverage (whey protein or soy) compared with an isocaloric carbohydrate alone drink.

MATERIALS AND METHODS

Participants

Seven physically active men (age 20.0 ± 0.9 years; height 167.7 ± 4.4 cm; body mass 56.4 ± 4.8 kg; and $\dot{V}_{2peak}$ 49.3 ± 0.3 L/min) with exercise habit > three times per week were recruited for this study. The participants signed informed consent after a verbal and written briefing on the procedures of this study including possible risks and discomforts involved. Then, they were completed a Physical Activity Readiness Questionnaire (PAR-Q) form prior to this study. Exclusion criteria are vegetarian, smokers, on a weight-reducing diet, consuming medication, or drugs, diagnosed with neurological, metabolic, and/or cardiovascular diseases, having an injury, and presenting high risk for performing maximal intensity exercises. University of Malaya Research Ethics Committee approved this study. The sample size was calculated using G-Power version 3.1.9.2 (Informer Technologies, Inc. United States) on a study by Romano-Ely et al. (2006). The G-Power indicated that a minimum sample of seven produced 95% CI with an effect size of $f = 1.10$, $\alpha = 0.05$, and $\beta = 0.80$.

Drink

Three isocaloric beverages (500 ml) were used in this study. The nutritional content of the drinks is shown in **Table 1**. RICE only beverage consists of 6% rice (30 g) and 4% cane sugar (20 g). SOY + RICE beverage contained 2% soybean (10 g), 4% rice (20 g), and 4% cane sugar (20 g). WHEY + RICE beverage contained 2% whey protein (10 g), 4% rice (20 g), and 4% cane sugar (20 g).

Study Design

The experimental design to assess the ergogenic effect of pre-exercise supplements (195–200 kcal) on high-intensity endurance

TABLE 1 | Nutritional content of pre-exercise drinks.

Macronutrients	RICE (50 g)	SOY + RICE (50 g)	WHEY + RICE (50 g)
Weight (g)			
Carbohydrate	44.3	38.5	36.8
Protein	1.8	4.8	9.2
Fat	0.2	1.8	0.8
Total	46.3	45.1	46.8
Calories (kcal)			
Carbohydrate	185.9	161.6	154.4
Protein	7.9	20.7	39.6
Fat	2.0	16.7	7.5
Total	195.8	199.0	201.5

performance (cycling time to exhaustion at 85% $\dot{V}_{2peak}$ and total work exerted) and recovery against the same exercise challenge is shown in **Figure 1**. Participants were randomized into one of the three beverage supplemented conditions in a counterbalanced order. The participants were fasted for 12 h before consuming 500 ml (1) rice mixed with soybean (SOY + RICE), (2) rice mixed with whey protein (WHEY + RICE), or (3) rice alone (RICE) as the control condition 2 h before the endurance performance test on a cycle ergometer (Day 1). Participants repeated the same experimental protocol with the same beverage on the next day (Day 2) to determine recovery efficacy. The three supplemented conditions were separated by a 1-week washout period. They were informed to refrain from taking any soy-based or whey protein-related supplements 2 days before the first trial and between Day 1 and Day 2 until the completion of all supplemented conditions. The participants were asked to limit themselves to activities of daily living and slow walking or cycling for personal transport 2 days before the trials and between Day 1 and Day 2 for each trial. All cycling trials with different supplements were performed under consistent experimental procedures under the same environmental conditions. A physician was on duty to monitor the safety of challenged participants during all the trials.

The participants were required to visit the laboratory on eight occasions. Each visit comprised of familiarization, sub-maximum cycling, and $\dot{V}_{2max}$ test, and three back-to-back cycling to exhaustion at 85% $\dot{V}_{2max}$ sessions with a 1-week period gap between them in a counter-balanced order. For the $\dot{V}_{2max}$ test, after a 30-min rest from the sub-maximum cycling test, the participant pedaled at 75 W for 1 min with 25 W increment every 1 min until volatile exhaustion. The $\dot{V}_{2max}$ was determined when the participant met at least two out of the following three criteria: (1) respiratory exchange ratio (RER) above 1.1, (2) VO_2 reached a plateau, and (3) 95% predicted maximum heart rate (HR_{max}). The maximum value of oxygen consumption was recorded as $\dot{V}_{2peak}$ if no plateau is observed. The aerobic power at 85% $\dot{V}_{2peak}$ of each participant was obtained using a regression formula between pedaling power and % $\dot{V}_{2peak}$.

Experimental Protocol

Participants arrived at the laboratory after a 12-h overnight fast, at approximately 8–9 h (Day 1). They were weighed using bioelectrical impedance analysis (InBody, United States) and a cannula (G-15, Venflon) was inserted in an antecubital vein by a phlebotomist. After a 10-min rest and blood collection, participants consumed one of the isocaloric RICE, SOY + RICE, or WHEY + RICE beverages. The participants were asked to stay within the testing area and remained sedentary (i.e., sitting, reading, and studying) for 2 h before a brief warm-up for 5 min. Then, participants were cycled on a Monark 839 E ergometer (Vansbro, Sweden) at a workload equivalent to 85% $\dot{V}_{2peak}$ until volitional exhaustion. Heart rate was monitored throughout the test using Polar FT4M (Polar Electro, Finland). Exhaustion is defined as the point at which participants can no longer maintain the cycling load. At this point, the rate of perceived exertion (RPE) was recorded using the Borg scale (Borg, 1982). The workload was recorded in kilopond per minute, and the total duration spent was used to calculate the total work done (kilopond). Blood samples were taken immediately (0 min) and 1 h post-exercise. To assess the magnitude of recovery against the exercise challenge from Day 1, the same protocol was repeated 24 h later (Day 2).

Free Radical Scavenging Capacity

Venous blood samples were collected into precooled appropriate tubes (EDTA, Heparin or plain) and centrifuged at 3,000 rpm for 15 min at 4°C before being assayed for ferric reducing antioxidant power (FRAP) (OxiSelect^{TM}, Inc., United States), glutathione (GSH and GSSG) (BioVision, United States), IL-6 (eBioscience, Vienna, Austria) with ELISA readers (Tecan Genios, Salzburg, Austria) while plasma creatine kinase (CK) was analyzed enzymatically using a benchtop DT-60IIanalyzer (Johnson and Johnson, NY, United States).

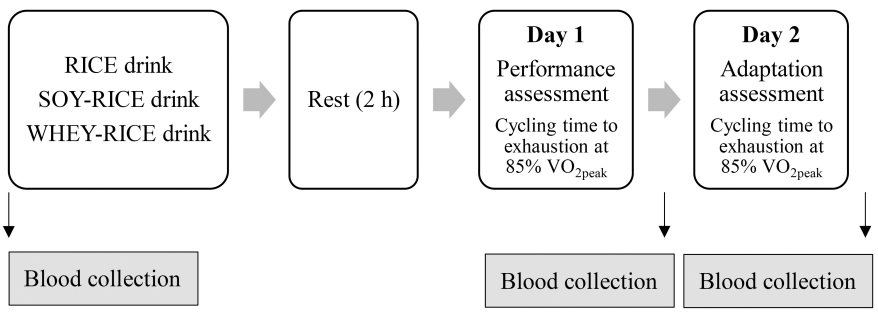

FIGURE 1 | The experimental design consisted of three isocaloric carbohydrate drinks (**Table 1**) supplemented 2 h before endurance performance test at 85% $\dot{V}_{2peak}$: RICE alone drink (n = 7), SOY-RICE drink (n = 7), and WHEY-RICE drink (n = 7) with a 7-day washout period between trials. The magnitude of fatigue recovery is indicated by performance improvement from Day 1 to Day 2 against the same cycling performance test. Arrow indicates the time of blood collection for measurements of creatine kinase (CK), interleukin-6 (IL-6), reduced-to-oxidized glutathione (GSH-to-GSSG ratio), and ferric reducing antioxidant power (FRAP). RICE alone drink (carbohydrate 95%, protein 4%, and fat 1%), SOY-RICE drink (carbohydrate 82%, protein 10%, and fat 8%), and WHEY-RICE drink (carbohydrate 76%, protein 20%, and fat 4%) in calorie.

Statistical Analyses

All values are expressed as a difference from baseline (mean ± SE). A one-way ANOVA with repeated measures was used for comparisons between three time points for endurance and plasma variables. A paired *t*-test with Bonferroni's correction was used to compare treatment differences between Day 1 and Day 2. The probability of a type I error less than 5% is considered statistically significant and 5–10% is considered moderately significant. Cohen's *d* was used to indicate the effect size of intervention on recovery (endurance performance improvement from Day 1 to Day 2).

RESULTS

On Day 1, high-intensity endurance performance (**Figure 2**) indicated by time to exhaustion (**Figure 3**) 2 h following the pre-exercise beverage ingestion was similar for the RICE, SOY + RICE, and WHEY + RICE supplemented conditions. The total work exerted (in kilopond) on Day 1 (**Figure 2A**) and RPE at exhaustion were similar among the three supplemented conditions (**Figure 2B**).

A significant difference between Day 1 and Day 2 indicates the magnitude of recovery from the Day 1 exercise. On Day 2, the total exerted cycling work increased by 5.7, 16.2, and 20.7% above Day 1 for the RICE, SOY-RICE, and WHEY-RICE supplemented conditions, respectively. Significant improvements in cycling time to exhaustion and total work exerted (in kilopond) from Day 1 were observed during the WHEY + RICE trial and to a moderate extent during the SOY + RICE trial. No improvement in cycling time to exhaustion and total workload between Day 1

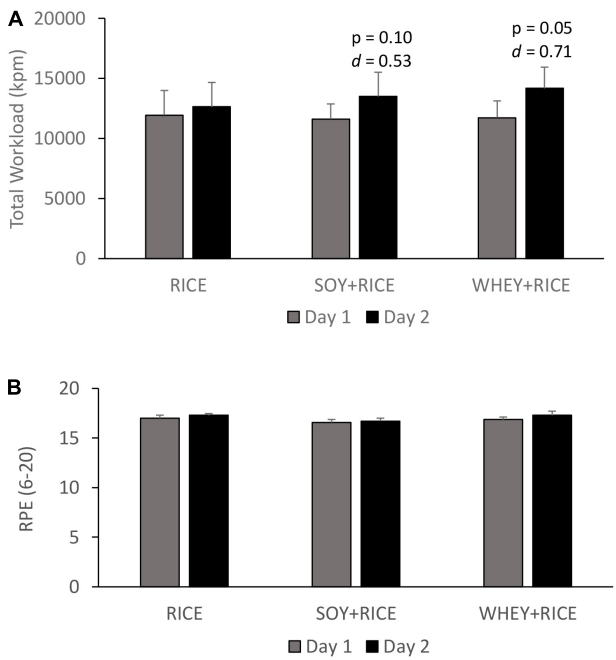

FIGURE 2 | Protein addition into carbohydrate drinks provides no immediate benefit in high-intensity endurance performance (85% $\dot{V}O_{2peak}$) than carbohydrate alone drink (n = 7). Total work exerted **(A)** was not immediately improved by protein addition, but recovery (performance on Day 2) was elevated only for the protein added condition. The rate of perceived exertion (RPE) at exhaustion was similar for the three supplemented trials **(B)**. RICE alone drink (carbohydrate 95%, protein 4%, and fat 1%), SOY-RICE drink (carbohydrate 82%, protein 10%, and fat 8%), and WHEY-RICE drink (carbohydrate 76%, protein 20%, and fat 4%) in calorie.

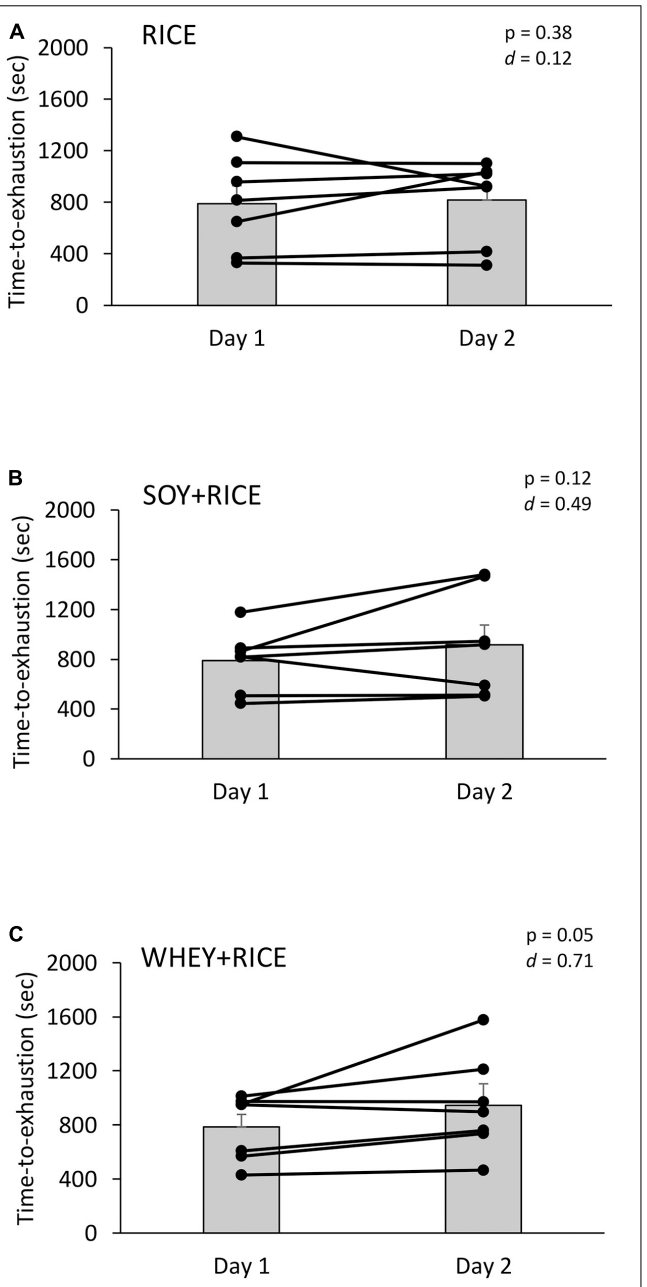

FIGURE 3 | Individual performance improvements from Day 1 to Day 2 against the same exhaustive cycling test at 85% $\dot{V}O_{2peak}$ for RICE alone drink (n = 7) **(A)**, SOY-RICE drink (n = 7) **(B)**, and WHEY-RICE drink (n = 7) **(C)**. RICE alone drink (carbohydrate, 95%, protein, 4%; fat, 1%), SOY-RICE drink (carbohydrate, 82%, protein, 10%; fat, 8%), and WHEY-RICE drink (carbohydrate, 76%, protein, 20%; fat, 4%) in calorie.

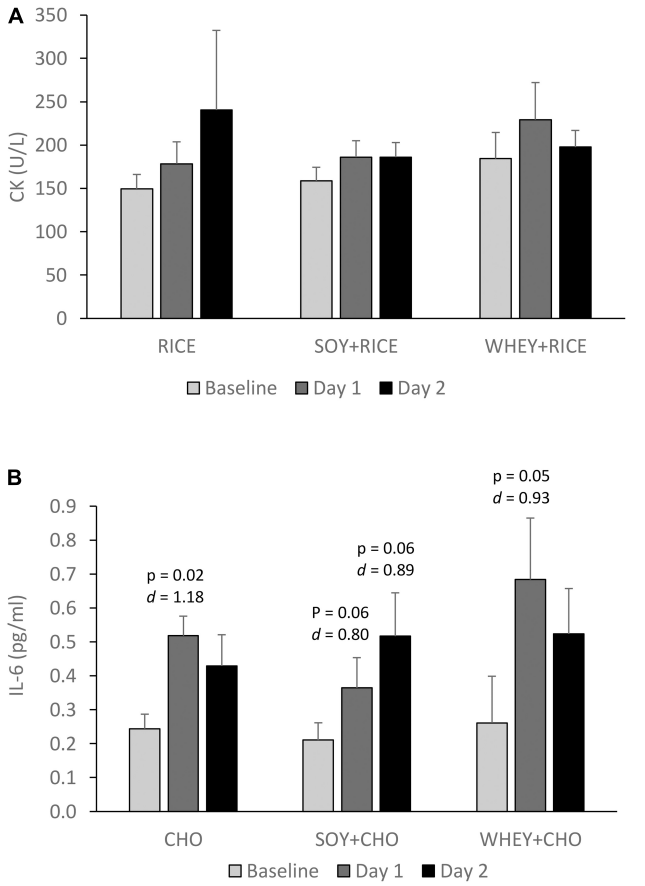

FIGURE 4 | Plasma IL-6 **(A)** and CK **(B)** responses 1 h after exercise are similar for RICE alone drink (n = 7), SOY-RICE drink (n = 7), and WHEY-RICE drink (n = 7) at Day 1 and Day 2. RICE alone drink (carbohydrate, 95%, protein, 4%; fat, 1%), SOY-RICE drink (carbohydrate, 82%, protein, 10%; fat, 8%), and WHEY-RICE drink (carbohydrate, 76%, protein, 20%; fat, 4%) in calorie.

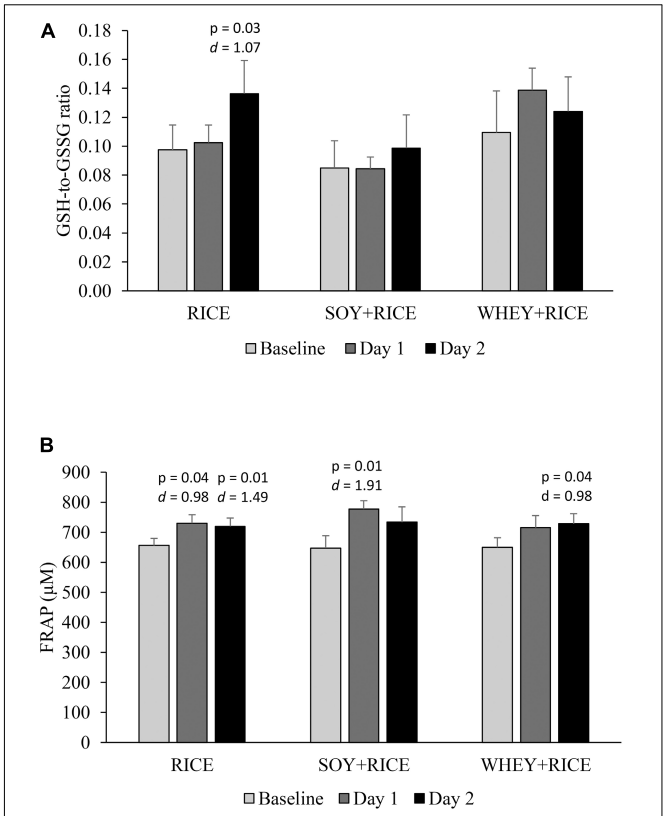

FIGURE 5 | Post-exercise antioxidant capacity (n = 7). Plasma reduced-to-oxidized glutathione (GSH-to-GSSG ratio) **(A)** and FRAP **(B)**, measured 1 h post-exercise, are similar for the three supplemented trials on the baseline, Day 1 and Day 2. Marginal time effect was found for FRAP but not for GSH-to-GSSG ratio **(B)**. RICE alone drink (carbohydrate 95%, protein 4%, and fat 1%), SOY-RICE drink (carbohydrate 82%, protein 10%, and fat 8%), and WHEY-RICE drink (carbohydrate 76%, protein 20%, and fat 4%) in calorie.

and Day 2 was observed in the RICE trial. The protein content of SOY + RICE was only half of the WHEY + RICE drink (**Table 1**). The magnitude of improvements was similar for the SOY + RICE and WHEY + RICE supplemented conditions.

Exercise-induced muscle damage and pro-inflammatory response, mirrored by plasma CK and IL-6 levels, were measured 1 h following exercise on Day 1 and Day 2 under the RICE, SOY + RICE, and WHEY + RICE supplemented conditions (**Figure 4**). No significant time effect was detected in plasma CK after the exhausted bout of cycling for the three supplemented conditions (**Figure 4A**). IL-6 was consistently elevated following exercise for the three supplemented conditions (**Figure 4B**). Differences in post-exercise IL-6 were not significant on Day 1 and Day 2 for all conditions.

Post-exercise free radical scavenging capacity was indicated by the GSH-to-GSSG ratio and FRAP in plasma, measured 1 h post-exercise (**Figure 5**). The GSH-to-GSSG ratio on Day 2 was increased above baseline for the RICE supplemented condition, but not significant for the SOY + RICE and WHEY + RICE supplemented conditions (**Figure 5A**). Post-exercise plasma FRAP under the RICE, SOY + RICE, and WHEY + RICE supplemented conditions are presented in **Figure 5B**. A trend of a small increase in plasma FRAP was observed 1 h after exercise regardless of supplemented conditions.

DISCUSSION

Pre-exercise carbohydrate supplementation (2–3 h prior to exercise) improves a time-to-exhaustion running performance (Chryssanthopoulos et al., 2002). This study asked the question of whether adding a small amount of protein provides immediate benefit for endurance performance and subsequent fatigue recovery on Day 2 against the same exercise challenge, compared with isocaloric carbohydrate alone drink. In this study, no immediate performance enhancement effect of protein addition was observed. However, endurance performance against the same cycling test was significantly improved on Day 2 when protein was included in the pre-exercise carbohydrate drink, without a significant difference between whey protein isolate

(20% improvement) and natural soy (16% improvement). Since protein is not the major fuel to sustain high-intensity endurance exercise, the finding of this study implicates a requirement of dietary nitrogen for post-exercise recovery.

The result of this study demonstrates a carry-over effect of pre-exercise protein supplementation for high-intensity endurance performance on the next day. The underlying mechanism for this delayed effect remains unclear. Exercise challenge causes tissue damage and triggers cell regeneration after mobilization of hematopoietic progenitor cells and endothelial progenitor cells into the sites of damage during inflammation (Krüger et al., 2015; Wu et al., 2019a,b; Lee et al., 2021). The cell regeneration process requires nitrogen source and time for DNA synthesis (Hosios et al., 2016), which might explain no immediate ergogenic effect of protein addition into the protein-containing carbohydrate drink, but a far-reaching improvement in high-intensity endurance performance on Day 2. We speculated that supplementing protein short after exercise can also produce a similar benefit. In a short-term training study, significant improvements in running performance with a less subjective feeling of performance capacity loss were observed when protein-containing carbohydrate supplements were orally given after exercise, compared with carbohydrate alone supplements (Hansen et al., 2015). It is generally known that amino acids and proteins exert a psychological effect on the brain under stressed conditions. Milk protein significantly improves mood and cortisol levels after stress among normal participants (Markus et al., 2000). Therefore, we could not preclude the possibility that the observed delayed effect on fatigue recovery is mediated by its dual effects on the brain and muscle.

Interleukin-6 is a pro-inflammatory cytokine produced as part of a signal that triggers healing after challenge. Exercise-induced recovery in endurance performance requires IL-6 (McGinnis et al., 2015; Marasco et al., 2018) and free radical production (Gomez-Cabrera et al., 2008) during inflammation. The inflammation process, involved with the elimination of injured tissue by phagocytosis followed by a protracted cell regeneration, is essential for recovery against physical challenge (Tidball, 2017). Increased leukocyte infiltration, satellite cell replenishment, and increased free radicals are normally observed in challenged muscle after aerobic cycling exercise without observable changes in plasma CK and GSH-to-GSSG ratio (Wu et al., 2019a,b). However, in this study, we do not find the difference in responses of IL-6, GSH-to-GSSG ratio, and FRAP against exercise among the three pre-exercise supplemented conditions, albeit better exercise recovery outcomes after consumption of protein-added rice supplements. This suggests that nutritional nitrogen source is a limiting factor for post-exercise recovery.

Soy supplementation has been shown to lower systemic inflammation (Mangano et al., 2013) and oxidative stress (Tikkanen et al., 1998; Jenkins et al., 2000) for non-exercise individuals. However, in this study, we could not observe the suppressive effect of soy addition on exercise-induced IL-6 elevation as well as the difference in antioxidant markers. The absence of decreased plasma GSH-to-GSSG ratio together with slightly elevated FRAP suggests that the oxidative stress produced during the exercise test is sufficiently accommodated by endogenous antioxidant capacity in these young men. In addition, the previously observed antioxidant effect of soy may not be mediated by the neutralization of free radicals.

It is worthy to note that the SOY-RICE drink used in this study has only 50% of protein of the WHEY-RICE drink. However, the performance improvement on Day 2 was similar for the SOY-RICE and WHEY-RICE drinks. We could not preclude the possibility that other ingredients in natural soy than protein play a role in facilitating the physiological recovery against the exercise challenge.

A major limitation of this study is the low sample size. Furthermore, the potential influence of different fat contents is another inevitable limitation for such type of dietary study. Therefore, the generalization of the knowledge produced from the current work should be interpreted with caution.

CONCLUSION

The result of this study demonstrated that consuming carbohydrate drinks (50 g) containing either natural soy or whey protein isolate 2 h before exercise provides no immediate benefit in performance enhancement at high intensity compared with isocaloric carbohydrate alone drink. However, fatigue recovery in endurance performance can be improved on Day 2 by including dietary protein into the pre-exercise carbohydrate drink. This study also provides encouraging evidence that natural protein-enriched soy supplementation, with less soy protein (10.6%), could produce a similar benefit in fatigue recovery as whey protein (19.7%).

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the University of Malaya Research Ethics Committee (UM.TNC2/RC/H&E/UMREC-115), and all participants gave written consent.

AUTHOR CONTRIBUTIONS

AT and C-YH, and C-HK designed the experiments. AT performed the experiments and statistical analyses. AT, S-HH, C-YH, and C-HK wrote the manuscript. All authors contributed to the article and approved the submitted version.

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Early Mental Health and Quality of Life in Discharged Patients With COVID-19

Jinzhuo Hu[1†], Yang Zhang[1†], Qingqing Xue[1], Yun Song[1], Fei Li[2], Ran Lei[2], Jinlun Wu[2] and Jinghua Qian[1]*

[1] School of Sports Medicine and Rehabilitation, Beijing Sport University, Beijing, China, [2] Rehabilitation Department, China Resources and Wisco General Hospital, Wuhan, China

Aim: This study aimed to analyze the early mental health (MH) and quality of life (QoL) of discharged patients with coronavirus disease 2019 (COVID-19), which can provide a scientific basis for the further development of intervention programs.

Methods: In total, 108 subjects participated in this study, including an experimental group (90 patients diagnosed with COVID-19 from March to April 2020 and hospitalized in Wuhan China Resources & WISCO General Hospital, Wuhan, China, 83.3%) and a control group (18 healthy participants, 16.7%). Their MH and QoL were measured through the 12-item Short Form Health Survey version 2 (SF-12v2), the Self-rating anxiety scale (SAS), the Self-rating depression scale (SDS), and the International Physical Activity Questionnaire (IPAQ). The results of questionnaires were compared between these two groups.

Results: (1) Comparison of anxiety status: among 90 discharged patients with COVID-19, 30 patients (33.3%) had a state of anxiety. Compared with healthy participants and the general population, patients with COVID-19 in the early stages of discharge had a higher incidence of anxiety and more severe anxiety symptoms ($P < 0.05$). (2) Comparison of depression status: among 90 discharged patients with COVID-19, 29 patients (32.2%) had a state of depression. Compared with healthy participants and the general population, patients with COVID-19 in the early stages of discharge had a higher incidence of depression and more severe depression symptoms ($P < 0.05$). (3) Comparison of QoL: 78 patients (86.7%) presented a decrease in physical health-related quality of life (HRQoL) and 73 patients (81.1%) presented a decrease in psychology-related QoL. The SF-12v2 physical component summary (PCS) and the SF-12v2 mental component summary (MCS) of patients were significantly lower than those of healthy people, especially in physical function (PF), vitality (VT), social function (SF), and mental health (MH) (all $P < 0.05$). (4) Gender differences in mental health and the QoL among patients with COVID-19: women had more severe anxiety/depression symptoms than men ($P < 0.05$). The scores of women in all dimensions of SF-12V2 were lower than those of men, and there were statistically significant differences between the two groups in PCS, PF, general health (GH), VT, and role-emotional (RE) ($P < 0.05$).

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Mallikarjuna Korivi,
Zhejiang Normal University, China

Reviewed by:
Yongxin Li,
Henan University, China
Xueqiang Wang,
Shanghai University of Sport, China

****Correspondence:***
Jinghua Qian
jh5727@163.com

† These authors have contributed equally to this work and share first authorship

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Conclusion: During the early phase after being discharged, patients with COVID-19 might experience negative emotions, such as anxiety or depression, and also problems with reduced QoL, especially among female patients. Therefore, an intervention plan should focus on strengthening psychological condition and improving physical function, and gender-specific rehabilitation programmes should be adapted to improve psychological status and QoL.

Keywords: coronavirus disease 2019, discharged patients, mental health, quality of life, the 12-item short form Health Survey version 2

INTRODUCTION

An outbreak of pneumonia of unknown cause occurred in Wuhan, Hubei Province, China, in December 2019. On February 11, 2020, the WHO officially named the pneumonia caused by the novel coronavirus as coronavirus disease 2019 (COVID-19) (1). Subsequently, many people in other countries worldwide were found to be infected with the respiratory infectious disease. As of March 31, 2020, COVID-19 had caused 862,234 confirmed infection cases and 42,424 deaths, posing an important threat to the lives and health of the global population (2). The main clinical characteristics of COVID-19 are fever, cough, and shortness of breath, and a proportion of patients may also suffer from new loss of taste or smell, diarrhea, nausea, vomiting, and other symptoms (3). As the most severely affected city in Hubei Province, the health consequences of these patients with COVID-19 have attracted worldwide attention and need to be evaluated urgently.

Due to isolation and lack of awareness of the consequences of the novel coronavirus, patients with COVID-19 have been under tremendous psychological pressure during the treatment against severe acute syndrome coronavirus 2 (SARS-CoV-2), which may bring them certain mental health problems, such as anxiety, depression, insomnia, and fear (4). A recent meta-analysis included 62 studies from 17 countries and found that the prevalence of anxiety and depression was the highest (56 and 55%) among patients with COVID-19 (5). Such mental health concerns may lead to both shorter- and longer-term problems, particularly when experienced in combination with other factors, such as poverty and insufficient healthcare services (6). However, the infectiousness of COVID-19 makes it difficult for researchers to reach patients directly and continuously. Most epidemic-related psychological studies are mainly concerned with ordinary residents and medical staff (7), while there are very few follow-up investigations of mental health among discharged patients and these are rarely compared with patients who were not infected with COVID-19.

In recent years, health-related quality of life (HRQoL) has aroused great interest among researchers. Studies have shown that COVID-19 can affect HRQoL of patients and general populations (8). The patients with COVID-19 who were admitted to hospitals during infection had a low QoL score in physical, psychological, and socials domains, and continued to have QoL issues even after recovery (9). However, only few studies have reported HRQoL of patients after discharge. One study has revealed that COVID-19 is associated with a substantial and measurable decrease in HRQoL, and the age and hospitalization status of participants were the key determinants of their COVID-19 health utility value (10). Further evidence suggests that, even though physical function was recovered, patients might still have mental disorders (11), which could affect them even after 1 year (12). Therefore, the impact of SARS-CoV-2 on the psychology and HRQoL of patients cannot be ignored.

Considering studies reporting the physical and psychological conditions of patients with COVID-19 after discharge from hospital are rare, the purpose of this study is to investigate the early psychology and QoL of clinically cured and discharged patients with COVID-19 in Wuhan, a city heavily affected by novel coronavirus 2019, so as to provide a basis for further scientific intervention plans.

METHODS

Subjects and Study Design

In total, 108 participants with and without COVID-19 were recruited in China Resources & WISCO General Hospital from March to April 2020, including an experimental group (90 patients diagnosed with COVID-19, 83.3%) and a control group (18 healthy participants, 16.7%). The inclusion and exclusion criteria of the experimental group are presented in **Table 1**. The control group recruited healthy people who were not infected with COVID-19 from healthcare workers and families of patients. The age and gender of the control group matched those of the experimental group, and the exclusion criteria were the same as those of the experimental group. Basic information of the two groups was collected, such as age, sex, disease type, educational status, and comorbidities of patients with COVID-19.

The mental health and Qol of all participants were measured through the 12-item Short Form Health Survey version 2 (SF-12v2), the Self-rating anxiety scale (SAS), and the Self-rating depression scale (SDS). The questionnaires were completed online and distributed in a WeChat group. The same IP address can be used only once and the questionnaire must be completed by the subject. Repeated questionnaires were eliminated. The obtained data were input into the "Questionnaire Star" system for real-time monitoring to ensure the accuracy of the data. All subjects signed the informed consent form.

Self-Rating Anxiety Scale

The Self-rating anxiety scale is a widely used self-rating tool for adults with anxiety symptoms. The scale consists of 20 items,

TABLE 1 | Article inclusion and exclusion criteria.

Inclusion criteria	Exclusion criteria
(1) A clear diagnosis of COVID-19	(1) Temperature >38°C
(2) Within 3–45 days after discharge (including patients in isolation sites after discharge and early home-based patients who have returned to the community)	(2) Heart rate >120 bpm or <40 bpm, systolic blood pressure <90 mmHg or >180 mmHg, respiratory rate >25 bpm or vital signs significant fluctuations calm state
(3) 18–80 years old	(3) Continuous oxygen therapy was needed
(4) Voluntarily agreed to join the queue and cooperate with the relevant assessments	(4) Patients with myocarditis, pulmonary hypertension, congestive heart failure, acute renal failure, fresh venous thromboembolic disease, unstable fracture, etc., who were not suitable for exercise
	(5) Conscious disorders, cognitive dysfunction, mental disorders, balance disorders, severe bone and joint diseases, and other impact assessment patients
	(6) Other conditions of inability to cooperate with rehabilitation treatment

TABLE 2 | Conversion table of each dimension of the 12-item Short Form Health Survey version 2 (SF-12v2).

Scale	Items	Score ranges	Conversion points
GH	1	1–5	(Actual score-1)/4*100
PF	2	2–6	(Actual score-2)/4*100
RP	2	2–10	(Actual score-2)/8*100
RE	2	2–10	(Actual score-2)/8*100
BP	1	1–5	(Actual score-1)/4*100
VT	1	1–5	(Actual score-1)/4*100
MH	2	2–10	(Actual score-2)/8*100
SF	1	1–5	(Actual score-1)/4*100

such as forward score and reverse score, and each item is scored at four levels. After the self-assessment, the total score was multiplied by 1.25 to get an integer. The higher the final score, the more severe the symptoms (13). According to the results of the healthy Chinese population, the cut-off value of SAS SD is 50 points, in which 50–59 is considered mild anxiety, 60–69 is considered moderate anxiety, and 69 or above is considered severe anxiety. The results need to be compared with the Chinese norm, which can provide a baseline for interpretation and comparison of the results (14).

Self-Rating Depression Scale

The Self-rating depression scale can effectively reflect the symptoms of depression and its severity and changes. The scale consists of 20 items, each of which corresponds to one symptom concerned, and is rated on a scale of 1–4. The raw score can be converted to an SDS Index score by multiplying the raw score by 1.25. According to the results of the Chinese norm, the cut-off value of SDS standard score is 53 points, of which 53–62 is considered mild depression, 63–72 is considered moderate depression, and 73 or above is considered severe depression (15). The results need to be compared with the Chinese norm, which can provide a baseline for interpretation and comparison of the results.

The 12-Item Short Form Health Survey Version 2

Studies have proved the applicability of SF-12v2 in the Chinese population (16). The SF-12v2 scale has 12 items, evaluating eight dimensions of HRQoL, such as general health (GH), physical functioning (PF), role-physical (RP), bodily pain (BP), vitality (VT), social functioning (SF), role-emotional (RE), and mental health (MH). GH, PF, RP, and BP can be calculated to obtain the physical component summary (PCS), while SF, RE, MH, and VT can be calculated to get the mental component summary (MCS). In the scoring calculation, corresponding weights should be given to each item according to the degree of impact on QoL (17) (**Table 2**). Each dimension should be converted into a percentage system, and the total physical health score and the total mental health standard score should be converted into normal-based score according to the standard where the mean is 50 and the SD is 10. A total score of more than 50 on the SF-12v2 scale indicates that the QoL is higher than that of the general population, while a score below 50 indicates that the QoL is lower than that of the general population.

Statistical Analysis

All valid data were entered into Excel 2016 after review, sorting, and coding. SPSS 25.0 software was used for statistical analysis. Descriptive analysis was conducted on the basic information of the research subjects. Age presented a normal distribution, reported by means ± SD. *T*-test was used for comparison between groups; qualitative data were presented as the number of cases (%), and x^2 test was used for comparison between the groups. Anxiety, depression, and QoL were compared between the patients with COVID-19 and healthy subjects using independent sample *t*-test and Wilcoxon signed-rank test. *P*-values < 0.05 were considered statistically significant.

RESULTS

In total, 111 questionnaires were collected, of which 108 were valid, with an effective rate of 97.3%.

Characteristics of Subjects

According to the questionnaire response, there were 108 subjects in this study, including 90 (83.3%) patients with COVID-19 and 18 (16.7%) healthy participants. Among the patients with COVID-19, the mean age was (50.8 years ± 12.5) including 40 men (44.4%) and 50 women (55.6%). In this study, 9 (10.0%) patients were mild type, 63 (70.0%) patients were ordinary type, 18 (20.0%) patients were severe type, but there were no critically ill patients. All the patients were cured and discharged. In addition, we examined the education status and comorbidities of patients with COVID-19, with 37 (41.1%) having a college education or above, 28 (31.1%) having a senior high school

TABLE 3 | The baseline characteristics of patients with COVID-19 and healthy participants.

	COVID-19 patients $n = 90$ (%)	Healthy participants $n = 18$ (%)	t/χ^2	*P*-value
Age-years (Mean ± SD)	(50.8 ± 12.5)	(51.3 ± 12.5)	−0.165	0.869
Gender				
Male	40 (44.4%)	9 (50.0%)	0.187	0.666
Female	50 (55.6%)	9 (50.0%)		
Clinical typing				
Mild	9 (10.0%)			
Ordinary	63 (70.0%)			
Severe	18 (20.0%)			
Educational status				
University and above	37 (41.1%)			
Senior high school	28 (31.1%)			
Junior high school and below	25 (27.8%)			
Comorbidities				
Yes	47 (52.2%)	5 (27.8%)	3.590	0.058
No	43 (47.8%)	13 (72.2%)		

education, and 25 (27.8%) having a junior high school education or below, and 47 (52.2%) having comorbidities (such as diabetes and hypertension). In the healthy participants, the mean age was (51.3 years ± 12.5), including nine men (50.0%), nine women (50.0%), and five participants (27.8%) with comorbidities. There was no statistical difference in general data between the two groups ($p > 0.05$) (**Table 3**).

Comparison of Anxiety With or Without COVID-19

Among patients with COVID-19, 30 (33.3%) had anxiety symptoms, of which 20 (22.2%) had mild anxiety, 7 (7.8%) had moderate anxiety, and 3 (3.3%) had severe anxiety. Among the healthy participants, 5 (27.8%) had anxiety symptoms, including 4 (22.2%) with mild anxiety and 1 (5.6%) with moderate anxiety. There was no significant difference in the frequency ratio of anxiety between the two groups although there was a difference in the raw scores ($P > 0.05$) (**Table 4**). Compared with healthy people, patients with COVID-19 had a significantly higher SAS score ($P < 0.05$). In addition, the anxiety levels in patients with COVID-19 were higher than the general population ($P < 0.001$) (**Table 5**).

Comparison of Depression With or Without COVID-19

Among patients with COVID-19, 29 (32.2%) had depressive symptoms, of which 23 (25.5%) had mild depression and 6 (6.7%) had moderate depression. Among healthy participants, three people (16.7%) had mild depression. There was no significant difference in the frequency ratio of depression between the two groups although there was a difference in the raw scores ($P > 0.05$) (**Table 6**). Compared with healthy participants, patients with COVID-19 had a significantly higher SDS score ($P < 0.05$). In addition, the depression levels in patients with COVID-19 were higher than the general population ($P < 0.001$) (**Table 7**).

Comparison of QoL With or Without COVID-19

The scores of patients with COVID-19 and healthy participants in eight dimensions are shown in **Table 8**. Compared with the healthy participants, the scores of all dimensions of patients with COVID-19 were lower, and there were significant differences between the two groups in PF, VT, SF, and MH ($P < 0.05$).

The average PCS score of patients with COVID-19 was (37.85 ± 12.63), of which 86.7% of patients (78 patients) scored <50 points. The average score of MCS was (38.81 ± 13.54), and 81.1% of the patients (73 patients) scored <50 points. The statistical results showed that the scores of the patients in both physiological and psychological fields were significantly lower than those of the healthy participants ($P < 0.05$).

Gender Differences in MH and the QoL Among Patients With COVID-19

Gender differences in MH and QoL among patients with COVID-19 are shown in **Table 9**. Women had more severe anxiety/depression symptoms than men ($P < 0.05$). The scores of women in all dimensions of SF-12v2 were lower than those of men, and there were statistically significant differences between the two groups in PCS, PF, GH, VT, and RE ($P < 0.05$).

DISCUSSION

In the face of newly emerging infectious diseases, the incidence of negative emotions, such as fear, sadness, and tension among the population increases (18). This study found that more than one-third of patients with COVID-19 had anxiety/depression. A meta-analysis also found similar results (11). This indicates that due to the long period of isolation and treatment, patients will have a sense of social alienation, anxiety, fear, and even pessimistic attitude about returning to society. However, although the incidence of COVID-19 anxiety/depression was higher than that of healthy people, the difference between the two groups was not statistically significant, which was considered to be related to the small sample size. In addition, because MH and psychosocial consequences of COVID-19 has a serious impact on various categories of people, the anxiety/depression incidence of healthy people may also increase (19).

The anxiety and depression levels in patients with COVID-19 were higher than the general population in this study. This suggests that a patient with COVID-19 may be more likely have severe anxiety/depression symptoms. In addition, we also found that the anxiety and depression of patients with COVID-19 are generally more severe than those of healthy people. This can indicate that the psychological problems of patients with COVID-19 are caused by COVID-19 infection. In addition, the difference between the two groups was small, which suggested that the COVID-19 pandemic also resulted in challenges for healthy people.

TABLE 4 | Incidence of anxiety reported by coronavirus disease 2019 (COVID-19) survivors and healthy participants.

	Self-rating anxiety scale (SAS)					
	Mild	**Moderate**	**Severe**	**None**	χ^2	***P*-value**
COVID-19 patients ($n = 90$)	20 (22.2%)	7 (7.8%)	3 (3.3%)	60 (66.7%)	0.211	0.646
Healthy participants ($n = 18$)	4 (22.2%)	1 (5.6%)	0 (0)	13 (72.2%)		

TABLE 5 | Average score of the Self-rating anxiety scale (SAS) reported by COVID-19 survivors, healthy participants, and the general population.

	COVID-19 patients ($n = 90$) mean (SD)	**Healthy participants ($n = 18$) mean (SD)**	**Mean difference between groups (95% CI)**	***P*-value**	**Effect size**
SAS score	45.72 (10.79)	39.72 (11.87)	6.00 (0.38–11.62)	0.036	0.52
	COVID-19 patients ($n = 90$) mean (SD)	**General population ($n = 2,249$) mean (SD)**	**Mean difference between groups (95% CI)**	***P*-value**	**Effect size**
SAS score	45.72 (10.79)	29.78 (0.46)	15.94 (15.49–16.39)	<0.001	2.09

TABLE 6 | Incidence of depression reported by COVID-19 survivors and healthy participants.

	Self-rating depression scale (SDS)					
	Mild	**Moderate**	**Severe**	**None**	χ^2	***P*-value**
COVID-19 patients ($n = 90$)	23 (25.5%)	6 (6.7%)	0 (0)	61 (67.8%)	1.741	0.187
Healthy participants ($n = 18$)	3 (16.7%)	0 (0%)	0 (0)	15 (83.3%)		

TABLE 7 | Average score of the Self-rating depression scale (SDS) reported by COVID-19 survivors, healthy participants, and the general population.

	COVID-19 patients ($n = 90$) mean (SD)	**Healthy participants ($n = 18$) mean (SD)**	**Mean difference between groups (95% CI)**	***P*-value**	**Effect size**
SDS score	47.28 (9.93)	41.94 (11.74)	5.34 (0.09–10.58)	0.046	0.49
	COVID-19 patients ($n = 90$) mean (SD)	**General population ($n = 2,249$) mean (SD)**	**Mean difference between groups (95% CI)**	***P*-value**	**Effect size**
SDS score	47.28 (9.93)	41.88 (10.75)	5.40 (3.14–7.66)	<0.001	0.52

It should be noted that the baseline difference in comorbidities between the experimental group and the control group was nearly significant ($P = 0.058$). This suggests that comorbidities may have an impact on the mental status of patients with COVID-19. Analyzing the clinical and epidemiological data of COVID-19 suggested that specific comorbidities increase the risk of infection with worse lung injury and death. The most common comorbidities reported up until now were hypertension, cardiovascular diseases, and diabetes (20). Additionally, a high proportion of patients with COVID-19 and other conditions in admitted intensive care unit (ICU) cases suggested comorbidities as a potential risk factor for patients with COVID-19 (21). Therefore, the meticulous management of patients with COVID-19 with comorbidities in contrast to without comorbidities is emphasized to control the jeopardy of life. Comorbid individuals must undertake vigilant preventive measures to protect themselves during the pandemic (22).

Evidence from the present study indicated that compared with the healthy population, patients with COVID-19 had lower SF-12v2 scores in all dimensions at the early stage of discharge, especially in PF, VT, SF, MH, PCS, and MCS. These six aspects indicated that the QoL of patients was generally reduced in the early stage after discharge. Individual level variables of COVID-19 anxiety and personal identity significantly predicted QoL (23). A sense of coherence as a marker of QoL may be considered as a psychological process influencing MH, which in turn may affect QoL as well (24).

TABLE 8 | Average score of SF-12v2 components reported by COVID-19 survivors and healthy participants.

SF-12v2 component	COVID-19 patients ($n = 90$) mean (SD)	Healthy participants ($n = 18$) mean (SD)	Mean difference between groups (95% CI)	*P*-value	Effect size
PF	73.89 (26.21)	93.06 (11.52)	−19.17 (−31.69 to −6.65)	0.003	−0.95
RP	58.06 (27.94)	67.36 (27.50)	−9.30 (−23.57 to 4.96)	0.199	
BP	55.83 (29.04)	69.44 (26.51)	−13.61 (−28.28 to 1.05)	0.069	
GH	45.28 (27.00)	55.56 (29.15)	−10.28 (−24.28 to 3.72)	0.149	
VT	58.89 (26.05)	73.61 (13.48)	−14.72 (−27.25 to −2.20)	0.022	−0.71
SF	36.94 (32.72)	75.00 (29.70)	−38.06 (−54.57 to −21.54)	<0.001	−1.22
RE	62.92 (24.96)	72.92 (24.72)	−10.00 (−22.76 to 2.76)	0.123	
MH	65.28 (21.02)	76.39 (15.39)	−11.11 (−21.46 to −0.76)	0.036	−0.60
PCS	37.85 (12.63)	46.56 (9.63)	−8.71 (−14.96 to −2.47)	0.007	−0.78
MCS	38.81 (13.54)	46.56 (11.90)	−7.75 (−14.55 to −0.94)	0.026	−0.61

TABLE 9 | Gender differences in mental health (MH) and the quality of life (QoL) among COVID-19 survivors.

Outcome variable	Men ($N = 40$) mean (SD)	Women ($N = 50$) mean (SD)	Mean difference between groups (95% CI)	*P*-value	Effect size
SAS	41.81 (8.41)	48.85 (11.52)	−7.04 (−11.36 to −2.71)	0.002	−0.70
SDS	44.97 (9.01)	49.13 (10.33)	−4.16 (−8.27 to −0.04)	0.048	−0.43
PCS	41.03 (10.51)	35.30 (13.68)	5.73 (0.52 to 10.95)	0.032	0.47
MCS	41.26 (12.58)	36.86 (14.08)	4.40 (−1.27 to 10.06)	0.127	
PF	81.88 (21.17)	67.50 (28.23)	14.38 (3.69 to 25.06)	0.009	0.58
RP	62.50 (25.63)	54.50 (29.42)	8.00 (−3.72 to 19.72)	0.179	
BP	62.50 (28.87)	50.50 (28.34)	12.00 (−0.05 to 24.05)	0.051	
GH	52.50 (27.62)	39.50 (25.30)	13.00 (1.89 to 24.11)	0.022	0.49
VT	66.25 (23.72)	53.00 (26.55)	13.25 (2.57 to 23.93)	0.016	0.53
SF	37.50 (32.52)	36.50 (33.20)	1.00 (−12.87 to 14.87)	0.886	
RE	69.06 (20.01)	58.00 (27.52)	11.06 (0.74 to 21.38)	0.036	0.46
MH	70.00 (61.50)	61.50 (20.80)	8.50 (−0.23 to 17.23)	0.056	

Studies have shown that gender was associated with MH and QoL for patients with COVID-19 (25, 26). Our study found that women have more severe psychological symptoms than men, which significantly affect their QoL. Findings from epidemiological studies indicate that women are at higher risk of psychological outcomes (27). Some researchers hypothesize that part of the increase in psychological stress among women may be due to their work being more affected by COVID-19 and the burden of home care (28, 29). Sex differences in self-reported stress are further reflected in the perceived need of psychological support services, which are often most evident in women (25). These findings call for active rehabilitation of patients with COVID-19 and highlight the difference in recovery between men and women.

Additionally, a reduction in physical activity participation is known to contribute to stress levels, which is strongly associated with QoL. Appropriate exercises (e.g., strength training, walking, lifting, and Qigong) are recommended behavioral strategies to promote the overall health of people (30). Exercise rehabilitation can enhance immune function, reduce the risk of infection, improve the prognosis, QoL, and the activity of daily living (31, 32). It is particularly emphasized that Qigong can relieve psychological stress, depression, and anxiety, and improve sleep quality (33).

In this study, the mental status of patients with COVID-19 is significantly reduced compared to that of the healthy participants. However, due to the small number of people in the control group, it is not possible to provide a more effective comparison to determine whether COVID-19 is the cause of mental status problems. Therefore, large sample size and high-quality randomized controlled studies should be conducted in the future. In this paper, healthy people were selected as the control group to explore the psychological status and QoL of post-discharge patients with COVID-19. However, to better exclude the impact of hospitalization on mental status, the hospitalized patients with other diseases could also be selected as the control group. Another limitation is that self-reported tools of anxiety and depression may not always be aligned with assessment by MH professionals. Our study used SAS and SDS to measure symptoms of anxiety and depression, which are

different from a clinical diagnosis and cannot measure severe psychiatric symptoms, such as suicidal ideation or psychotic experience. Finally, this study cannot reveal causality. Large-scale prospective, longitudinal studies are recommended to better describe the predictors of psychological disorders and QoL in patients with COVID-19.

CONCLUSIONS

Patients with COVID-19 have negative emotions, such as anxiety or depression and problems related to physical or psychological QoL in the early stage after discharge. Considering the negative impact of depression and anxiety on daily life and health outcomes, timely screening and appropriate interventions, such as online psychological counseling tailored for concerns specific to different genders, especially female patients, are urgently needed to reduce the likelihood of emotional disturbances after discharge. Meanwhile, patients should insist on rehabilitation training to improve their physical function and thus improve their QoL.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Medical Ethics Committee of China Resources & Wisco General Hospital. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

JH, YZ, and JQ: conceptualization and writing-original draft preparation. JH, QX, YS, FL, RL, and JW: methodology, data curation, and investigation. JH, YZ, and JQ: writing-review and editing. JQ: supervision, funding acquisition, and project administration. All authors contributed to the article and approved the submitted version.

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SYSTEMATIC REVIEW
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Effect of Exercise Training on Serum Transaminases in Patients With Nonalcoholic Fatty Liver Disease: A Systematic Review and Meta-Analysis

Feng Hong[1†], Yubo Liu[2,3†], Veeranjaneya Reddy Lebaka[4], Arifullah Mohammed[5], Weibing Ye[2], Biqing Chen[1] and Mallikarjuna Korivi[2*]*

[1]Department of Sports Operation and Management, Jinhua Polytechnic, Jinhua, China, [2]Exercise and Metabolism Research Center, College of Physical Education and Health Sciences, Zhejiang Normal University, Jinhua, China, [3]Key Laboratory of Intelligent Education Technology and Application of Zhejiang Province, Zhejiang Normal University, Jinhua, China, [4]Department of Microbiology, Yogi Vemana University, Kadapa, India, [5]Faculty of Agro-Based Industry, Universiti Malaysia Kelantan, Kota Bharu, Malaysia

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Leonardo Alexandre Peyré-Tartaruga, Federal University of Rio Grande do Sul, Brazil

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Stefano Ministrini, University of Zurich, Switzerland
Liang Dai, Peking University, China

***Correspondence:**
Biqing Chen
357564752@qq.com
Mallikarjuna Korivi
mallik.k5@gmail.com

[†]These authors have contributed equally to this work

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Background/Purpose: Nonalcoholic fatty liver disease (NAFLD) constitutes a spectrum of liver diseases associated with various metabolic disorders. Exercise interventions reportedly manage the clinical outcomes of NAFLD, but their efficacy depends on exercise as well as characteristics of patient. We hypothesized that exercise could alleviate the elevated transaminases level, which may be associated with the characteristics of patients (age/bodyweight/sex) or exercise variables (frequency/intensity/duration). Therefore, we examined the effect of exercise on serum transaminases, and identified the variables influencing transaminases in NAFLD patients.

Methods: Article search was conducted using electronic databases (PubMed, Web of Science, EMBASE, ScienceDirect, Google Scholar) until December 2021. Studies that involved examination and comparison of the effect of an exercise intervention on alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels in NAFLD/nonalcoholic steatohepatitis patients were included. We calculated pooled effect upon a meta-analysis, determined correlations (between transaminases and characteristics of patients/exercise) by meta-regression, and assessed the influencing variable through subgroup analysis.

Results: A total of 18 studies (22 trials) with 1098 NAFLD patients (exercise = 568; control = 530) were included. The pooled outcomes revealed that exercise intervention significantly decreased both ALT (p = 0.004) and AST (p = 0.001) levels in NAFLD patients. Meta-regression analysis showed decreased ALT (coef. = 1.138, $p < 0.01$) and AST (coef. = 0.459, p = 0.041) after intervention was correlated with the age of patients. Particularly, patients aged 30–39 years (MD: −25.89 U/L, 95% CI: −36.40 to −15.37, $p < 0.00001$) and 40–49 years (MD: −12.17 U/L, 95% CI: −20.38 to −3.96, p = 0.004) represented a substantial decrease in ALT levels. Additionally, the 50–59 years age group tended to have decreased ALT levels (MD: −3.94 U/L, 95% CI: −8.19 to 0.31, p = 0.07); however, patients above 60 years did not respond (p = 0.92) to exercise intervention. In contrast, exercise-induced AST reduction was found in only the 30–39 years age group (MD: −11.92 U/L, 95% CI: −16.78 to −7.06, $p <$

0.00001) and not in patients under the 40–49 (p = 0.19), and 50–59 groups (p = 0.12) and above 60 years (p = 0.15).

Conclusion: Our findings suggest that the age of NAFLD patients may be an important variable in improving the levels of serum transaminases, and clinically young patients may have greater benefits from exercise than older patients.

Keywords: physical actvity, fatty liver, older age group, NAFLD therapy, transaminase

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is a component of metabolic syndrome which is characterized by excessive accumulation of hepatic fat (≥5%) and is not caused by excessive consumption of alcohol, overuse of hepato-toxic medications, or other chronic liver disorders (Friedman et al., 2018). NAFLD constitutes a spectrum of liver disorders that gradually progress from simple steatosis to nonalcoholic steatosis (NASH), fibrosis, cirrhosis, and liver cancer (Bhala et al., 2011). The global prevalence of NAFLD is projected to increase from 25 to 33.5%, and the number of patients in the United States is expected to reach 100 million by 2030 (Estes et al., 2018; Younossi et al., 2018). Clinically, NAFLD is associated with several metabolic comorbidities, including obesity, hyperlipidemia, insulin resistance, type 2 diabetes, and hypertension (Younossi et al., 2016). The existence of these comorbidities further exacerbates the disease burden, and patients with severe NAFLD remain at a high risk of cardiovascular diseases (CVDs) (Targher et al., 2016). The prevalence of NAFLD is higher among older adults with obesity and diabetes; thus, an aging population may suffer severely from the disease (Estes et al., 2018). NAFLD patients with evidence of NASH and advanced fibrosis are highly susceptible to adverse outcomes, including overall mortality and liver-specific morbidity and mortality (Cotter and Rinella, 2020).

NAFLD patients with comorbidities are susceptible to the infection and severity of the coronavirus disease 2019 (COVID-19), during the ongoing global pandemic (Portincasa et al., 2020). A cross-sectional analysis from the US demonstrated that age (>75 years) and body mass index (BMI, >40 kg/m^2) of COVID-19 patients are strong predictors of hospitalization (Petrilli et al., 2020). A retrospective study from China demonstrated that NAFLD patients had a greater risk of COVID-19 progression, more likelihood of abnormal liver function from admission to discharge, and longer viral shedding time than patients without NAFLD (Ji et al., 2020). Nevertheless, no efficient medical therapy has been endorsed yet for NAFLD treatment. Therefore, to mitigate the NAFLD pathology, effective strategies are necessary for slowing down the disease progression, especially in older patients.

The American Association for the Study of Liver Diseases (AASLD) and other studies have indicated that lifestyle changes, including exercise and dietary interventions associated with weight loss, could be an integral part of NAFLD treatment (Chalasani et al., 2012; Abenavoli et al., 2018). For instance, a higher level of habitual physical activity (PA) is associated with lower intrahepatic fat content, which suggests an inverse correlation between PA and NAFLD outcomes (Perseghin et al., 2007). A clinical trial showed that both aerobic and resistance exercise trainings (8 weeks) equally reduced hepatic fat content and the levels of liver function biomarkers, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in NAFLD patients. However, the benefits of aerobic exercise were independent of weight loss and decreasing BMI (Shamsoddini et al., 2015). Contrarily, resistance exercise (8 weeks) reportedly reduced liver fat content, promoted fat oxidation, and improved glucose control independent of weight loss in NAFLD patients (Hallsworth et al., 2011). A recent study showed weight-loss-independent benefits of exercise represented by reduced liver steatosis, stiffness, and levels of liver enzymes (AST and ALT) in Japanese men with NAFLD (Oh et al., 2021).

Although either type of exercise has been documented to alleviate NAFLD complications, the degree of exercise benefits may be attributed to the characteristics of patients (age, BMI, and sex differences) or exercise variables (frequency, intensity, duration). In this context, a meta-analysis of randomized controlled trials (RCTs) demonstrated that either type of PA significantly reduced intrahepatic lipid content and blood ALT and AST levels in NAFLD patients. Specifically, patients with high BMI at baseline were represented by an intense decline of liver fat content, while exercise effects were not modified by intensity variables (Orci et al., 2016). Another meta-analysis concluded that decreased intrahepatic triglyceride content after exercise is independent of weight change, but exercise benefits are greater when weight loss occurs in NAFLD patients (Sargeant et al., 2018). A recent meta-analysis of 11 articles reported improved ALT and AST levels, and decreased BMI after long-term exercise in Chinese NAFLD patients, whereas the effects of patient characteristics or exercise variables remain inconclusive (Gao et al., 2021).

So far, no meta-analysis revealed the effect of an exercise intervention on serum transaminase (ALT/AST) levels in NAFLD patients of different age groups. In this study, we conducted a systematic review and meta-analysis of RCTs, and explored the effect of an exercise intervention on transaminases in adult NAFLD/NASH patients. Meta-regression analysis was conducted to discover whether changes in transaminase levels are associated with patient characteristics (age, BMI, and sex) or exercise variables (frequency, intensity, duration). Thereon, subgroup

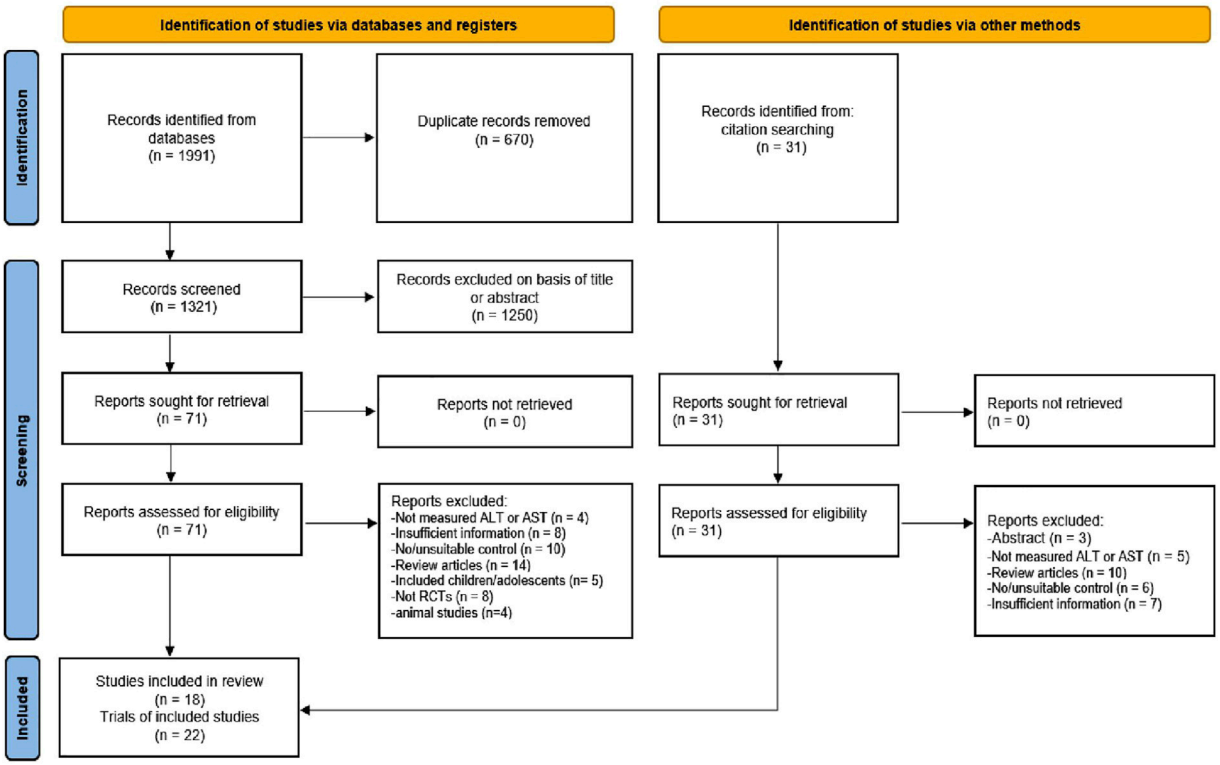

FIGURE 1 | Flowchart of the study selection according to the latest Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA).

analysis was performed to identify the most sensitive age group of patients in response to exercise intervention.

MATERIALS AND METHODS

This study was conducted following the latest Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Moher et al., 2009; Page et al., 2021).

Search Processes

We systematically searched electronic databases, including PubMed, Web of Science, EMBASE, ScienceDirect, and Google Scholar from inception to December 2021. The specific keywords, such as "non-alcoholic fatty liver disease" OR "NAFLD" OR "non-alcoholic steatohepatitis" OR "NASH" OR "fatty liver" OR "hepatic steatosis" AND "exercise" OR "physical activity" OR "aerobic exercise" OR "resistance exercise" OR "strength training" along with "randomized controlled trial" OR "RCT" OR "RCTs" were used to search the articles. The detailed search strategy implemented on the PubMed database has been presented as supplementary data (**Supplementary Table S1**). In addition, we manually searched the lists of references to obtain other suitable articles.

Inclusion and Exclusion Criteria

The inclusion criteria were: 1) All participants in the trials were adults, diagnosed with NAFLD or NASH; 2) the studies should be RCTs with parallel-groups; 3) the intervention group had undergone any type of exercise intervention (such as aerobic, strength, or mobility exercises), whilst the control group had not undergone any exercise intervention; 4) the duration of exercise was 4 weeks or more; and 5) ALT and/or AST levels were assessed per- and post-intervention. The exclusion criteria were: 1) studies conducted on animals; 2) insufficient information of participants (number, age, and BMI), exercise variables (frequency, intensity, duration), and/or outcome measures, and 3) non-original research articles (protocols, meta-analyses, systematic reviews). Based on the inclusion/exclusion criteria, two authors (FH and YL) independently reviewed and assessed the relevant articles. Initially, the titles and abstracts of the identified articles were screened for relevance. Then, the full-text of particular articles were obtained and carefully assessed for the inclusion criteria. Any disagreements were resolved by discussion with another author (MK) to obtain consensus. The detailed study selection process according to the PRISMA guidelines is described in **Figure 1**.

Extraction of the Data

Two authors (FH and YL) independently extracted the data using an Excel spreadsheet, followed by verification by review authors (BC and WY). Further cross-checking and in-depth analyses on data were performed by other three authors (VRL, AM, and WY). From each study, the following were extracted:

1) the first author's last name, year of publication, and country of publication; 2) details of intervention protocol (frequency, intensity, type of exercise, time/duration); 3) characteristics of participants (number, sex, baseline age, and BMI), and 4) pre- and post-intervention means and standard deviations (SD) of clinical outcomes (ALT and AST levels). The outcome values from eligible studies were reported as mean with SD. Standard errors and 95% confidence interval (CI) provided in the included trials were converted to SD using an equation.

Quality Assessment for the Included Trials

The quality of the included articles was evaluated according to the Cochrane risk of bias tool (Higgins et al., 2011). The Cochrane risk of bias assessment tool consists of the following items: 1) random sequence generation and allocation concealment (selection bias); 2) blinding of participants/personnel (performance bias); 3) blinding of outcome assessments (detection bias); 4) incomplete outcome data (attrition bias); 5) selective reporting (reporting bias); and 6) other sources of bias. The quality of each domain was rated as "low risk," "high risk," or "unclear," indicated with green (+), red (−), and yellow (?) colors and symbols, respectively. The quality of trials was assessed by two authors (FH and YL), and discrepancies were resolved through discussion with a third reviewer (MK) to reach a consensus.

Statistical Analysis

Cochrane Collaboration's Review Manager (RevMan 5.3., Copenhagen, and Denmark) was used to statistically analyze the effect of exercise on clinical outcomes (ALT and AST levels) of the NAFLD/NASH patients. The mean difference (MD) and 95% CI were calculated to decide the magnitude of influence of the interventions on the outcomes. To determine heterogeneity, I^2 statistics was used, with $I^2 \geq 50\%$ representing high heterogeneity and $I^2 < 50\%$ representing low heterogeneity. The fixed effect model was used to pool the study results when the heterogeneity was low. The random effect model was used to pool the study results when the heterogeneity was high. Based on the heterogeneity significance (pooled outcomes), we performed a meta-regression analysis to identify the correlation between the characteristics of participants (baseline age, BMI, and sex) or exercise variables (frequency, intensity, duration) and changes in clinical outcomes (ALT and AST levels) using the STATA version 12.0 (StataCorp, College Station, TX, United States). The dynamics in ALT and AST levels after exercise were found to correlate with age, and not with BMI, sex, and exercise frequency, intensity, and duration. Hence, to identify the effective age group, we categorized the trials into four subgroups, including patients under 30–39 years, 40–49 years, 50–59 years, and more than 60 years old groups. Sensitivity analysis was performed by conducting a meta-analysis after removing each study sequentially to ascertain if one study biased the pooled results. If the estimate after deleting a study fell outside the 95% CI of the combined effect, the study was considered to have biased the pooled results. The STATA was further used for constructing funnel plots and performing Egger's test to examine the potential bias in the included RCTs.

RESULTS

Search Results and the Selection of Studies

Through systematic search, we identified a total of 2022 articles, of which 1991 were from electronic databases (PubMed, Web of Science, EMBASE, ScienceDirect, and Google Scholar) and 31 were from other sources (i.e., a manual search of the reference lists of included studies and related reviews). After removing duplicates (670), the 1,321 records were retrieved for further assessment. The titles and abstracts of 1,321 articles were examined for suitability, leading to an exclusion of another 1,250 articles. Then, the remaining 102 (71 + 31) articles were carefully reviewed as per the inclusion/exclusion criteria, and 84 articles were excluded for valid reasons. Among them, nine articles did not report ALT or AST level, 15 articles had insufficient information, 16 reported unsuitable controls, 24 were review articles, five included children or adolescents, eight were not RCTs, four were animal studies, and three were only abstracts. Finally, 18 articles were included in the systematic review. Of these 18 articles, one was a three-armed study (3 trials), and two were two-armed studies (4 trials). Thus, a total of 22 trials were included in the meta-analysis. The stages of the article search and study selection process are depicted in **Figure 1**.

Summary of the Included Studies and Characteristics of the Patients

Among the 18 RCTs, seven studies were from England (Hallsworth et al., 2011; Pugh et al., 2013; Pugh et al., 2014; Hallsworth et al., 2015; Shojaee-Moradie et al., 2016; Houghton et al., 2017; Whyte et al., 2020), four from China (Wong et al., 2013; Dong et al., 2016; Cheng et al., 2017; Yao et al., 2018), two from the US (Promrat et al., 2010; Sullivan et al., 2012), two from Iran (Shamsoddini et al., 2015; Nikroo et al., 2017), two from Saudi Arabia (Abd El-Kader et al., 2016; Abdelbasset et al., 2019), and one from Brazil (Rezende et al., 2016). All included RCTs were published between 2010 and 2020. Five studies recruited only males, one recruited only females, eight recruited combinations of both sexes, and in four studies, the ratios were not reported. RCTs can provide the most reliable evidence on the effectiveness of intervention (Evans, 2003). To attain high-quality data, we therefore included RCTs in our meta-analysis.

The 21 trials of 18 studies included a total of 1,098 patients diagnosed with NAFLD/NASH. The exercise intervention and control groups were composed of 568 and 530 patients, respectively. The baseline mean age of patients in the trials ranged from 38.67 to 61.28 years, and their mean baseline BMI was from 25.46 to 37.1 kg/m^2. For the type of exercise, 11 trials included aerobic exercise, three trials resistance exercise, two trials high-intensity interval training (HIIT), and four trials a combination of aerobic

TABLE 1 | Characteristics of the included articles.

Study	Country	Participants (M/F)		Age (Y)	BMI	Exercise type	Intensity	Frequency (t/wk)	Duration (wk)
		Exercise	Control						
Whyte et al. (2020)	England	15 (15/0)	12 (12/0)	57.4	31.6	AE	40–60% HRR	4–5	16
Abdelbasset et al. (2019)	Saudi Arabia	16 (10/6)	16 (9/7)	54.4	36.3	HIIT	80–85% VO_{2max}	3	8
Yao et al. (2018)	China	AE:29 (7/22)	31 (13/18)	AE: 61.28	AE:25.46	AE	AE:60–70% HRmax	3	22
		RE:31 (16/15)		RE:55.8	RE26.86	RE	RE:60–70% 1RM		
Cheng et al. (2017)	China	AE:22 (5/17)	CO:18 (4/14)	AE:59	AE:27.3	AE	60–75% VO_{2max}	2–3	36.9
		AE + DT:23 (7/16)	DT:22 (6/16)	AE + DT:60	AE + DT:26.4				
Houghton et al. (2017)	England	12 (nr)	12 (nr)	54	33	AE + RE	RPE 14–18 (hard–very hard)	3	12
Nikroo et al. (2017)	Iran	12 (12/0)	11 (11/0)	38.67	30.37	AE	55–60% HRR	3	8
Dong et al. (2016)	China	130 (130/0)	139 (130/0)	56.68	26.04	AE	60–80% target heart rate (170-age)	3–4	103
Abd El-Kader et al. (2016)	Saudi Arabia	50 (34/16)	50 (36/14)	50.78	32.35	AE	65–75% HRmax	3	12
Rezende et al. (2016)	Brazil	19 (0/19)	21 (0/21)	56.2	34.1	AE	VAT up to 10% below RCP	2	24
Shojaee-Moradie et al. (2016)	England	15 (15/0)	12 (12/0)	52.4	31.6	AE + RE	40–60% HRR	4–5	16
Hallsworth et al. (2015)	England	11 (nr)	12 (nr)	54	31.5	HIIT	RPE 16–17 (very hard)	3	12
Shamsoddini et al. (2015)	Iran	AE: 10 (10/0)	10 (10/0)	AE: 39.7	AE: 28.1	AE	AE: 60–75% HRmax	3	8
		RE: 10 (10/0)		RE: 45.9	RE: 30.6	RE	RE: 50–70% 1RM		
Pugh et al. (2014)	England	13 (7/6)	8 (4/4)	50	30	AE	30–60% HRR	3–5	16
Pugh et al. (2013)	England	6 (nr)	5 (nr)	45	31	AE	30–60% HRR	3–5	16
Wong et al. (2013)	China	77 (41/36)	77 (31/46)	51	25.5	AE + RE	Moderate-intensity	3–5	51
Sullivan et al. (2012)	United States	12 (4/8)	6 (1/5)	48.6	37.1	AE	45–55% VO_{2max}	5	16
Hallsworth et al. (2011)	England	11 (nr)	8 (nr)	52	32.3	RE	50–70%1RM	3	8
Promrat et al. (2010)	United States	21 (14/7)	10 (8/2)	48.9	33.9	AE + RE	Moderate-intensity	nr	48

Note: M/F, male/female; Y, years; t/wk, times/week; AE, aerobic exercise; RE, resistance exercise; AE + RE, combination of aerobic and resistance exercises; HIIT, high-intensity interval training; Co., control; DT, diet; nr, not reported; VO2max, maximal oxygen uptake; HRR, heart rate reserve; HRmax, maximal heart rate; 1RM, one-repetition maximum; RPE, ratings of perceived exertion (6–20); VAT, ventilatory anaerobic threshold; RCP, respiratory compensation point.

and resistance exercises. The frequency of exercise varied from 2 to 5 times per week, and the duration of exercise was ranged from 8 to 103 weeks. The overview of patients and exercise characteristics are presented in **Table 1**.

Exercise Decreases Both ALT and AST Levels in NAFLD/NASH Patients

The effect of an exercise intervention on ALT changes was evaluated in the patients from 22 trials. The pooled results of meta-analysis showed that exercise intervention substantially decreased (p = 0.004) ALT levels in patients. The MD in the reduction of ALT levels was -5.27 U/L (95% CI: −8.84 to −1.70), and the heterogeneity was I^2 = 80% (**Figure 2**). Next, we identified 16 trials that addressed the effects of exercise on AST levels of NAFLD/NASH patients. Similar to ALT levels, we found exercise intervention significantly decreased (p = 0.001) AST levels in patients. The average reduction of AST levels was −4.93 U/L (95% CI: −7.94 to −1.91), and the heterogeneity was I^2 = 78% (**Figure 3**). The heterogeneity of both ALT and AST levels indicates that the included trials revealed diverse effects of exercise on ALT and AST changes in adult NAFLD patients.

Association Between Transaminases and Characteristics of Patients/Exercise Variables

Prevalence of NAFLD is more common among older and obesity adults. Therefore, we performed a meta-regression analysis to determine the source of heterogeneity using age, BMI, and sex variables. We found that the baseline BMI, BMI reduction, and sex differences were not correlated with the reduction of either ALT or AST levels in NAFLD/NASH patients (**Table 2**). Noteworthy, the 'age' of patients significantly correlated with an exercise-induced reduction of ALT ($p < 0.01$) and AST (p = 0.041) levels (**Table 2**). These findings revealed that the beneficial effects of exercise on transaminases are correlated with the "age" of NAFLD patients.

Next, we assumed that the exercise characteristics (frequency, intensity, duration) may also be involved in the reduction of ALT or AST levels in NAFLD patients. We performed meta-regression analyses and found that changes in the levels of transaminases were not correlated with exercise frequency (ALT, p = 0.872; AST, p = 0.147) or exercise intensity (ALT, p = 0.076; AST, p = 0.325). Similarly,

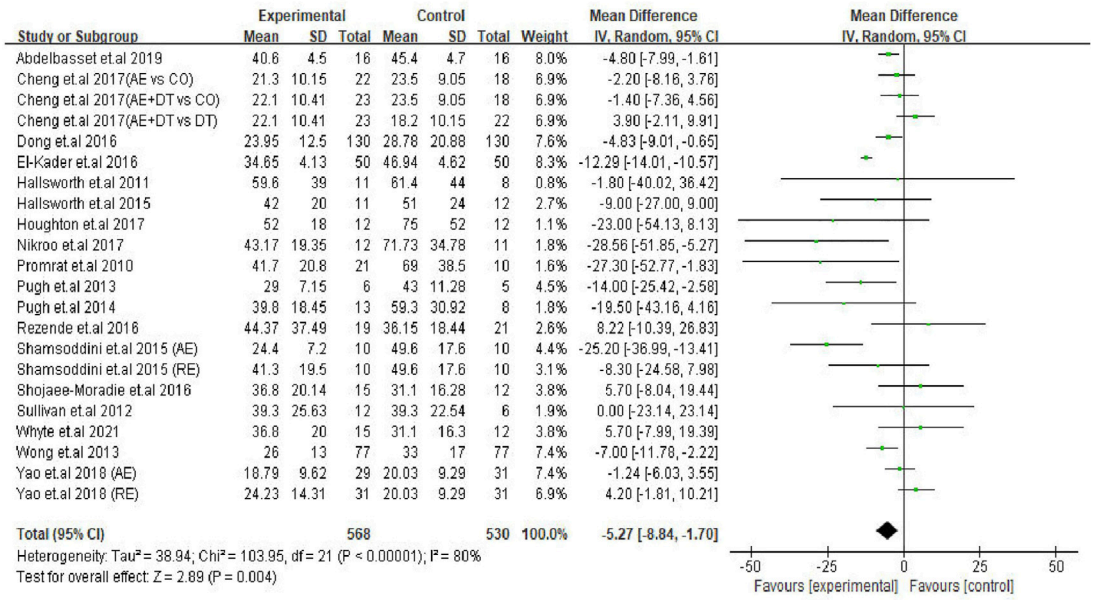

FIGURE 2 | Pooled outcomes of exercise intervention on ALT levels in NAFLD/NASH patients. SD, standard deviation; 95% CI, 95% confidence interval.

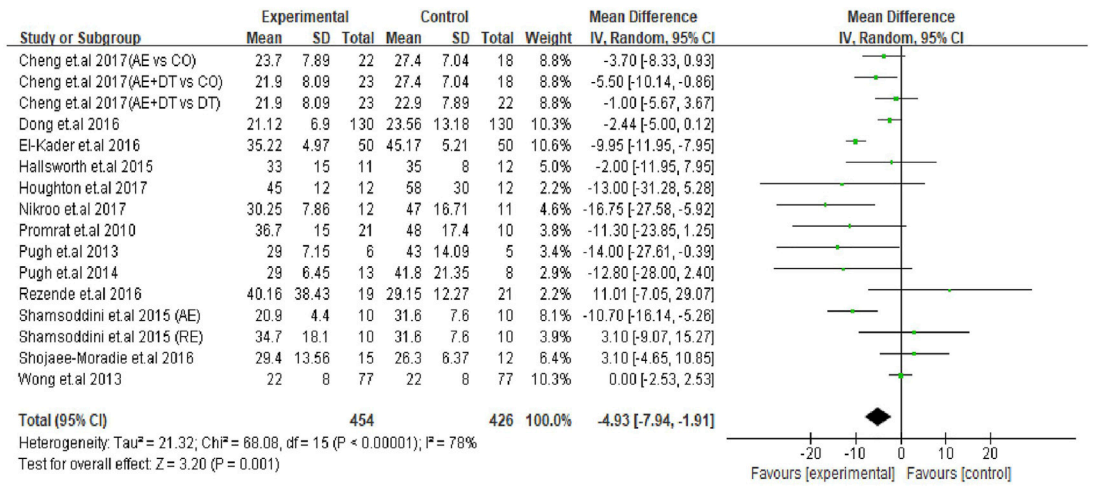

FIGURE 3 | Pooled outcomes of exercise intervention on AST levels in NAFLD/NASH patients. SD, standard deviation; 95% CI, 95% confidence interval.

the regression analysis showed that decreased ALT (p = 0.508) and AST (p = 0.218) levels were not associated with the duration of exercise in NAFLD patients (**Supplementary Table S2**).

Exercise Intervention Decreases ALT Levels in Different Age Groups of NAFLD Patients

After finding a significant correlation between age and the levels of transaminases, we categorized the patients of the trials (22) into four age subgroups; including 30–39 years (two trials), 40–49 years (four trials), 50–59 years (13 trials) and ≥60 years old (three trials) groups to identify the most sensitive age group of patients in response to exercise intervention. The prevalence of NAFLD can be seen in adults at the age of early thirties. With advancing age, the risk or severity of the disease would be progressing every 10 years (Kalra et al., 2013; Hu et al., 2018; Tobari and Hashimoto, 2020). A meta-analysis showed a gradual increase of NAFLD incidence in adults of various age groups, including 30–39, 40–49, 50–59 and 60–69 (Younossi et al., 2016). In our study, the average age of patients in trials ranged from 38 to 61 years. Therefore, we assumed that classifying of the patients into four subgroups would be appropriate and imperative to investigate the dynamics of serum transaminases after exercise.

TABLE 2 | Meta-regression analysis to identify the effective variables.

	Characteristics of patients	Coefficient	Standard error	T-Value	*p*-Value
ALT	Baseline BMI	−0.4281872	0.5619431	−0.76	0.455
	Age	1.138232	0.2380615	4.78	0.000[a]
	Sex (17 trials)	−10.23261	6.730051	−1.52	0.149
	BMI reduction (19 trials)	−3.137282	2.17553	−1.44	0.167
AST	Baseline BMI	−0.6276582	0.5291798	−1.19	0.255
	Age	0.459831	0.2045216	2.25	0.041[a]
	Sex (11 trials)	−2.131885	5.426621	−0.39	0.704
	BMI reduction (13 trials)	−2.288405	1.974409	−1.16	0.271

[a]*Represents statistical significance.*

Subgroup analysis results revealed that young patients were highly responsive to exercise intervention compared with the older, as indicated by improved ALT levels. The decreased ALT level were seen in two age groups, i.e., 30–39 years (MD: −25.89 U/L, 95% CI: −36.40 to −15.37, $p < 0.00001$, $I^2 = 0\%$) and 40–49 years (MD: −12.17 U/L, 95% CI: −20.38 to −3.96, $p = 0.004$, $I^2 = 0\%$), whilst the ALT levels in patients in 50–59 years age group tended to decrease with exercise intervention (MD: −3.94 U/L, 95% CI: −8.19 to 0.31, $p = 0.07$, $I^2 = 81\%$). In contrast, the ALT levels in older adults (≥60 years) were not responsive to

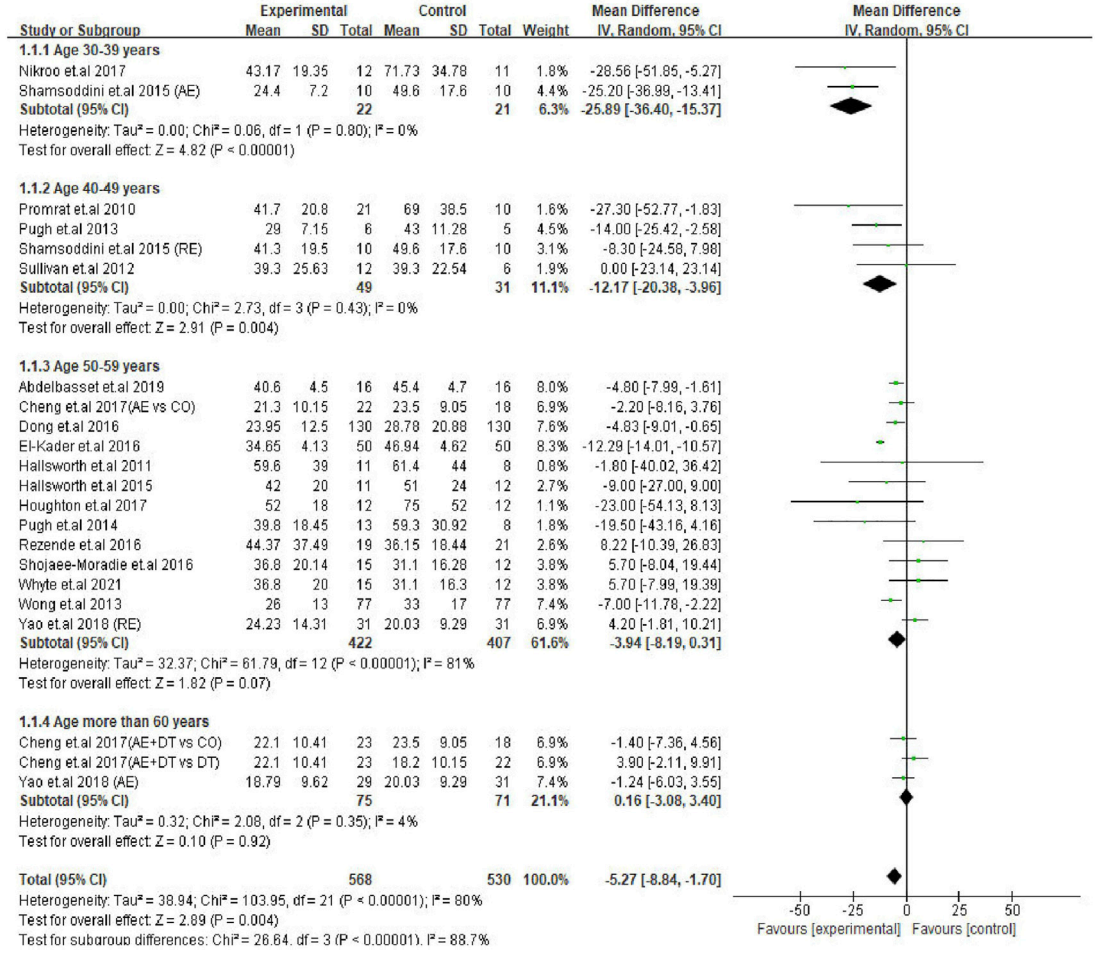

FIGURE 4 | Subgroup analysis of exercise intervention on changes in ALT levels in different age groups of NAFLD/NASH patients. SD, standard deviation; 95% CI, 95% confidence interval.

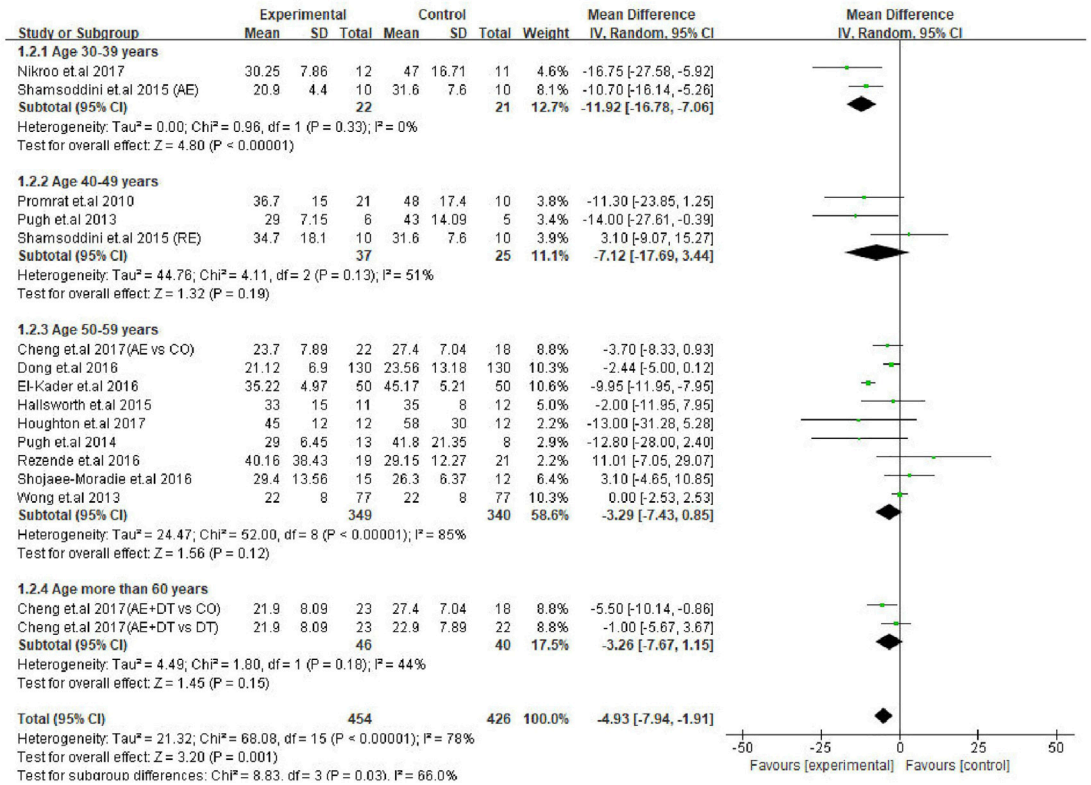

FIGURE 5 | Subgroup analysis of exercise intervention on changes in AST levels in different age groups of NAFLD/NASH patients. SD, standard deviation; 95% CI, 95% confidence interval.

exercise intervention (MD: 0.16 U/L, 95% CI: −3.08 to 3.40, p = 0.92, I^2 = 4%). It appears that the effect size of ALT reduction was bigger in younger patients (30–39 years; MD: −25.89 U/L) and gradually lessened as age advanced. Furthermore, the test for subgroup differences showed significant (p < 0.00001) differences among the groups (**Figure 4**).

Exercise Intervention Decreases AST Levels in Young Patients but Not in Older Patients

Next, a subgroup analysis was conducted for AST changes to identify the age groups of patients responsive to exercise intervention. The results showed that patients in the 30–39 years age group were represented by a significant decrease in AST levels (MD: −11.92 U/L, 95% CI: −16.78 to −7.06, p < 0.00001, I^2 = 0%). Interestingly, exercise intervention could not alter the AST levels in patients of 40–49 years (MD: −7.12 U/L, 95% CI: −17.69 to 3.44, p = 0.19, I^2 = 51%), 50–59 years (MD: −3.29 U/L, 95% CI: −7.43 to 0.85, p = 0.12, I^2 = 85%) and ≥60 years (MD: −3.26 U/L, 95% CI: −7.67 to 1.15, p = 0.15, I^2 = 44%) age groups. On the other hand, the differences between each subgroup reached statistical significance (p = 0.03), which emphasizes the correlation between the age of patients and degree of AST change (**Figure 5**).

Summary of Risk of Bias

Risk of bias assessment for the 22 trials is presented in **Figure 6**. For the selection bias, the highest number of studies (22 trials) reported to have low risk of random sequence generation, and ten trials were judged to have a low risk of allocation concealment. As physical exercise was the primary intervention method among all the included trials, it may not have been feasible to adopt the blind method; therefore, several studies were judged as having a high risk of performance bias (19 trials), and a few studies were judged as having a high risk of detection bias (9 trials). However, reporting of such a high risk of performance bias and detection bias does not indicate a compromised quality of the study (Schindhelm et al., 2006; Senior, 2012; Liu et al., 2021). In addition, an attrition bias was identified in five trials, and a reporting bias was identified in six.

We then performed sensitivity analysis by removing each study sequentially to determine if one study biased the pooled results. We found that the pooled results did not vary substantially. The MD upon reduction of ALT levels was from −4.20 (95% CI: −7.32 to −1.09) to −5.90 (95% CI: −9.44 to −2.36), while the MD upon reduction of AST levels was from −4.07 (95% CI: −6.70 to −1.43) to −5.48 (95% CI: −8.52 to −2.44). The sensitivity analysis using STATA software did not show any significant impact on the total effect size of ALT (combined estimate = −5.266, 95% CI: −8.834 to −1.698) and AST (combined estimate = −4.924, 95% CI: −7.937 to −1.91) (**Supplementary**

Figure S1). A funnel plot was produced to ascertain whether significant publication bias was present. Symmetry in the funnel plot shows the absence of publication bias (**Supplementary Figure S2**). Furthermore, Egger's test also indicated no publication bias for both ALT (p = 0.240) and AST (p = 0.929) changes after intervention.

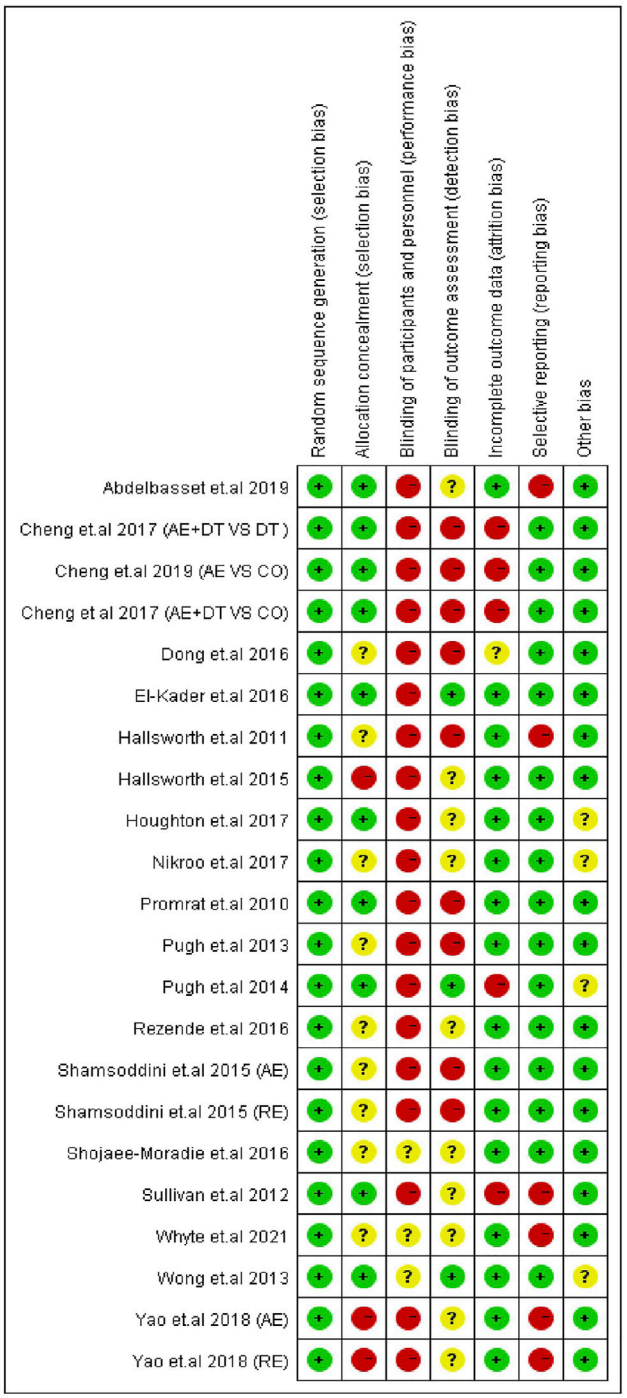

FIGURE 6 | Risk of bias summary of the included studies.

DISCUSSION

To the best of our knowledge, this is the first systematic review and meta-analysis to explore the exercise-induced benefits on serum transaminases in NAFLD patients among different age groups. Meta-analysis of 18 RCTs (22 trials) revealed that exercise intervention significantly decreased both ALT and AST levels. Through meta-regression analysis, we identified "age" as a key factor that influences the exercise-induced beneficial effects on transaminases. Specifically, exercise caused a significant reduction of ALT levels in patients aged 30–39 and 40–49 years, while a decreased tendency was seen in patients aged 50–59 years. Contrarily, ALT levels in patients aged 60 years and above were not responsive to exercise intervention. On the other hand, exercise-induced reduction of AST levels was noticed only in the 30–39 years age group and not in other age groups (40–49, 50–59, and ≥60 years). Taken together, exercise training is effective in reducing the levels of transaminases in NAFLD patients, irrespective of type, but its efficacy is progressively reduced along with aging.

NAFLD is the most common liver disease, and its incidence rate is continually rising (Huang et al., 2021). This chronic disease does not only lead to advancing stages of liver damage and death, but it is also closely associated with the incidence and/or progression of type 2 diabetes and CVDs (Cotter and Rinella, 2020; Deprince et al., 2020). Typically, NAFLD is a metabolic disorder caused by insulin resistance, which causes too many free fatty acids to enter the liver, and promotes the production and accumulation of total triglycerides (TG) in the liver. Accumulation of a large amount of TG in the liver subsequently leads to the excessive production of pro-inflammatory cytokines and free radicals, which then impairs liver function (Rolo et al., 2012). On one hand, a sedentary lifestyle or physical inactivity is an important reason for the incidence of NAFLD and elevation of levels of liver biomarkers, including transaminases. On the other hand, lifestyle modification or regular PA is beneficial for patients in decreasing the clinical outcomes and disease burden (Chalasani et al., 2012; Golabi et al., 2020). A study from Israel reported that regular PA is associated with a lower prevalence of NAFLD (Zelber-Sagi et al., 2008). A recent longitudinal analysis (10.6 years) identified that longer duration of total PA and moderate-to-vigorous PA are associated with a lower all-cause mortality and cardiovascular mortality in NAFLD patients (Kim et al., 2021). Another RCT from China reported that 6- or 12-months of moderate exercise and vigorous-moderate exercise effectively decreased intrahepatic TG contents in NAFLD patients (Zhang et al., 2016). In insulin-resistant individuals, exercise training has been shown to improve skeletal muscle glycogen synthesis with a concomitant decrease of *de novo* lipogenesis and hepatic TG synthesis (Rabøl et al., 2011). It has been claimed that exercise can reduce hepatic fat or TG content in patients through improved insulin resistance, fatty acid oxidation, and mitochondrial function and the activation of inflammatory or antioxidant cascades (van der Windt et al., 2018)

Both transaminases, ALT and AST, are commonly used as biomarkers to determine the physiological status and function of

the liver. The ALT is primarily produced in the cytoplasm of hepatocytes, while AST is mainly produced in the mitochondria of hepatocytes. Upon hepatocyte damage, the permeability of cell membrane increases, and ALT is released into the blood. If hepatocytes are seriously damaged or persist, the mitochondria in hepatocytes will be damaged, and AST will be released into the blood (Schindhelm et al., 2006; Senior, 2012). Previous studies have focused on the effect of exercise on transaminases in NAFLD patients, but the results are equivocal. For instance, 8 weeks of aerobic exercise and resistance training were equally effective in reducing the ALT and AST levels as well as hepatic fat content in the NAFLD patients, but the efficiency of aerobic exercise was independent of weight loss (Shamsoddini et al., 2015). Another study showed that resistance training (8 weeks) decreased liver fat and improved fat oxidation in NAFLD patients without affecting body weight or whole-body fat mass (Hallsworth et al., 2011). Fealy et al. reported a significant decrease in ALT levels, but not AST levels and intrahepatic fat contents, in NAFLD patients after 7 days of treadmill exercise (Fealy et al., 2012). The main reasons for this discrepancy may be factors, including characteristics of patients (i.e., age, BMI, and sex) or exercise intervention (i.e., frequency, intensity, and duration).

The pooled outcomes of our meta-analysis of 22 RCTs showed exercise intervention to significantly decrease both ALT and AST levels in NAFLD patients. A previous meta-analysis reported an exercise-induced decrease of transaminase levels in NAFLD patients, and such decrease correlated with the baseline BMI of patients (Orci et al., 2016). Conversely, another meta-analysis of 10 trials concluded that either exercise intervention alone or in combination with diet had no effect on ALT levels in NAFLD patients (Keating et al., 2012). In a recent meta-analysis, Wang et al. categorized the included trials into two groups based on intervention duration and found that the longer duration of exercise (≥4 months) tended to decrease the ALT and AST levels in NAFLD patients, while the shorter duration (<4 months) had no effect (Wang et al., 2020). A network meta-analysis revealed aerobic exercise to be effective in improving the ALT and AST activities compared with progressive resistance exercise in NAFLD patients (Zou et al., 2018). These previous meta-analyses clearly demonstrated that factors including the bodyweight of patients, type of exercise, and duration of exercise are involved in controlling AST and ALT levels in NAFLD patients. Nevertheless, the influence of "age" on exercise-induced alternations of ALT and AST levels remains inconclusive in NAFLD patients.

Identification of the variables that are critically involved in the manifestation of liver pathology is vital in the treatment of NAFLD. Literature revealed that the prevalence of NAFLD can be seen in adults at the age of early thirties, and the disease burden would be increased for every 10 years (Kalra et al., 2013; Hu et al., 2018; Tobari and Hashimoto, 2020). In this context, a meta-analysis reported progressively increased incidence of NAFLD in adults of various age groups, such as 30–39 (22.43%), 40–49 (26.53%), 50–59 (27.40%), and 60–69 (28.90%) years (Younossi et al., 2016). These findings emphasize that the prevalence of NAFLD is increasing in adults for every 10 years of age from 30 to 69 years. In our study, we found "age" of patients to significantly correlate with the exercise-induced reduction of ALT and AST levels in patients but not the BMI of patients. Thus far, there are no reports which delineate the role of the age of patients on exercise-induced improvements of transaminase levels; thus our findings signify the clinical vitality of "age" in the treatment of NAFLD. We then classified the trials into four age subgroups based on a previous study (Younossi et al., 2016). We further emphasized that compared to older patients, younger patients were highly responsive to exercise intervention in the improvement of transaminases. This was evidenced by a substantial decrease in ALT levels in both 30–39 to and 40–49 years age groups and a substantial decrease in AST levels in 30–39 years age group of patients.

With increasing age, the structure and function of liver change considerably in older NAFLD patients, and that is related to the significant damage of hepatic metabolism and detoxification (Bertolotti et al., 2014). A study from Japan found age to be significantly associated with the development of NASH in male NAFLD patients and progression of fibrosis in female NASH patients (Shima et al., 2015). Similarly, a prospective study conducted on 44 NAFLD patients (mean age 36.5 years) showed significantly decreased ALT and AST levels after exercise (Baba et al., 2006). In another RCT, NAFLD patients aged 44.16 years showed decreased ALT and AST levels after aerobic exercise (Draz et al., 2020). In contrast, older NAFLD patients with pre-diabetes were unable to revert their transaminases even after 8 months of exercise combined with dietary intervention (Cheng et al., 2017). These findings reveal the importance of age in attenuating the levels of liver function biomarkers and pathology. It is key to note that younger people generally play an important role in social and economic development. However, owing to busy schedules, PA of young individuals may decline, which results in a large amount of fat accumulation. Excessive fat accumulation in the liver eventually causes inflammation and oxidative injury to hepatocytes and decreases liver function. Therefore, decreasing transaminase levels in young or older NAFLD patients is necessary to prevent and/or reverse the liver damage.

LIMITATIONS

The included articles in this study were obtained through a comprehensive systematic search of major electronic databases and manual search. However, the records identified from grey literature sources did not fulfill our inclusion criteria for meta-analysis. Although some adult NAFLD patients would invariably have used drugs along with exercise intervention to control the clinical manifestations of the disease, we did not consider or adjust the drug effect on reported outcomes. Furthermore, two trials were included more than once in our analysis, and this may have promoted bias in the results. In the subgroup analysis, the relative weights of four subgroups appeared to be different from each other. Such differences in weights may be due to the number of trials and/or number of patients included in certain age groups. Although exercise has significant effect on transaminases, the small effect size in this study may not strongly convey the

practical application of intervention in the clinical management of NAFLD. Further analysis with an adequate number of studies and a precise classification of age subgroups may be warranted to evaluate the intervention effect exercise on transaminases.

CONCLUSION

The evidence from our systematic review and meta-analysis showed age to be one of the key variables in improving the serum transaminase levels of NAFLD patients. Specifically, exercise can decrease both ALT and AST levels in young NAFLD patients, while with aging, this efficacy was progressively reduced (in older patients). Therefore, NAFLD patients should undergo adequate exercise sessions as soon as possible upon diagnosis of the disease.

DATA AVAILABILITY STATEMENT

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

AUTHOR CONTRIBUTIONS

FH, YL, BC, and MK conceptualized and designed the study. FH, YL, and BC conducted article search and screening the titles. FH and YL performed statistical analyses. VL, AM and WY helped in organization and interpretation of data. FH, YL. BC, and MK reviewed the full-text articles, extracted the data and analyzed. FH and YL drafted the manuscript. BC and MK revised and finalized the manuscript. All authors read and approved the submission.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2022.894044/full#supplementary-material

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

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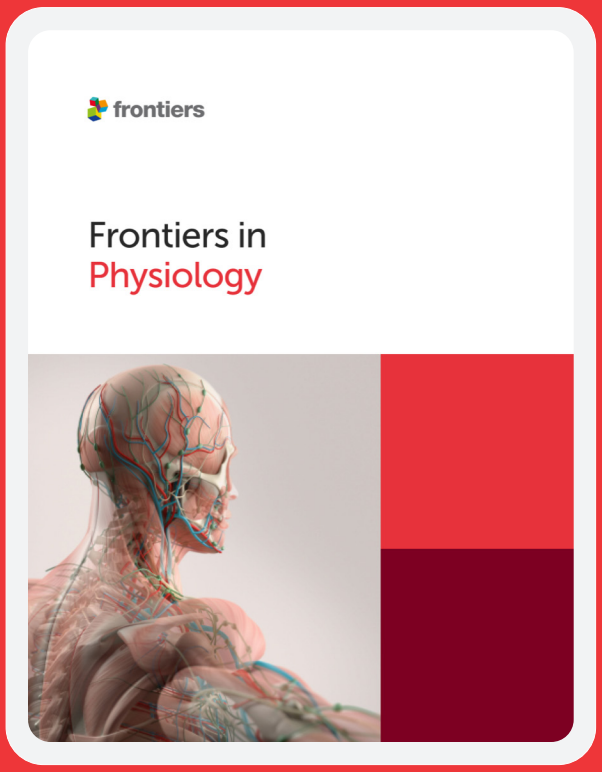

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