REVIEW

The Impact of Aerobic and Anaerobic Exercise Interventions on the Management and Outcomes of Non-Alcoholic Fatty Liver Disease

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Summary

Non-alcoholic fatty liver disease (NAFLD) is a metabolic disorder that includes non-alcoholic hepatic steatosis without or with moderate inflammation and non-alcoholic steatohepatitis (NASH), characterized by necroinflammation and a more rapid progression of fibrosis. It is the primary pathological basis for hepatocellular carcinoma. With its prevalence escalating annually, NAFLD has emerged as a global health epidemic, presenting a significant hazard to public health worldwide. Existing studies have shown that physical activity and exercise training have a positive effect on NAFLD. However, the extent to which exercise improves NAFLD depends on the type, intensity, and duration. Therefore, the type of exercise that has the best effect on improving NAFLD remains to be explored. To date, the most valuable discussions involve aerobic and anaerobic exercise. Exercise intervenes in the pathological process of NAFLD by regulating physiological changes in cells through multiple signaling pathways. The review aims to summarize the signaling pathways affected by two different exercise types associated with the onset and progression of NAFLD. It provides a new basis for improving and managing NAFLD in clinical practice.

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Introduction

Non-alcoholic fatty liver disease (NAFLD) includes non-alcoholic steatosis with or without moderate inflammation, as well as non-alcoholic steatohepatitis (NASH), which is distinguished by necroinflammation and a faster rate of fibrosis advancement than non-alcoholic fatty liver disease [1]. NAFLD is defined as the accumulation of fat in the liver with no known secondary cause. This condition is usually diagnosed after secondary causes such as excessive alcohol consumption, chronic hepatitis C virus infection, hemochromatosis, etc. NAFLD is characterized by the accumulation of fat in the liver without these known secondary causes. In addition, the spectrum of disease in NAFLD ranges from simple hepatic steatosis (non-alcoholic fatty liver) to non-alcoholic steatohepatitis (NASH), which is characterized by necroinflammation and a more rapid progression of fibrosis than simple fatty liver [2]. As a metabolic disorder, it is a major cause of chronic fatty liver disease and liver cancer [3,4]. NAFLD and type 2 diabetes share a complex and bidirectional relationship. NAFLD is associated with insulin resistance, a key feature of type 2 diabetes. In individuals with NAFLD, the accumulation of fat in the liver can cause inflammation and damage, further impairing insulin signaling and exacerbating insulin resistance [5]. It is reported to threaten approximately 25 % of the world's population, with a prevalence of 52.34/1000 people annually in Asia [4,6]. Patients with mild disease are advised to control

their diet and exercise to reverse the disease course. Patients with NAFLD who received n-3-PUFA medication for a full year showed improvements in their plasma lipid profile, a significant drop in gamma-glutamyltransferase activity, and a reduction in liver fat in those who lost weight [7]. Another piece of data indicated that statin therapy significantly reduces cardiovascular disease morbidity and mortality while also significantly improving or curing NAFLD/NASH in humans. Strong statin administration seems to be a safe and successful way to save the lives of NAFLD/NASH patients [8]. Medications for type 2 diabetes mellitus (T2DM) include sodium glucose cotrans-porter-2 inhibitors, glucagon-like peptide-1 rs11eceptor agonist and thiazolidinediones have been shown to improve steatosis based on the correction of metabolic abnormalities. Somatostatin reduces blood glucose levels and liver fat accumulation and acts as an anti-inflammatory in advanced NAFLD [9]. Despite the combination of other drugs, such as the lipid-lowering drug statin and the metabolic disorder-improving drug orlistat, the safety profile remains to be investigated.

Modern lifestyle changes, especially prolonged sedentary behavior and a lack of exercise in the context of nutritional enrichment, are key risk factors for NAFLD [10]. Studies have proven that inactivity contributes to the first stage of NAFLD, namely benign steatosis [10,11]. Thus, exercise is regarded as one of the options to prevent NAFLD. Numerous studies have shown a negative correlation between NAFLD and exercise intensity [12-15]. Current evidence suggests that both resistance exercise and aerobic exercise can improve NAFLD. Exercise has an ameliorative effect on intrahepatic triglycerides (IHTG) [16]. A study demonstrated that regular moderate-intensity aerobic exercise significantly improved cardiometabolic and health status in NAFLD patients [17]. Furthermore, available evidence demonstrates that vigorous exercise significantly reduces IHTG in patients with NAFLD [18]. However, Zhang *et al.* also mentioned that NAFLD patients are unable to exercise vigorously [18]. Therefore, exercise in the context of reducing the risk of NAFLD needs to be tightly controlled to achieve the desired results. This remains the great challenge of the present. There are differences in the beneficial effects of different types of exercise on the fatty liver. For example, the differences in the impact of long-term low-intensity aerobic exercise and short-term high-intensity anaerobic exercise on patients with fatty livers remain to be explored. Moreover, the optimal exercise protocol for determining the prevention,

treatment, and prognosis of fatty livers remains unclear. Therefore, finding better ways to exercise to complement the pharmacological treatment of NAFLD is a long and challenging process.

Aerobic exercise

Aerobic exercise improves metabolic mechanisms in NAFLD by enhancing insulin sensitivity and decreasing fatty acid transport to the liver and adipose tissue. Aerobic exercise actually increases fat metabolism, which is beneficial for individuals with NAFLD. This increased oxidation helps to reduce the accumulation of fat in the liver, thereby mitigating steatosis. The study by Rector *et al.* supports this finding, demonstrating that daily exercise is a good way to reduce the accumulation of fat in the liver. Daily exercise in Otsuka Long-Evans Tokushima Fatty rats, a model of NAFLD, leads to an increase in hepatic fatty acid oxidation and prevents the development of NAFLD [19-22]. Previous research has shown that aerobic exercise has a beneficial preventive effect on NAFLD and related diseases. Aerobic exercise reduced very low-density lipoprotein triglyceride levels and liver cell damage markers, such as aspartate aminotransferase and alanine aminotransferase (ALT) [23]. After 16 weeks of moderate-intensity aerobic exercise in NAFLD patients, fatty liver-related markers such as triglycerides and lipoprotein metabolism were improved [24]. Similarly, another study showed that short-term aerobic exercises reduced circulating markers of hepatocyte apoptosis [25]. Ongoing research is investigating the combined use of drugs or drug active ingredients with aerobic exercise for NAFLD treatment. Notably, Lycium barbarum poly-saccharides combined with aerobic exercise have shown promise as a potential treatment for NAFLD by maintaining gut microbiota homeostasis, thereby restoring gut barrier function and benefiting liver health [26]. According to the current recommendations, NAFLD patients should aim for at least 150 min of moderate-intensity aerobic exercise per week, which can be beneficial for both obese and lean individuals [27]. Current research data support the beneficial effects of aerobic exercise in the adult population. Additionally, aerobic exercise experiments in mice found that aerobic exercise can affect multiple signaling pathways and inhibit pathological activities associated with NAFLD. Therefore, the effect of aerobic exercise on NAFLD can be further investigated by analyzing its associated numerous signaling pathways.

CNPY2/PERK pathway in aerobic exercise

        Canopy fibroblast growth factor signaling regulator 2 (CNPY2), a secreted protein, belongs to the Canopy family (CNPY1, 2, 3, and 4). Furthermore, it promotes smooth muscle cell proliferation and migration to enhance angiogenesis, being highly expressed mainly in cardiomyocytes [28-30]. CNPY2 is a novel unfolded protein response (UPR) promoter. High levels of CNPY2 induce vascular endothelial cell injury by activating protein kinase RNA-like endoplasmic reticulum kinase (PERK) signaling pathway. In a study of patients with NASH, the degree of angiogenesis was positively correlated with the degree of hepatic fibrosis, suggesting that angiogenesis plays a vital role in the progression of NASH [31]. Several studies have now demonstrated that deletion of CNPY2 blocks the PERK-C/EBP homologous protein (CHOP) pathway [32]. PERK/CHOP, a downstream signaling pathway of the UPR, is involved in endoplasmic reticulum stress (ERS) to induce hepatic injury and steatosis [32]. In addition, a positive feedback loop exists between CNPY2 and the PERK/CHOP axis, as shown in Figure 1. CHOP promotes the transcription of CNPY2 and exacerbates the UPR. PERK is thus activated to promote the transcription of CHOP. Moreover, Liu *et al.* showed that the CNPY2-PERK pathway was involved in the up-regulation of vascular endothelial growth factor expression after hypoxia/reoxygenation injury in human umbilical vein endothelial cells [33].
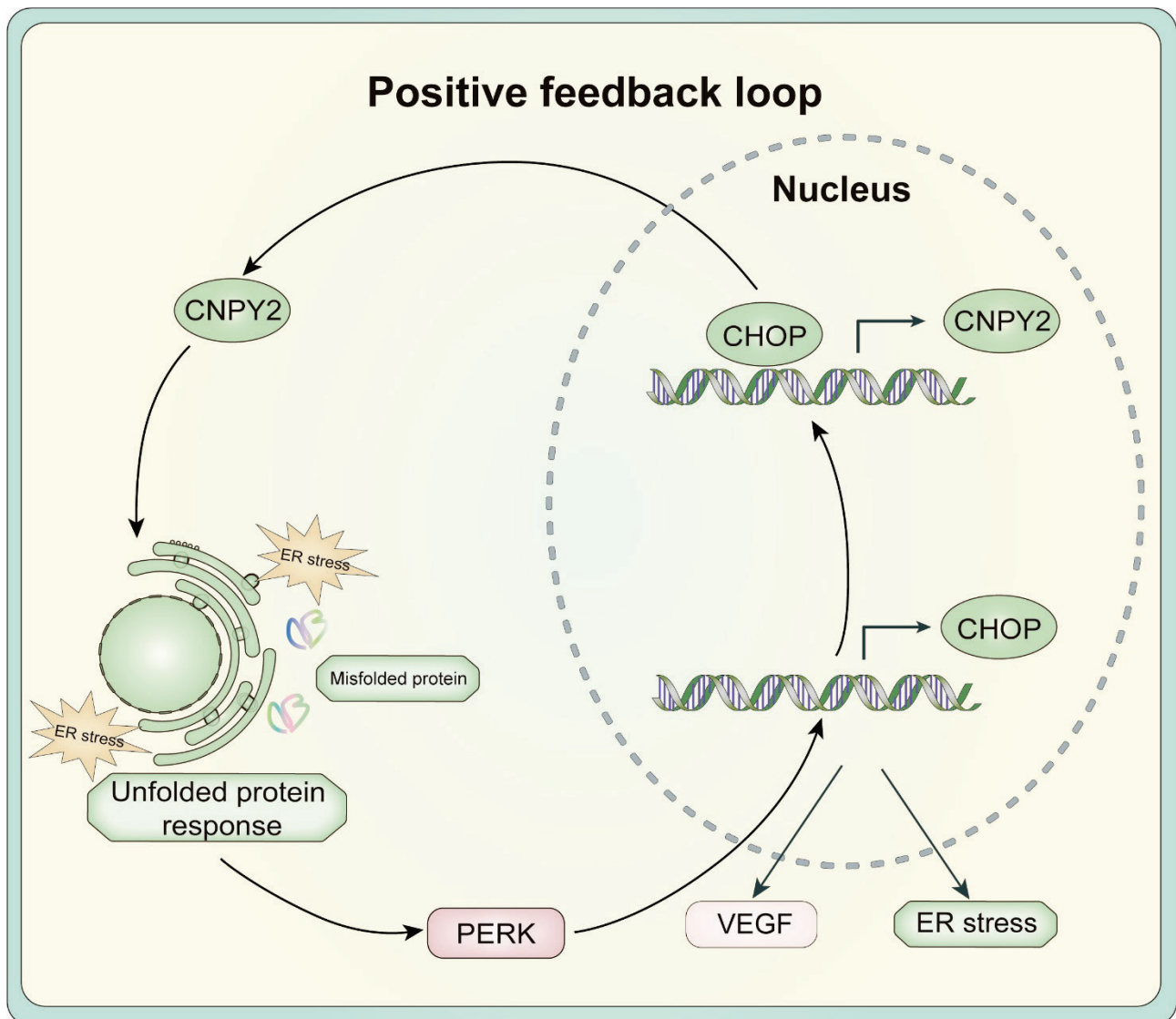

Fig. 1. The positive feedback loop between CNPY2 and the PERK/CHOP axis. Canopy fibroblast growth factor signaling regulator 2 (CNPY2) upregulation elicits an unfolded protein response (UPR). It activates the downstream protein kinase RNA-like endoplasmic reticulum kinase (PERK)/C/EBP homologous protein (CHOP) axis, which in turn promotes CNPY2 transcription. This process upregulates vascular endothelial growth factor (VEGF) and leads to increased endoplasmic reticulum stress (ERS).

Aerobic exercise ameliorates NAFLD by down-regulating the CNPY2-PERK pathway [34]. High-fat diet (HFD)-induced NAFLD mice showed a significant reduction in hepatic lipid density after aerobic exercise [34]. Additionally, CNPY2-PERK expression decreased [34]. However, Li *et al.* found that although aerobic exercise improved liver function in NAFLD mice, it had no positive effect on normal livers. It may be related to the shorter duration of running training as well as the level of intensity, thus requiring in-depth research.

AMPK pathway in aerobic exercise

AMP-activated protein kinase (AMPK), as an energy sensor, is a heterotrimer consisting of an α-subunit, a β-subunit, and a γ-subunit [35,36]. AMPK activation up-regulates ATP-producing pathways, facilitating glucose uptake, and inhibiting ATP-depleting pathways associated with glucose synthesis [35,36]. AMPK activation is mediated by phosphorylation of its

Thr^{172} site on the α-subunit. Hepatic ATP is reduced in patients with NAFLD, but there is evidence that hepatic AMPK activity may be reduced [37-40]. Other factors are therefore crucial in controlling hepatic AMPK activity during NAFLD. AMPK activity is measured by two indicators of the AMPK phosphorylation site (Thr^{172}) and its target acetyl-CoA carboxylase (ACC) phosphorylation site (Ser^{79} and Ser^{221}) [41,42]. Hasumi *et al.* found that activation of AMPK resulted in increaseed phosphorylation of AMPK (T^{172} AMPKα), ACC (Ser^{79}-ACC), and inhibition of fatty acid synthesis [43]. Aerobic exercise upregulated AMPK Thr^{172} phosphorylation and peroxisome proliferator-activated receptor α (PPARα) protein expression, reducing the progression of macrovascular steatosis and inflammation in obese mice. They all contributed to the NAFLD amelioration, as shown in Figure 2. PPARα is associated with hepatic lipid metabolism and is closely related to NAFLD. PPARα is a transcriptional regulator involved in
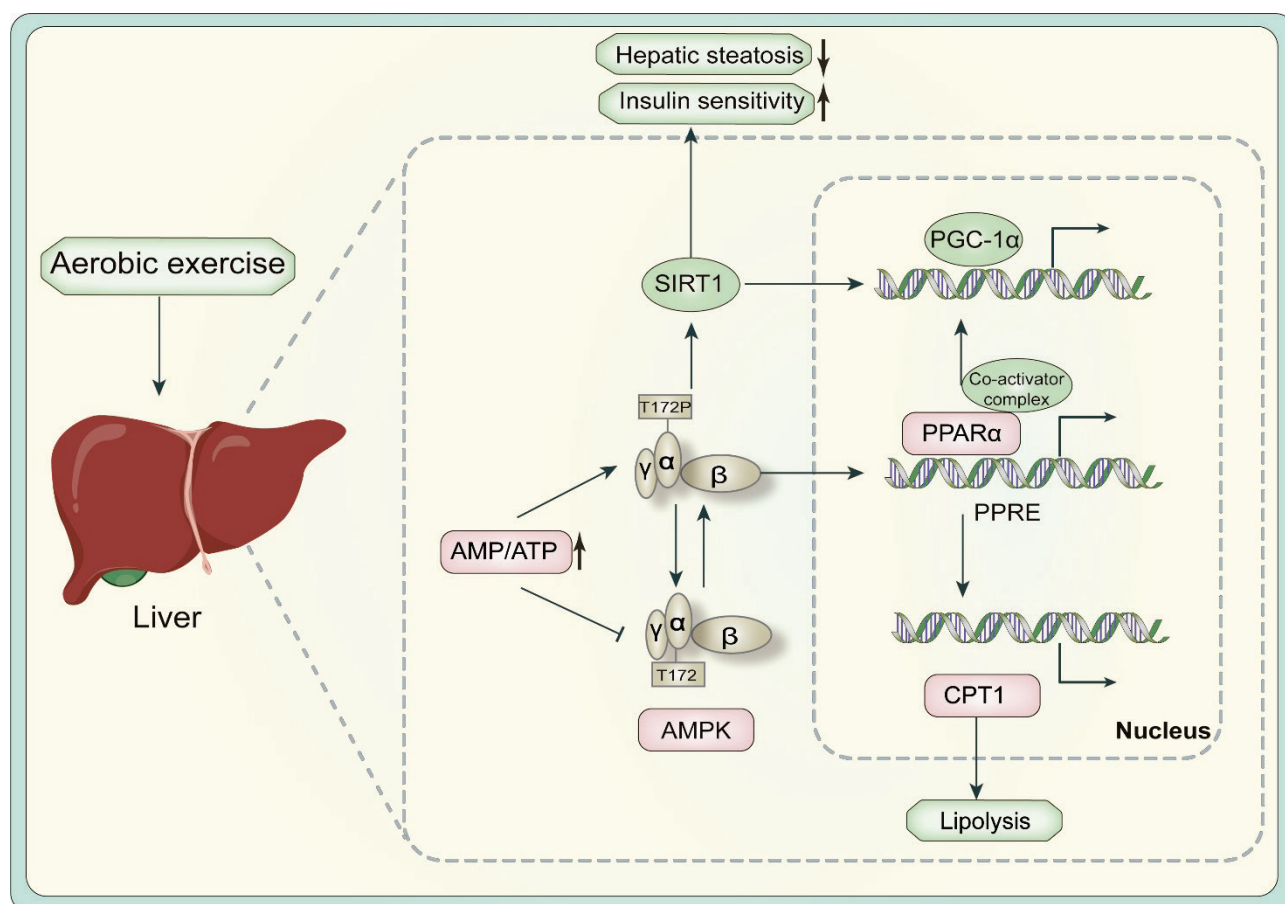

Fig. 2. The molecular mechanism of aerobic exercise affects the AMPK axis in hepatocytes. During exercise, a rise in the AMP/ATP ratio promotes AMP-activated protein kinase (AMPK) phosphorylation. It results in peroxisome proliferator-activated receptor α (PPARα) binding at the peroxisome proliferator response elements (PPREs) site, which promotes peroxisome proliferator-activated receptor co-activator-1α (PGC-1α) transcription. It also upregulates carnitine palmitoyl transferase 1 (CPT1) transcription, contributing to lipolysis. Moreover, the AMPK/sirtuin1 (SIRT1) axis improves insulin sensitivity and reduces hepatic steatosis.

mitochondrial β-oxidation, fatty acid transport, and hepatic gluconeogenesis [44]. It recognizes and binds to peroxisome proliferator response elements (PPREs) located in the promoter region of target genes, such as peroxisome proliferator-activated receptor co-activator-1α (PGC-1α). In livers lacking PPARα, transcription of carnitine palmitoyl transferase 1 (CPT1)-related genes are impaired, leading to the accumulation of excess fatty acids derived from lipolysis. These fatty acids are esterified to form triglycerides, which then accumulate in the liver during steatosis, contributing to the development of obesity [44]. Aerobic exercise stimulates AMPK/sirtuin1 (SIRT1) signaling and downstream regulation of lipid metabolism to attenuate hepatic steatosis and insulin resistance [45]. Furthermore, Bai *et al.* found that aerobic exercise and vitamin E combinational use can improve NAFLD in rats, leading to a reduction in liver fat accumulation [46]. Its combined treatment was the most effective [46]. Aerobic exercise promotes ACC phosphorylation and reduces fatty acid synthesis by activating the AMPK pathway [46]. This process also reduces oxidative stress, contributing to anti-inflammatory effects [46]. The results of a study demonstrated that exercise slows the progression of non-alcoholic liver disease, shows lower triglyceride and tumor necrosis factor-alpha expression, and that exercise improves biochemical and histological parameters of NAFLD and blocks the progression of fibrosis and tumorigenesis associated with enhanced activation of AMPK signaling, and facilitates hepatic autophagy [47].

In conclusion, as exercise intensity and duration increase, there is a corresponding enhancement in AMPK phosphorylation. It is our belief that the activation of AMPK through aerobic exercise is an important factor in the amelioration of NAFLD. Nonetheless, further research is required to establish the optimal levels of exercise intensity and duration that maximize the therapeutic benefits for individuals with NAFLD.

Mogat1 pathway in aerobic exercise

Monoacylglycerol O-acyltransferase 1 (MOGAT1) is an enzyme involved in lipid metabolism that catalyzes the conversion of monoacylglycerol to diacylglycerol, which is a key step in the synthesis of triglycerides. It is highly expressed in adipocytes and plays a crucial role in triglyceride synthesis [48]. Mogat1 expression is critical in the pathogenesis and progression of NAFLD [49,50]. PPARγ, a transcription factor that activates the expression of adipogenic genes, has deficient expression in normal liver. In contrast, PPARγ is highly expressed in the liver of NAFLD mice and stimulates triglyceride synthesis mainly by upregulating Mogat1 expression.

Recent studies have shown that regular aerobic exercise can have an inhibitory effect on Mogat1 [50]. In HFD-induced NAFLD mice after moderate-intensity aerobic activity, fatty liver accumulation was dramatically reduced, accompanied by a remarkable decrease in Mogat1 expression [50]. In addition, NAFLD mice that underwent aerobic exercise did not deteriorate but also improved, even when HFD persisted. It suggests that aerobic exercise can independently inhibit Mogat1 and alleviate NAFLD status without dietary restrictions. Interestingly, Mogat1 down-regulation had a positive effect not only on NAFLD but also on alcoholic hepatic steatosis [51]. Yu *et al.* found that hepatic lipid accumulation could be inhibited by the knockdown of Mogat1 [51]. However, a recent study showed that reduction of Mogat1, while inhibiting lipid accumulation in fatty liver, was associated with drastic side effects [52]. Kim *et al.* attempted to knock down Mogat1 expression in a NAFLD mouse model using antisense oligonucleotides (ASO). Although knocking down Mogat1 reduced hepatic triglyceride accumulation, it exacerbated ischemia/reperfusion injury (IRI) and inflammation to prolong the time to regression, which increased mortality in mice [52]. This suggests that Mogat1 may be essential for liver regeneration after IRI. Besides, the physiological role of hepatic Mogat1 in triacylglycerol (TAG) synthesis has been controversial. This is because it is believed that the primary pathway for triacylglycerol formation in the liver is the glycerol-3-phosphate pathway, not the Mogat1 pathway. For example, it has been reported that knockout of Mogat1 in obese mice does not affect steatosis. Collectively, aerobic exercise may reduce lipid aggregation by suppressing Mogat1. It offers a promising target for the treatment of NAFLD. However, the safety of how to target Mogat1 to treat NAFLD remains to be investigated.

SRA pathway in aerobic exercise

Long non-coding RNAs (lncRNAs) are a large and diverse group of RNAs that are usually germline-specific and regulate a wide range of biological functions [53]. Steroid receptor RNA activator (SRA) is an RNA an emerging regulatory factor, it can affect various coactivator belonging to the lncRNAs [54]. As physiological functions, such as regulating adipose tissue differentiation and glucose uptake [55,56]. Liu *et al.* found that SRA is highly expre-
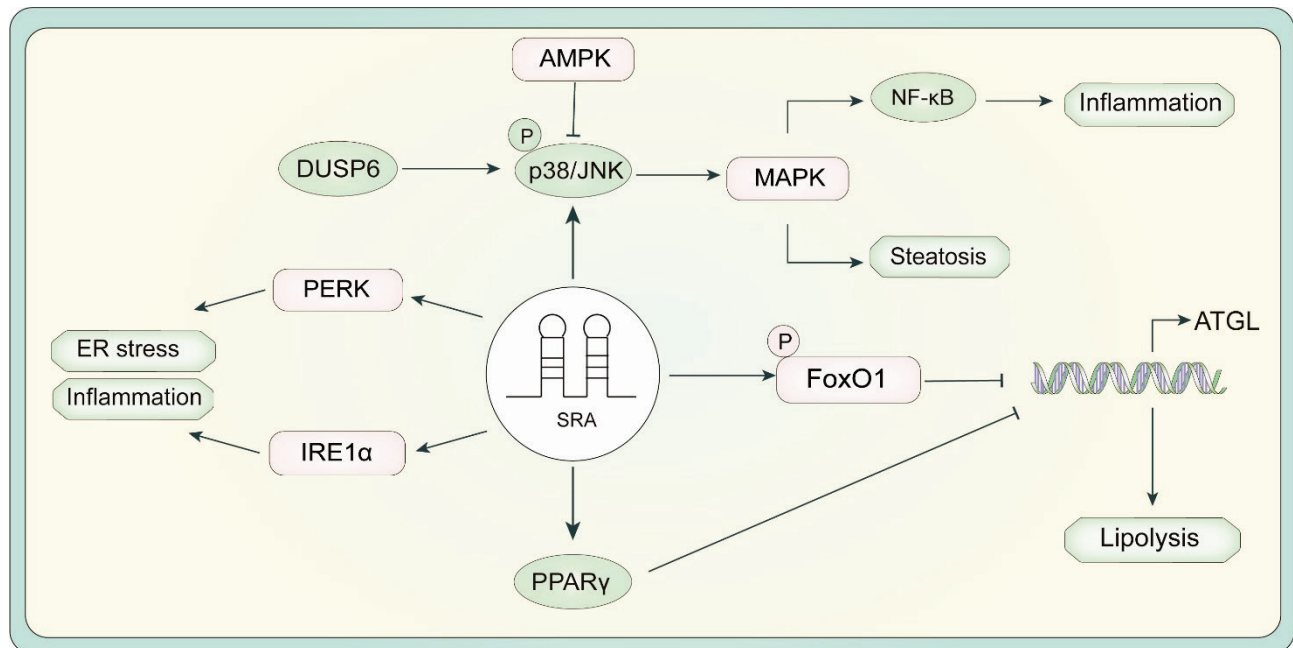
Fig. 3. The central pivotal role of SRA in NAFLD occurrence and progression. Steroid receptor RNA activator (SRA) upregulates protein kinase-like endoplasmic reticulum kinase (PERK) and endoplasmic reticulum transmembrane kinase 1α (IRE1α) to promote endoplasmic reticulum stress and inflammation. SRA promotes the phosphorylation of forkhead box protein O1 (FoxO1) to inhibit adipose triglyceride lipase (ATGL) expression, thereby preventing lipolysis. SRA is also involved in steatosis by regulating the p38/C-Jun amino-terminal kinases (JNKs) signaling pathway. p38/JNK is regulated by upstream factors dual-specificity phosphatase 16 (DUSP16) and AMP-activated protein kinase α1 (AMPKα1).

ssed in adipose tissue and protects against high-fat diet-induced obesity [56]. However, SRA seems to play a central pivotal role in NAFLD, as shown in Figure 3. SRA upregulates protein kinase-like endoplasmic reticulum kinase (PERK) and endoplasmic reticulum transmembrane kinase 1α (IRE1α), promoting ERS and inflammation. SRA is also involved in steatosis by regulating the p38/JNK signaling pathway. In addition, SRA was reported to inhibit the expression of adipose triglyceride lipase (ATGL) and suppress the trans-activation of the ATGL promoter *via* PPARγ [54]. ATGL specifically hydrolyses the first ester bond of triglycerides, contributing to lipid hydrolysis. Therefore, it is thought to play a delaying role in diseases such as AS and NAFLD.

A recent study has demonstrated the significant impact of aerobic exercise on liver lipid metabolism, particularly in the context of hepatic steatosis. Wu *et al.* observed that aerobic exercise in high-fat diet-fed mice not only effectively reduced blood cholesterol levels, which are elevated due to the diet, but more crucially, it led to a substantial decrease in hepatic triglycerides. This reduction in liver triglycerides is a pivotal finding, as it addresses the condition of hepatic steatosis. The exercise regimen was found to inhibit the expression of steroid receptor RNA activator, which is associated with improved lipid metabolism and a reduction in inflammation through the upregulation of ATGL activity [57,58]. Gang Chen *et al.* reveal a novel function of SRA in promoting hepatic steatosis through repression of ATGL expression. Forkhead box protein O1 (FoxO1) is an upstream transcription factor for ATGL expression, and its phosphorylation downregulates ATGL expression. ATGL expression was significantly reduced in mouse liver on a high-fat diet. However, exercise significantly reduced FoxO1 phosphorylation [54]. Therefore, the current speculation is that SRA can regulate ATGL expression through FoxO1 phosphorylation. This opens up the potential to find new targets for NAFLD.

p38/JNK pathway in aerobic exercise

A recent study found that aerobic exercise negatively regulates p38/JNK-MAPK signaling, which leads to improved obesity in mice, confirming previous findings [58]. C-Jun amino-terminal kinases (JNKs) and p38MAPK are members of the mitogen-activated protein kinase (MAPK) family that play essential roles in lipid regulation and inflammation response as shown in Figure 3 [58,59]. Studies have shown that HFD-induced metabolic stress stimulates the synergistic activation of the UPR and the p38/JNK signaling pathway. p38/JNK axis is closely related to excessive stress and

inflammation. Numerous studies have demonstrated that inhibition of p38/JNK-MAPK activity attenuates hepatic steatosis and inflammation [60-62]. It is now considered a potential target for developing anti-inflammatory therapeutic drugs, especially for NAFLD. For example, Zhang et al. found that AMP-activated protein kinase α1 (AMPKα1) overexpression attenuates NAFLD in a hepatocyte model by inactivating the p38MAPK pathway [60]. In addition, the inhibition of JNK activity by its inhibitor JM-2 had a facilitative effect on treating NAFLD [61]. Because the investigators found that liver inflammation and hepatocyte apoptosis were more severe in mice on a regular high-fat diet than in obese mice treated with JM-2 [61]. Similar findings were reported in a previous study in which Wu et al. found that JNK activity was inhibited in dual-specificity phosphatase 16 knockout mice, and hepatic steatosis was effectively alleviated in mice [62].

The p38/JNK axis has different physiological effects at different developmental stages. Overall, the current study suggests that aerobic exercise can regulate hepatic lipids that affect p38/JNK-MAPK signaling, but the mechanisms remain fully explored. Further investigation is warranted to dissect the intricate interactions between exercise, hepatic lipid metabolism, and the MAPK signaling cascade. This could involve examining the activation states of p38 and JNK in response to different types and durations of aerobic exercise, as well as the downstream transcriptional effects on genes involved in lipid metabolism. Understanding these mechanisms could lead to the development of targeted interventions for the prevention and treatment of metabolic disorders, leveraging the beneficial effects of physical activity on hepatic function and systemic metabolism.

Nrf2 pathway in aerobic exercise

Nuclear factor erythroid 2-related factor 2 (Nrf2) is an important transcription factor that belongs to the family of fundamental region leucine zipper transcription factors [63]. It is important to emphasize that the Nrf2 signaling pathway can regulate anti-inflammatory effects [64]. Numerous studies have shown that NAFLD can be mitigated through the Nrf2 signaling pathway. Yu et al. demonstrated that aescin (Aes) can activate and enhance the Nrf2 pathway to promote autophagy and attenuate NAFLD [65]. The expression of Nrf2 and its downstream proteins was significantly increased in the tissues of mice treated with Aes [65]. In addition, there is evidence that both acetylated stropharia rugoso-annulata polysaccharides and Pterostilbene, can activate Nrf2 and promote

autophagy in a mouse model of chronic NAFLD [66,67].

Swimming exercise reduced lipid accumulation in the liver of HFD-fed zebrafish and ameliorated pathological changes. In addition, swimming enhanced the anti-apoptotic effect of the organism, accompanied by a rise in the expression of the anti-apoptotic factor B-cell lymphoma-2 (bcl2) and a decrease in the pro-apoptosis gene expression such as caspase3 and bcl2-associated X (Bax). Zou et al. found that sirtuin1/AMPK signaling-mediated lipid metabolism and anti-inflammatory responses were activated after aerobic exercise, enhancing NRF2 activation [45]. This demonstrates that aerobic exercise can alleviate NAFLD in zebrafish through the Nrf2 signaling pathway [45]. Taken together, the current intervention of aerobic exercise in NAFLD through the Nrf2 signaling pathway remains to be investigated in depth. From the available evidence, we can speculate that Nrf2 is a potential target for treating NAFLD.

Heat shock protein in aerobic exercise

Heat shock protein (HSP) is a family of proteins produced by cells under stress conditions. The relationship between HSP60, HSP70, HSP90, and NAFLD was elaborated on in previous experiments. HSP60, also known as HSPD1, is a 60 kDa chaperone protein found mainly in the mitochondrial matrix [68]. The overexpression of HSP60 reduced HFD-induced fat accumulation and liver mass, as well as hepatocellular steatosis and thus NAFLD [68]. A study showed that N-acetylcysteine fed to mice on an HFD significantly elevated the expression of HSP60 and HSP70 in the mice's tissue, attenuating hepatocellular steatosis in the mice [69]. It validated the previous findings of an inverse relationship between HSP60 and HSP70 and the NAFLD progression [69]. However, in an earlier study in an animal model, it was shown that attenuation of fibrosis in NAFLD hepatitis could be achieved through a mechanism of HSP60 downregulation [70]. HSP70, a heat shock protein family member, is best characterized by its anti-inflammatory effects [71]. An earlier study examined NAFLD scores in 95 severely obese adult patients (75 females/20 males) who underwent bariatric surgery. The results showed that HSP70 (HSP72+HSP73) protein levels were negatively correlated with the progression of NAFLD [71]. However, it was demonstrated that HSP70 protein expression was upregulated in the livers of obese mice in the HFD group, and lipid droplets were significantly increased in overexpressing HSP70 cells [72]. This is paradoxical to the present conclusions. In combination, it is unclear whether

HSP70 is upregulated under compensatory conditions to counteract steatosis. HSP90 is a common molecular chaperone in HSP. It has a broader distribution of actions than HSP70 and consists of two main isoforms, HSP90α and HSP90β [73]. In a study conducted by researchers, tissue and blood samples were analyzed from 68 obese or overweight children and a control group of 10 children matched for sex and age [73]. The findings revealed a notably increased expression of Heat Shock Protein 90 (HSP90) in both the tissues and blood samples of the obese group. These results imply a potential role for HSP90 as a biomarker for Non-Alcoholic Fatty Liver Disease (NAFLD), given the observed positive correlation between HSP90 levels and the presence of the disease. This discovery may pave the way for the development of diagnostic tools and therapeutic strategies targeting HSP90 in the context of NAFLD [73]. In another report, Zheng *et al.* found higher levels of HSP90β than HSP90α by examining liver specimens from 20 patients with NAFLD [74]. Furthermore, the knockdown of HSP90β in mice resulted in 21 % of cellular triglyceride and 33 % of total cholesterol reduction in mouse tissues, whereas the knockdown of HSP90α did not seem to affect it [74]. Therefore, the knockdown of HSP90β could achieve improved lipid homeostasis. A recent report found that the HSP90 inhibitor 17-AAG promoted the maintenance of hepatic mitochondrial homeostasis and prevented mitochondrial depletion, which inhibited the progression of NAFLD in mice [75].

In an experiment, 120 male college students were divided into a control group (C), a resistance exercise group, a high-intensity interval training group (HIIT), and an aerobic exercise group. Except for group C, which did not exercise, the other groups exercised at different intensities 3 days a week for 8 weeks. Comparative analysis of the expression levels of HSP70 in blood before and after exercise revealed the greatest decrease in HSP70 in the aerobic exercise group [76]. However, the role of HSP70 in NAFLD needs to be further explored. Current relevant studies have focused on the changes in HSP70 before and after exercise, while it remains to be demonstrated by more new evidence whether other isoforms in the heat shock protein family, such as HSP60 and HSP90, similarly cause changes.

Anaerobic exercise

The American College of Sports Medicine defines anaerobic exercise as a high-intensity physical activity of short duration. It is fueled by energy within contracted muscles, does not use inhaled oxygen as an energy source, and is characterized by lactic acid build-up [77,78]. Current research suggests that anaerobic exercise is strongly associated with cardiovascular disease [78]. For instance, it has been shown that anaerobic exercise can reduce arterial vasodilation by modulating the ratio of collagen to elastin. Besides, anaerobic exercise can affect NK cells, T cells in the blood. Comparison of the blood results before and after freediving revealed a significantly higher percentage of CD69-expressing NK cells and a significantly higher percentage of $V\delta1^+$ and $V\delta2^+\gamma\delta$ T cells that also expressed CD69 [79]. In addition, freediving has been shown to activate AMPK and increase AMP levels [79]. AMPK can be directly involved in regulating cytotoxicity and cytokine production [80]. The experiment was limited by the small number of subjects and the short duration of the experiment. Current reports suggest that prolonged anaerobic exercise in athletes may activate intracellular hypoxia-inducible factor (HIF). In addition, the effect of anaerobic exercise on NAFLD is unclear. Therefore, further discussion is needed.

The HIF transcription factor is the master regulator of the cellular response to hypoxia. It coordinates the transcriptional program to ensure optimal functional, metabolic, and vascular adaptation to oxygen shortages [81]. The HIF family is a heterodimer consisting of a transcriptional regulatory subunit, HIF-α (HIF-1α, HIF-2α, and HIF-3α), and a ligand-binding subunit, HIF-β. HIF-α is a transcription factor with a helix-loop-helix structure that regulates genes involved in proteins of the hypoxic homeostatic response in a cell-specific manner. In past reports, HIF has been closely related to inflammation [82]. Karhausen *et al.* found that HIF-1α mutant mice had a higher mortality rate, slower body weight recovery, and significantly more severe colitis [82,83]. Another experiment revealed that hypoxia plays a very important role in the pathogenesis of rheumatoid arthritis (RA), which in turn promotes synovial inflammation through HIF-1α [89]. The HIF family of transcription factors plays a central role in the cellular regulation of the response to hypoxia, as well as important regulatory roles in inflammatory and immune responses. Future studies need to further reveal the specific mechanisms of action of HIF family members in different inflammatory diseases, as well as their interactions with other signaling pathways, so as to provide a theoretical basis for the development of new therapeutic strategies [84].

Similarly, there is evidence of a strong association between HIF and NAFLD. Some previous reports favored an important role for HIF-2α in the regulation of lipid metabolism, but more evidence has yet to emerge to elucidate this accurately [85]. Many experiments have demonstrated that HIF-2α is associated with steatosis [86]. Under hypoxic conditions, upregulation of HIF-2α is accompanied by lipid accumulation and activation of the PI3K/AKT/mTOR pathway [87]. Knockdown of HIF-2α in adipose hepatocellular carcinoma ameliorates triglyceride accumulation and steatosis [87]. Moreover, Chen *et al.* found that hypoxia upregulates HIF-2α to inhibit fatty acid β-oxidation and induce hepatic lipogenesis *via* PPARα, thereby exacerbating the progression of NAFLD. HIF-2α levels were found to be higher in the cytoplasm and nucleus of NAFLD hepatocytes [88]. Improvement in NAFLD is associated with the down-regulation of hepatocyte histidine-rich glycoprotein (HRGP) production, which is positively correlated with HIF-2α expression [89]. Yu *et al.* found a positive correlation between hepatic HIF-2α expression and the progression of NAFLD [88]. In addition, oxygen therapy (OT) alleviated hypoxia and inhibited the hepatic HIF-2α signaling pathway to alleviate NAFLD in a mouse model [88]. It indicated that OT may be a feasible approach for the treatment of NAFLD.

Hepatocyte HIF-1 mediates an increase in hepatic fibrosis, mainly due to hypoxia in liver tissue during hepatic steatosis [90]. The angiotensin II receptor antagonist losartan significantly reduced obesity-enhanced macrophage M1 activation and inhibited hepatic steatosis [91]. Macrophage M1 activation is required for HIF-1α expression in the liver and epididymal white adipose tissue of obese mice [91]. Furthermore, HIF-1α-mediated mitochondrial dysfunction was reversed by losartan [91]. It provides a potential target for losartan in the treatment of NAFLD *via* targeting HIF-1α. Yet another study found that HIF-1α silencing promotes lipid accumulation in NAFLD cells, accompanied by significant increases in triglycerides and apolipoprotein B (ApoB) levels [92]. In addition, HIF-1α deletion markedly elevated the activation of oxidative stress and exacerbated the inflammatory response in NAFLD cells [92]. This view suggests that HIF-1α is involved in the regulation of lipid metabolism in NAFLD through activation of the PPAR-α/angiopoietin-like 4 signaling pathway [92]. It is supported by other studies. HIF-1 mediates the PPARα/PGC-1α pathway in NAFLD by activating lipin1 expression and nuclear

accumulation [93]. It prevents excessive accumulation of fat in hepatocytes.

It is well known that anaerobic exercise can affect the distribution of HIFs. Sprinting exercise under hypoxic conditions increased the expression of HIF-1α protein after exercise [94]. In addition, another report conducted a hypoxic exercise intervention in 47 hyper-tensive patients. The results showed that the expression level of HIF-1α was significantly higher in both the intermittent hypoxic resting breathing group and intermittent hypoxic training group (IHT) groups [95]. AMPK interacts with other signaling molecules such as HIF-1α (hypoxia-inducible factor 1-alpha), which is involved in the adaptation to low oxygen conditions. During anaerobic exercise, the interaction between AMPK and HIF-1α can influence the metabolic response and adaptation to exercise [96].

The vast majority of the only research evidence available examines the relationship between HIF-1α and anaerobic or hypoxic exercise, with very little relevant research on HIF-2α. More new evidence on whether anaerobic or hypoxic exercise affects the distribution of HIF-2α is yet to emerge. In addition, the current opinion only confirms that HIF-2α appears to be positively associated with the progression of NAFLD. The role of HIF-1, on the other hand, is still ambiguous. On the one hand, overexpression of HIF-1 in hepatocytes promotes hepatocyte fibrosis. However, the knockdown of HIF-1, in turn, promotes hepatocyte lipid accumulation and inflammatory responses. It suggests that HIF-1 is indispensable in hepatocytes, but its role cannot yet be clarified. Numerous factors need to be further investigated in the association between anaerobic exercise and NAFLD.

Summary and prospects

Overall, current reports show that a combination of lifestyle modifications can significantly promote the improvement of NAFLD. In this review, by studying and summarizing the effects of various lifestyle interventions on NAFLD, we present a comprehensive overview in Table 1. We found that aerobic exercise can improve NAFLD through various signaling pathways. Additionally, anaerobic exercise may affect HIF-1α; however, its relationship with NAFLD, which is primarily associated with HIF-2α, requires further investigation with new evidence. Beyond exercise, dietary changes have been shown to be crucial in managing NAFLD. Adopting

a balanced diet that is low in saturated fats, refined sugars, and cholesterol, and rich in fiber, antioxidants, and omega-3 fatty acids can lead to significant improvements in liver health. Weight reduction, even modest weight loss of 5-10 % of the initial body weight, has been associated with improvements in liver enzymes and hepatic steatosis. Furthermore, adequate sleep and stress management are essential components of a healthy lifestyle that can contribute to the overall well-being of individuals with NAFLD. The key signaling pathways summarized in this review, along with these lifestyle interventions, may provide potential therapeutic targets for improving and preventing NAFLD in the future.

Conflict of Interest
There is no conflict of interest.

Acknowledgements

Table 1. The signaling pathways of aerobic and anaerobic exercise on NAFLD.

Exercise type	Key factors	Mechanism	Reference
Aerobic exercise	CNPY2/PERK	Aerobic exercise ameliorates NAFLD by down-regulating the CNPY2-PERK pathway. There are positive feedback loops between CNPY2 and PERK/CHOP axis to promote steatosis.	[27]
	AMPK	Aerobic exercise promotes AMPK phosphorylation. AMPK activates the PPARα/PGC-1α axis and the PPARα/CPT1 axis to promote lipolysis. The AMPK/SIRT1 axis improves insulin sensitivity and attenuates hepatic steatosis.	[39,40]
	Mogat1	Aerobic exercise reduces lipid aggregation by inhibiting Mogat1.	[49]
	SRA	Aerobic exercise inhibited the expression of SRA. SRA upregulates PERK and IRE1α to promote ERS and inflammation. SRA promotes the phosphorylation of FoxO1 to inhibit ATGL expression, thereby preventing lipolysis. SRA is involved in steatosis by regulating the p38/JNK signaling pathway.	[53,56]
	p38/JNK	Aerobic exercise negatively regulates the p38/JNK axis. p38/JNK axis is strongly associated with ERS and inflammation. DUSP16 knockout mice have suppressed JNK activity.	[57,58]
	Nrf2	Aerobic exercise upregulates SIRT1/AMPK signaling to enhance NRF2 activation.	[72]
	HSP	Possible inverse association between HSP60 and HSP70 and NAFLD progression. HSP90 may be a marker for NAFLD. However aerobic exercise significantly reduced blood concentrations of HSP70.	[77,78]
Anaerobic exercise	HIF-1	Sprinting under hypoxic conditions increases HIF-1α protein expression. HIF-1 is essential in hepatocytes, but its role is unclear.	[97]
	HIF-2	Whether anaerobic exercise affects HIF-2α distribution is unclear. HIF-2α appears to be positively associated with the progression of NAFLD.	[93]

CNPY2, canopy fibroblast growth factor signaling regulator 2; PERK, protein kinase RNA-like endoplasmic reticulum kinase; PPARα, peroxisome proliferator-activated receptor α; PGC-1α, peroxisome proliferator-activated receptor co-activator-1α; CHOP, C/EBP homologous protein; CPT1, carnitine palmitoyl transferase 1; AMPK, AMP-activated protein kinase; SIRT1, sirtuin1; Mogat1, Monoacylglycerol O-acyltransferase 1; SRA, Steroid receptor RNA activator; IRE1α, endoplasmic reticulum transmembrane kinase 1α; FoxO1, Forkhead box protein O1; ATGL, adipose triglyceride lipase; JNK, C-Jun amino-terminal kinase; DUSP16, dual-specificity phosphatase 16; Nrf2, Nuclear factor erythroid 2-related factor 2; NAFLD, non-alcoholic fatty liver disease; HSP, Heat shock protein; HIF, Hypoxia-inducible factor.

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