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Invited Review

The Effects of Physical Exercise on Fatty Liver Disease

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The increasing prevalence of obesity has made nonalcoholic fatty liver disease (NAFLD) the most common chronic liver disease. As a consequence, NAFLD and especially its inflammatory form nonalcoholic steatohepatitis (NASH) are the fastest increasing etiology of end-stage liver disease and hepatocellular carcinoma. Physical inactivity is related to the severity of fatty liver disease irrespective of body weight, supporting the hypothesis that increasing physical activity through exercise can improve fatty liver disease. This review summarizes the evidence for the effects of physical exercise on NAFLD and NASH. Several clinical trials have shown that both aerobic and resistance exercise reduce the hepatic fat content. From clinical and basic scientific studies, it is evident that exercise affects fatty liver disease through various pathways. Improved peripheral insulin resistance reduces the excess delivery of free fatty acids and glucose for free fatty acid synthesis to the liver. In the liver, exercise increases fatty acid oxidation, decreases fatty acid synthesis, and prevents mitochondrial and hepatocellular damage through a reduction of the release of damage-associated molecular patterns. In conclusion, physical exercise is a proven therapeutic strategy to improve fatty liver disease.

Key words: Nonalcoholic fatty liver disease (NAFLD); Nonalcoholic steatohepatitis (NASH); Aerobic exercise; Resistance exercise; Lipid metabolism

INTRODUCTION

For millions of years, humans needed to invest significant physical effort in gathering food to meet their nutritional needs, which has hard-wired our metabolism to efficiently store nutrients at rare moments of caloric abundance. However, in the current day and age, no physical activity is required to obtain a daily caloric load for most people in developed as well as developing countries. The resulting obesity epidemic has caused nonalcoholic fatty liver disease (NAFLD) to rapidly become the most common etiology of chronic liver disease[1–3]. NAFLD can progress to nonalcoholic steatohepatitis (NASH), which places patients at risk for developing end-stage liver disease (i.e., cirrhosis) in which hepatocellular carcinoma (HCC) may develop[4]. Of great concern is the observation that HCC can also develop in noncirrhotic NASH[5]. Of all common cancers in the US, HCC is the only tumor with an increasing mortality[6].

Diet and lifestyle modification leading to weight loss of 10% or more has been proven to be an effective strategy to achieve resolution of NASH in >90% of patients[7]. Unfortunately, greater than 50% of patients included in clinical trials have not been able to meet this weight loss threshold[8]. Likewise, treatment with medications, such as pioglitazone, vitamin E, or the bile acid derivative obeticholic acid, has been effective only in up to 45% of patients[9,10].

Physical inactivity and its related reduced cardiorespiratory fitness have been associated with increased NASH severity[11]. Among obese people, sedentary individuals have increased risks of having a fatty liver in comparison with weight-matched physically active individuals[12]. These data provide support for the hypothesis that increasing physical activity through exercise, defined as a planned, structured, and repetitive physical activity with a specific intensity, frequency, and duration, has

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beneficial effects on NAFLD. Theoretically, it is also a cheap intervention with both therapeutic and preventive value. Simultaneously, exercise can reduce risk factors for cardiovascular disease in NASH patients, such as diabetes and hypertension[13]. The American Gastroenterological Association, the American Association for the Study of Liver Diseases, and American College of Gastroenterology all recommend physical exercise as a treatment for NAFLD[14]. The current recommendations do not specify what exercise regimen is most beneficial, and the mechanisms by which exercise affects the liver remain, at least in part, unknown. Here we aim to review the existing evidence for the effects of physical exercise on NAFLD, as well as the mechanistic principles that have been elucidated through human trials and basic scientific studies.

EFFECTS OF EXERCISE ON BIOPSY-PROVEN NASH

The true outcomes of fatty liver disease are end-stage liver disease (ESLD) and HCC. No studies with longitudinal follow-up have been performed to evaluate the effect of exercise on these outcomes, and such studies would probably not be feasible either. However, the liver's remarkable regeneration capacity can result in the reversibility of steatohepatitis. It is most probably correct to assume that with recovery of NASH, the risks of developing ESLD and HCC are reduced as well. A small number of studies have used postintervention liver biopsy to evaluate the effect of exercise on the histologic reversibility of NASH. Eckard et al. performed a randomized controlled study on the effect of 6 months of various lifestyle modifications on NASH, including exercise and dietary modifications[15]. In this trial, the effect of a moderate exercise regimen (20- to 60-min routine, 4–7 days/week, including both resistance and aerobic training) with or without dietary intervention (unrestricted diet vs. low-fat diet vs. moderate fat/low-carbohydrate diet) was compared to a group undergoing no intervention. In each of the intervention groups there was a significant decrease in the NASH Activity Score (NAS), a histological grading of NASH[15]. Similarly, a randomized control trial (RCT) of 31 NASH patients reported that 48 weeks of intensive lifestyle intervention (moderate-intensity exercise with a goal of >200 min per week, reduced calorie diet, and behavioral guidance) led to a 2.4 point reduction in the NAS on postintervention liver biopsy, a significantly greater reduction than in the control arm[16]. Another study evaluated 120 candidates for living liver donation who were encouraged to do aerobic exercise and restrict their calorie intake to 25 cal/kg, after liver biopsy showed at least 30% steatosis. On repeat biopsy after a median of 10 weeks of intervention, steatosis was improved in >85% of patients[17]. We can infer from these results that exercise has a beneficial effect on the reversal of histologically proven fatty liver disease. However, because dietary interventions aiming at weight loss were included as well, these studies do not provide direct evidence that the effect on the liver was mediated by exercise or through the effect of weight loss.

EFFECTS OF EXERCISE ON NONINVASIVE MEASURES OF NASH

Although liver biopsy is the gold standard for diagnosis and grading of NAFLD, its risk of complications, potential to obtain nonrepresentative samples, and cost deter its widespread use[18]. Liver biopsy has therefore infrequently been used in the evaluation of the efficacy of exercise on NAFLD. Alternatively, several noninvasive techniques have been developed to assess liver fat content[19], and their advantages and shortcomings are summarized in Table 1. In general, these techniques measure liver fat content or liver stiffness as an indication of fibrosis and do not necessarily distinguish NASH from simple steatosis. Liver stiffness measured by magnetic resonance (MR) elastography, however, appeared to closely correlate with a diagnosis of NASH on liver biopsy and had a sensitivity of 94% and a specificity of 73% to discern NASH from simple steatosis[20].

Over the past decades, several trials have been performed using these surrogate endpoints to estimate the effect of physical exercise on NASH. Randomized trials reported since 2005 are summarized in Table 2. In 2012, Keating et al. performed a meta-analysis of 12 trials (11 of them randomized) investigating the effect of exercise on liver fat content. In the pooled analysis, 439 subjects were included. A small reduction in liver fat was seen, however, only if studies that looked at diet and exercise were left out of the analysis[21]. Most studies included were small ($n = 14$–45 in 11 studies with 1 study of 130 subjects), and exercise regimens were often short (in 7 studies 10 weeks or shorter), which are potential reasons why the reported effect was limited.

Since Keating and colleagues' meta-analysis, additional and larger RCTs have been done that clearly demonstrate the beneficial effect of physical exercise on NASH. Golabi et al. conducted a systematic review of these studies published between 2011 and 2016. In this work, only trials of at least 8-week intervention were included. On reviewing eight randomized trials, the effect of physical exercise on the reduction of hepatic fat content was assessed. With the use of MR spectroscopy or liver biopsy, a pooled analysis of a total of 433 adult participants revealed a 30.2% reduction in hepatic fat as a result of the exercise intervention, and a 49.8% reduction in liver fat resulting from exercise combined with dietary intervention[22].

Whitsett et al. conducted a systematic review of 18 studies[23]. Besides randomized trials, prospective and

Table 1. Noninvasive Modalities for Assessment of Fatty Liver Disease

Modality	Technique	Advantages	Shortcomings
US	Increased echogenicity makes steatotic livers appear brighter than spleen and kidney	Low cost; widely available; reasonable sensitivity/ specificity	Lower performance with steatosis <30% and in morbidly obese patients; operator dependent
US-CAP	Measures the degree of ultrasound attenuation by hepatic fat using vibration control transient elastography	Can roughly distinguish steatosis categories	Overlap between stages; not validated in large patient cohorts
CT	Decreased attenuation of fatty liver (10 HU less than spleen, or liver attenuation <40 HU)	Widely available	Not sensitive for detecting mild steatosis (5%–30%); radiation exposure
MRS	Protons in triglycerides resonate with specific spectral peaks	High sensitivity; correlates strongly with the histological fat percentage	Not widely available; increased cost, cannot be used as a screening tool
MRE	Contrast MRI with a low frequency vibration source to assess stiffness	High sensitivity for fibrosis; differentiates between steatosis and NASH	Not widely available; long procedural time; low image resolution
Transient elastography	Velocity of electric shear indicates liver stiffness	Short procedure time; can be done at bedside; immediate results	Mainly looks at fibrosis; operator dependent; difficult to get accurate and valid results (requires at least 10 measurements)

US, ultrasound; US-CAP, ultrasound with controlled attenuation parameter; CT, computed tomography; MRS, magnetic resonance spectroscopy; MRE, magnetic resonance elastography.

well-conducted retrospective cohort studies were also included. The included studies together evaluated more than 6,000 patients with NAFLD, with two studies in particular having a study population greater than 1,000. The intervention duration varied greatly from 1 to 52 weeks, and the most commonly employed imaging modality to determine change in hepatic steatosis was hydrogen-MR spectroscopy (H-MRS). The authors concluded that exercise significantly improves hepatic fat content.

A recent randomized trial not included in the above systematic reviews, but worth mentioning because of its relatively large size, was conducted by Wong et al. In this study of 145 NASH patients, lifestyle intervention (aerobic exercise, resistance exercise, and dietary restriction) demonstrated a 64% remission rate (i.e., achievement of <5% intrahepatic triglyceride content) in the intervention group, compared to a 20% remission rate in the control arm, which underwent no intervention[24].

EXERCISE REGIMEN AND INTENSITY

Several studies addressed which modality, intensity, and duration of exercise are most efficacious in ameliorating NASH. A retrospective trial of 813 biopsy-proven NAFLD patients asking them to self-report on their physical activity status found that only those patients who met the vigorous exercise criteria, corresponding to 7 or more metabolic equivalents[25], had a decreased odds ratio of developing NASH[26]. Those patients who doubled the time of recommended vigorous exercise further decreased the adjusted odds for advanced fibrosis[26]. Another self-reported retrospective trial from Japan, on the physical activity of 1,149 patients with fatty liver disease, corroborated this finding. Vigorous physical activity showed a significant preventative effect in the progression of fatty liver to NASH[27]. Modified high-intensity interval training (HIIT) of five cycles of high-intensity cycling followed by 3-min recovery periods, three times/week for 12 weeks demonstrated reduction in liver fat and improvement in early diastolic filling in 23 NAFLD patients compared to standard controls[28]. This improvement in early diastolic filling is beneficial to NAFLD patients, as it has been well documented that cardiorespiratory fitness, independent of visceral fat, is a predictor of liver fat[11,29,30]. These studies establish that vigorous exercise results in significant reduction in hepatic steatosis.

A number of studies tried to establish scientific evidence for this matter in an experimental setting. A recent study of 48 overweight and obese patients compared aerobic exercise regimens of various doses and intensities[31]. Patients were randomly assigned to low-intensity/ high-volume, high-intensity/low-volume, low-intensity/ low-volume, or no exercise. Each exercise group experienced significant reduction in liver fat, but there was no significant difference between the different regimens. This led to the conclusion that aerobic exercise, even if done at low intensity and low volume, would have a beneficial effect on the reduction of liver fat[31]. Several randomized trials provide evidence that aerobic exercise indeed reduces hepatic fat content at different intensities and frequencies[32–44].

Table 2. Randomized Controlled Trials of Exercise and the Effect on Nonalcoholic Fatty Liver Disease (NAFLD)

Reference	*n*	Exercise Intervention	Main Results
Bacchi et al., 2013[45]	40	AE vs. RE, 3×/week for 16 weeks	Equal effects on reducing intrahepatic fat
Balducci et al., 2015[110]	606	AE + RE vs. control, 2×/week for 12 months	Reduced fatty liver index
Cassidy et al., 2013[39]	28	AE vs. controls, 3×/week for 12 weeks	Decreased hepatic lipid content, improvement in cardiac function
Cuthbertson et al., 2016[58]	69	AE vs. control, 3–5×/week for 16 weeks	Decreased hepatic lipid content, improved peripheral insulin sensitivity
Eckard et al., 2013[15]	56	AE vs. AE + low fat diet, 4–7×/week for 6 months	Decrease in NASH activity score on liver biopsy
Finucane et al., 2010[32]	100	AE vs. control, 1×/week for 12 weeks	Decreased hepatic lipid content, improved cardiorespiratory fitness
Goodpaster et al., 2010[33]	130	Diet + AE for 6 months vs. diet + AE for 12 months, 5×/week	12-Month intervention resulted in greater decrease in hepatic fat content, with equal reduction in insulin resistance
Hallsworth et al., 2011[46]	21	RE vs. control, 3×/week for 8 weeks	Decreased hepatic lipid content, improved insulin resistance
Hallsworth et al., 2015[28]	29	High intensity AE vs. control, 3×/week for 12 weeks	Decreased hepatic lipid content, improved cardiorespiratory fitness
Houghton et al., 2017[40]	24	AE + RE vs. control, 3×/week for 12 weeks	Decreased hepatic lipid content and plasma triglycerides
Larson-Meyer et al., 2008[34]	23	Diet + AE vs. diet vs. control, 5×/week for 6 months	Decreased hepatic lipid content
Lee et al., 2012[41]	45	AE vs. RE vs. control, 3×/week for 12 weeks	Decreased hepatic lipid content. RE improved insulin sensitivity
Levinger et al., 2009[111]	55	RE vs. control, 3×/week for 10 weeks	No reduction in ALT/AST or inflammatory markers
Monteiro et al., 2015[42]	32	AE vs. AE + RE vs. control, 3×/week for 20 weeks	Decreased hepatic fat content
de Piano et al., 2012[105]	58	AE vs. AE + RE, 3×/week for 12 months	AE + RE results in reduced ALT and insulin resistance, and increased adipokine levels
Promrat et al., 2010[16]	31	AE + diet vs. control, 1×/week for 48 weeks	Decrease in NASH activity score on liver biopsy
Pugh et al., 2013[103]	20	AE vs. control, 3×/week for 16 weeks	No difference in hepatic fat content. Improved ALT/AST levels
Pugh et al., 2014[104]	31	AE vs. control, 3×/week for 16 weeks	No difference in hepatic fat content. Improved ALT/AST levels. Improved cardiovascular risk factors
Shah et al., 2009[35]	18	Diet + AE/RE vs. diet, 3×/week for 6 months	Comparable decrease in hepatic lipid content and insulin resistance
Shoojaee-Moradie et al., 2007[54]	17	AE vs. control, 3×/week for 6 weeks	No difference in intrahepatic fat content. Decreased circulating FFA, increased insulin sensitivity
Skrypnik et al., 2016[106]	44	AE vs. AE + RE, 3×/week for 3 months	AE + RE results in greater reduction in ALT and AST
Slentz et al., 2011[43]	196	AE vs. RE vs. AE + RE, 3×/week for 8 months	Regimens including AE result in greater reduction in hepatic fat content, ALT and insulin resistance
Straznicky et al., 2012[112]	63	Diet + AE vs. diet vs. control, 300 min/week for 12 weeks	Decreased insulin resistance, ALT, γ-GT; no significant differences between diet + AE and diet alone
Sullivan et al., 2012[36]	18	AE vs. control, 3×/week for 16 weeks	Decreased hepatic lipid content, decreased circulating FFA, improved insulin sensitivity

(*continued*)

Table 2. (Continued)

Reference	*n*	Exercise Intervention	Main Results
Tamura et al., 2005[37]	14	Diet + AE vs. diet, 5–6×/week for 2 weeks	Decreased hepatic lipid content in both groups. Improved insulin sensitivity in AE group
Thompson et al., 2009[62]	41	AE vs. control, 4×/week for 24 weeks	Decreased IL-6, ALT, and FFA
Wong et al., 2013[24]	154	AE vs. control, 3×/week for 12 months	Decreased hepatic lipid content
Yoshimura et al., 2014[113]	33	Diet + AE vs. diet, 300 min/week for 12 weeks	Equal decrease in hepatic lipid content
Zelber-Sagi et al., 2014[38]	82	RE vs. control, 3×/week for 12 weeks	Improved steatosis and inflammation
Zhang et al., 2016[44]	220	AE vs. control, 150 min/week for 12 months	Decreased hepatic lipid content. Effect disappeared when adjusted for weight loss

AE, aerobic exercise; RE, resistance exercise.

Bacchi et al. conducted an RCT comparing the effects of resistance training versus aerobic training in 31 NASH patients over a 4-month period. In both arms of the trial, there was a significant reduction in liver fat on MRS. However, there was no difference between the two exercise regimens[45]. Lee et al. also found a similar beneficial effect of resistance exercise compared with aerobic exercise[41]. Hallsworth et al. reported on 19 patients with NAFLD who were either subjected to 8 weeks of resistance training or no exercise. A significant reduction in liver fat was achieved[46]. Zelber-Sagi et al. also found a significant effect of resistance exercise on hepatic fat content[38]. On the contrary, in 29 obese/overweight adults who were randomized to either 8 weeks of resistance exercises or sham control exercise regimen no significant difference in liver fat by MR spectroscopy was achieved[47]. A randomized trial including 196 subjects revealed that regimens including aerobic exercise resulted in greater reduction in hepatic fat content than a resistance exercise program[43].

In conclusion, although various exercise regimens have been shown to affect liver fat content, there is no definitive evidence to recommend one regimen over another. Aerobic exercise has shown an effect on hepatic fat content in many studies, but resistance exercise may provide an option to patients unable to perform aerobic exercises, for example, due to a limited cardiorespiratory reserve. It is therefore emphasized in recent practice guidelines that the choice of training for patients with NAFLD should be tailored based on patients' preferences and on the highest likelihood of continuation by the individual patient in the long term[19,48].

THE EFFECT OF EXERCISE ON THE LIVER IS INDEPENDENT OF WEIGHT LOSS

Importantly, the decrease in the hepatic fat content was achieved even when overall weight loss was not observed in a multitude of studies[15–18,22,31,45–47], which is consistent with the idea that exercise has a direct effect on the liver. However, the mechanisms by which exercise reduces liver fat are still greatly unknown. The next sections of this review summarize the available evidence on possible metabolic and molecular pathways involved in the reduction of hepatic fat by exercise.

EFFECTS ON INSULIN RESISTANCE AND FREE FATTY ACIDS

In order to begin to understand the molecular pathways that are involved in the effect of physical exercise on fatty liver disease, it is worth looking at other outcome measures than hepatic fat content. For example, insulin resistance is thought to be a driving force in NASH and its related metabolic syndrome[49]. Improving insulin resistance is among the mechanisms by which physical exercise has been proposed to improve NASH. In support of this hypothesis, several studies in humans have reported on the beneficial effect of exercise on insulin resistance[35–37,45,46,50–54]. For example, in a comparative trial of 16 weeks of aerobic versus resistance training, Bacchi et al. used a euglycemic clamp technique to demonstrate that both programs substantially increased insulin sensitivity, along with an improvement in other markers of the metabolic syndrome, like HbA1C and visceral fat[45].

Mechanistically, insulin resistance in adipose tissue results in an incomplete suppression of lipase, leading to enhanced lipolysis and release of free fatty acids (FFAs), which are elevated in serum of NAFLD patients[55,56] and are taken up by the liver[57]. An improvement in insulin resistance is thought to reduce this flux of FFA to the liver (Fig. 1). In a randomized trial of 69 NAFLD patients, it was shown that 4 months of physical exercise predominantly affects peripheral rather than hepatic insulin resistance[58]. Studies on the acute metabolic effects of exercise in 15 prediabetic adults confirmed that the improvement in insulin sensitivity mainly occurs in adipose and muscle tissues[59]. Although some studies did not detect a significant reduction in fasting serum FFA[46,58], several others have demonstrated a resulting decrease in

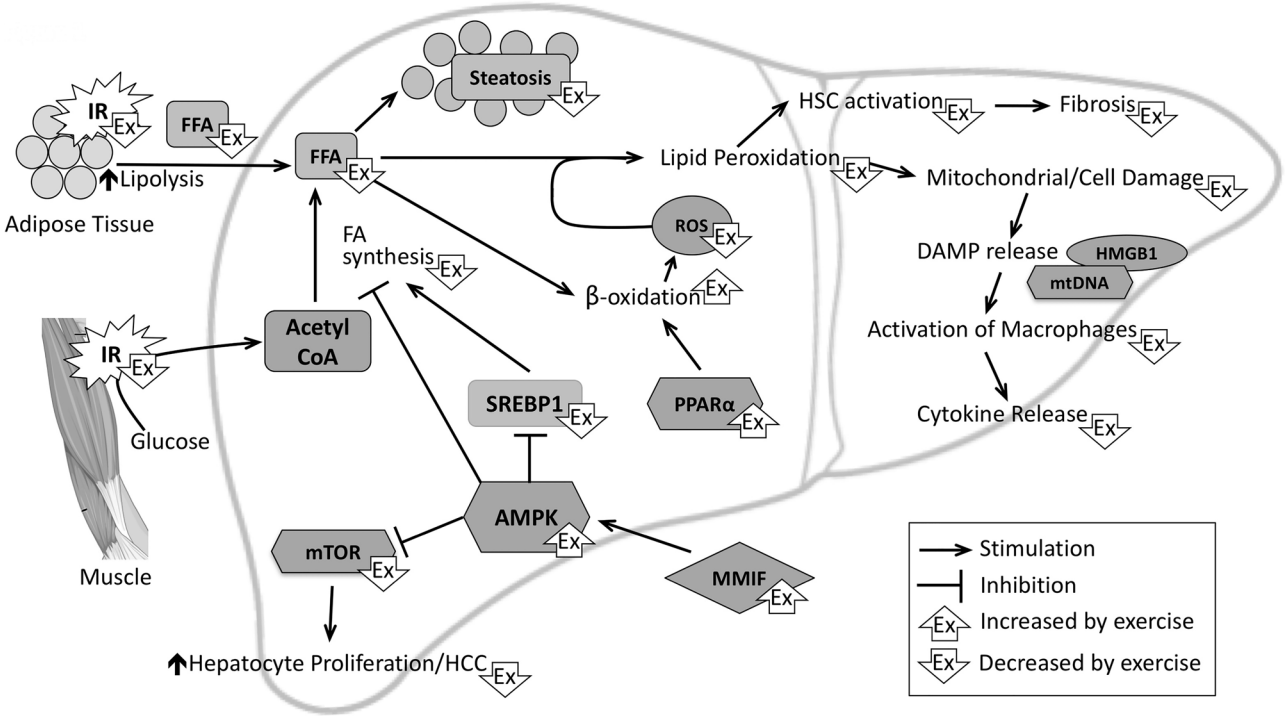

Figure 1. Schematic overview of metabolic and molecular pathways involved in the pathobiology of nonalcoholic steatohepatitis (NASH) and the effects of physical exercise thereon. Peripheral insulin resistance causes an increase in delivery of glucose and FFA to the liver. FA synthesis further increases FFA levels. When the mechanisms for FA storage as triglycerides (steatosis) and metabolism (β-oxidation) become overwhelmed, ROS production increases, resulting in mitochondrial and hepatocyte damage, DAMP release, and amplification of inflammation. Exercise affects these pathways at multiple levels, as indicated. Of note, multiple other pathways are involved in the pathogenesis of NASH. As the effects of exercise have not been investigated on these pathways, they are not included in this diagram. AMPK, AMP-activated protein kinase; DAMP, damage-associated molecular pattern; FA, fatty acid; FFA, free fatty acids; HCC, hepatocellular carcinoma; HMGB1, high-mobility group box-1; HSC, hepatic stellate cell; IR, insulin resistance; MMIF, macrophage migration inhibitory factor; mtDNA, mitochondrial DNA; mTOR, mammalian target of rapamycin; PPARα, peroxisome proliferator-activated receptor-α; ROS, reactive oxygen species; SREBP-1, sterol regulatory element-binding protein 1.

FFA in human patients[36,52,54,59–62], as well as in experimental rodent models of NASH[63,64]. In addition, it has been shown that FFA in return increases insulin resistance in skeletal muscle and that physical exercise can direct FFA into triglyceride formation with a resulting increase in insulin sensitivity[65].

EXERCISE AND FATTY ACID SYNTHESIS

Insulin resistance in skeletal muscle is the cause of diversion of glucose to the liver for FFA synthesis (de novo lipogenesis)[66]. In addition to storage into triglycerides leading to steatosis, FFAs are considered the metabolically and immunologically active form of fat contributing to cell damage and inflammation that are characteristic of NASH (Fig. 1)[67,68]. Animal models have shown that the main transcription factor controlling lipogenesis, sterol regulatory element-binding protein 1 (SREBP-1), is elevated in the pathobiology of NASH[69–71]. The best available evidence that physical exercise can modify de novo synthesis of FFA in human patients with fatty liver disease is reported by Oh et al., who exposed middle-aged, sedentary, obese men to exercise regimens in various intensity and frequency. They found that 12 weeks of either resistance or high-intensity aerobic exercise led to a decrease in the expression of SREBP-1c in circulating peripheral blood mononuclear cells (PBMCs)[52,72]. As PBMCs have the same embryonic origin as liver cells, it is felt that they accurately represent the changes in hepatocytes.

In lipogenesis, acetyl-coA derived from the Krebs cycle gets converted into long-chain fatty acids facilitated by a number of enzymes, such as acetyl-CoA carboxylase (ACC), fatty acid synthase (FAS), elongases, and stearoyl-CoA desaturase 1 (SCD1) (Fig. 2). Although evidence in humans is limited (changes in levels of these enzymes in the studies by Oh et al. were nonsignificant), several rodent studies have shown that exercise reduces expression levels of FAS, elongases, and SCD1 in fatty livers, resulting in decreased levels of FFA and reversal of steatosis[69,73–77]. Exercise also increased phosphorylation

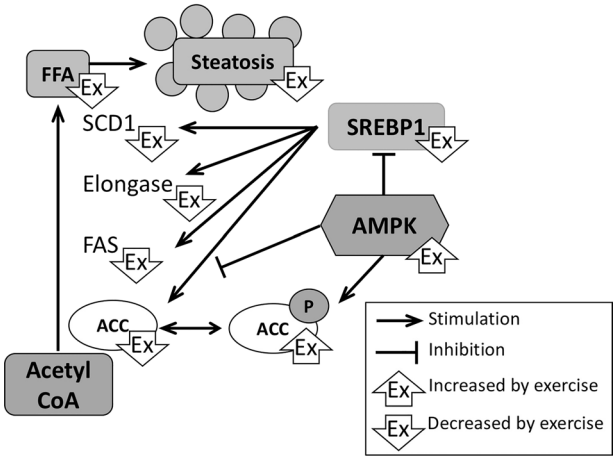

Figure 2. Effects of physical exercise on hepatic fatty acid synthesis. In NASH, the increase in glucose delivery to the liver results in increased FFA synthesis. Exercise reduces the expression of various enzymes that mediate the conversion of acetyl-coA to FFA. An increase in AMPK by exercise stimulates the phosphorylation and therefore inactivation of these enzymes (here depicted for ACC) and of SREBP-1, which is a main transcription factor for expression of ACC, FAS, elongase, and SCD1. ACC, acetyl coenzyme A carboxylase; AMPK; FAS, fatty acid synthase; FFA; SCD1, stearoyl coenzyme A desaturase 1; SREBP-1. P denotes phosphorylation.

of ACC, resulting in its inactivity[76,77]. Epigenetic mechanisms (e.g., reduction of DNA hypermethylation) have been proposed to be responsible for the beneficial effect of physical exercise on the metabolic pathways including de novo lipogenesis[78].

EFFECTS ON FATTY ACID OXIDATION AND MITOCHONDRIA

The liver can neutralize metabolically and immunologically active FFA in three major pathways: esterification of FFA into triglycerides and sequestration into lipid droplets (steatosis), excretion in very-low-density lipoprotein, and fatty acid oxidation in hepatocyte mitochondria (β-oxidation). β-Oxidation was found to be increased in human NASH, as measured by fasting serum β-hydroxybuturate levels[79]. However, structural defects to liver mitochondria, such as the loss of cristae, were observed simultaneously[79], indicating that a compensatory increase in β-oxidation may lead to mitochondrial damage and dysfunction in the long term. Indeed, in other studies, a positive correlation between NASH severity and reduced liver mitochondrial performance was observed[80,81]. Haus et al. measured fatty acid oxidation in PBMCs before and after a 7-day aerobic exercise course in 17 NAFLD patients. The participants were found to have a significant increase in fatty acid oxidation, as measured with indirect calorimetry[50].

In rodent studies, it was confirmed that running improves liver mitochondrial function and increased palmitate oxidation in freshly excised livers, concomitantly with an increase in hepatic carnitine palmitoyl-CoA transferase 1 (CPT-1)[76,77,82,83], an enzyme necessary for transport of FA from the cytosol across the mitochondrial membrane (Fig. 3). Other investigators confirmed these findings and demonstrated that exercise increased CPT-2, acyl-coenzyme A dehydrogenase (ACD), and trifunctional enzyme, which are rate-limiting enzymes in fatty acid oxidation in the liver[64]. In mice, Gonçalves et al. demonstrated with electron microscopy that exercise caused an improvement in abnormal liver mitochondria[84]. Exercise studies in rodents have also shown an improvement in mitochondrial respiration[84] and an increase in cytochrome C[76], indicating that, in addition to β-oxidation, downstream oxidative phosphorylation is enhanced by aerobic exercise. Peroxisome proliferator-activated receptor-α (PPARα) regulates the expression of enzymes responsible for mitochondrial fatty acid oxidation and thereby stimulates β-oxidation in the liver (Fig. 3). Several studies have shown that exercise increases PPARα[73,77], which may indicate that exercise, in part, acts along a mechanism similar to the action of thiazolidinediones[9].

However, when β-oxidation becomes overwhelmed by the abundance of FFA and mitochondria sustain damage, reactive oxygen species (ROS) are formed and create double bonds in polyunsaturated FA (lipid peroxidation), resulting in toxic metabolites capable of causing further mitochondrial damage (Fig. 3). In the pathology of human NASH, multiple markers of this oxidative stress

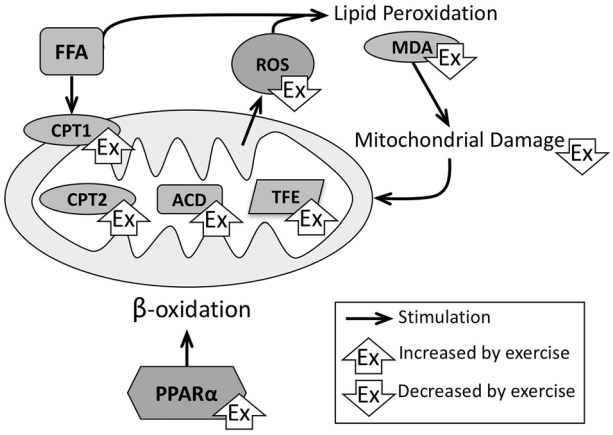

Figure 3. Effects of NASH and exercise on mitochondrial function. When β-oxidation fails to appropriately neutralize the access in FFA, ROS formation leads to lipid peroxidation products, which in return cause more mitochondrial damage. Physical exercise stimulates PPARα, which has beneficial effects on multiple aspects of β-oxidation and therefore improves mitochondrial quality and function. ACD, acyl coenzyme A dehydrogenase; CPT1/2, carnitine palmitoyl coenzyme A transferase 1/2; FFA; MDA, malondialdehyde; PPARα; TFE, trifunctional enzyme.

have been shown to be elevated[85–89]. Multiple clinical trials have shown that exercise reduces ROS formation by various methodologies. The studies by Oh et al. revealed that 12-week exercise programs significantly reduced serum levels of the thiobarbituric acid-reactive substances that reflect the levels of ROS production and lipid peroxidation[60,90,91]. This was corroborated by a trial that established 7 days of aerobic exercise was sufficient to significantly reduce the ROS levels in PBMCs during an oral glucose challenge in 17 NASH patients, measured using chemiluminescence[50]. In rats, Hu et al. measured improved serum levels of the lipid peroxidation marker malondialdehyde (MDA) after 8 weeks of treadmill exercise. With mass spectrometry, they found carbonylation (indicative of oxidative damage) to eight mitochondrial proteins, which was improved by exercise[92].

THE ROLE OF AMPK IN EXERCISE-MEDIATED IMPROVEMENT OF LIVER LIPID METABOLISM

When ATP consumption is increased during physical exercise, the formation of ADP and AMP is sensed by AMP-activated protein kinase (AMPK). AMPK shifts liver lipid metabolism away from FFA synthesis through phosphorylation (and thereby suppression) of ACC and FAS, and of SREBP-1 to reduce expression of these lipogenic enzymes (Fig. 2). In addition, AMPK increases fatty acid oxidation through activation of CPT-1[93]. Several basic scientific studies have documented that exercise activates AMPK-regulated pathways of lipid metabolism in the liver. Treadmill exercise resulted in increased levels of phosphorylated AMPK and ACC in livers of mice[77,94,95]. Moon et al. showed that this effect can be mediated by macrophage migration inhibitory factor, a cytokine of which metabolic effects are increasingly becoming known[94] (Fig. 1).

EFFECTS ON HEPATOCYTE DAMAGE AND ACTIVATION OF INFLAMMATION

The activation of inflammation and the innate immune system, and the role of macrophages as predominant effector immune cells in NASH are well established[96]. Innate immune activation is thought to be a consequence of hepatocyte damage induced by the above-described toxic consequences of FFA. Indeed, elevations in alanine aminotransferase (ALT) and aspartate aminotransferase (AST) as markers of hepatocyte damage positively correlate with NASH severity[97]. With cellular damage, the release of damage-associated molecular patterns (DAMPs) such as high-mobility group box-1 protein (HMGB1) and mitochondrial DNA can activate pattern recognition receptors on macrophages[98,99], which are a major source of inflammatory cytokines leading to amplification of the inflammation.

In several human trials, various exercise regimens reduced the serum levels of ALT and AST[43,100–107]. In some studies, a direct correlation between reduced ROS and improvement in transaminase levels was found[60]. In a randomized trial comparing aerobic versus resistance training, 12 weeks of aerobic exercise resulted in a greater reduction in ALT and AST than resistance training[100]. In a small study in 15 obese women who failed a lifestyle intervention program, it was found that 24 weeks of a combination of voluntary and electrically stimulated movement of the quadriceps and hamstrings was also able to significantly lower ALT levels[60]. Contrarily, the meta-analysis of randomized trials by Keating et al. was unable to detect an effect of exercise on ALT, possibly because patients in most included trials had baseline ALT levels that already fell within the normal range[21].

In several studies, levels of serum cytokines and inflammatory markers (IL-6, IL-8, TNF-α, ferritin, CRP) were significantly reduced, concurrently with a decrease in liver transaminases[51]. In addition, Oh et al. demonstrated that a high-intensity aerobic exercise program caused a reduction in TLR4, TLR5, CD11b, and CD14 expression on PBMCs, indicating an improvement in innate immune activation. Among the high-intensity aerobic group, they reported an increase in nuclear respiratory factor 2 (nrf2), a transcription factor that inhibits the macrophage inflammatory response[52].

We can infer from these trials that physical exercise, or even electrically stimulated exercise, has a beneficial effect on hepatocellular damage and the consequent inflammatory activation in NAFLD patients.

EFFECTS ON PROGRESSION OF NASH TO FIBROSIS AND HCC

Besides the effects on the development of NASH, some studies have investigated the effects of exercise on downstream outcomes, such as fibrosis and progression to liver cancer. In rats with NASH, a treadmill running regimen reduced markers of fibrosis (collagen 1α1 mRNA, α-smooth muscle actin, and fibrosis scores) through decreased activation of hepatic stellate cells, which activation by lipid peroxidation products is thought to be the cause of fibrosis in the pathobiology of NASH (Fig. 1)[108].

In a mouse model of NASH that progresses to HCC, Piguet et al. demonstrated that exercise-induced AMPK decreased mammalian target of rapamycin (mTOR) signaling, which reduced hepatocyte proliferation and tumor formation (Fig. 1)[95]. Although not in the setting of NASH, mice with diethylnitrosamine-induced primary liver cancer had a reduction in tumor burden when doing voluntary treadmill training, also indicating the beneficial effect of exercise on liver cancer progression[109].

CONCLUSIONS

From the available literature, it is evident that physical exercise has a beneficial effect on NAFLD. Various regimens of aerobic and resistance training have been shown to reduce hepatic fat content through improvements in insulin resistance, liver fatty acid metabolism, liver mitochondrial function, and activation of inflammatory cascades. These data provide justification for the current guidelines that recommend an exercise regimen that fits with the patient's individual abilities and preferences, in order to facilitate long-term compliance with a more active lifestyle[14].

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