The effect of acute and chronic exercise on hepatic lipid composition

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Abbreviations: ^{1}H-MRS, proton magnetic resonance spectroscopy; a-v, arteriovenous; ACC1, acetyl-CoA carboxylase 1; CRF, cardiorespiratory fitness; CVD, cardiovascular disease; DAG, diacylglycerol; DNL, *de novo* lipogenesis; ELOVL, fatty acid elongase; IHL, intra-hepatic lipid; MUFA, monounsaturated fatty acids; NAFLD, non-alcoholic fatty liver disease; PC, phosphatidylcholine; PPARα, peroxisome proliferator-activated receptor alpha; PUFA, polyunsaturated fatty acids; SCD-1, stearoyl-CoA desaturase-1; SFA, saturated fatty acids; SREBP-1c, sterol regulatory element-binding protein-1c; T2DM, type 2 diabetes mellitus; TAG, triacylglycerol

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Abstract

Exercise is recommended for those with, or at risk of non-alcoholic fatty liver disease (NAFLD), owing to beneficial effects on hepatic steatosis and cardiometabolic risk. Whilst exercise training reduces total intra-hepatic lipid in people with NAFLD, accumulating evidence indicates that exercise may also modulate hepatic lipid composition. This metabolic influence is important as the profile of saturated (SFA), monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA) dramatically affect the metabolic consequences of hepatic lipid accumulation; with SFA being especially lipotoxic. Relatedly, obesity and NAFLD are associated with hepatic PUFA depletion and elevated SFA. This review summarises the acute (single bout) and chronic (exercise training) effects of exercise on hepatic lipid composition in rodents (acute studies: *n*=3, chronic studies: *n*=13) and humans (acute studies: *n*=1, chronic studies: *n*=3). An increased proportion of hepatic PUFA after acute and chronic exercise is the most consistent finding of this review. Mechanistically, this may relate to an enhanced uptake of adipose-derived PUFA (reflecting habitual diet), particularly in rodents. A relative decrease in the proportion of hepatic MUFA after chronic exercise is also documented repeatedly, particularly in rodent models with elevated hepatic MUFA. This outcome is related to decreased hepatic stearoyl-CoA desaturase-1 activity in some studies. Findings regarding hepatic SFA are less consistent and limited by the absence of metabolic challenge in rodent models. These findings require confirmation in well-controlled interventions in people with NAFLD. These studies will be facilitated by recently validated magnetic resonance spectroscopy techniques, able to precisely quantify hepatic lipid composition *in vivo*.

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Introduction

The dual health burdens of obesity and type 2 diabetes mellitus (T2DM) have prompted a widespread increase in the prevalence of non-alcoholic fatty liver disease (NAFLD). Globally, it is now estimated that 32% of adults are living with NAFLD[1], with the prevalence being higher in people with coexisting obesity and T2DM[2]. NAFLD is a generic term representing a spectrum of hepatic pathologies, whose primary feature is an excessive accumulation of intra-hepatic lipid (IHL)[3]. In an increasing proportion of individuals, this pathology progresses through association with additional hepatocyte stresses, initiating hepatic inflammation (non-alcoholic steatohepatitis [NASH]) and fibrogenesis[4,5]. Whilst this advanced hepatic pathology is more strongly linked with cardiovascular and liver-related morbidity and mortality[6,7], elevated IHL (simple steatosis) is associated with impaired insulin sensitivity, dysregulated substrate metabolism and heightened cardiovascular disease (CVD) risk[8,9].

Although it is widely appreciated that IHL is associated with metabolic dysfunction, growing evidence suggests that hepatic lipid accumulation as triacylglycerol (TAG) *per se* may not be inherently harmful[10,11]. Indeed, pre-clinical studies demonstrate that the promotion of hepatic TAG synthesis is protective against lipotoxicity and insulin resistance[12,13]. Instead, the accumulation of other lipids, and/or lipid intermediates, are more directly linked to metabolic dysfunction[11,14]. Therefore, the composition of hepatic lipids may be more discriminatory when considering the relationship between IHL and cardiometabolic health. Notably, evidence demonstrates that saturated fatty acids (SFA), and their preferential incorporation into various lipid species, are more deleterious than monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA)[14,15]. In contrast, MUFA are preferentially partitioned into esterification and/or oxidative pathways[16], whilst PUFA, particularly of the *n*-3 series, are preferentially incorporated into structural lipids[17]. This notion has many parallels with skeletal

muscle biology, where it is recognised that insulin resistance is promoted more aggressively by SFA compared with unsaturated fatty acids[18].

The association between NAFLD and a range of chronic conditions (e.g., T2DM, CVD, certain cancers) has focused efforts to identify effective therapeutic strategies. Lifestyle therapies currently assume prominence as no pharmaceuticals are licenced for NAFLD treatment[19,20]. Exercise is one strategy that can positively impact many aspects of NAFLD pathophysiology[21]. This includes improvements to insulin sensitivity (adipose, hepatic, and skeletal muscle)[22–24] and the inhibition of hepatic inflammatory signalling and fibrogenesis[25–27]. Notably, meta-analyses demonstrate that exercise training reduces IHL in people with NAFLD through weight-dependent and weight-independent mechanisms[22]. At present, the impact of exercise on the composition of hepatic lipids has received less attention. Historically, the requirement to perform repeated liver biopsy and histological analyses underpins the lack of evidence in this area (in humans). However, recent developments in proton magnetic resonance spectroscopy (^{1}H-MRS) have facilitated growing interest[28,29]. Specifically, semi-quantitative indices of hepatic lipid saturation, unsaturation, and polyunsaturation can be generated by quantifying multiple proton peaks within the ^{1}H-MRS spectra, each representing different functional groups within lipid fatty acid chains[30,31].

This narrative review summarises rodent and human studies evaluating the impact of exercise on hepatic lipid composition. To provide a complete understanding, this review includes interventions employing both acute (single bout) and chronic (exercise training two or more times per week for at least one week) exercise protocols. The review covers all six major categories of lipids (glycerolipids, fatty acyls, glycerophospholipids, sphingolipids, sterol lipids and prenol lipids). Given stark differences in methodology, rodent and human

intervention studies are presented separately in each section. Across all studies, lipid composition changes are primarily discussed in the context of relative changes to the fatty acid composition of individual lipid species, however, changes in the contribution of different lipid species to the total IHL pool are also discussed to provide wider context. With the intention for stimulating further enquiry, this review concludes by highlighting relevant areas worthy of future investigation.

The acute effect of exercise on total hepatic lipid content

Under normal circumstances (excluding starvation), ATP regeneration during acute exercise is fuelled by a mixture of carbohydrates and lipids, with the relative contribution determined by factors including exercise intensity, exercise duration, individual training status and dietary intake (recent and habitual)[32]. The relative contribution of lipids to exercise metabolism is highest when the intensity is low-to-moderate (<65% maximum aerobic capacity), the duration is prolonged, individuals are regularly active/trained, and exercise is undertaken when fasted. In these circumstances, the demand for lipids is met predominantly via the oxidation of circulating non-esterified fatty acids (NEFA; derived from adipose tissue TAG lipolysis), and the breakdown of intramuscular TAG[33,34]. Prolonged bouts of exercise reduce the TAG content in skeletal muscle of lean and healthy individuals, however, impaired metabolic flexibility in those with obesity and T2DM is associated with an impaired utilisation of intramuscular lipid during exercise[35].

Over several decades, numerous rodent studies have characterised changes in IHL (total liver TAG) after single bouts of exercise, along with mechanistic detail about biochemical activity in related metabolic pathways[36–47]. More recently, data from five human studies have been published[48–52], all of which employed continuous exercise protocols (90 to 120 min) of

moderate-intensity (50 to 65% of maximum oxygen uptake). In direct contrast to the changes observed in skeletal muscle, in both rodents and humans the evidence is consistent in showing that IHL increases in response to acute exercise (relative increase 5 to 42% in humans). This response is transient, with elevated levels of IHL detected shortly after exercise cessation[49,51], and/or in the immediate hours into recovery[48,50,52]. Conversely, elevated levels of IHL are not apparent on the day after exercise[45]. This response is seen in both healthy individuals and in people with excess adiposity and NAFLD[48].

Exercise elicits an increase in circulating NEFA concentrations resulting from the promotion of adipose tissue lipolysis and inhibition of fatty acid re-esterification within adipose tissue[53]. Therefore, an increase in the hepatic uptake of circulating NEFA likely explains the short-term expansion of IHL stores, serving as a buffer to prevent lipotoxicity when muscular oxidative capacity is exceeded. Several studies have shown that inhibiting this exercise-related increase in circulating NEFA, for example via nicotinic acid administration[47] or pre-exercise glucose feeding[48], prevents an increase in IHL. *De novo* synthesis of hepatic fatty acids (termed *de novo* lipogenesis [DNL]) cannot explain the documented increase in IHL in response to exercise[45], as fatty acid oxidation (upregulated by exercise) and fatty acid synthesis pathways are reciprocal. Moreover, hepatic very-low-density lipoprotein production does not appear to change after acute exercise[54].

The acute effect of exercise on hepatic lipid composition

In contrast to total IHL content, fewer studies have investigated the effect of acute exercise on hepatic lipid composition. At present, the evidence base is limited to three rodent studies[38,39,55] and one human study[52]. The protocols employed in these studies are heterogenous and therefore it is difficult to draw any firm conclusions.

Rodent studies

Sen et al[39] were the first to describe the acute effect of exercise on hepatic lipid composition. In this study, male Wistar rats were assigned to one of six groups for an 8-week supplementation period. Three groups received a fish oil supplement whilst the other three groups received a soy oil supplement. For each supplement, two out of three groups also received vitamin E supplements, with one of these groups additionally being exposed to an exhaustive bout of exercise prior to being sacrificed. The authors assessed a selection of unsaturated fatty acids in the total hepatic lipid pool and reported that acute exercise, irrespective of dietary assignment, had minimal effects on hepatic lipid composition. Conversely, it was reported that combined fish oil and vitamin E supplementation alleviated exercise-induced oxidative stress in the liver. Given that a non-supplementation control group was not included in the study, a potential interaction of these supplements with exercise acting to mask potential changes in hepatic lipid composition cannot be discounted.

Two more recent animal studies have detected changes in the hepatic lipidome when measured in the hours after acute exercise. In the first study, Hu et al[38] found that the total hepatic lipid pool was elevated 3 h after an intensive endurance exercise protocol in C57BL/6J mice, which corresponds with the findings seen in humans[48–52]. Moreover, significant increases in seven hepatic TAG molecules were found to underpin the overall increase in hepatic TAG content, specifically those containing unsaturated fatty acids rather than SFA. Significant reductions in three phosphatidylcholine (PC) molecules were detected, however, these were not abundant in the PC pool and most PC molecules were unaltered by exercise. These data may indicate that acute exercise does not alter structural lipids within the liver but can alter the composition of lipids used in energy metabolism.

In another study utilising C57BL/6J mice, Henderson et al[55] undertook further lipidomic analyses of samples previously used for assessment of total IHL content[45]. In this study, the total (absolute) IHL pool was elevated 3 h after exercise (continuous and interval exercise) but not when measured 24 h after exercise, which mirrors the observations seen in humans. Conversely, exercise did not affect the total content of other major lipid classes (diacylglycerol [DAG], cholesterol ester, cardiolipin, lysophosphatidylcholine, PC, phosphatidylethanolamine, phosphatidylserine, sphingomyelin). Interestingly, it was also shown that the fatty acid profile of TAG (relative abundance of SFA, MUFA and PUFA) remained unchanged after exercise, whereas the fatty acid profile within DAG changed significantly. Specifically, the relative abundance of SFA was reduced after high-intensity interval exercise whilst the relative abundance of total PUFA and *n*-6 PUFA were increased; and these changes remained evident on day two. It must be noted that these later responses were modest in magnitude, and the authors highlight that the increased enrichment of DAG with PUFA may reflect the exercise-induced release of dietary fatty acids stored in adipose tissue, given the high amounts of *n*-6 in the habitual diet of mice. In support of this notion, mechanistic data from human hepato-splanchnic arteriovenous (a-v) difference experiments show a positive a-v difference for MUFA and PUFA after acute exercise, potentially indicating an augmented release from adipose tissue TAG and uptake by the liver[56]. Conversely, the a-v difference for SFA were either unchanged (<C18) or negative (≥C18).

Preclinical rodent models enable researchers to examine lipidomic responses to exercise in tightly controlled laboratory experiments and provide detailed mechanistic insight. However, caution must be taken when translating the findings of these models, particularly when extrapolating data from small, homogenous rodent populations to the wider human population who exhibit greater heterogeneity in terms of genetics, lifestyle behaviours and health status.

Whilst similarities exist between species in factors such as simple enzyme kinetics and hormonal regulation[57], other important physiological and metabolic differences between rodents and humans must be considered. Specifically, at rest, hepatic gene expression profiles across NAFLD stages differ between species[58] and the fatty acid composition of tissue and plasma membranes are reported to contain lower SFA and higher PUFA in rodents compared to humans[59], likely reflecting differences in habitual diet composition[60]. Moreover, resting metabolic rate, heart rate and oxygen consumption are considerably greater in rodents compared to humans, leading to higher energetic cost[61] and differences in hepatic substrate usage[60] at a specific exercise intensity. Interestingly, acute exhaustive exercise matched for both intensity and duration leads to similar blood biochemical responses in rodents and humans[61]. Therefore, similar comparative studies are warranted for hepatic lipid parameters and the translation of findings between species must be done with caution.

Human studies

To date, only Johnson et al[52] have investigated the effect of acute exercise on hepatic lipid composition in humans. They sought to determine whether IHL was depleted by single bouts of exercise and whether recent dietary intake (high-fat diets) mediated effects. In this trial, six healthy, endurance trained cyclists completed two conditions in a cross-over design. In one condition, participants consumed a ‘standard’ mixed diet (50% carbohydrate, 35% fat, 15% protein) for three days before exercise procedures, whereas in the other condition an iso-energetic, high-fat diet (~85% fat, ~15% protein, negligible carbohydrate) was consumed. On the next day, 90 min of moderate-intensity cycling (65% of peak oxygen uptake) was undertaken in the fasted state. ^{1}H-MRS (1. 5 T) was used to measure total IHL (percentage of the methylene resonance to water) and an index of lipid saturation (fraction of allylic functional groups to the sum of allylic, methylene and methyl functional groups) before exercise and then

30 min and 4.5 h after-exercise. The authors found that short-term dietary manipulation did not impact hepatic lipid composition, possibly because IHL composition is thought to be influenced by adipose tissue lipid composition, which requires a longer intervention to provoke change. Additionally, although the absolute amount of IHL increased after exercise, the saturation of IHL was unchanged. These data suggest that in humans, processes impacting the creation and/or desaturation of SFA in the liver may not be influenced by single bouts of exercise.

It must be noted, however, that total IHL content in the aforementioned study was very low (0.3%) in the endurance-trained cyclists[52], and it has previously been shown that successful quantification of hepatic lipid composition using ^{1}H-MRS is poor at IHL percentages <6.7%[62]. Therefore, further acute exercise studies are required in human populations with elevated hepatic steatosis where accurate quantification via ^{1}H-MRS is more successful. Whilst responses of total IHL to acute exercise are similar in individuals with overweight or NAFLD[48], differences in composition changes between trained versus sedentary populations are currently unknown. The exercise-induced increase in IHL is suggested to primarily derive from the increased mobilisation of circulating NEFA[48], which in turn, reflects the fatty acid composition of adipose tissue (via enhanced lipolysis)[35]. Given that visceral adiposity is associated with lower fatty acid unsaturation[63], it could be postulated that the composition of the increased IHL with acute exercise may differ in individuals with visceral obesity. Additional studies are required to confirm this hypothesis.

The effect of exercise training on total hepatic lipid content

In recent years, multiple systematic reviews and meta-analyses have been conducted to summarise the effects of exercise training on total IHL content in humans[22,64–66]. The literature included in these studies span a wide range of training durations (7 days to 6 months), frequencies (2 to 7 times per week), intensities (45 to 85% of maximum heart rate) and modalities (aerobic, high-intensity interval training [HIIT], resistance, combined)[22,64–66]. Collectively, these studies show that exercise training produces a 10 to 40% relative reduction in total IHL content, independent of significant reductions in body mass. Nevertheless, in line with current clinical practice guidelines for lifestyle modifications[67], greater reductions are observed when greater weight loss is achieved such that each 1% relative reduction in body mass is associated with approximately 1% absolute reduction in IHL[22]. These beneficial effects are seen in both individuals with and without NAFLD[68] and can be maintained in the long-term (i.e. 12 months) when exercise participation is continued[69]. Regarding different exercise modalities, resistance exercise produces similar reductions to aerobic exercise through alternate and complementary mechanisms[69]; whilst HIIT is equally as effective in reducing total IHL when compared to moderate-intensity continuous training[70,71].

Given that exercise training reduces IHL in the absence of weight loss, it is thought that exercise may act through other systemic and hepatic mechanisms in addition to inducing negative energy balance[72]. One key mechanism may be through the ability of exercise to enhance insulin sensitivity in multiple tissues[22,23]. In adipose tissue, this manifests as improved insulin-mediated suppression of lipolysis thereby reducing the supply of circulating NEFA to the liver[23]. Furthermore, greater glucose uptake with enhanced skeletal muscle insulin sensitivity is associated with lower circulating glucose and insulin concentrations, both of which are direct activators of hepatic DNL[21]. In support, daily exercise in rodents with NAFLD downregulates key genes and enzymes involved in the hepatic DNL pathway[72]. A greater

skeletal muscle uptake of circulating NEFA and TAG with exercise training may also act to divert lipids away from the liver[21]. In addition to reducing hepatic lipid supply, exercise training may also enhance hepatic lipid disposal by increasing fatty acid oxidation[21]. Indeed, in the same rodent study by Rector et al[72] in which hepatic DNL was suppressed, daily exercise also upregulated markers of oxidative phosphorylation and mitochondrial biogenesis.

The effect of exercise training on hepatic lipid composition

Rodent studies

The effect of exercise training on hepatic lipid composition has been examined in several rodent experiments. Although some themes are apparent, it is challenging to aggregate these data given the heterogeneity of methods employed. This includes variation in the animal models/species, experimental diets, exercise protocols and reporting of hepatic lipid composition (absolute concentration versus relative [%] contribution). Moreover, some studies have reported lipid composition data in whole liver tissue[73–77] whereas other studies have extracted certain lipid classes[78–85]; or characterised lipid composition in isolated mitochondria[83–85]. The range of fatty acids analysed also varies between studies. To help summarise these data, the following section is sub-divided into studies reporting the effect of exercise training on lipid composition in whole liver tissue, individual lipid classes/species and mitochondria. Across all studies, we have focused most directly on the individual effect of exercise interventions, rather than accompanying dietary interventions.

Lipid composition in whole liver

We identified five studies comparing the proportion of SFA, MUFA and PUFA in whole liver tissue (i.e. lipid species not discriminated) in rodents who had undergone exercise training

versus their untrained counterparts[73–77]. Table 1 outlines the key details and findings of these experiments. Generally, across these studies, exercise interventions were 4-10 weeks in duration and comprised of treadmill running protocols which provided a progressive overload stimulus. Rodents consumed standard diets in three studies[73,76,77], whereas a corn-starch diet was consumed in two others (as the control diet)[74,75]. Findings from these studies were mixed, however, an elevated percentage of hepatic PUFA was identified in four out of five studies[73,75–77]. Among these, Wirth et al[73] were the first to document an increased relative abundance of hepatic PUFA in trained animals. Concomitantly, a reduction in the relative abundance of hepatic MUFA, and a lower percentage of fatty acids with shorter chain lengths were found. Therefore, the authors speculated that exercise training may have enhanced fatty acid elongation and reduced mono-desaturation (i.e., the desaturation of SFA to MUFA). Interestingly, in the same experiment, pair-feeding (dietary energy restriction to match the energy deficit created by exercise) produced opposing effects, with an increased relative abundance of hepatic MUFA identified alongside an increase in the relative amounts of fatty acids with shorter chain lengths. Although a mechanistic explanation for these findings was not sought, these data imply that energy restriction may increase mono-desaturation of SFA. Overall, an important finding highlighted by this study is that exercise training may have a direct effect on hepatic lipid composition independent from the associated energy deficit.

Research consensus suggests that obesity and related metabolic diseases are associated with an increased percentage of hepatic SFA and MUFA, at the expense of PUFA[31]. However, recent evidence indicates that exercise training may attenuate this response. Specifically, when comparing lean Zucker rats and Zucker rats with obesity, Martínez et al[76] documented an increase in relative hepatic MUFA composition and a reduction in hepatic PUFA, in the animals with obesity, compared to lean controls. This occurred concomitantly with an increase

in indices of stearoyl-CoA desaturase-1 (SCD-1) activity (responsible for the conversion of SFA to MUFA) and reduced fatty acid elongase (ELOVL) 5 activity (responsible for fatty acid elongation to long-chain PUFA). These changes may have contributed to the altered phenotype; and serve to protect the liver from an accumulation of toxic SFA. Importantly, when comparing trained versus untrained rodents with obesity, the relative percentages of hepatic PUFA and SFA (C18:0) were higher whilst MUFA were lower. Again, these differences matched altered indices of SCD-1 and ELOVL5, which in the rodents with obesity were reduced and increased, respectively (versus lean counterparts). These findings may indicate that when challenged metabolically (specifically via obesity), exercise training alters hepatic lipid composition by influencing enzymes involved in fatty acid metabolism. These mechanisms are yet to be investigated in humans.

The findings of a more recent study highlight the importance of diet composition when investigating the impact of interventions, such as exercise training, on hepatic lipid composition[77]. In this study, a specific increase in the relative percentage of hepatic *n*-6 PUFA was identified in trained BALB/cAnHsd mice, compared to their untrained counterparts. This difference was primarily related to an increase of linoleic acid (C18:2 [*n*-6]), an essential fatty acid not synthesised endogenously. Notably, although background diet was not reported, both groups of animals (in the placebo group) received a sunflower oil supplement, containing a high proportion of linoleic acid (~60%). It is therefore possible that frequent exercise facilitates the uptake of dietary lipid, particularly fatty acids released from adipose tissue, in response to each bout of exercise. In support, an aforementioned study[73] found positive associations between lipid composition in rodent liver and adipose tissue, but not between the liver and serum, skeletal or cardiac muscle.

Insert Table 1 here

Findings were more variable regarding the proportion of SFA and MUFA in whole liver tissues (without lipid species discrimination) with studies reporting that the relative abundance of hepatic SFA is higher[76], lower[73,77] or not different[74,75] between trained and untrained rodents. Similarly, the relative abundance of hepatic MUFA was lower in some studies[73,76] yet no different in others[74,75]. The findings from three studies provide some evidence that exercise training may prompt a reduction in fatty acid mono-desaturation and an increase in chain elongation[73,76,77]. Specifically, indices of mono-desaturation (e.g., C16:1/16:0 or C18:1/C18:0) were reduced in two studies[73,76], whilst the gene expression of hepatic SCD-1 was reduced in the other[77]. This occurred concomitantly with a reduced relative percentage of hepatic MUFA in two studies[73,76]. It cannot be discounted, however, that an increase in poly-desaturation explains these findings[73]. With regards to SFA, the mixed findings may be related to enhanced chain elongation promoted by exercise in certain contexts. Specifically, enhanced indices of chain elongation were reported in one study[76], whilst an upregulated gene expression of elongase enzymes was apparent in another[77]. This may explain why the proportion of the most abundant SFA, palmitate (C16:0), is reduced after exercise in some studies[73], whereas the proportion of longer even-chain fatty acids (≥C18) is increased[73,76].

Lipid composition in specific lipid classes

Five studies were identified which characterised the effect of exercise training on hepatic fat composition within certain lipid classes[78–82]. These classes included TAG[78,80–82], DAG[78,79,81], ceramide[79,81], phospholipids[82] and cholesterol esters[80]. For context, it should be noted that TAG represents the major lipid class within the liver, followed by phospholipids, and much smaller proportions of other lipid classes[86,87]. Overall, study interventions were 4-17 weeks in duration

and involved a mix of exercise modalities (treadmill running, swimming, voluntary wheel running). In four studies, rodents were fed a standard chow diet[78–80,82]. In the other study, a high-fat, high-sucrose diet was fed[81]. Table 2 describes the key features and findings of studies included in this section.

Insert Table 2 here

Findings regarding the effect of exercise training on the lipid composition within hepatic TAG are mixed and generally limited by the use of healthy rodent models (absence of metabolic challenge). Regarding hepatic PUFA in TAG, two studies report higher proportions in trained versus untrained rodents[80,81], whilst one study reports no difference[82]. In the latter study[82], a comparatively less potent training stimulus (voluntary wheel running for eight weeks) was administered in already metabolically healthy rodents. Interestingly, the increase observed in the former two studies[80,81] was specifically related to linoleic acid (C18:2 [*n*-6]). Given that this is an essential fatty acid which cannot be endogenously synthesised in mammals, these data support the aforementioned notion that repeated exercise may have enhanced the uptake of adipose tissue-derived PUFA from the circulation, which is reflective of habitual dietary intake. This cannot be confirmed, however, as the contribution of fatty acid sources to the hepatic TAG pool was not investigated in these studies.

Out of four studies reporting hepatic MUFA within TAG[78,80–82], two studies observed decreased proportions relative to total TAG in trained rodents[78,81], whilst one documented an increase[80] and the other reported no change[82]. Interestingly, in the studies which identified decreased MUFA, both documented reduced proportions of palmitoleic acid (C16:1) and oleic acid (C18:1)[78,81]. Furthermore, trained rodents exhibited reduced SCD-1 activity in both studies,

suggesting that training directly inhibited mono-desaturation. In the one study reporting an increased proportion of hepatic MUFA, it was suggested that an enhanced uptake of unsaturated fatty acids from adipose tissue may be explanative[80]. Three studies reported findings regarding SFA, with one documenting reduced percentages of SFA in trained rodents[80] and the others reporting no difference[81,82]. The mechanisms responsible for the aforementioned reduction in SFA percentage were not investigated. However, the study by Townsend et al[81] observed reductions in markers of DNL (acetyl CoA-carboxylase 1 [ACC1] and sterol regulatory element-binding protein-1c [SREBP-1c]) and increases in markers of fatty acid oxidation (which can inhibit DNL) with exercise training; therefore, direct modulation of SFA is theoretically possible.

Two studies have reported that the fatty acid composition in hepatic DAG was no different in trained versus untrained rodents[78,81]. Conversely, in one study, both a high-sucrose diet and exercise training were linked with altered hepatic DAG composition in a non-obese animal model with metabolic dysfunction[79]. Specifically, rodents fed a high-sucrose diet had increased proportions of MUFA, and decreased PUFA, within their hepatic DAG. Conversely, trained rodents had lower proportions of SFA and MUFA; and higher PUFA. These data may indicate that exercise training modulates hepatic DAG composition in metabolically-compromised animals. In support, whilst no statistical differences were reported in the study by Jackson et al [78], the percentage of MUFA (C16:1 and C18:1) in DAG was visually lower with exercise training in the ovariectomized rodents only, which exhibit higher visceral fat and impaired glycaemic control compared to their wild-type counterparts.

Besides glycerolipids, less attention has been given to the interaction between exercise training and hepatic lipid composition in particular lipid species. Whilst ceramide may be an important

regulator of hepatic insulin sensitivity[88], only two studies have examined the impact of exercise training on hepatic lipid composition within this species[79,81]. Both studies reported no influence of dietary interventions (high-sucrose and skimmed milk powder) or exercise training on either total hepatic ceramide content or fatty acid composition. Regarding hepatic phospholipids and cholesterol esters, only single studies have reported on each. In trained versus untrained rodents, a reduction in the proportion of MUFA, and an increase in the proportion of PUFA was found in both phospholipids[82] and cholesterol esters[80]. Notably, elevated PUFA in each of these studies was due to essential fatty acids, indicating that enhanced dietary uptake of these lipid species occurs in response to repeated bouts of exercise.

Lipid composition in mitochondrial membranes

Mitochondria are vital organelles in cellular metabolism and mitochondrial alterations are suggested to contribute to the development and progression of NAFLD/NASH[89]. One factor is the lipid composition of mitochondrial membranes which is thought to influence membrane structure, integrity, and function. We identified three studies which investigated whether diet composition and exercise training influenced the lipid composition specifically in mitochondrial membranes[83–85]. The earliest two reports were conducted by the same research group and utilised similar protocols[83,84]. Herein, Wistar rats were divided into groups which were supplemented with either sunflower oil (high in PUFA) or olive oil (high in MUFA), before being further sub-divided into sedentary and exercise training groups. Interventions lasted eight weeks, with animals running on treadmills for five days per week (~65 to 70% of aerobic capacity). Findings were consistent in showing that dietary fatty acid composition directly modulated the lipid composition of mitochondrial membranes; with higher proportions of MUFA in the olive oil-supplemented groups, and higher proportions of PUFA in the sunflower oil-supplemented groups. In both studies, the proportion of MUFA was lower in

485 trained versus untrained rodents, whilst the proportion of PUFA was higher in trained animals
486 irrespective of diet[83,84], including total *n*-3 and *n*-6 PUFA[84]. These studies did not investigate
487 mechanisms of action, however, the authors speculated that lower MUFA with exercise
488 training may be related to preferential oxidation. Findings were less consistent regarding SFA
489 as the relative percentage was higher in trained animals irrespective of diet in one study[84], and
490 not statistically different in the other[83]. In a third study, Gonçalves et al[85] reported a reduced
491 proportion of SFA with eight weeks of exercise training alongside an elevated proportion of
492 PUFA (C18:2 [*n*-6]). Notably, these alterations were more prominent in the group with dietary-
493 induced NASH and were accompanied by preserved mitochondrial membrane integrity and
494 fluidity.
495
496 As noted previously, despite some general trends emerging across both the whole liver and
497 specific hepatic lipids, some inconsistencies are evident, particularly within the SFA fraction
498 of the different hepatic lipids. These discrepancies may be related to key methodological
499 differences in factors such as exercise modality, dietary intake, metabolic status and additional
500 supplementation[60]. For example, the voluntary wheel running provides rodents with a more
501 modest exercise stimulus with less experimental control over exercise intensity and duration,
502 whilst forced treadmill running offers greater experimental control but introduces additional
503 stresses which may impact metabolic responses[60]. Furthermore, standard chow diets were fed
504 in some studies which typically comprise of low amounts of fat (<10%) and high PUFA[90],
505 whereas more extreme high-fat and/or high-sucrose diets were fed in others. The latter
506 obesogenic diets are used to induce the steatotic and metabolic features of NAFLD[90] and
507 responses may therefore differ between metabolically healthy versus metabolically unhealthy
508 rodents.
509

Human studies

Evidence from human studies regarding the interaction between exercise training and hepatic lipid composition is limited. Although cross-sectional studies are not a primary focus of this review, it is worthwhile noting that relationships between physical activity, cardiorespiratory fitness (CRF) and hepatic lipid composition have been identified in two recent reports, each using ^{1}H-MRS to assess indices of hepatic lipid composition[91,92]. Specifically, in adults with suspected NAFLD, Erickson et al[91] identified a moderate-to-large (r = 0.49) association between objectively-measured CRF and an index of hepatic lipid polyunsaturation. These findings are supported by our own preliminary data in a sample of men with NAFLD, with and without impaired glucose regulation[92]. Herein, CRF was positively associated with the hepatic unsaturation (r = 0.44) and polyunsaturation indices (r = 0.47), whilst an inverse relationship was apparent for the saturation index (r = -0.44). Similar associations, in terms of direction and magnitude, were apparent between these lipid composition indices and device-measured physical activity (moderate- and moderate-to-vigorous-intensity). One important consideration when interpreting these cross-sectional relationships is that healthy lifestyle behaviours typically co-exist, therefore, the confounding influence of healthy dietary habits cannot be separated from the influence of physical activity itself.

Johnson et al[93] were the first to examine the impact of exercise training on hepatic lipid composition in humans using ^{1}H-MRS. Nineteen sedentary adults with obesity completed four weeks of aerobic exercise training (cycling) or stretching (control). Exercise training sessions were fully supervised, occurred three times per week, and were progressive in intensity (50 to 70% of aerobic capacity). Despite a 21% (relative) reduction in total IHL, the intervention did not affect the hepatic saturation index, whilst indices of lipid unsaturation were not reported in this study.

The effect of seven consecutive days of aerobic exercise on hepatic lipid composition was examined by Haus et al[94]. In this study, 17 men and women with obesity and NAFLD performed 50-60 min of supervised moderate-intensity (80 to 85% of maximum heart rate) exercise (walking/jogging) each day. The intervention did not impact body weight or total IHL, however, the polyunsaturated lipid index was 28% higher after the exercise intervention. These data raise the possibility that short-term exercise training may favourably alter the hepatic lipid profile in humans.

A more recent study examined the impact of a combined diet and physical activity intervention on hepatic lipid composition in people with obesity and NASH[95]. Participants were randomised to a meal replacement intervention, or a lifestyle change intervention, each lasting 24 weeks. In the meal replacement arm, participants substituted two daily meals with meal replacement shakes (1000-1200 kcal $\times$ d^{-1}) for the first six weeks and one daily meal (1500-1700 kcal $\times$ d^{-1}) for the remaining 18 weeks. In the lifestyle change arm, participants consumed a balanced hypo-energetic diet and undertook supervised moderate-intensity (60 to 75% of aerobic capacity) exercise training, initially once per week and progressing to twice per week after six weeks. Both the lifestyle change and meal replacement interventions resulted in reductions in body weight (-9.1 kg versus -6.4 kg) and total IHL (-77% versus -56%). Moreover, the saturated hepatic lipid component was significantly reduced after the lifestyle change intervention only, whilst the fraction of unsaturated lipids was unchanged after both interventions.

The heterogenous nature of the study designs utilised by the three available human studies limits the ability to compare findings between studies. This includes the wide range of

intervention durations (seven days to 24 weeks) and the differing degrees of NAFLD severity between participants. Specifically, the cohort recruited by Deibert et al.[95] possessed a more severe liver pathology i.e. NASH, whilst IHL content was two-fold lower in the study by Johnson et al.[93], potentially contributing to the lack of observed response. Additionally, the inclusion of a hypo-energetic diet as part of the lifestyle intervention by Deibert et al.[95] and the substantial accompanying weight loss confound the ability to isolate the independent effects of chronic exercise/physical activity. Consequently, more exercise training studies in populations with NAFLD and with longer intervention periods (>4 weeks) are essential for a consensus to be reached.

Differences in the assessment method of hepatic lipid composition also limit comparisons to the rodent literature and are worthy of consideration. Specifically, the semi-quantitative nature of the ^{1}H-MRS technique describes the ratio of hepatic lipid saturation/unsaturation, whilst the biochemical analysis of rodent liver tissue quantifies the relative abundance of different fatty acids[96]. Recent efforts have therefore been made to develop equations to enable the expression of lipid composition as percentages of SFA, MUFA and PUFA[96] which future ^{1}H-MRS studies may seek to employ. Nevertheless, the technique correlates closely with gold-standard measurements in human adipose tissue samples[96] and circumvents the need for repeat liver biopsies. Alternative non-invasive methods using chemical-shift-encoded magnetic resonance imaging are currently being explored which offers greater spatial coverage and accessibility compared to ^{1}H-MRS[96]. Currently, however, this technique is less established than ^{1}H-MRS owing to limited application to human interventional research and validation against lipidomic analysis[97,98].

Discussion

This review summarises findings regarding the acute and chronic effect of exercise on hepatic lipid composition. Whilst some themes are apparent, findings are heterogenous due to stark methodological differences in the available pre-clinical research and lack of human experimental studies. Nonetheless, in an acute setting, whilst evidence is consistent in showing that total IHL is transiently elevated, rodent studies demonstrate that the proportion of hepatic PUFA may be augmented. An increased proportion of hepatic PUFA is also the most consistent finding in studies investigating the impact of exercise training on hepatic lipid composition. Several studies have also identified that the proportion of hepatic MUFA is reduced in trained versus untrained rodents, potentially related to a reduction in hepatic SCD-1 activity. Data are less consistent regarding the impact of exercise training on hepatic SFA. Figure 1 summarises the findings from this review and highlights possible mechanisms by which exercise may impact hepatic lipid composition. Given that only four acute exercise studies were identified containing heterogenous methods and conflicting findings, the following section primarily discusses key findings and potential mechanisms relating to the exercise training literature.

Insert Figure 1 here

Polyunsaturated fatty acids

The most consistent finding in this review was an increase in the proportion of hepatic PUFA with exercise. This finding was particularly apparent when looking at intervention studies, with most rodent experiments documenting this effect. In one experiment, this effect was apparent in rodents with obesity but not rodents that were lean, suggesting that exercise may combat relative hepatic PUFA depletion commonly seen in obesity[76]. In human studies, an increase in the hepatic PUFA fraction in more active individuals with obesity and/or NAFLD has also been found in cross-sectional and intervention studies[91,92,94]. Interestingly, whilst many studies

identified an increase in the proportion of total hepatic PUFA, some studies were able to provide a more detailed analysis of the hepatic lipidome. A specific increase in PUFA within the *n*-6 family (total *n*-6, C18:2 [*n*-6], C20:4 [*n*-6]) was identified in many of these studies[76,77,81]. Given that these are essential fatty acids, this finding may suggest a potential diet-exercise interaction, whereby repeated bouts of exercise may enhance the uptake of PUFA from dietary sources and/or augment adipose tissue lipolysis. Preferential partitioning of PUFA into oxidation pathways has previously been shown in humans[99–101]. Taken together, chronic adaptations in hepatic fatty acid oxidation may also play a role in the exercise-induced changes in hepatic fatty acid composition. The metabolic relevance of these changes is not clear. Whilst PUFA are generally thought to confer favourable metabolic effects in hepatocytes[102], it is recognised that many of the bioactive metabolites of *n*-6 fatty acids, such as pro-inflammatory eicosanoids, can be metabolically harmful[103]. As the ratio between *n*-6 and *n*-3 PUFA is important for metabolic health[104], further mechanistic work is needed to understand the physiological relevance of the exercise-induced increase in hepatic *n*-6 PUFA and the subsequent implications for metabolic and liver-related health.

Monounsaturated fatty acids

Although based almost entirely on rodent studies, a reduction in the proportion of hepatic MUFA in the trained state was another reoccurring finding in this review. MUFA can be desaturated from SFA and are thought to be preferentially incorporated into TAG for storage in order to protect hepatocytes from SFA-induced lipotoxicity[105,106]. Consequently, an increase in hepatic MUFA is associated with obesity and/or NAFLD[31]. Indeed, in our review, an elevation in hepatic MUFA was seen in rodent models exhibiting obesity[76,78]. Interestingly, in these studies, the proportion of hepatic MUFA was reduced in the trained versus untrained state, only in animals with elevated baseline MUFA. This response was consistent with a reduction

635 in SCD-1 activity, implied from surrogate indices [73,76] and gene expression[78]. This may imply
636 that exercise training reduces hepatic MUFA accumulation by downregulating key pathways
637 of MUFA synthesis; however, it must be noted that not all studies documented a reduction in
638 SCD-1 expression in trained animals[77,81]. Nonetheless, as hepatic SCD-1 is upregulated by
639 energetic excess, particularly SFA and carbohydrate[106]; it is theoretically possible that exercise-
640 related energy expenditure indirectly impairs the activity of SCD-1. In support, exercise-trained
641 rodents exhibited an upregulated expression of peroxisome proliferator-activated receptor
642 alpha (PPARα) and its target genes which are known inhibitors of SCD-1 activity[76,81].
643 Alternatively, an upregulation of elongation activity may be another explanation for the
644 exercise-related reduction in hepatic MUFA and concomitant increase in hepatic PUFA;
645 however, data are scarcer regarding this hypothesis. As such, three studies found that exercise
646 training was associated with either increased longer-chain fatty acids[73], indices of chain
647 elongation[76] or gene expression of elongation enzymes[77], whilst one study reported no
648 differences in indices of chain elongation[82].
649
650 Saturated fatty acids

651 Saturated hepatic lipids, and their associated bioactive lipid intermediates, are thought to be
652 more deleterious owing to their tendency to promote oxidative and endoplasmic reticulum
653 stress, apoptosis, and insulin resistance[31]. The proportion of SFA in the liver are elevated in
654 obesity and/or NAFLD and associated with increased DNL (predominantly producing
655 palmitate)[107]. Given that lipogenic pathways are stimulated by glucose, insulin, and high energy
656 availability, it is possible that exercise may be able to reduce hepatic SFA through a reduction
657 in hepatic DNL. Indeed, exercise training is an effective means of improving glycaemic control
658 in those with dysglycaemia[108]. Moreover, exercise training generally facilitates modest weight
659 loss, which has been shown to reduce DNL in people with obesity and NAFLD[107]. Further

support for this contention comes from rodent studies demonstrating that exercise training combats IHL accumulation whilst concomitantly suppressing key enzymes and transcription factors involved in fatty acid synthesis (ACC1, fatty acid synthase, SREBP-1c)[27,109]. Whilst some studies support a reduction in the proportion of hepatic SFA with exercise training[73,77,79,80], others show no differences[74–78,81,82] or even an increase[73,76,80]. As previously alluded to, exercise training may influence fatty acid mono-desaturation, and an increase in the proportion of hepatic SFA could theoretically result from a blunted desaturation of SFA to MUFA.

Future directions

To date, most evidence relating to exercise and hepatic lipid composition has been derived from rodent studies owing to the challenges associated with quantifying hepatic lipid composition in humans. More clinical studies are therefore needed to determine whether findings translate to humans. Given the invasiveness and risks associated with the liver biopsy technique, further development of non-invasive alternatives, such as the ^{1}H-MRS technique, is essential to advancing current knowledge in the area. Although this technique is semi-quantitative, and only provides indices of hepatic fat composition, it permits the conduct of bespoke, tightly controlled, intervention studies. Whilst obesity and T2DM pharmacotherapies (such as GLP-1 and GIP receptor agonists and SGLT2 inhibitors) have shown promise in NAFLD/NASH treatment[110–112] and are currently undergoing further clinical trials (NCT04166773, NCT05364931, NCT04639414, NCT04822181), lifestyle therapies remain the cornerstone for the management of NAFLD[20]. Therefore, future studies should seek to determine how exercise impacts on hepatic lipid composition in key populations with obesity and related cardiometabolic disease, and how changes in hepatic lipid composition may influence both cardiometabolic and liver-related health. Studies capable of enhancing

685 understanding of the interaction between dietary intake, exercise and energy balance are also
686 necessary to inform future clinical practice guidelines.

Perspective

Guidelines for the management of non-alcoholic fatty liver disease (NAFLD) recommend that exercise is performed on a regular basis. This recommendation is founded on knowledge that exercise training reduces liver fat, the hallmark feature of NAFLD. Besides the absolute amount of liver fat, developing evidence suggests that exercise training may additionally benefit people with NAFLD by altering the composition of liver fat (proportions of saturated, unsaturated, and polyunsaturated fatty acids). This is relevant because obesity and NAFLD are associated with an elevation in the proportion of hepatic saturated and monounsaturated fatty acids; whilst polyunsaturated fatty acids are reduced. This lipid phenotype is linked with a more adverse metabolic health profile. The summarised findings from this review of rodent and human data indicate that exercise training may combat this phenotype by enhancing the proportion of hepatic polyunsaturated fatty acids and reducing monounsaturated fatty acids (the saturated fatty acid response to exercise is situation specific). The implication of these findings is that exercise training may confer an added but currently unrecognised benefit for people living with excessive liver fat.

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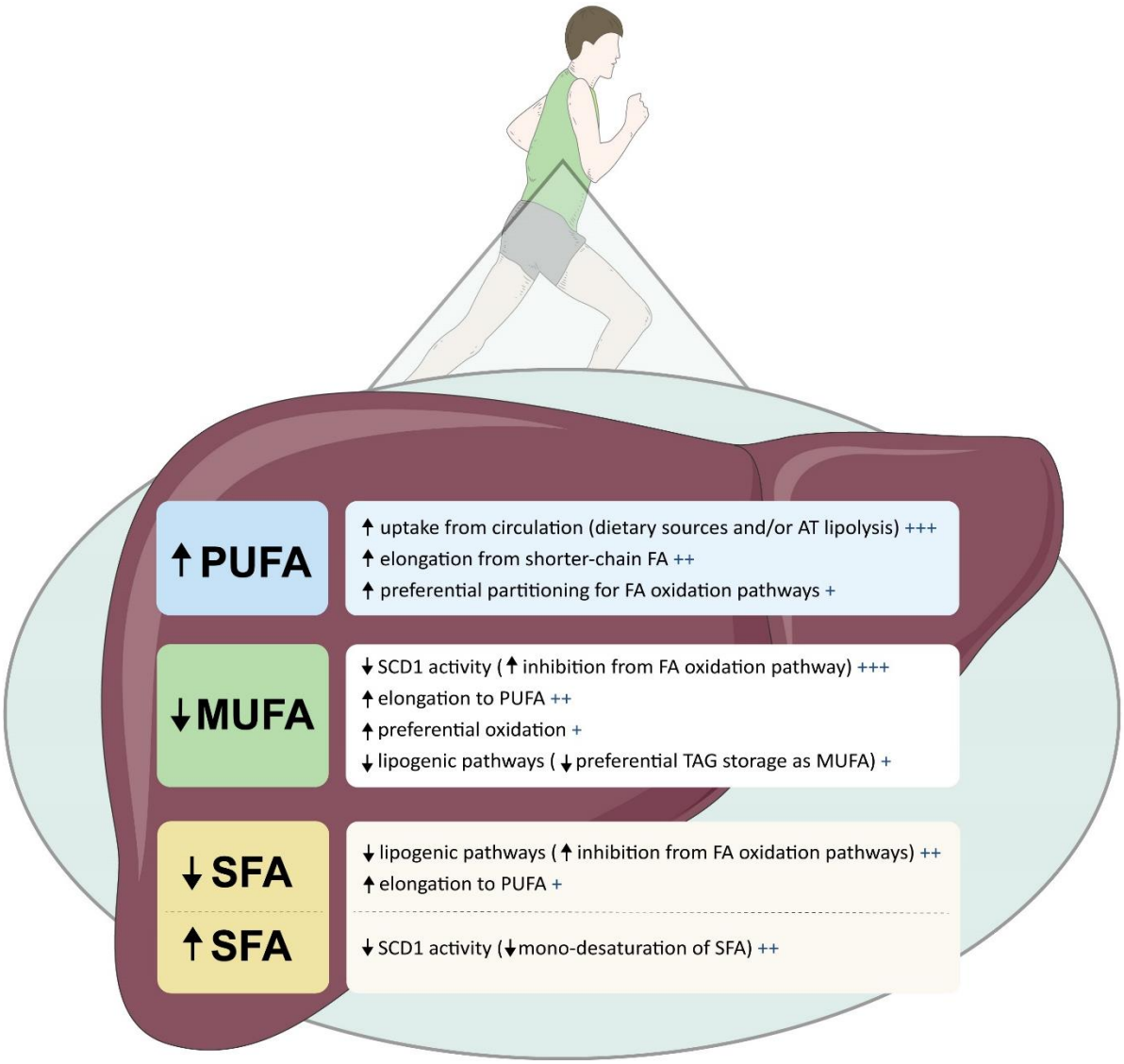

Figure 1. The potential mechanisms by which exercise may influence hepatic lipid composition. The proportion of hepatic PUFA may be increased by an enhanced uptake of circulating PUFA derived from AT lipolysis and/or the habitual diet. Furthermore, increased elongation from shorter-chain fatty acids (as indicated by elevated activity and/or gene expression of ELOVL enzymes) could also explain an increased proportion of hepatic PUFA and decreased proportion of hepatic MUFA and/or SFA. Alternatively, PUFA may be preferentially retained for partitioning into oxidation pathways which are upregulated with exercise. Consequently, the upregulation of transcription factors involved in fatty acid oxidation, such as PPARα and its target genes, exerts an inhibitory influence on hepatic SCD-

1. In turn, this could theoretically reduce the mono-desaturation of SFA to MUFA, potentially leading to a decreased proportion of MUFA and increased proportion of SFA. However, these transcription factors also exert an inhibitory effect on key enzymes and transcription factors involved in the *de novo* lipogenesis of hepatic SFA, such as ACC1, fatty acid synthase and SREBP-1c, which would support a reduced proportion of hepatic SFA. The preferential oxidation of hepatic MUFA may be another mechanism whereby exercise may decrease the proportion of hepatic MUFA.

ACC1, acetyl-CoA carboxylase 1; AT, adipose tissue; ELOVL, fatty acid elongase; FA, fatty acid; MUFA, monounsaturated fatty acids; PPARα, peroxisome proliferator-activated receptor alpha; PUFA, polyunsaturated fatty acids; SCD-1, stearoyl-CoA desaturase; SFA, saturated fatty acids; SREBP-1c, sterol regulatory element-binding protein-1c; TAG, triacylglycerol.

\+ theoretical mechanism

++ mechanism supported by evidence from a limited number of studies

+++ mechanism supported by evidence from multiple studies

1101 **Table 1. Rodent studies characterising the effect of exercise training on hepatic lipid composition in whole liver tissue.**

Ref.	First author (year of publication)	Experimental design	Animal model / diet	Intervention duration	Exercise protocol	Key findings
73	Wirth (1980)	Three groups: 1-Trained 2-Pair-fed (to trained group, -24% food restriction) 3-Freely fed	Sprague Dawley Rats (male) Commercial rat pellets (5% fat, 23% protein, 55% carbohydrate [by weight])	8 weeks	Rodent treadmill 5 days per week 20 m/min for 15 min 2x per day → 32 m/min for 90 min 1 x per day	Compared to pair fed animals, training: - ↓ SFA (C16:0) - ↑ SFA (C18:0) - ↓ MUFA (C16:1, C18:1) - ↑ PUFA (C18:2, C18:3, C20:3, C20:4, C22:4, C22:5) - ↓ shorter-chain FAs (total C14, C16) - ↑ longer-chain FAs (total C18, C20, C22) The same pattern of response was apparent for trained animals compared with freely feeding, however, the size of differences between groups was smaller (the group comparison was not analysed statistically)
74	Fiebig (1998)	Four groups (cornstarch diet was the control diet): 1 - Cornstarch diet-sedentary	Sprague Dawley Rats (female) 2 weeks – all rats consumed a cornstarch diet, after which 50% continued on cornstarch (50% by weight) and the other 50% consumed a high	10 weeks	Rodent treadmill 5 days per week 15.5 m/min (0%) for 10 min per day → 25 m/min (10%) for 2 h per day	Fructose feeding vs cornstarch (control) - ↑ C16:0, C16:1, C18:1 - ↓ C18:0, C18:2, C20:4

		2 - Cornstarch diet-exercise 3 - Fructose diet-sedentary 4 - Fructose diet exercise	fructose diet (50% by weight) (isoenergetic)			Exercise training did not alter the composition of these hepatic fatty acids in either diet group <u>Miscellaneous</u> FAS activity >50% lower in the trained vs untrained cornstarch diet groups
75	Fiebig (2002)	Lean and obese rodents each allocated to two groups: 1 – Exercise 2 – Sedentary	Obese Zucker rats (female) Lean Zucker rats (female) 2 weeks powdered chow followed by 50% cornstarch diet (by weight)	4 weeks	Rodent treadmill 5 days per week 15.5 m/min (0%) for 10 min per day → 18 (obese) / 20 m/min (lean) (0%) for 100 min	<u>Obese vs lean</u> - Obesity ↓ PUFA (C18:2, C20:4), ↑ MUFA (C16:1, C18:1) - SFA (C16:0 ↑, C18:0 ↓) <u>Obese animals – trained had:</u> - C18:3↓ - C20:4↑ No between group differences in C16:0, C16:1, C18:0, C18:1, C18:2 <u>Lean animals – trained had:</u> - C18:3↓ - C20:4↑

						No between group differences in C16:0, C16:1, C18:0, C18:1, C18:2 <u>Miscellaneous</u> Obesity associated with ↑ FAS activity which was attenuated by training
76	Martínez (2018)	Lean and obese rodents each allocated to four groups 1 - Control diet-sedentary 2 - Control diet-exercise 3 - Lentil protein hydrosylate diet-sedentary 4 - Lentil protein hydrosylate diet-exercise	Obese (fa/fa) Zucker rats (male) Lean Zucker rats (male) Standard rodent diet (4% fat, 14% protein). Lentil protein hydrosylate (intervention diet) or water (control diet) administered by gavage	8 weeks	Combined aerobic interval and strength training 5 days per week 60 min of work per session Aerobic intervals (30 min): 4 min moderate and 3 min vigorous intensity repetitions Strength training: 8 x 2 min bouts of incline running (10-20% grade) at a moderate speed with 1 min of rest	Obese vs lean phenotype – obese animals had: - SFA (no different) - MUFA↑ - PUFA ↓ Comparison of trained animals to sedentary animals consuming the control diet only: <u>Obese animals</u> - SFA ↑ - MUFA ↓ - PUFA ↑ <u>Lean animals</u>

						- SFA ↓ - MUFA (no different) - PUFA (no different) <u>Miscellaneous</u> Obese animals – trained displayed ↓ indices of SCD-1 activity and ↑ ELOVL5 activity Lean animals – trained displayed ↓ ELOVL5 activity, ↑ desaturase-elongase activity Training also ↓ expression of lipogenic genes (*Srebf1*, *Fasn*, *G6pd*) and ↑ expression of lipolytic genes (*Ppara, Acox1, Cpt1a*)
77	Mika (2019)	Four groups: 1 - Placebo-sedentary 2 - Conjugated linoleic acid-sedentary 3 - Placebo trained 4 - Conjugated linoleic acid trained	BALB/cAnNHsd mice (male) Main diet not reported Conjugated linoleic acid supplement administered by gavage (35 µg/day). 50:50 ratio of C18:2 c9, t11 and C18:2 t10, c12). Placebo	6 weeks	Rodent treadmill 5 days per week 3.2 m/min for 15 min → 4.8 m/min for 60 min	Comparing placebo trained vs placebo sedentary only: -Total even chain SFA (no different) (C20:0 ↓) -Total odd chain SFA ↓ (C19:0 ↓) -Total PUFA (*n*-6) ↑ -Total PUFA (*n*-3) (no different) (C18:3 ↑) -Total MUFA (no different)

			group given the same amount of sunflower oil			<u>Miscellaneous</u> Training not associated with alterations in indices of lipid desaturation (C18:1/C18:0) or elongation (C18:0/C16:0); however, gene expression was higher in the trained group for SCD-1 and ELOVL6

ELOVL5, fatty acid elongase 5; ELOVL6, fatty acid elongase 6; FA, fatty acid; FAS, fatty acid synthase; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SCD-1, stearoyl Co-A desaturase; SFA, saturated fatty acid

Table 2. Rodent studies characterising the effect of exercise training on hepatic lipid composition in specific lipid classes.

Ref.	First author (year of publication)	Lipid class measured	Experimental design	Animal model / diet	Intervention duration	Exercise protocol	Key findings
80	Simko (1970)	TAG Cholesterol ester	Two groups: 1 - Swimming training 2 - Untrained	Wistar rats (male) 'natural pelleted diet' (9% fat, 50% carbohydrate)	~17 weeks	Swimming 6 days per week 1 h per day	Swim trained rats vs. untrained rats <u>TAG</u> SFA ↓ MUFA ↑ PUFA ↑ <u>Cholesterol ester</u> SFA ↑ MUFA ↓ PUFA ↑
82	Petridou (2005)	TAG Phospholipids	Two groups: 1 - VWR (trained) 2 - No access to running wheel (untrained)	Wistar rats (male) All rodents provided with standard rodent chow (3.5% fat [linoleate 40%, palmitate 28%, oleate 21%])	8 weeks	*Ad libitum* access to running wheel	Free access to running wheel: <u>TAG</u> SFA (no different to untrained) MUFA (no different to untrained) PUFA (no different to untrained)

							<u>Phospholipids</u> SFA (no different to untrained) MUFA ↓ PUFA – no different as a whole but some individual species were ↑ (C18:2 *n*-6, C18:3 *n*-6, C18:3 *n*-3) <u>Miscellaneous</u> Training did not impact enzymatic indices of fatty acid chain elongation or desaturation
78	Jackson (2011)	TAG DAG Measurements limited to: C16:0, C16:1, C18:0, C18:1	Four groups: 1 - Ovariectomised VWR (trained) 2 - Ovariectomised untrained 3 - Sham surgery VWR (trained) 4 - Sham surgery untrained	C57BL/6J mice (female) All rodents provided with standard rodent chow (4.5% fat, 23% protein, 6% fibre)	8 weeks	*Ad libitum* access to running wheel	Impact of ovariectomy: ↑ in C16:1 and C18:1 in ovariectomised rodents vs sham surgery; no impact on C16:0 or C18:0 Ovariectomy ↑ hepatic expression of SCD-1 Impact of exercise training in ovariectomised animals:

							↓ C16:1 and C18:1 but no effect on C16:0 or C18:0 Exercise training ↓ hepatic expression of *scd-1* Comparing sham surgery animals only: <u>TAG</u> SFA (C16:0, C18:0) - no difference between groups MUFA (C16:1, C18:1) - no difference between groups <u>DAG</u> SFA (C16:0, C18:0) - no difference between groups MUFA (C16:1, C18:1) - no difference between groups <u>Miscellaneous</u> VWR ↓ C18:1/C18:0 (desaturase index) but did not alter the C16:1/C16:0 index VWR did not impact hepatic *scd-1* expression

79	Škop (2015)	DAG Ceramide	Four groups: 1 - Standard diet VWR (trained) 2 - Standard diet, no running wheel access 3 – High-sucrose diet VWR (trained) 4 – High-sucrose diet, no running wheel access	HHTg (genetically dyslipidemic) rats (male) Standard diet (7% fat, 23% protein, 5% fibre, 43% starch) High-sucrose diet (20% v/w sucrose solution)	4 weeks	*Ad libitum* access to running wheel	<u>DAG</u> The proportion of hepatic PUFA (C18:2) was ↑ in both dietary groups which performed VWR The proportion of hepatic SFA (C16:0, C18:0) and MUFA (C18:1) were both ↓ in the trained groups irrespective of diet <u>Ceramide</u> Voluntary wheel running had no effect on liver ceramide in either dietary group
81	Townsend (2020)	TAG DAG Ceramide	Four groups: 1 - Casein (control diet) -sedentary 2 - Casein (control diet) - exercise 3 - Skimmed milk powder - sedentary 4 - Skimmed milk powder-exercise *iso-energetic diets	Sprague-Dawley rats (male) High-fat, high-sugar (HF-HS) diet for 8 weeks to induce obesity (41% fat, 10% protein, 49% carbohydrate) At 12 weeks of age, sole protein source changed to either non-fat skimmed milk	6 weeks	Motorised treadmill 5 days per week 10 m/min (0%) for 60 min → 20 m/min (5%) for 60 min	<u>TAG</u> SFA - not affected by exercise or diet MUFA - ↓ by exercise training irrespective or diet PUFA - ↑ by exercise training irrespective of diet. Effect related to C18:2 *n*-6 and not *n*-3 PUFA <u>DAG</u> Neither exercise nor diet influenced the fatty acid composition of DAG

				powder (intervention diet) or casein (control diet)			<u>Ceramide</u> Neither exercise nor diet influenced the fatty acid composition of ceramide <u>Miscellaneous</u> Trained animals had reduced indices of SCD-1 activity (C16:1/16:0, C18:1/C18:0) but this was not matched by hepatic *scd-1* gene expression or protein content Trained animals displayed reduced indices of lipogenesis (C16:0 to C18:2 *n*-6, *Acc1* & *Srebp1c* expression) and enhanced indices of fatty acid oxidation (PPARα, CPT1 and β-HAD expression)

β-HAD, beta-hydroxyacyl-CoA dehydrogenase; CPT1, carnitine palmitoyltransferase 1; DAG, diacylglycerol; HHTg, hereditary hypertriacylglycerolemic; MUFA, monounsaturated fatty acid; PPARα, peroxisome proliferator-activated receptor alpha; PUFA, polyunsaturated fatty acid; SCD-1, stearoyl Co-A desaturase; SFA, saturated fatty acid; TAG, triacylglycerol; VWR, voluntary wheel running

1120