Tuning fatty acid oxidation in skeletal muscle with dietary fat and exercise

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Abstract | Both the consumption of a diet rich in fatty acids and exercise training result in similar adaptations in several skeletal muscle proteins. These adaptations are involved in fatty acid uptake and activation within the myocyte, the mitochondrial import of fatty acids and further metabolism of fatty acids by β-oxidation. Fatty acid availability is repeatedly increased postprandially during the day, particularly during high dietary fat intake and also increases during, and after, aerobic exercise. As such, fatty acids are possible signalling candidates that regulate transcription of target genes encoding proteins involved in muscle lipid metabolism. The mechanism of signalling might be direct or indirect targeting of peroxisome proliferator-activated receptors by fatty acid ligands, by fatty acid-induced NAD^+-stimulated activation of sirtuin 1 and/or fatty acid-mediated activation of AMP-activated protein kinase. Lactate might also have a role in lipid metabolic adaptations. Obesity is characterized by impairments in fatty acid oxidation capacity, and individuals with obesity show some rigidity in increasing fatty acid oxidation in response to high fat intake. However, individuals with obesity retain improvements in fatty acid oxidation capacity in response to exercise training, thereby highlighting exercise training as a potential method to improve lipid metabolic flexibility in obesity.

Skeletal muscle of lean individuals has the ability to shift to increased levels of fatty acid oxidation in response to a high-fat diet (HFD)[1–6]. Likewise, a hallmark of aerobic exercise training is an increased capacity of skeletal muscle to oxidize fatty acids[7,8]. These shifts in substrate utilization towards increased fatty acid oxidation in skeletal muscle in response to high fat intake and exercise training are orchestrated by a plethora of tightly coordinated molecular events, which are reliant on substrate fluxes and adaptations within the lipid metabolic system. The increased availability of fatty acids under the conditions of HFD and/or exercise training might lead to fatty acids acting as a signal, which seemingly induces the expression of proteins in the lipid metabolic pathways, thereby increasing the fatty acid metabolic capacity. Some of the signals and adaptations occurring in response to high fat intake and aerobic exercise training are shared by both stimuli, whereas others are not. Of note, lean individuals (that is, those with BMI 18–25 kg/m^2) can increase fatty acid oxidation when fat availability increases; however, individuals with obesity (that is, those with BMI >30 kg/m^2) have an impaired ability to regulate fatty acid oxidation when the availability of fatty acids fluctuates[9,10]. Thus, in individuals with metabolic disturbances, improvements in the capacity of skeletal muscle to oxidize fatty acids and to match fatty acid oxidation to fluctuations in fatty acid availability are probably important in order to re-establish a healthy metabolism.

This Review discusses how a HFD and aerobic exercise training, via the stimulation of lipid metabolism, regulate common signalling pathways and subsequent adaptations in skeletal muscle. In addition, the impairment in skeletal muscle fatty acid oxidation capacity in obesity will be highlighted as will the effect of exercise training in reversing this dysfunction. The Review focuses on the response to aerobic exercise training at different intensities, as this type of exercise relies on aerobic metabolism that extracts energy in the form of ATP from energy substrates, such as fatty acids. Human studies are emphasized in the present Review.

Lipid metabolic adaptations

Exercise training and high fat intake in humans lead to adaptations in lipid metabolism in skeletal muscle. The following section highlights such adaptations, with evidence from human studies.

Delivery of fatty acids to skeletal muscle. The delivery of circulating fatty acids to skeletal muscle is increased postprandially following the consumption of dietary fat, but also during aerobic exercise and in recovery from exercise. Fatty acids can be supplied from lipolysis of triacylglycerol stored in adipose tissue and from muscle

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Key points

- Both high fat intake and aerobic exercise training increase the abundance and activity of several lipid metabolic proteins in skeletal muscle related to fatty acid uptake, handling and mitochondrial import.
- Mitochondrial biogenesis is induced primarily by aerobic exercise training and not by high fat intake in humans, probably due to increased ATP turnover occurring only during exercise.
- Fatty acid availability seems to be a key signal for adaptations in muscle proteins involved in lipid metabolism as fatty acids act as ligands for peroxisome proliferator-activated receptors and through β-oxidation-driven sirtuin 1 signalling.
- Obesity is characterized by impairments in fatty acid oxidation capacity, but aerobic exercise training is a potent tool to restore such impairments by induction of lipid metabolic proteins in muscle.
- An efficient capacity to handle and oxidize fatty acids, and the ability to adapt fatty acid utilization to fatty acid availability, seem to be of great importance for both lipid and glucose homeostasis and insulin action.

lipoprotein lipase (mLPL)-mediated hydrolysis of triacylglycerol present in circulating VLDLs and chylomicrons (FIG. 1). As mLPL is important for the breakdown of circulating triacylglycerol, it is appropriate to consider whether mLPL activity is influenced by a HFD and aerobic exercise training. In accordance, following 3 days or 4 weeks of a HFD in recreationally active men (fat comprising 70% or 54% of total daily energy intake (E%), respectively), mLPL activity increased by 175% and 80%, respectively, compared with pre-intervention activity[11,12]. For the purposes of this Review, recreationally active is defined as people who do not do formal exercise but are active in their day-to-day life. In regard to exercise training, studies have shown that maximal mLPL activity was higher in endurance-trained versus non-trained individuals of both sexes[13,14]. In addition, when an exercise training programme was performed for 8 weeks by men who were moderately active at baseline, mLPL activity was increased by 47–70%[15,16].

The skeletal muscle uptake of albumin-bound fatty acids that are released from the adipose tissue, and of fatty acids derived from the hydrolysis of triacylglycerol in plasma, is mediated by lipid binding proteins as well as by passive diffusion (FIG. 1). Most studies have focused on CD36, a fatty acid translocase mediating transport of fatty acids into cells. A large portion of CD36 is, in the basal resting and fasting state, stored in intramyocellular compartments, whereas a remaining part is present at the sarcolemma, the outer membrane of the muscle cell, to mediate basal fatty acid uptake[17]. When a hypercaloric HFD (fat comprising 77E%) was consumed for 3 days in lean men, *CD36* mRNA was upregulated in skeletal muscle; however, total and sarcolemmal levels of the CD36 protein were unaffected[18]. By contrast, CD36 total protein content of skeletal muscle was increased by 17% after 5 days and by 30% after 4 weeks of a HFD (fat comprising 65E%) in endurance-trained men[19]. These findings suggest a time-dependency in the upregulation of CD36.

In contrast to the dietary regulation, muscle total CD36 protein content was similar between regularly endurance-trained and untrained individuals — both women and men[13], suggesting no effect of exercise training on CD36 protein. However, when daily moderate intensity aerobic exercise training was conducted for 9 days in untrained women and men, the CD36 protein content of skeletal muscle was increased by 36%[20], which is potentially related to the high training frequency. When high-intensity aerobic interval training (HIIT; defined as interval exercise at 90% of peak oxygen uptake, three times per week) was performed, the CD36 protein content of skeletal muscle was increased after 6 weeks in untrained women[21], and also in recreationally active men and women[22]. Of note, in the study of the adaptations to HIIT in untrained women[21], muscle biopsy samples obtained under resting conditions showed that CD36 protein content in the sarcolemma did not increase following training, whereas an increase was obtained in the mitochondrial levels of CD36 protein by 30% and 51% following HIIT for 2 and 6 weeks, respectively[21]. Interestingly, higher muscle total CD36 protein content was evident in women compared with men, irrespective of training status[13], suggesting a sex-specific regulation of CD36 protein.

Another family of membrane-associated fatty acid transport proteins is the fatty acid binding protein (FABP) family, which is divided into two groups: plasma-associated proteins anchored at the outer surface of the cell membrane (FABPpm), operating in a coordinated fashion with CD36; and cytosolic proteins (FABPc), functioning as cytosolic shuttle proteins for fatty acid activation of the nuclear peroxisome proliferator-activated receptors (PPARs)[23]. Interestingly, FABPpm protein content of skeletal muscle increased with moderate intensity aerobic exercise training in men, observed both in cross-sectional and interventional studies[13,24,25]; however, this effect was not seen in trained compared with untrained women[13]. By contrast, when recreationally active women or untrained women performed HIIT for 2 and 6 weeks, respectively, FABPpm protein content did increase by 25–48%[21,22,26]. Changes in FABPc protein abundance in muscle have not been demonstrated in endurance-trained women and men compared with untrained individuals[13], whereas higher FABPc protein and mRNA content of skeletal muscle have been observed in both untrained and trained women compared with men[13,27].

The fatty acid transport proteins (FATPs) are another group of membrane proteins that facilitate the import of fatty acids (FIG. 1). When a HFD (fat comprising 62–64E%) was consumed for 4–6 weeks by untrained men, an increase in FATP1 and FATP4 protein abundance was observed in skeletal muscle, along with increases in FABPpm, FABPc and CD36 total protein contents[2,28]. Furthermore, 8 weeks of moderate intensity aerobic exercise training upregulated FATP4 protein by 33% in the skeletal muscle of untrained men, which was associated with increased fatty acid oxidation during exercise[25]. By contrast, FATP1 protein content was decreased[25]. In recreationally active men and women, 6 weeks of HIIT was not followed by an increase in FATP4 protein content in skeletal muscle[22]. Once fatty acids are within the myocyte, it seems that FATPs act via fatty acyl-CoA synthetase (ACS) that activates the incoming fatty acids by the formation of fatty acyl-CoAs, which in turn can be directed towards cellular metabolic

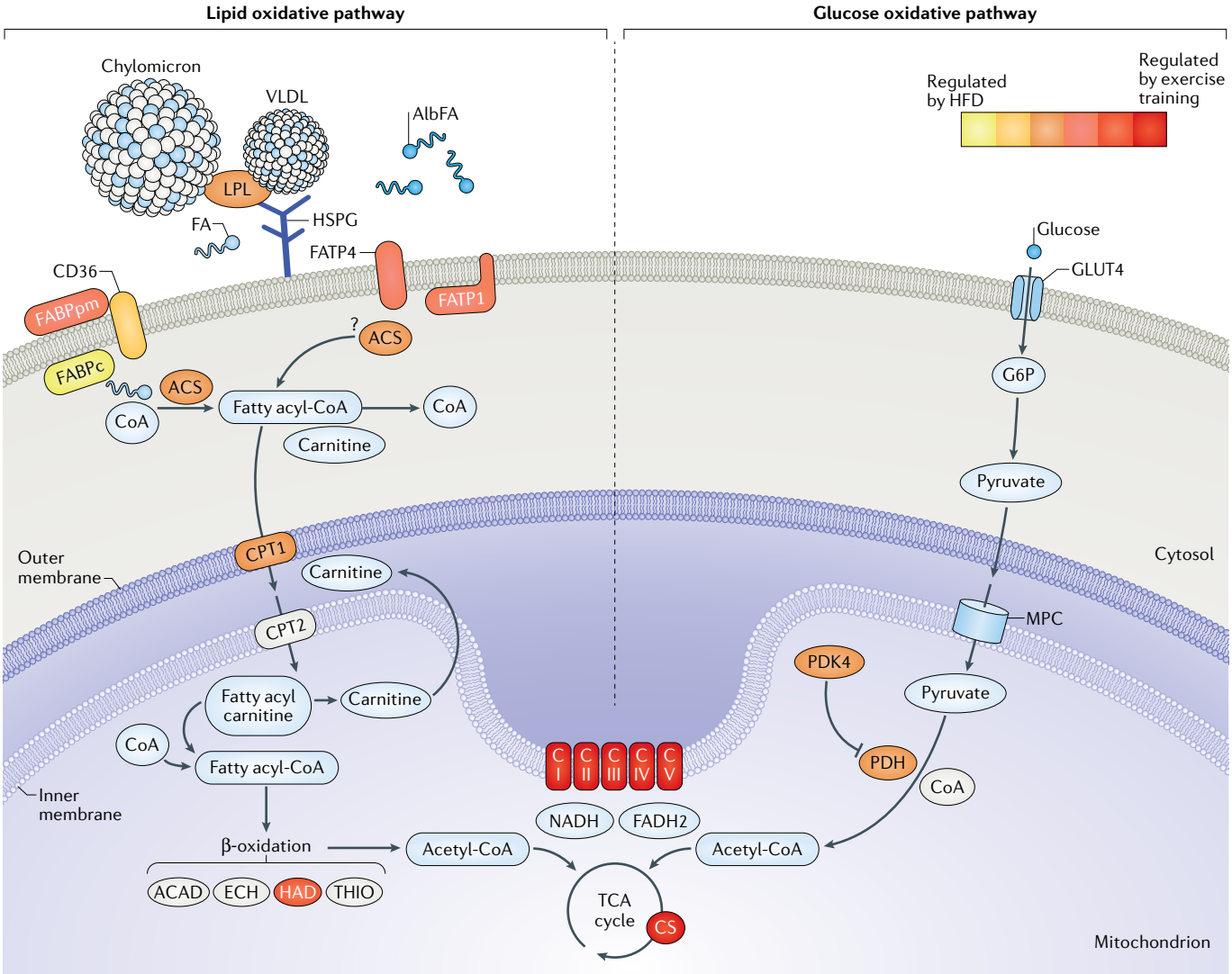

Fig. 1 | **Skeletal muscle adaptations to high dietary fat intake and aerobic exercise training.** The figure summarizes known adaptations in skeletal muscle proteins involved in lipid metabolism and fatty acid oxidation, in response to a high-fat diet (HFD) or aerobic exercise training. Proteins indicated in yellow are increased in abundance or activity in skeletal muscle in response to HFD. Proteins indicated in red are increased with aerobic exercise training. Proteins indicated with orange are increased both by a HFD and exercise training. The degree of yellow–red colouring indicates the respective regulation by HFD and aerobic exercise training, as obtained from studies in human skeletal muscle. ACAD, acyl-CoA dehydrogenase; ACS, acyl-CoA synthetase; AlbFA, albumin-bound fatty acids; CI–V, oxidative phosphorylation complex I–V; CPT1/2, carnitine palmitoyltransferase 1/2; CS, citrate synthase; ECH, enoyl-CoA hydratase; FA, fatty acid; FABPc, fatty acid binding protein at the cytosol; FABPpm, fatty acid binding protein at the plasma membrane; FADH2, flavin adenine dinucleotide 2; FATP1/4, fatty acid transport 1/4; G6P, glucose-6-phosphate; GLUT4, glucose transporter 4; HAD, β-hydroxy-acyl-CoA dehydrogenase; HSPG, heparan sulfate proteoglycan; MPC, mitochondrial pyruvate carrier; LPL, lipoprotein lipase; PDH, pyruvate dehydrogenase complex; PDK4, pyruvate dehydrogenase kinase 4; TCA, tricarboxylic acid; THIO, thiolase.

pathways for oxidation or storage[29]. One study demonstrated a considerable upregulation of *ACS* mRNA in skeletal muscle following 8 weeks of exercise training in untrained men[30]; however, the regulation of ACS protein expression by high fat intake or training awaits further investigation.

Mitochondrial fatty acid import, acetyl-CoA selectivity and β-oxidation. Maximal activity of carnitine palmitoyltransferase 1 (CPT1), an enzyme that is essential to the carnitine-shuttle import of long-chain fatty acids into mitochondria (FIG. 1), was increased in skeletal muscle by 38% and 44% following 15 days and 4 weeks of a HFD (fat comprising 69E%), respectively, in endurance-trained men[31,32]. In relation to exercise training, maximal CPT1 activity was 20–89% greater in endurance-trained men compared with sedentary men[33–35]. Furthermore, aerobic training for 8 weeks in sedentary men increased maximal CPT1 activity by 250% along with decreased malonyl-CoA sensitivity of CPT1 (REF.[36]). These findings indicate an increased capacity for the import of fatty acids into mitochondria both with a HFD intake and exercise training.

The regulation of carbohydrate-derived acetyl-CoA availability to the tricarboxylic acid (TCA) cycle from glycolysis-produced pyruvate is mediated by pyruvate dehydrogenase (PDH), an enzyme complex linking glycolysis with oxidation. The activity of PDH will

reciprocally affect β-oxidation-derived acetyl-CoA oxidation. Pyruvate dehydrogenase kinase (PDK) is an upstream kinase of PDH and inhibits the activity of PDH (FIG. 1). PDK4 is the main PDK isoform present in human skeletal muscle, and *PDK4* mRNA levels were substantially increased in skeletal muscle after 3 days of a hypercaloric HFD (fat comprising 78E%) in recreationally active men, resulting in a metabolic shift towards increased whole-body fatty acid oxidation[37]. Notably, when the same individuals consumed a hypercaloric carbohydrate-rich diet (carbohydrate comprising 80E%) for 3 days, *PDK4* mRNA was reduced to undetectable levels. Other eucaloric studies show increased levels of *PDK4* mRNA after 2–5 days of a HFD (fat comprising 50–69E%) in untrained men[38–40] and in untrained men and women[41]. In accordance, threefold to fivefold increases in skeletal muscle PDK activity were observed after 1–6 days of a HFD (fat comprising 63–73E%) in moderately trained men and women[42–44].

As described above, PDK4 is an inhibitor of PDH. In accordance, PDH activity was reduced by 50–71% after 2–5 days[3,43,45–47] and by 75% following 4 weeks[48] of a HFD (fat comprising 59–75E%) in untrained to moderately trained men. These findings demonstrate covalent (PDK4-mediated) suppression of PDH activity, and hence downregulation of glycolysis-derived acetyl-CoA oxidation, within a few days of high fat intake, allowing increased fatty acid oxidation. In the context of exercise training, 7 weeks of aerobic exercise training increased PDH-E1α protein content and PDH total activity by 30% in skeletal muscle of previously untrained men, concomitant with an increase in total PDK activity[49]. PDK4 protein content was also higher in skeletal muscle of endurance-trained compared with untrained men[50]. The training-induced adaptations in PDK thus mimic the upregulation of PDK activity with high fat intake, thereby leading to an increased potential for PDH inhibition in both high-fat-exposed or trained muscle, facilitating increased fatty acid oxidation at the expense of glucose oxidation.

β-Hydroxy-acyl-CoA dehydrogenase (HAD) has been proposed to be the rate-limiting enzyme of β-oxidation, a four-step process where fatty acids are sequentially catabolized to generate acetyl-CoA for the TCA cycle and reduced coenzymes used in oxidative phosphorylation. Several studies show an unchanged maximal HAD activity in skeletal muscle following high fat intake. For example, maximal HAD activity was unchanged after 3–6 days of a HFD (fat comprising 55–70E%) in untrained men[1,51] and in a mix of moderately trained men and women[44]. Even following 2–4 weeks of a HFD (fat comprising 54–69E%), maximal HAD activity remained unchanged in untrained[48,52] and trained men[12,31]. However, when a HFD (fat comprising 62E%) was consumed for 7 weeks, a 26% increase in maximal HAD activity was obtained in both untrained and trained men[53], suggesting that long-term high fat intake is necessary for this adaptation. With regard to exercise training, most studies show an increase in maximal HAD activity by ~20–40% following several weeks of whole-body moderate intensity aerobic exercise[25,54]. Notably, when exercise training was allocated to a single muscle group (that is, the knee extensors) for ≥2 weeks, an increase of 18–38% in maximal HAD activity in the exercised vastus lateralis muscle was repeatedly shown in untrained men[7,55,56]. In addition, 6 weeks of HIIT increased maximal HAD activity (20–32%) in skeletal muscle of untrained men and women[21,22,57].

The TCA cycle, oxidative phosphorylation and mitochondrial function. The enzyme citrate synthase resides as the gateway into the TCA cycle, converting acetyl-CoA to citrate (FIG. 1). Several studies have documented that untrained or trained men who consume a HFD (fat comprising 54–69E%) for 5 days to 7 weeks show no alterations in maximal citrate synthase activity[12,31,44,48,51–53,58]. Similarly, the protein content of oxidative phosphorylation complexes was unchanged after 3 days to 6 weeks of a HFD (fat comprising 65–72E%) consumed by untrained[1,2] and trained men[58]. In contrast to diet, aerobic exercise training has consistently been shown to upregulate maximal citrate synthase activity. This finding is supported in a systematic review of published data, containing 69 intervention groups who undertook aerobic exercise training at different intensities, showing an average increase of 33% in maximal citrate synthase activity[59]. In addition, the protein content and maximal activity of oxidative phosphorylation complexes increased after 6–10 weeks of aerobic exercise training in previously untrained men and women[60–62].

In regard to mitochondrial volume and respiratory function, high fat intake seems to have no effect on either of these parameters in human skeletal muscle (BOX 1); this observation is in accordance with the lack of a HFD-induced regulation of mitochondrial enzyme activities and protein contents in the TCA cycle and oxidative phosphorylation. By contrast, aerobic exercise training exerts potent effects on mitochondrial volume and respiration capacity (BOX 1).

In contrast to the observations with high fat intake in humans, research has shown that ad libitum HFD feeding of rodents is associated with skeletal muscle upregulation of mitochondrial DNA copy number as well as maximal citrate synthase, HAD and CPT1 activities, and increased protein contents of oxidative phosphorylation complexes[63–66]. These findings could lead to the interpretation that a HFD is a potent mitochondrial stimulus. However, these findings have not been replicated in humans.

Collectively, the current literature suggests several similarities in the regulation of lipid metabolic adaptations to high fat intake and aerobic exercise training. However, the response to a HFD and exercise training until now has only been studied on a fairly limited number of proteins in muscle (BOX 2). A 2018 proteomics analysis of the gastrocnemius muscle in mice revealed that when mice were fed a HFD (fat comprising 60E%), while either exercise training or remaining sedentary for 20 weeks, a considerable overlap between muscle lipid metabolic proteins regulated by a HFD and exercise training was obtained[67]. These findings support the notion that a HFD increases fatty acid metabolic adaptations in a similar manner to exercise training.

Box 1 | **Mitochondrial volume and function**

Beyond the regulation of specific mitochondrial enzyme activities and protein contents, mitochondrial volume and respiration rate provide an additional level of regulation for lipid metabolism. Thus, it is important to assess the effect of fat availability from diet and exercise training on mitochondrial volume and function. Mitochondrial volume measured with electron microscopy remained unchanged after trained[175] and untrained[176] men consumed a high-fat diet (HFD; fat comprising 41–53% total daily energy intake (E%)) for 4–5 weeks compared with pre-intervention measurements. As a measure of mitochondrial function, the rate of maximal ATP production in isolated mitochondria from moderately trained men and women was unchanged after 6 days of HFD (fat comprising 63E%) with the addition of either glycolytic or lipid substrates or intermediates from the tricarboxylic acid (TCA) cycle when compared with pre-intervention measurements[44].

In permeabilized muscle fibres from endurance-trained men, lipid and glycolytic supported respiration was decreased rather than improved after 5 days of HFD (fat comprising 67E%)[58]. Furthermore, 2.5 weeks of HFD (fat comprising 55E%) consumed by untrained men did not alter mitochondrial respiration with TCA cycle intermediates or ADP as substrates, or uncoupled respiration, when compared with a control diet[52]. No strong evidence thus supports improvements (or decrements) in mitochondrial volume or respiratory function after high fat intake when measured in skeletal muscle biopsy samples obtained in the resting state.

As described in the main text, aerobic exercise training potently upregulates maximal activities in several mitochondrial proteins such as β-hydroxy-acyl-CoA dehydrogenase[7,55,56], citrate synthase[59] and oxidative phosphorylation complexes[60–62]. The training-induced mitochondrial adaptations arise in combination with increased mitochondrial volume[177,178], resulting in an increased mitochondrial respiration capacity following aerobic exercise training when measured in permeabilized muscle fibres of human volunteers[61,62].

Together, these findings highlight that improvements in mitochondrial content, volume and function are specific to exercise training, and probably related to the considerable increased energy turnover during exercise only. This hypothesis explains why a high fat intake in itself does not increase exercise endurance without engaging in simultaneous exercise training.

Metabolic effects of adaptations

The lipid metabolic adaptations to a HFD and aerobic exercise training, which increase the capacity of skeletal muscle for fatty acid oxidation, lead to several functional improvements. For example, 3 days of a HFD (fat comprising 70E%)[1] or 7–10 days of daily aerobic exercise training[1,68] increased maximal fatty acid oxidation flux in vitro in muscle homogenates obtained from untrained women and men compared with pre-intervention measurements. When measured in vivo in human volunteers, the intake of eucaloric HFDs for 1–6 weeks increased fasting whole-body fatty acid oxidation[2,3,69,70]. For aerobic exercise training, it generally applies that the last exercise bout is associated with increased plasma levels of fatty acids and elevated fatty acid oxidation for up to 24–36 h (reviewed elsewhere[71]), depending on the intensity and duration of the prior exercise bout. Therefore, to avoid confounding effects from the last exercise bout, the effect of training on resting fatty acid oxidation should be assessed at a time point beyond 36 h. However, few studies have measured the respiratory exchange ratio, an indicator of the specific fuel (that is, glucose or fatty acids) metabolized, in sufficient separation from the last exercise bout and, for many studies, there is no specification of this parameter.

The composition of the diet in the 36-h period after an exercise bout is also of major importance for the determination of resting fatty acid oxidation; therefore, it is not possible from the available data to assess whether basal, fasting fatty acid oxidation is increased by exercise training. However, when men and women underwent an aerobic exercise training programme for 8–12 weeks, and then carried out a similar bout of aerobic exercise as before the training intervention and at the same absolute workload, fatty acid oxidation was increased in both men and women[7,8,72,73], leading to improved physical performance[74,75]. In women, fatty acid oxidation was even improved when exercise was performed at the same relative intensity (that is, 65% of pretraining and post-training peak oxygen uptake) after 12 weeks of moderate intensity exercise training as before[76].

An increased capacity for fatty acid oxidation in muscle also results in other metabolic benefits. In accordance, 6 weeks of a HFD (fat comprising 64E%) consumed by young men led to 45% lower postprandial excursion of plasma levels of triacylglycerol following a high-fat meal when compared with pre-intervention levels, accompanied by ~45% higher postprandial fatty acid oxidation[2]. Exercise training also seems to improve the ability of humans to handle and clear triacylglycerol from the circulation, as trained men and women (investigated 12–36 h after the last exercise bout) have lower postprandial circulating levels of triacylglycerol in response to high-fat meals compared with sedentary, lean men and women[77–81]. However, this finding could still be driven partly by the last exercise bout, as no effect of 4–12 weeks of aerobic exercise training was obtained on postprandial lipaemia in lean men and women when they were assessed 48 h or 60 h after exercise[82,83]. When one-legged knee extensor exercise was performed for 8 weeks in young men, beneficial effects on the blood lipoprotein profile were obtained, which to a large extent could be explained by training-induced adaptations in the lipid metabolic pathway in skeletal muscle, even within a single leg[16].

Collectively, it seems clear that lipid metabolic adaptations in muscle translate into an increased ability for individuals to dispose lipids from the circulation into skeletal muscle. Furthermore, such adaptations increase fatty acid handling within skeletal muscle when needed, with several beneficial outcomes.

Fatty acid availability as a signal

Traditionally, exercise training adaptations in metabolic proteins are thought to be initiated by contraction-induced mechanical stress and challenges in energy (for example, changes to the AMP to ATP ratio), redox (for example, the NAD to NADH ratio), oxidative stress and ionic homeostasis (for example, Ca^{2+} flux) leading to increased transcription of exercise-responsive lipid metabolic genes[84–88]. Such signalling pathways are not substantially affected by high fat intake, suggesting other potentially overlapping mechanisms.

While eating a HFD, plasma and intramyocellular fatty acid availability are repeatedly increased during the postprandial periods. For example, following a large fat-rich meal, postprandial plasma concentrations of triacylglycerol are typically elevated to 2–3 mmol/l in normolipidaemic individuals[2]. Furthermore, the consequent LPL activation in the muscle capillary bed increases intramyocellular fatty acid availability[89].

Indeed, metabolic imaging by PET and PET-CT showed that meal-derived fatty acids accumulate in muscle over a 6-h postprandial period[90].

Circulating fatty acid levels are also increased when aerobic exercise is performed to ~600–1,000 μmol/l, but importantly, if no food is ingested in the hours following exercise, a further increase in plasma fatty acid levels to ~1,500–2,100 μmol/l is obtained during the first few hours following exercise, depending on exercise intensity and duration[71,91–96]. As a result of the high circulating fatty acid availability, intramuscular fatty acyl-CoA has been shown to increase approximately fourfold during 2 h of moderate intensity exercise[97].

Under conditions of exercise, an increased fatty acid oxidation revealed from respiratory exchange ratio measurements suggests an increased fatty acid flux into skeletal muscle; isotopic turnover studies and measurement of substrate exchange across the exercising legs revealed that 60–75% of the exercise-induced increase in whole-body fatty acid rate of disposal was accounted for in the exercising muscle[98], and 60–100% of fatty acids taken up into exercising muscle were directly oxidized (reviewed elsewhere[99]). Interestingly, a higher fatty acid flux into skeletal muscle was obtained during exercise in trained women than in trained men[76,98]. In addition, during recovery from endurance exercise, fatty acid uptake and oxidation by the leg was increased 1.5 h into recovery compared with pre-exercise resting conditions[100]. On the contrary, if plasma fatty acid levels were suppressed during post-exercise recovery by intake of high-carbohydrate meals in humans, the increase in mRNA content of lipid metabolic genes as *PDK4*, *LPL*, *CPT1* and *CD36* was blunted 8 h into recovery[101].

All together, these observations make fatty acids an attractive and shared physiological signal in the regulation of the lipid oxidative system in skeletal muscle (FIG. 2).

Fatty acid signalling candidates

Fatty acids and PPAR signalling. Fatty acids and their acyl-CoA derivatives serve as important ligands for PPARs leading to PPAR activation and DNA transcription by PPAR promoter binding to target genes in lipid metabolism[102] (FIG. 2). The family of PPARs consists of three members encoded by distinct genes; PPARα, PPARδ and PPARγ. PPARα and PPARδ are both expressed in skeletal muscle, PPARδ as the dominant isoform[102]. PPARδ activation has been shown to increase muscle fatty acid oxidation and to regulate several genes implicated in fatty acid uptake and metabolism in rodent models[102]. In agreement, administration of a PPARδ agonist in mice increased, dose-dependently, maximal fatty acid oxidation in muscle, supported by higher gene expression of LPL, fatty acid transport proteins (that is, FATP1, FABPpm and CD36) and β-oxidation enzymes (for example, HAD)[103]. Moreover, 4 weeks of PPARδ agonist-mediated activation or exercise training both increased maximal fatty acid oxidation at rest and during submaximal exercise in wild-type mice, but not in PPARδ muscle-specific knockout mice (PPARδmKO); the phenotype in wild-type mice is probably mediated via a PPARδ-dependent PDK4-mediated PDH inhibition of glucose oxidation[104]. Additionally, in rats, 6 days of administration of a PPARδ agonist increased fatty acid oxidation at rest[105] and during muscle contractions[106], which occurred concomitantly with increased HAD activity and PDK4-mediated PDH inhibition[105]. Together, data indicate that PPARδ activation driven by circulating fatty acids results in increased protein expression of lipid metabolizing enzymes and transporters[107].

The most abundant fatty acids in plasma are palmitic acid, oleic acid and linoleic acid, and they are all ligands for the PPARs[108]. When investigated in vitro, the binding and activation potential of fatty acids on the PPARs depend on fatty acid saturation status, as unsaturated fatty acids are more potent activators of PPARα and PPARδ than saturated fatty acids[109]. In particular, the n-3 polyunsaturated fatty acid α-linolenic acid is a strong activator of PPARα[109,110]. Importantly, 3 days of a hypercaloric, monounsaturated and polyunsaturated HFD (fat comprising 78E%) increased human skeletal muscle mRNA expression of the PPAR target genes *CPT1* and *PDK4* (REF.[37]), thereby supporting a potent role of unsaturated fatty acids in PPAR activation in human muscle. Fatty acids contributing to the intramuscular pool can be plasma-derived, or can also stem from hydrolysis of intracellular triacylglycerol. Although not investigated in detail in skeletal muscle, deletion of *ATGL* (ATGL catalyses the initial step in triacylglycerol hydrolysis) in the heart of mice substantially decreased PPAR target gene expression and this impairment was reversed by PPAR agonist treatment[111], suggesting that muscle lipolysis is an important step in the delivery of ligands to PPARs.

Sirtuin 1 signalling. The type III NAD^+-dependent deacetylase sirtuin 1 (SIRT1) regulates lipid oxidative gene transcription in muscle, primarily via deacetylation-mediated activation of PPARγ co-activator 1-α (PGC1α)[112,113] (FIG. 2). Both activation and overexpression of SIRT1 increases maximal fatty acid oxidation in mouse muscle[112,114,115]. Intake of hypercaloric[116] or eucaloric[40] HFDs (fat comprising 50E%) for 2–5 days by human volunteers led to deacetylation of PGC1α in human skeletal muscle, indicative of increased SIRT1

Box 2 | **Protein adaptations in skeletal muscle**

Proteins in skeletal muscle that are engaged in tissue uptake, handling and metabolism of circulating fatty acids are transcriptionally induced by both high fat intake and aerobic exercise training, within days or longer. Likewise, rapid transcription of the mitochondrial upstream kinase of pyruvate dehydrogenase, pyruvate dehydrogenase kinase 4, and post-translational regulation of the downstream pyruvate dehydrogenase complex are also induced by high fat intake and aerobic exercise training; these changes account for short-term metabolic shifts in oxidation of energy substrates when fatty acid and carbohydrate availability changes. By contrast, mitochondrial biogenesis is induced primarily by aerobic exercise training and not by high fat intake in humans, probably due to increased energy expenditure and ATP production during exercise, which is not the case in relation to eucaloric, high fat intake (BOX 1).

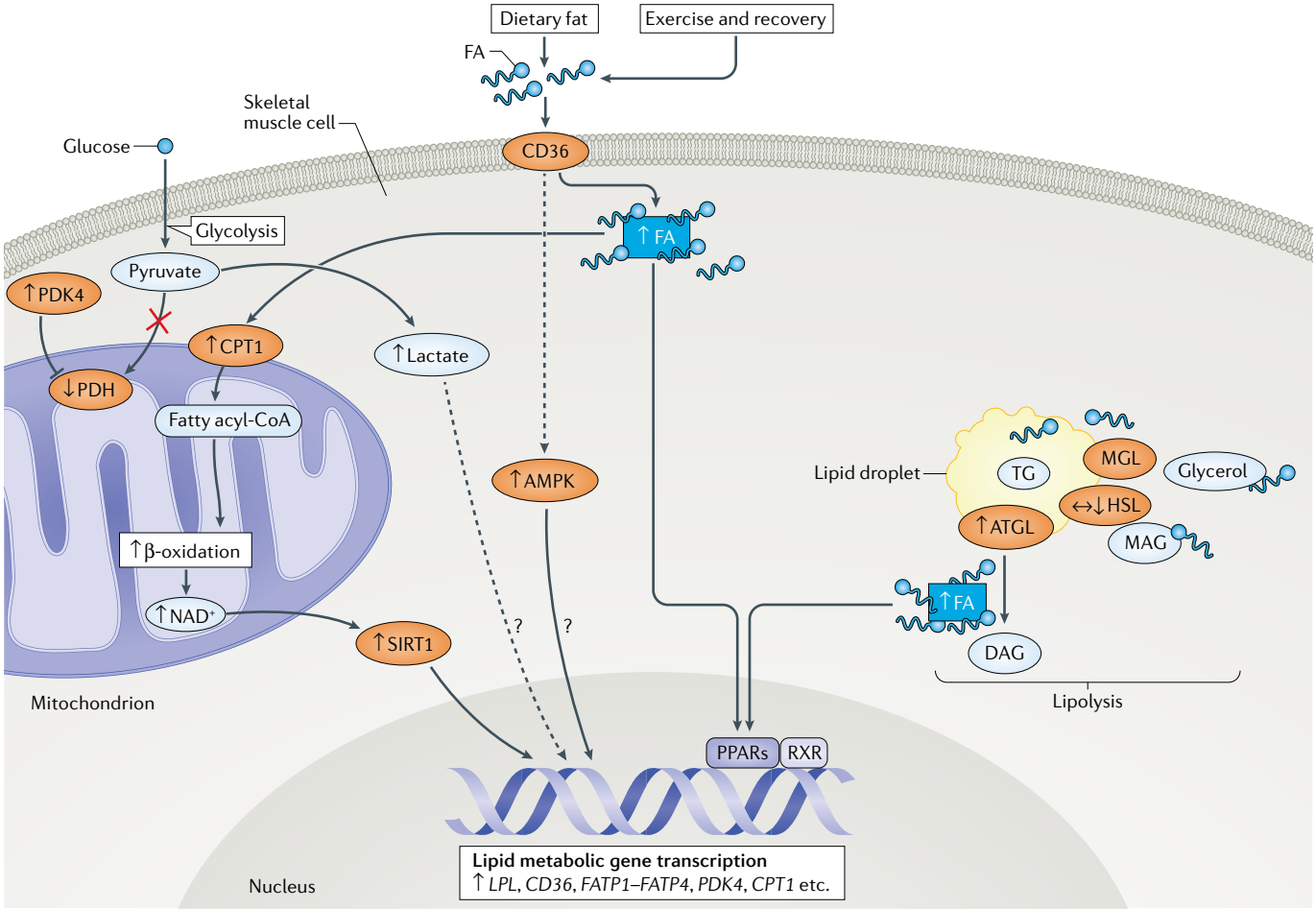

Fig. 2 | **A model suggesting fatty acids as a signal for lipid metabolism adaptations.** Increases in circulating fatty acid levels occur repeatedly during the day after meals, and during and following acute aerobic exercise bouts; these changes increase the intramyocellular availability of fatty acids, which is suggested to mediate lipid metabolic adaptations. Fatty acids might signal to regulate such adaptations via different mechanisms. For example, fatty acids are released from triacylglycerol (TG) located in muscle lipid droplets from the first step of lipolysis mediated by adipose triglyceride lipase (ATGL). These fatty acids can act directly as ligands for the peroxisome proliferator-activated receptor (PPAR) signalling in the nucleus; however, fatty acids taken up in muscle cells might also be ligands for PPARs. In addition, intramyocellular fatty acid availability of saturated fatty acids directly drives β-oxidative flux, which activates sirtuin 1 (SIRT1) signalling via NAD^+ production. Furthermore, fatty acids interact with CD36 in the sarcolemma, enabling AMP-activated protein kinase (AMPK) to be activated. Finally, intramyocellular fatty acid availability leads to decreased pyruvate dehydrogenase kinase 4 (PDK4) activity and thereby pyruvate dehydrogenase (PDH) complex inhibition, which leads to a fuel switch towards fatty acid oxidation at the expense of glucose metabolism, followed by increased lactate production. Dotted arrows are used when evidence of regulation is suggestive and not conclusive yet. Proteins are shown in orange; substrates and metabolites are shown in light blue. CPT1, carnitine palmitoyltransferase 1; DAG, diacylglycerol; FA, fatty acid; FATP1/4, fatty acid transport protein 1/4; HSL, hormone sensitive lipase; LPL, lipoprotein lipase; MAG, monoacylglycerol; MGL, monoacylglycerol lipase; RXR, retinoid X receptor.

activity. Other human studies examining the effects of short-term HFD interventions showed increased expression of the lipid metabolic genes *PDK4*, *HAD* and *CD36* in human skeletal muscle[19,40]. In the context of exercise, acute aerobic exercise activated SIRT1 in mouse muscle[117,118]. Furthermore, skeletal muscle SIRT1 activity was also increased by 6 weeks of HIIT in untrained men[119]. Whereas SIRT1 might be dispensable for mitochondrial biogenesis in response to exercise training, as suggested in the conditional SIRT1 knockout mouse[120,121], this finding does not exclude that SIRT1 could contribute to induction of genes with protein products involved in the lipid metabolic pathway in response to a HFD.

The activity of SIRT1 is reliant on the provision of its substrate NAD^+. A high β-oxidative flux leading to increased production of NAD^+ is probably the signal mediating the effect of fatty acid availability on SIRT1-mediated lipid metabolic gene transcription. In support of this hypothesis, inhibition of fatty acid oxidation in vitro, in C2C12 myotubes, blocked the induction of SIRT1 activity and lipid metabolic genes in response to fatty acids[122]. In 2019, we showed that 3 days of a hypercaloric HFD (fat comprising 78E%) increased SIRT1 protein content and activity in skeletal muscle biopsy samples from untrained men, concomitant with an increased fatty acid oxidation rate and lowered triacylglycerol storage in muscle[123]. This finding suggests that SIRT1 acts as a sensor of fatty acid β-oxidative flux, leading to regulation of mitochondrial and fatty acid utilization genes. Interestingly, in men who consumed a hypercaloric HFD (fat comprising 78E%), activation of

SIRT1 in skeletal muscle was only observed when saturated fatty acid was ingested, whereas an unsaturated HFD did not activate SIRT1 (REF.[123]). It might therefore be speculated that the fatty acid signalling pathway to transcription of lipid metabolic genes is dependent on the degree of fatty acid saturation, where saturated fatty acids signal via β-oxidation-derived NAD^+-dependent SIRT1 activation.

By contrast, unsaturated fatty acids might also signal directly, independently of β-oxidation rate, via the PPARs. The oxidation of saturated palmitic acid was greater than that of monounsaturated oleic acid in primary human myotubes in vitro[124,125]; however, human studies with oral intake of labelled fatty acids combined with indirect calorimetry have shown that the oxidation of the saturated palmitic acid and stearic acid was slightly less than the monounsaturated oleic acid and polyunsaturated linoleic acid and linolenic acid during the postprandial period[126–128]. In addition, when labelled fatty acid oxidation was measured across the forearm during aerobic exercise, skeletal muscle showed some preference for oleic and linoleic acids compared with palmitic acid[129]. This finding suggests that unsaturated fatty acids actually seem to have an increased oxidative disposal rate in vivo.

AMP-activated protein kinase signalling. AMP-activated protein kinase (AMPK) might be another regulator of lipid metabolic protein expression. This notion is based on findings in conditional AMPK$\alpha_1\alpha_2$ knockout mice, which have reduced protein expression of CD36 and FABPpm[130], decreased *FATP1* and *FATP4* gene expression[131] and reduced expression of several mitochondrial genes with protein products involved in the TCA cycle and oxidative phosphorylation[131,132]. AMPK is well described as being activated in human muscle during exercise[133]. The question is whether increased fatty acid availability associated with a HFD can also stimulate AMPK.

Turning to in vitro studies, both unsaturated and saturated fatty acids acutely increased AMPK activation in myotubes[134,135]. The activation of AMPK by increased fatty acid availability has been proposed to be mediated indirectly via fatty acids interacting with CD36. This model is based on findings in L6 myotubes that CD36 knockdown abolished fatty acid-induced AMPK activation[134] (FIG. 2). In humans, acute lipid infusion elevated the plasma levels of fatty acids several-fold; however, AMPK phosphorylation was not different compared with the control (saline infusion)[136,137]. By contrast, 5 days of a HFD (fat comprising 50–68E%) increased muscle AMPKα_1 and AMPKα_2 activity in lean trained men[138] and AMPK phosphorylation in untrained lean men and women, and men and women with obesity[116]. Besides increased AMPK activity, longer periods (6 weeks) of HFD (fat comprising 64E%) consumption increased AMPKα_2 protein content of skeletal muscle in untrained men who were slightly overweight[2]. These findings suggest some role of fatty acid availability in AMPK activation. It should be noted, however, that the role of fatty acids as direct or indirect activators of AMPK in human skeletal muscle during high fat intake or aerobic exercise needs to be verified (FIG. 2).

Importantly, a potential role of AMPK in regulating lipid metabolic proteins and thereby fatty acid oxidative capacity should be distinguished from the proposed acute role of AMPK activation in the regulation of mitochondrial substrate selection and rate of fatty acid oxidation. Whereas AMPK seems dispensable in the regulation of fatty acid oxidation acutely at rest and during exercise[139–141], an acute role in the regulation of fatty acid oxidation in muscle during recovery from exercise is suggested from findings of decreased fatty acid oxidation during exercise recovery in mice lacking AMPKα_2 (REF.[118]).

Fatty acids as a signal — summary. In summary, besides being a major energy source, fatty acids, induced by dietary fat intake and aerobic exercise, seem to signal to trigger transcription of lipid metabolic genes by several mechanisms, which is probably related to the saturation degree of the fatty acids. First, fatty acids can act directly as a signal as PPAR ligands, which is a process governed mainly by unsaturated fatty acids. Second, saturated fatty acids can activate SIRT1 via β-oxidative flux. In addition, fatty acids are suggested to interact with CD36 in the sarcolemma, which can in turn lead to AMPK activation. Another potential signalling molecule shared between high fat intake and exercise is production of intramyocellular lactate, which results from the high fatty acid availability during these stimuli (FIG. 2). Lactate has been recognized in the signalling for exercise-induced mitochondrial adaptations[142–144] (BOX 3).

Lipid metabolic inflexibility in obesity

The adaptations to high fat intake and exercise training that have been described so far were obtained in lean, healthy individuals and apply to untrained and endurance-trained volunteers. Most known adaptations were obtained in both women and men, but of note is that several lipid metabolic proteins are more abundant in the skeletal muscle of women than that of men, independently of high fat intake or exercise training (higher levels of CD36, FABPpm and FABPc). The question is whether similar adaptations occur in obesity.

When fatty acid oxidation was assessed before and after a high-fat meal, with meal caloric content adjusted for body size, in a mix of women and men with obesity ($n=701$) and lean women and men ($n=113$), both fasted and postprandial whole-body fatty acid oxidation were reduced with increasing BMI[5]. Furthermore, 1 week ad libitum intake of a HFD (fat comprising 52E%) resulted in a positive association between fatty acid intake and 24-h whole-body fatty acid oxidation in lean men and women, but not in individuals with obesity[4]. When postprandial fatty acid oxidation was studied with a palmitic acid tracer in lean men and women, and men and women with overweight or obesity, a negative correlation was observed between meal-derived whole-body palmitic acid oxidation and the degree of obesity[145]. When fatty acid oxidation was measured in vitro in muscle homogenates obtained by biopsy before and after 3 days of a HFD (fat comprising 70E%), muscle fatty acid

Box 3 | **Lactate as a signalling molecule?**

Lactate is recognized as a signalling molecule for exercise-induced mitochondrial adaptations[142–144]. When fatty acid availability is experimentally increased by lipid plus heparin infusion in humans, pyruvate dehydrogenase (PDH) complex activity in skeletal muscle was inhibited and lactate release increased by 60–120%, suggesting increased intramyocellular lactate production[136,137]. Following a period of high fat intake, skeletal muscle PDH activity was reduced by 50–71%, which was associated with 65–100% increased muscle lactate content in the resting fasted state[43,46]. Furthermore, forearm lactate release increased by 400% following a 61E% high-fat meal in lean men[179]. In addition to dietary fatty acids, exercise is a very potent stimulus for lactate production, with intramyocellular lactate content increasing with exercise intensity[180].

When muscle lactate accumulation was reduced during exercise training bouts by administration of the pyruvate dehydrogenase kinase (PDK) inhibitor (and hence PDH activator) dichloroacetate in mice, increased muscle β-hydroxy-acyl-CoA dehydrogenase (HAD) activity and CD36 protein content (which both occurred in saline-treated control mice) were not induced after 4 weeks of exercise training[181]. Accordingly, a single injection of lactate increased peroxisome proliferator-activated receptor-γ co-activator 1-alpha and PDK4 mRNA expression in mouse muscle[182]. Furthermore, when lactate was administered prior to exercise bouts during a 3-week training intervention, citrate synthase, HAD and oxidative phosphorylation complex activities in mouse muscle increased to a greater extent than with training alone[183].

Lactate could therefore have an important regulatory role in lipid metabolic adaptations. The mechanisms by which lactate induces gene transcription in muscle await to be elucidated, but have been suggested to occur via extracellular signal-regulated kinase and AMP-activated protein kinase activation[184], involving lactate-mediated reactive oxygen species production within the muscle[144] and via circulating lactate-induced release of an adipokine, transforming growth factor-β2 (REF.[185]). In addition, in 2019 lactate was found to affect metabolic gene transcription by lactate-derived lactylation of histone lysine residues serving as an epigenetic modification directly stimulating gene transcription[186].

oxidation was increased in lean men, but not in men with obesity[1]. Together, the data suggest that increased dietary fatty acid availability does not induce the same switch to muscle fatty acid oxidation in people with obesity as it does in lean individuals, which is why the obese state can be characterized by a state of lipid metabolic inflexibility. Decreased fatty acid oxidation in response to dietary fat in obesity might result from insufficient adaptations in muscle.

Several aspects of the fatty acid metabolic capacity differ between skeletal muscle from lean individuals and those with obesity when assessed independently of increased exposure to dietary fat. In obesity, the capacity for fatty acid oxidation is found to be lower, which is why intramyocellular handling of fatty acids towards mitochondrial oxidation might not match increased fatty acid availability. In skeletal muscle of women with obesity, maximal CPT1 activity was 27–35% lower than in lean women[146] and was comparable to a mix of men and women with obesity[27]. Furthermore, the muscle CPT1 activity to FABPpm protein ratio in individuals with obesity was half that obtained in lean individuals, suggesting that in obesity fatty acids can be taken up from the plasma but that muscle has a reduced capacity for further oxidation[27]. In line with this hypothesis, an increased intramyocellular content of long-chain fatty acyl-CoA was observed in the skeletal muscle of men[147] and women[148] with obesity compared with lean men[147] and women[148]. Furthermore, maximal fatty acid uptake was higher in incubated muscle strips from women with obesity than in those from lean women, concomitant with greater fatty acid re-esterification to intramyocellular triacylglycerol[149,150] and higher intramyocellular levels of triacylglycerol in the women with obesity[150]. These findings support the observed decreased capacity for mitochondrial import and oxidation of fatty acids in obesity relative to uptake of fatty acids. This hypothesis is also consistent with findings of a greater maximal sarcolemmal fatty acid transport rate due to greater sarcolemmal CD36 protein content, demonstrated in skeletal muscle of women with obesity compared with lean women[150].

Within the process of β-oxidation, maximal HAD activity was reported to be 17–65% lower in skeletal muscle of women with obesity than in that of lean women[146,151]. Likewise, when mitochondrial function was assessed in permeabilized muscle fibres, fatty acid-supported ADP-stimulated respiration was 35% lower in muscle fibres from men with obesity and insulin resistance than in lean men[152]. This phenotype is observed along with findings of lower maximal fatty acid oxidation obtained in muscle homogenates[68,146] and muscle strips in women with obesity[148] and men with obesity[147] than in lean individuals.

Together, it turns out that although skeletal muscle from individuals with obesity possesses an improved protein-facilitated apparatus for transmembrane fatty acid transport and uptake, the potential for intracellular fatty acid handling towards oxidation is impaired due to reduced capacity for mitochondrial import, β-oxidation and fatty acid-derived respiration. This pathophysiological state could direct an increased fraction of fatty acids to storage rather than oxidation in obesity.

Lipid metabolic adaptations in obesity

A limited number of studies have assessed the molecular adaptations to high fat intake in the skeletal muscle of lean participants and individuals with obesity in a parallel design. One such study reported that 5 days of a HFD (fat comprising 65E%) did not increase *PPARA* and *PDK4* mRNA content in skeletal muscle of men and women with obesity, whereas expression of these genes was increased in lean individuals[41]. This finding could suggest that both the signal and transcriptional adaptations to a HFD are impaired in obesity, but more research is needed to solve this phenomenon. The mechanisms explaining the seemingly insufficient adaptation to handle increased fatty acids in obesity might result from an inherent predisposition or from long-term exposure to increased adiposity.

Physical activity levels are a main determinant in regulating the adaptation of fatty acid oxidation to the availability of lipid, as well as glucose, substrates[153]. Whereas individuals with obesity present some rigidity in adapting fatty acid oxidation to high fat intake, their adaptability to respond to exercise training seems to be intact. Hence, 7 days (in women[68]) and 10 days (in men[1]), respectively, of daily aerobic exercise training increased maximal fatty acid oxidation in muscle homogenates obtained from individuals with obesity to the same extent as in lean individuals. Of note, 6 weeks of low-volume HIIT did not alter maximal fatty acid oxidation in muscle homogenates from healthy individuals with obesity when compared with pre-intervention

levels[154], suggesting that a certain amount of training volume is needed to improve fatty acid oxidative capacity in obesity. At the molecular level, 12 weeks of moderate intensity aerobic exercise training similarly increased maximal HAD, citrate synthase and oxidative phosphorylation complex activities in muscle by 20–25% in both sedentary women with obesity and sedentary lean women[155]. Maximal HAD and citrate synthase activities were also increased by 50–60% and 75–85%, respectively, in sedentary healthy men with obesity and type 2 diabetes mellitus following 8 weeks of moderate intensity aerobic interval exercise training[156]. Moreover, HAD protein content and activity increased by 30% following 6–8 weeks of HIIT in healthy men with obesity[157,158] and in men with obesity and type 2 diabetes mellitus[158]. The described mismatch between fatty acid uptake and oxidation in obesity seems to be improved after exercise training, as 72% increased maximal fatty acid oxidation in muscle homogenates obtained from men with obesity after 8 weeks of aerobic exercise training occurred concomitantly with 42% lowering of intramyocellular concentrations of triacylglycerol[159].

The adaptations to exercise training in these studies were all obtained under conditions of body weight maintenance. Notably, it seems that even a pronounced diet-induced weight loss (14–40% of body weight) was not associated with an improvement in maximal fatty acid oxidation in the skeletal muscle of individuals who formerly had severe obesity[10,68,160] or after Roux-en-Y gastric bypass-mediated weight loss (21–29% of body weight)[161].

Taken together, exercise training seems to be an effective tool to improve fatty acid oxidation capacity in both healthy women and men with obesity and those with insulin resistance and obesity, independent of weight loss.

Improved lipid metabolic capacity

It has been debated whether it is metabolically favourable to increase fatty acid oxidation[162,163] or whether fatty acid oxidation should rather be downregulated to

Table 1 | **Human intervention studies on the effect of eucaloric high-fat diets on whole-body insulin sensitivity**

Ref.	Design	Participants[a]	Diet intervention	Indirect calorimetry	Insulin sensitivity[b]
174	Crossover; 2×3 weeks; HF vs LF	$n=8$ (3 M, 5 F); 37±3 years; BMI 24±1.6 kg/m^2	HF: 50E% fat; LF: 20E% fat	NA	↔
70	Crossover; 2×3 weeks; HF vs CON	$n=10$ (M); 27±2 years; BMI 24±1 kg/m^2; moderately trained	HF: 75E% fat; CON: 35E% fat	↓ Glucose oxidation; 7.8±1.1 to 2.5±1.3 µmol/kg/min	↔
89	Crossover; 2×16 days; HF vs LF	$n=25$ (15 M, 10 F); 29±1 years; BMI 22.1±0.3 kg/m^2	HF: 50E% fat; LF: 25E% fat	NA	↔
69	Crossover; 3×11 days; HF vs MF vs LF	$n=6$ (M); 29–55 years; BMI 21–26 kg/m^2	HF: 83E%; MF: 41E% fat; LF: 0E% fat	↓ Glucose oxidation; ~10 to ~3 µmol/kg/min	↔
6	Crossover; 2×14 days	$n=7$ (2 M, 5 F); 51–65 years; with obesity; BMI 27–36 kg/m^2; T2DM; untrained	HF: 89E% fat; 0E% CHO; LF: 0E% fat; 89E% CHO	↓ RER; 0.79±0.01 to 0.73±0.01	↔
173	1×2 days; HF vs pre-intervention	$n=6$ (M); 23±1 years; BMI 21.6±0.8 kg/m^2; untrained	HF: 45E% fat	NA	↔
3	Crossover; 2×6 days; HF vs CON	$n=10$ (M); 26±3 years; BMI 23.7±0.9 kg/m^2	HF: 77E% fat; CON: 33E% fat	↓ RER; 0.79±0.01 to 0.75±0.01	↑ 10% glucose Rd
52	Parallel; 1×2.5 weeks; HF vs CON	$n=11$ CON (M); $n=10$ HF (M); 24±1 and 24±1 years; BMI 23.2±0.2 and 23.0±0.4 kg/m^2; untrained	HF: 55E% fat; CON: 28E% fat	RER; 0.84±0.02 and 0.81±0.01 (NS)	↔
172	Parallel; 1×3 weeks; HF vs LF	$n=10$ HF (M); $n=10$ LF (M); 54±2 years and 56±3 years; sedentary and with overweight; BMI 29.3±0.6 and 28.3±0.5 kg/m^2	HF: 49E% fat; LF: 22E% fat	RER; 0.81±0.01 and 0.81±0.01 (NS)	↔
171	Crossover; 4 weeks HF vs 10 days CON	$n=10$ (7 M, 3 F); 36±3 years; with obesity; BMI 33.6±1.3 kg/m^2; normal glucose tolerance	HF: 55E% fat; CON: 35E% fat	NA	↓ Glucose Rd at a low and a 4-times higher insulin infusion rate
170	Crossover; 2×1 weeks; HF vs CON	$n=23$ (F); 34±1 years; with overweight; BMI 29.7±0.9 kg/m^2	HF: 50E% fat; CON: 30E% fat	RER; 0.90±0.01 and 0.87±0.01 ($P=0.1$)	↑ GIR (+10%)
2	Parallel; 1×6 weeks; HF-PUFA and HF-SFA vs pre-intervention	$n=9$ in HF-PUFA (M); $n=9$ in HF-SFA (M); 32±6 years and 33±1 years; BMI 25.8±2.0, 27.0±2.7 kg/m^2; sedentary	HF-PUFA: 64E% fat (32E% PUFA); HF-SFA: 64E% fat (37E% SFA)	↓ RER; 0.82±0.01 to 0.75±0.01 (PUFA); 0.82±0.02 to 0.73±0.01 (SFA)	↔

↔, Unchanged with the high-fat (HF) diet intervention; ↓, decreased with the HF diet intervention; ↑, increased with the HF diet intervention; CHO, carbohydrate; CON, control diet; E%, % of total daily energy consumed; F, female patients; GIR, glucose infusion rate; glucose Rd, glucose rate of disappearance; LF, low-fat (high-carbohydrate) diet; M, male patients; MF, moderate-fat diet; NA, not assessed; NS, not significant; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid; RER, respiratory exchange ratio; T2DM, type 2 diabetes mellitus. [a]Participant data are shown as mean ± s.e.m., unless stated otherwise. [b]Whole-body insulin sensitivity was measured by the hyperinsulinaemic–euglycaemic clamp.

allow for glucose oxidation[164–166]. One group[167] proposed that increased fatty acid availability for mitochondrial β-oxidation acutely downregulates glucose oxidation and hence tissue glucose disposal by virtue of substrate competition.

In 2020, we revealed in mice that although acute inhibition of fatty acid oxidation lowered fasting blood levels of glucose due to increased glucose utilization in peripheral tissues, the accompanying increase in circulating levels of fatty acids induced glucose intolerance, and long-term inhibition of fatty acid oxidation led to hepatic steatosis and whole-body insulin resistance[168]. This finding emphasizes the importance of sufficient fatty acid oxidation for whole-body fatty acid and glucose homeostasis. Accordingly, fasting fatty acid oxidation is inversely associated with insulin-stimulated glucose disposal rate during a hyperinsulinaemic–euglycaemic clamp in both lean individuals and in individuals with obesity and type 2 diabetes mellitus[169], which highlights that inefficient regulation of fatty acid oxidation might be associated with impaired insulin action.

The importance of fatty acids as nutrients in maintaining metabolic health is demonstrated by our 2018 findings, in which 6 weeks of a HFD (fat comprising 64E%) mediated high fatty acid oxidation in sedentary men with slight overweight, which was accompanied by preservation of insulin action and lowering of hepatic glucose output and plasma levels of lipids[2]. To systematically assess whether intake of high-fat, low-carbohydrate diets is associated with altered insulin action, we evaluated the available evidence from human HFD interventions, in which insulin sensitivity was measured by the gold-standard hyperinsulinaemic–euglycaemic clamp. As summarized in TABLE 1, 11 studies revealed that eucaloric HFDs with fat intake in the range of 45E% to 83E% administered for 2 days to 6 weeks during weight maintenance in both lean individuals and in individuals with overweight or obesity increased fatty acid oxidation and preserved or improved, rather than impaired, whole-body insulin action[2,3,6,52,69,70,89,170–174].

Collectively, a high capacity to handle and oxidize fatty acids, and the ability to adapt fatty acid utilization to fatty acid availability, seems to be of great importance for insulin action and substrate homeostasis, and thereby is an important aspect of metabolic health.

Conclusions

In lean individuals, both high fat intake and aerobic exercise training potently increase the abundance and activity of protein machinery related to fatty acid uptake, handling and oxidation in skeletal muscle. A major signal that appears to have a role in the shared regulation of enzymes and proteins in muscle fatty acid oxidation is the increase in plasma levels of fatty acids that occurs in response to a HFD, and during aerobic exercise and recovery. In accordance, fatty acids act directly as ligands for PPAR signalling, and intramyocellular fatty acid availability drives β-oxidative flux, which activates SIRT1 signalling via NAD^+ production. Some evidence suggests that fatty acids interact with CD36, which in turn activates AMPK. Collectively, gene transcription of target genes encoding proteins in the lipid oxidative pathway will be mediated. Moreover, intramyocellular fatty acid availability leads to increased PDK4 activity and thereby PDH inhibition, which leads to a fuel switch towards fatty acid oxidation at the expense of glucose metabolism. Inhibition of PDH also increases lactate production, which has been proposed to play a part in signalling, mediating adaptations in skeletal muscle metabolic proteins.

Skeletal muscles in individuals with obesity display a reduced capacity for the intracellular handling of fatty acids towards oxidation, including decreased mitochondrial fatty acid transport and capacity for β-oxidation. Moreover, a defective adaptive increase in fatty acid oxidation is evident in individuals with obesity when exogenous fat availability is increased. Of great translational importance, aerobic exercise training seems to be an effective tool to improve fatty acid oxidation and lipid metabolic inflexibility in both healthy and insulin-resistant individuals with obesity. This effect on substrate metabolism seems to be independent of energy deficit and weight loss. Finally, human diet interventions reveal that increasing fatty acid availability by HFDs, while maintaining energy balance, results in maintained or improved insulin sensitivity. This finding underlines that high ability or capacity to handle and oxidize fatty acids is of great importance for peripheral insulin action, fat balance and substrate homeostasis, and hence is a crucial aspect of metabolic health.

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