

# The Role of Membrane Fatty-Acid Transporters in Regulating Skeletal Muscle Substrate Use during Exercise

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## Abstract

While endogenous carbohydrates form the main substrate source during high-intensity exercise, long-chain fatty acids (LCFA) represent the main substrate source during more prolonged low- to moderate-intensity exercise. Adipose tissue lipolysis is responsible for the supply of LCFA to the contracting muscle. Once taken up by skeletal muscle tissue, LCFA can either serve as a substrate for oxidative phosphorylation or can be directed towards esterification into triacylglycerol. Myocellular uptake of LCFA comprises a complex and incompletely understood process. Although LCFA can enter the cell via passive diffusion, more recent reports indicate that LCFA uptake is tightly regulated by plasma membrane-located transport proteins (fatty acid translocase [FAT/CD36], plasmalemmal-located fatty acid binding protein [FABPpm] and fatty acid transport protein [FATP]). Depending on cardiac and skeletal muscle energy demands, some of these LCFA transporters can translocate rapidly from intracellular pools to the plasma membrane to allow greater LCFA uptake. This translocation process can be induced by insulin and/or muscle contraction. However, the precise signalling

pathways responsible for activating the translocation machinery remain to be elucidated. This article will provide an overview on the effects of diet, acute exercise, and exercise training on the expression and/or translocation of the various LCFA transporters in skeletal muscle tissue (FAT/CD36, FABPpm, FATP).

Fatty acids are important substrates for skeletal muscle energy metabolism, especially during low- to moderate-intensity exercise.<sup>[1-5]</sup> Even though long-chain fatty acids (LCFA) have many functions, such as building blocks for phospholipids or precursors for several intracellular signalling molecules, they primarily serve as a substrate source. Previous studies have consistently shown that LCFA form the main substrate source during sustained low- to moderate-intensity exercise.<sup>[2,6,7]</sup> LCFA can originate from three distinct endogenous stores. Most LCFA are stored as triacylglycerol in adipose tissue, comprising >95% of total endogenous energy storage.<sup>[8]</sup> These LCFA can be released into the circulation and are mainly transported bound to albumin due to their low plasma solubility. In addition, some LCFA are incorporated as triacylglycerol in circulating lipoprotein particles. Furthermore, part of the LCFA are stored as intramyocellular triacylglycerol (IMTG) within skeletal muscle fibres. Given the fact that skeletal muscle has a limited LCFA storage capacity, and no ability for *de novo* LCFA synthesis, muscle tissue mainly relies on plasma LCFA uptake.<sup>[8,9]</sup> Adipose tissue provides the major source of LCFA supply for subsequent skeletal muscle uptake.<sup>[3,5,10]</sup> Once taken up, LCFA can either serve as a substrate for oxidative phosphorylation or can be re-directed towards esterification into triacylglycerol.

Myocellular LCFA uptake comprises a complex and yet incompletely understood process. To reach the mitochondria for oxidation within skeletal muscle, plasma-derived LCFA need to pass the endothelium, interstitial space, plasma membrane, cytosol and finally the two mitochondrial membranes. There has been much debate on the predominant mechanism(s) and the rate-limiting step of LCFA entry into skeletal muscle tissue. Traditional arguments contend that LCFA enter the cell via

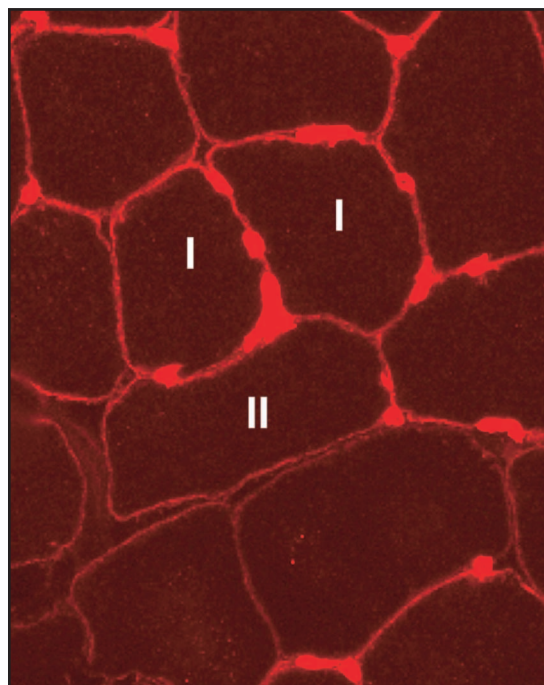
gradient-driven passive diffusion, while the more contemporary view is that LCFA uptake is facilitated and tightly regulated by plasma membrane-located LCFA transporters.<sup>[11-17]</sup> More recent reports indicate that both mechanisms are operative,<sup>[18]</sup> with the view that LCFA membrane translocation is the rate-limiting step in LCFA uptake and that LCFA transporters are needed for efficient transport.<sup>[18,19]</sup> The latter studies applied giant sarcolemmal vesicle methodology on saturation and competitive inhibition to show that protein-mediated transport forms the prevailing factor modulating the balance between LCFA uptake and oxidation.<sup>[14-25]</sup> Currently, three membrane-bound and two intracellular-located lipid-binding proteins have been postulated to be involved in LCFA uptake and mitochondrial LCFA supply in skeletal muscle tissue.<sup>[13,14,25,26]</sup> The role of these transporters in regulating LCFA uptake is not yet fully understood, but it is suggested that they are involved in both the acute and chronic skeletal muscle adaptive responses to exercise<sup>[21,22,27-29]</sup> and/or instrumental in the aetiology of insulin resistance and/or type 2 diabetes mellitus.<sup>[25,30,31]</sup> In this article, we will focus on the exercise/contraction-stimulated role and regulation of fatty acid translocase (FAT/CD36), plasma membrane fatty acid-binding protein (FABPpm), fatty acid transport protein (FATP), cytoplasmic fatty acid-binding protein (FABPc) and acyl coenzyme A binding protein (ACBP) on skeletal muscle fuel selection in humans.

## 1. Sarcolemmal Long-Chain Fatty Acid (LCFA) Uptake and Cytoplasmic Transport

### 1.1 Fatty Acid Translocase

The most studied membrane LCFA transporter is FAT/CD36. It is a heavily glycosylated integral

membrane protein of 88 kD, with two predicted transmembrane domains and 85% homology to glycoprotein IV or CD36 of human blood platelets and leucocytes.<sup>[32,33]</sup> Traditionally, FAT/CD36 was thought to be located exclusively at the sarcolemma, but recent reports show that it is also located in an endosomal pool,<sup>[16,22]</sup> as well as on skeletal muscle mitochondrial membranes.<sup>[34-36]</sup> The latter indicates a role of FAT/CD36 in mediating LCFA transfer into the mitochondria and regulating oxidation.<sup>[34-36]</sup> FAT/CD36 knock-out mice were shown to have a markedly decreased oleate uptake, which suggests a critical role for FAT/CD36 in skeletal muscle LCFA uptake.<sup>[15]</sup> In line with this, it has also been reported that LCFA esterification and oxidation rates are ~3-fold greater in the oxidative muscle of wild type versus knock-out mice.<sup>[37]</sup> Conversely, over-expression of FAT/CD36 has been shown to increase palmitate oxidation rates in contracting soleus muscle.<sup>[38]</sup> However, it seems unlikely that FAT/CD36 channels LCFA to different metabolic fates as most studies have been performed on cell lines.<sup>[39]</sup> It is more likely that, downstream of the LCFA transporters, regulation by insulin- and contraction-mediated signalling pathways leads to either oxidation or esterification.<sup>[19]</sup> Increases in FAT/CD36 mRNA and/or protein content of human vastus lateralis muscle have been reported in conditions of increased plasma LCFA availability, such as after 5 days on a high-fat diet,<sup>[40,41]</sup> a single bout of glycogen-depleting exercise,<sup>[42]</sup> and after short-term ( $\leq 2$  weeks) exercise training.<sup>[28]</sup> In line with its role of increasing LCFA oxidation, sarcolemmal FAT/CD36 expression has been shown to be more abundant in type I versus type II muscle fibres (figure 1).<sup>[43-46]</sup> Because plasma LCFA availability in type I and II fibres is most likely equivalent, differences in FAT/CD36 fibre-type protein content are likely associated with the difference in oxidative capacity or IMTG contents between fibre types.<sup>[2,46,47]</sup> The latter differences in fibre-type specific IMTG contents might also explain the reported gender differences in FAT/CD36 expression<sup>[27]</sup> as skeletal muscle tissue tends to contain a higher percentage of type I muscle fibres in females compared with males.<sup>[45]</sup> In addition, the gender-specific differ-

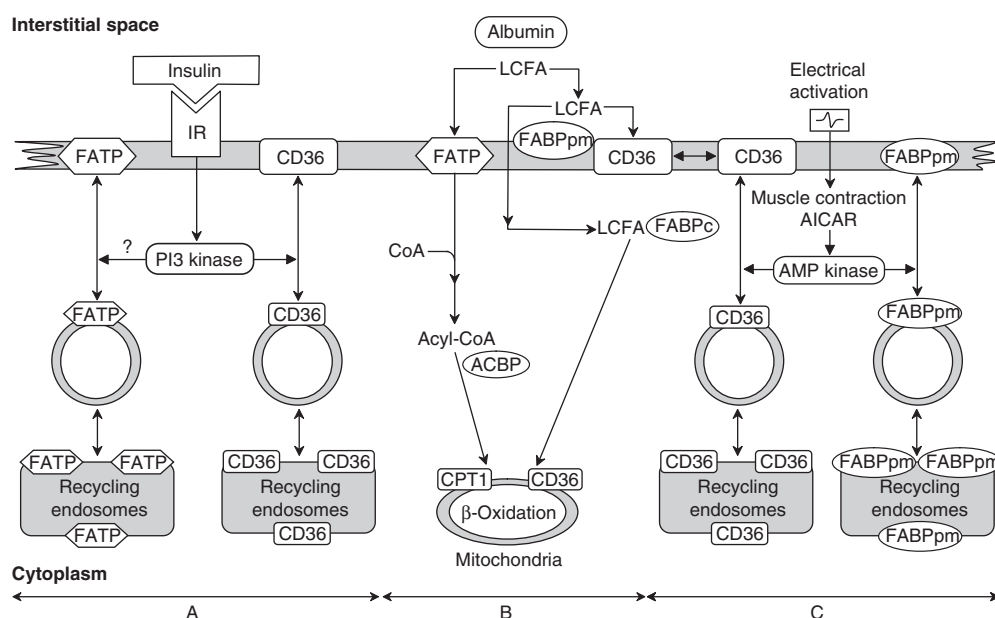


**Fig. 1.** Fatty acid translocase (FAT/CD36) distribution in human type I and II muscle fibres as visualized by immuno-histochemical staining of muscle cross-section.<sup>[44]</sup>

ences in skeletal muscle FAT/CD36 content could also partly be due to the greater LCFA flux in females versus males.<sup>[48,49]</sup> In short, skeletal muscle FAT/CD36 protein content depends on muscle fibre-type composition, gender and training status. Such factors should be considered when reviewing literature on the role of LCFA transporters on skeletal muscle substrate use.

### 1.2 Plasmalemmal-Located Fatty Acid Binding Protein

FABPpm is a 43 kDa protein located peripherally on the plasma-membrane and identical to mitochondrial aspartate aminotransferase (mAspAT) [figure 2].<sup>[50,51]</sup> Studies measuring FABPpm have applied two different rabbit polyclonal antisera to FABPpm.<sup>[52,53]</sup> Although mAspAT is recognized by the FABPpm antibody, Turcotte et al.<sup>[54]</sup> have shown that the effect of this detection is minimal, since 90–95% of the FABPpm contained in the total



**Fig. 2.** Schematic representation of putative long-chain fatty acid (LCFA) uptake mechanism by passive diffusion and facilitated by LCFA transporters. Section A represents the insulin-induced translocation pathway from endosomal pools via vesicles to the sarcolemma, while section C represents the contraction-induced translocation. Section B represents the membrane-located LCFA uptake mechanism. **ACBP** = acyl-CoA binding protein; **AICAR** = 5-aminoimidazole-4-carboxamide 1- $\beta$ -D-ribofuranoside; **AMP** = adenosine monophosphate; **CD36** = fatty acid translocase; **CoA** = coenzyme A; **CPT1** = carnitine palmitoyl transferase; **FABPc** = cytoplasmic-located fatty acid binding protein; **FABPpm** = plasmalemmal-located fatty acid binding protein; **FATP** = fatty acid transport proteins; **IR** = insulin receptor; **PI3 kinase** = phosphatidylinositol-3 kinase.

homogenate is from the skeletal muscle plasma membrane. However, recently FABPpm also has been reported to be present within an endosomal pool in the cytoplasm in rat cardiac myocytes.<sup>[55]</sup> Studies that inhibit palmitate uptake by using antibodies against FABPpm,<sup>[56]</sup> or increase palmitate uptake by overexpression of FABPpm in rat skeletal muscle,<sup>[57,58]</sup> clearly indicate that FABPpm contributes to increased sarcolemmal LCFA transport, but not to LCFA transport into mitochondria.<sup>[58]</sup> FABPpm has been suggested to be co-localised with FAT/CD36 and functions as an important LCFA transporter together with FAT/CD36.<sup>[24]</sup> Sarcolemmal FABPpm expression is more abundant in type I versus type II muscle fibres in rats, but this remains to be verified in human muscle tissue.<sup>[23]</sup> A 4-week high-fat diet,<sup>[40]</sup> prolonged fasting,<sup>[54]</sup> endurance exercise training,<sup>[27,29,59]</sup> or 2 weeks of high-intensity aerobic interval training,<sup>[60]</sup> have each been reported to increase muscle FABPpm protein content. Inter-

estingly, and opposite to gender-specific FAT/CD36 expression, the exercise training-induced increase in FABPpm expression was observed only in endurance-trained males as opposed to females.<sup>[27,59]</sup> In contrast to skeletal muscle FAT/CD36 transport protein expression, no gender differences were reported for FABPpm content in vastus lateralis muscle when comparing groups of non-trained subjects.<sup>[26]</sup>

### 1.3 Fatty Acid Transport Proteins

FATP is a 63 kDa LCFA transport protein, that forms an integral membrane protein with six predicted transmembrane domains.<sup>[27,61-64]</sup> So far, six isomers of FATP have been identified, of which FATP1 and FATP4 are expressed predominantly in skeletal muscle tissue.<sup>[27,61,63,65]</sup> Recently, FATP1 has also been reported to be present in an endosomal pool in rat cardiac myocytes.<sup>[55]</sup> Although FATP1 is thought to function primarily as a fatty acyl-CoA

synthase for very LCFA,<sup>[66]</sup> Wu et al.<sup>[67]</sup> recently reported that FATP1 also serves as an insulin-sensitive skeletal muscle LCFA transporter in diet-induced obese rats. Similar to FAT/CD36 and FABPm, FATP1 protein content was higher in type I versus type II rat muscle fibre homogenates.<sup>[68]</sup> For now, data on human skeletal muscle FATP1 expression are scarce. Overexpression of FATP1 in human muscle cell culture moderately enhanced palmitate uptake, but also showed a greater intracellular localisation.<sup>[69]</sup> Kiens et al.<sup>[27]</sup> were the first to report human skeletal muscle FATP1 mRNA data in biopsies taken at rest in a cross-sectional study of trained and non-trained subjects, but showed no significant influence of training status or gender. Our laboratory confirmed these mRNA results and extends on those findings by showing no significant differences in skeletal muscle FATP1 protein content between sedentary and endurance-trained older men.<sup>[29]</sup> Emerging data thus far suggest that the role of FATP1 and FATP4 in human exercise-induced skeletal muscle lipid metabolism is of relatively minor importance.

#### 1.4 Intracellular Fatty Acid Transport

Once LCFA are taken up into the cell they can either be esterified into long-chain fatty acyl CoA (LCFA-CoA) or transported as LCFA to the mitochondria for  $\beta$ -oxidation (figure 2). Intracellular transport of LCFA-CoA is mediated by binding to cytoplasmic ACBP,<sup>[70-72]</sup> while LCFA are transported via binding to FABPc.<sup>[26,73]</sup> Toxic effects of increased intracellular levels of hydrophobic LCFA can be prevented by the binding of LCFA to FABPc. Binding of LCFA to these binding proteins is believed to be an important factor in regulating free intracellular LCFA concentration. LCFA are thought to be extracted directly from the plasma membrane by the cytoplasmic binding proteins. Interestingly, FABPc is believed to serve a permissive function rather than being obligatory for fatty acid transport as FABPc knock-out mice show only a 45–50% reduction in skeletal<sup>[74]</sup> or cardiac muscle LCFA uptake.<sup>[75]</sup>

## 2. LCFA Transport Protein Translocation

Recent studies suggest that LCFA transporter content within the plasma membrane represents a more important factor determining LCFA uptake than total muscle LCFA transporter expression, except for FABPm.<sup>[16,22,27,42,55,59,65,76]</sup> Similar to the insulin- and contraction-dependent intracellular translocation of the glucose transporter protein 4 (GLUT4),<sup>[77]</sup> it has been become clear that LCFA transport proteins can translocate from endosomal pools to the plasma membrane to mediate LCFA influx (figure 2). In line with this, FAT/CD36 translocation in rat skeletal muscle and cardiac myocytes can be induced by muscle contraction as well as by insulin.<sup>[55,76,78,79]</sup> Translocation of FABPm in rat cardiac myocytes can only be induced by contraction.<sup>[55]</sup> However, recently Han et al.<sup>[80]</sup> showed for the first time insulin- and contraction-induced translocation of FABPm in rat skeletal muscle. Recent studies have also shown that insulin can induce FATP1 translocation in both 3T3 L1 adipocytes and rodent skeletal muscle (figure 2),<sup>[67,81]</sup> but not in rodent cardiac myocytes.<sup>[55]</sup> Currently, there is no evidence for contraction-induced FATP1 translocation. As relatively large amounts of muscle tissue are needed to allow the assessment of membrane-bound LCFA transporters, insulin- and contraction-induced translocation has hardly been studied in human skeletal tissue.<sup>[65]</sup>

### 2.1 Insulin-Induced Translocation

Subcellular fractionation of insulin-stimulated rat cardiac myocytes or insulin-perfused rat hindlimb muscle have shown that insulin can rapidly increase sarcolemmal FAT/CD36 content by ~1.5-fold, at the concomitant expense of intracellularly stored FAT/CD36.<sup>[78-80]</sup> The insulin-mediated translocation pathway of FAT/CD36 is to a major part comparable with the insulin-mediated translocation pathway of GLUT4.<sup>[82]</sup> Phosphatidylinositol-3 kinase (PI-3 kinase)-Akt signalling shows to be required for translocation of FAT/CD36 as inhibition of PI-3 kinase by wortmannin has been shown to completely block the translocation effect of insulin (figure 2).<sup>[79]</sup> In obese and type 2 diabetes patients,

under basal conditions, elevated basal LCFA uptake rates are correlated with increased plasma membrane FAT/CD36 content.<sup>[25]</sup> The permanent relocation of FAT/CD36 at the plasma membrane is most likely related to elevated levels of lipid intermediates that impair FAT/CD36 cycling between the cytoplasmic and sarcolemmal LCFA transporter pools by disturbing the PI3-kinase signalling pathway.<sup>[25,82]</sup> In accordance, FABPpm translocation was found not to be regulated via PI3-kinase signalling,<sup>[56]</sup> and FABPpm sarcolemmal protein content was not upregulated in either obese and/or type 2 diabetes subjects.<sup>[25]</sup> However, Han et al.<sup>[80]</sup> recently reported insulin-induced translocation of FABPpm in rat skeletal muscle. Recently, Bandyopadhyay et al.<sup>[65]</sup> reported insulin-stimulated translocation of FAT/CD36 and FATP4 in skeletal muscle of lean subjects and of rosiglitazone-treated type 2 diabetes patients.

## 2.2 Contraction-Induced Translocation

Contraction-induced translocation in electrically stimulated rat skeletal muscle and cardiac myocytes not only shows an acute upregulation of LCFA uptake, but also results in rapidly increased sarcolemmal FAT/CD36<sup>[76,77,83]</sup> and FABPpm<sup>[80]</sup> content after contraction. *De novo* FAT/CD36 protein synthesis did not seem to occur during 30 minutes of contraction. During post-contraction recovery, LCFA uptake returned to baseline values and FAT/CD36 was cycled back from the sarcolemma to the intracellular pools.<sup>[76]</sup> Adenosine monophosphate-kinase (AMPK) is involved in contraction-induced translocation of FAT/CD36 and FABPpm,<sup>[83,84]</sup> while for FAT/CD36 also Erk1/2 signalling may play a role.<sup>[85]</sup> AMPK plays an important regulatory role in fat metabolism by regulating malonyl-CoA levels and subsequently carnitine palmitoyl transferase (CPT1) activity.<sup>[86]</sup> Sarcolemmal FAT/CD36 and FABPpm protein contents can be increased by activation of AMPK by either physiological (contraction) or pharmaceutical (5-aminoimidazole-4-carboxamide 1- $\beta$ -D-ribofuranoside; AICAR) interventions. The latter stimulates LCFA uptake and subsequent oxidation.<sup>[55,79,81]</sup> The effects

of both insulin and contraction on FAT/CD36 translocation have been reported to be additive in rat cardiac myocytes,<sup>[79]</sup> which implies that there are at least two different pools of intracellular FAT/CD36 (figure 2) analogous to GLUT4.<sup>[87]</sup> So far, the exact signalling pathways responsible for the contraction- and insulin-stimulated translocation machinery of LCFA transporters remain to be elucidated. Recently, phosphorylation of Akt substrate (AS160) has evolved as a potential part of the translocation machinery.<sup>[88]</sup>

It has become clear that the translocation of sarcolemmal LCFA transporters plays a vital role in regulating LCFA flux. Thus, the existing literature should be evaluated on whether the generated LCFA transporter data represents either sarcolemmal or intracellular LCFA transporters, or both. Therefore, in the following sections, we will indicate whether studies evaluated either sarcolemmal/intracellular and/or total LCFA transporter content.

## 3. Influence of Diet on LCFA Transport Protein Expression

Skeletal muscle accounts for nearly 40% of total bodyweight and, from a quantitative point of view, forms the most important site for LCFA uptake and utilization.<sup>[89,90]</sup> In theory, nutritional and exercise intervention strategies that enhance fat utilization during exercise should spare muscle and liver glycogen stores and, as such, improve endurance performance capacity. Indeed, specific 'fat adaptation' protocols, where subjects consume a high-fat diet with ~70% of total energy intake being derived from dietary fat while training, have been explored as a means to augment fat use during competition with the hopes of improving performance.<sup>[91-93]</sup> In order to examine the effects of elevated plasma LCFA levels on the expression and/or protein content of fatty acid transporters, rats were fed a chronic high-fat diet.<sup>[94-96]</sup> The length and saturation of the LCFA turned out to be important for the regulation of FABPc. A high-fat diet composed mainly of saturated LCFA showed no effects on FABPc,<sup>[93,94]</sup> while ingestion of a high-fat diet composed of mainly *n*-3 LCFAs markedly increased heart and skeletal

muscle FABPc content.<sup>[96]</sup> In support of this, Roepstorff et al.<sup>[40]</sup> reported that male subjects consuming a high-fat diet for 4 weeks (high content of *n*-3 FA and *n*-6 FA) showed increased total FABP, FABPpm and FAT/CD36 protein content in crude membrane extracts of human skeletal muscle. Total FABPpm protein content showed no upregulation after 1 week of adaptation to a high-fat diet. Similar findings have been reported by Cameron-Smith et al.<sup>[41]</sup> Skeletal muscle biopsies taken at rest in well trained male subjects showed no changes in FABPpm, but upregulated FAT/CD36 mRNA and total protein content after 5 days of adaptation to a high-fat diet. Taken together, these data show that the total expression of FAT/CD36 can be rapidly upregulated on a high-fat diet, while FABPpm and FABPc tend to be more resistant to such changes. However, there is still no convincing evidence that this dietary and training regime provides exercise performance benefits, since most competitive athletes perform at such a high workload that endogenous carbohydrate remains the predominant fuel. In addition, a high-fat diet tends to downregulate glycogenolysis and, thus, could even impair high-intensity performance capacity.<sup>[97]</sup>

#### 4. Exercise and LCFA Transporter Content

Skeletal muscle contraction and the adrenergic response to exercise strongly increases LCFA release from adipose tissue and augments muscle LCFA uptake and oxidation rates.<sup>[89,90]</sup> The presence of a large LCFA gradient across the plasma membrane suggests that increased LCFA uptake is mediated by LCFA transporters, rather than by LCFA diffusion.<sup>[98]</sup> However, the specific mechanisms regulating LCFA transporter-mediated LCFA uptake and subsequent oxidation during exercise remains unclear. Only a few studies have examined the effect of acute or prolonged exercise on LCFA transporters in human skeletal muscle. Thus far, the proper evaluation of the effects of exercise on LCFA transporter content and/or translocation in human muscle tissue has been hampered by the limited amount of skeletal muscle tissue obtained by percutaneous

muscle biopsy collection *in vivo* in humans. Therefore, most exercise studies have applied either giant vesicles (rodent studies) or crude membrane extracts of total homogenates (rodent and human studies). However, due to modifications of previously published fractionation protocols, Bandyopadhyay et al.<sup>[65]</sup> have recently measured LCFA transporter content in both plasma membrane and microsomal fractions using only 30–40mg of skeletal muscle tissue.

##### 4.1 Effects of Single-Bout Exercise

In humans, LCFA transporter content has been shown to respond acutely to exercise, at least on the mRNA level. A single 90-minute bout of exercise (60% maximal oxygen uptake [ $\dot{V}O_{2max}$ ]) has been shown to increase skeletal muscle mRNA levels of FAT/CD36 (~25%) and FABPpm (~15%).<sup>[27]</sup> In contrast, Tunstall et al.<sup>[28]</sup> showed no significant changes in FAT/CD36 mRNA levels after a single 60-minute exercise bout (63%  $\dot{V}O_{2max}$ ). This apparent discrepancy might be attributed to differences in training intensity and/or time of biopsy collection, as in the latter study the biopsy was taken 24 hours after exercise at which an upregulation of FAT/CD36 mRNA might have been no longer apparent. To investigate LCFA transporter content and translocation, Bonen et al.<sup>[76]</sup> evaluated the effect of acute exercise on sarcolemmal FAT/CD36 protein content in giant vesicles prepared from electrically stimulated (30 minutes) rat hind-limb muscle. They observed that contraction induced a maximal 75% increase in LCFA uptake, in line with a 40% greater FAT/CD36 content in the plasma membrane. This was accompanied by a comparable reduction in intracellular FAT/CD36 content, while total FAT/CD36 content did not change during the 30-minute contraction period.

In humans, total FAT/CD36 protein content was measured in crude membrane extracts of skeletal muscle biopsies obtained directly after a single bout of glycogen-depleting cycling exercise (3–4.5 hours) and showed a significant increase in FAT/CD36 protein content.<sup>[42]</sup> Recently Holloway et al.<sup>[36]</sup> also reported an increased mitochondrial FAT/

CD36 content following 120 minutes of cycling at ~60%  $\dot{V}O_{2\max}$ . This mitochondrial upregulation might be attributed to either translocation from an endosomal pool or from *de novo* FAT/CD36 protein synthesis.

In summary, FAT/CD36 translocates to the plasma membrane following an acute bout of exercise within a timeframe of <30 minutes.<sup>[76]</sup> More prolonged exercise activities lead to an upregulation of FAT/CD36 mRNA and total protein content.<sup>[42]</sup> Recent improvements in the methodology for subcellular fractionation of muscle tissue will allow future studies to elucidate the effects of exercise on translocation of LCFA transporters in humans.<sup>[65]</sup>

#### 4.2 Effects of Short-Term Exercise Training

Effects of short-term ( $\leq 2$  weeks) exercise training on the expression of LCFA transporters have also been reported. Seven days of chronic electrical stimulation of rat hind-limb muscle has been shown to increase FAT/CD36 and FABPpm protein content in the plasma membrane, and was accompanied by a greater LCFA uptake rate.<sup>[99,100]</sup> A comparable study by Campbell et al.<sup>[34]</sup> also reported a greater (47%) total FAT/CD36 expression with a concomitant increase in mitochondrial FAT/CD36 content. Conversely, the importance of LCFA transporter translocation was eloquently shown by the fact that 7 days of denervation showed a ~25–30% decrease in FAT/CD36 and FABPpm protein content in the plasma membrane without alterations to total muscle LCFA transporter content. These findings were accompanied by a 39% decrease in fat oxidation rate.<sup>[100]</sup> In human skeletal muscle, 9 days of repeated exercise (60 minutes of cycling per day at 63%  $\dot{V}O_{2\max}$ ) was shown to increase total fat oxidation rates by 24%, which was accompanied by a similar increase in FAT/CD36 mRNA and total protein content.<sup>[30]</sup> In contrast, another study implementing 2 weeks of high-intensity interval training showed an upregulation in skeletal muscle FABPpm protein content (25%) after exercise, without changes in total FAT/CD36 protein content.<sup>[60]</sup> Despite the absence of any changes in FAT/CD36 expression, whole-body fat oxidation rates were

substantially greater following training (36%). Comparable results on unchanged fatty acid transporter content were seen after sprint training and detraining.<sup>[101]</sup>

Taken together, FAT/CD36 tends to form the main LCFA transporter that is translocated and upregulated during the early adaptive response to short-term exercise training or following an acute bout of exercise. The published data seem to imply that the apparent adaptive response in FAT/CD36 content is instrumental to allow the rapid upregulation of LCFA uptake and subsequent oxidation during prolonged exercise. The impact of exercise training on LCFA transporter translocation remains to be established.

#### 4.3 Effects of Endurance Exercise Training

More prolonged endurance exercise training (>3 weeks) augments skeletal muscle fat oxidative capacity through the proliferation of capillaries and the increase in mitochondrial density, leading to a greater capacity to utilize plasma LCFA during exercise in the endurance-trained state.<sup>[102]</sup> It has been speculated that endurance training is associated with an upregulation of LCFA transporter content in skeletal muscle tissue, but this hypothesis remains equivocal due to a paucity of data. Plasma membrane FABPpm content of oxidative skeletal muscle was increased in rats after 8 weeks of treadmill training and was correlated with greater palmitate uptake.<sup>[103]</sup> Crude membrane fractions derived from muscle biopsy samples taken at rest after 3 weeks of intense one-legged endurance training in young untrained male subjects showed a significant (49%) increase in total skeletal muscle FABPpm protein content. In contrast, in the untrained leg, no changes were observed.<sup>[58]</sup> The greater mixed muscle protein FABPpm content in male endurance-trained subjects was also reported in a cross-sectional study comparing male and female subjects with minimal 2 years of endurance-training history versus sedentary controls.<sup>[27]</sup> Our laboratory confirmed these FABPpm data when comparing LCFA transporter mRNA and total protein content in elderly ( $57 \pm 1$  years) endurance-trained male subjects who had a

cycling history of >25 years with sedentary healthy and type 2 diabetes patients.<sup>[29]</sup> In contrast, a group of young ( $25 \pm 1$  years) trained male athletes showed no upregulation of total skeletal muscle FABPpm.<sup>[31]</sup> The only significant difference between subjects in these studies was age, so perhaps a certain consistent and long-term amount of training is needed to increase total skeletal muscle FABPpm content.<sup>[29,31]</sup> Furthermore, the upregulation of FABPpm by endurance training appears to be gender specific, as the increased FABPpm content was only shown in endurance-trained males.<sup>[27]</sup> However, in contrast to acute or short-term exercise, there was no effect of endurance training on muscle FAT/CD36 protein content.<sup>[27,29,31]</sup> In accordance, FATP1 mRNA and protein data showed no significant influence of training status or gender,<sup>[27,29]</sup> while muscle FABPc and ACBP protein also showed no changes after training.<sup>[27]</sup>

In summary, these data suggest that skeletal muscle FABPpm expression is upregulated in the endurance-trained state and is likely instrumental to allow greater LCFA oxidation rates. To date, no data on LCFA transporter translocation to the sarcolemma have been obtained following long-term exercise training intervention studies in humans.

## 5. Perspectives

Exercise intensity determines the relative contribution of either carbohydrate or fat oxidation to total energy production.<sup>[7,104]</sup> At higher exercise intensities (>70%  $\dot{V}O_{2max}$ ) fat oxidation is decreased and only 10% of total energy expenditure is being delivered by non-plasma-derived LCFA oxidation.<sup>[104]</sup> Mitochondrial LCFA uptake is mainly regulated by CPT1 activity, which is inhibited by malonyl-CoA.<sup>[105]</sup> At higher exercise intensities there is an increased production of  $H^+$  that reduces the intracellular pH. Lower pH inhibits mitochondrial LCFA uptake due to either a decreased activity of CPT1 as well as an altered sensitivity of CPT1 for malonyl-CoA,<sup>[106,107]</sup> and/or due to a reduction in free carnitine availability.<sup>[1]</sup> Recently, FAT/CD36 was also identified on mitochondria isolated from rat and human skeletal muscle and found to be involved in

LCFA oxidation.<sup>[34-36]</sup> Interestingly, CPT1 was shown to immunoprecipitate together with FAT/CD36, suggesting that FAT/CD36 and CPT1 are physically paired,<sup>[108]</sup> while the oxidative capacity of the tissues (heart >> red muscle > white muscle) follows mitochondrial FAT/CD36 protein content.

It has been hypothesized that exercise-induced increases in fatty acid oxidation occur as a result of an increased ability to transport LCFA into mitochondria. This can be due to decreased inhibition by malonyl-CoA on CPT1 and some further co-involvement of FAT/CD36 in submaximal exercise, when fat oxidation rates are highest.<sup>[36]</sup> It is suggested that FAT/CD36 may be playing a role downstream of CPT I activity, possibly in the transfer of palmitoyl-carnitine from CPT I to carnitine-acylcarnitine translocase.<sup>[35]</sup> The recent findings of mitochondrial located FAT/CD36 indicates a role in LCFA transfer into the mitochondria. These data provide new insight regarding human skeletal muscle mitochondrial LCFA transport, and suggest that FAT/CD36 could be involved in the cellular and mitochondrial adaptations resulting in improved and/or impaired states of LCFA oxidation.<sup>[35]</sup> However, it remains unclear which regulatory mechanism(s) are involved and most dominant. The FAT/CD36 pool is particularly interesting<sup>[109]</sup> as FAT/CD36 can either be directed to the plasma or the mitochondrial membranes to increase LCFA uptake or oxidation, respectively. Future research studies should aim to unravel the various mechanisms responsible for regulating insulin and/or contraction-induced LCFA transport protein translocation.

## 6. Conclusion

Exercise training in combination with optimal nutritional strategy is of major importance to optimize skeletal muscle fuel selection and performance. Endogenous fat stores, from a quantitative point of view, form the most important substrate source during low- to moderate-intensity exercise. LCFA uptake is primarily regulated by transport proteins FAT/CD36 and FABPpm. The role of FATP1 and FATP4 during exercise conditions remains unclear. All three LCFA transporters are lo-

cated in the plasma membrane, and to a lesser extent in endosomal pools, and/or within the mitochondrial membranes. Translocation of these transport proteins to the plasma membrane can be induced by either insulin (FAT/CD36, FATP1) or muscle contraction (FAT/CD36, FABPpm). High-fat diet, acute exercise or short-term exercise training can rapidly upregulate plasma membrane FAT/CD36 content by either translocation (<30 minutes) or by *de novo* transport protein synthesis (>60 minutes), showing an early adaptive response to acute exercise or dietary intervention to allow greater LCFA uptake and/or subsequent oxidation. In contrast, total skeletal muscle FABPpm protein content is only upregulated after a period of >4 weeks on a high-fat diet or after prolonged endurance exercise training (>3 weeks). Recent studies report the presence of FAT/CD36 in mitochondria, co-localized with CPT1. This finding will likely increase our understanding in lipid handling and metabolic regulation. Although the complex regulation of the LCFA transporter translocation machinery remains to be elucidated, rodent models indicate that AMPK may play a pivotal role in exercise-induced translocation. Future studies are warranted to confirm these findings in humans.

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