Symposium 2: The fatty acid transporters of skeletal muscle

Studies of plasma membrane fatty acid-binding protein and other lipid-binding proteins in human skeletal muscle

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The first putative fatty acid transporter identified was plasma membrane fatty acid-binding protein (FABPpm). Later it was demonstrated that this protein is identical to the mitochondrial isoform of the enzyme aspartate aminotransferase. In recent years data from several cell types have emerged, indicating that FABPpm plays a role in the transport of long-chain saturated and unsaturated fatty acids. In the limited number of studies in human skeletal muscle it has been demonstrated that dietary composition and exercise training can influence the content of FABPpm. Ingestion of a fat-rich diet induces an increase in FABPpm protein content in human skeletal muscle in contrast to the decrease seen during consumption of a carbohydrate-rich diet. A similar effect of a fat-rich diet is also observed for cytosolic fatty acid-binding protein and fatty acid translocase/CD36 protein expression. Exercise training up regulates FABPpm protein content in skeletal muscle, but only in male subjects; no significant differences were observed in muscle FABPpm content in a cross-sectional study of female volunteers of varying training status, even though muscle FABPpm content did not depend on gender in the untrained state. A higher utilization of plasma long-chain fatty acids during exercise in males compared with females could explain the gender-dependent influence of exercise training on FABPpm. The mechanisms involved in the regulation of the function and expression of FABPpm protein remain to be clarified.

Abbreviations: FABPc, cytosolic fatty acid-binding protein; FABPpm, plasma membrane fatty acid-binding protein; FAT, fatty acid translocase; LCFA, long-chain fatty acids; mAspAT, mitochondrial isoform of aspartate aminotransferase.

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membrane (Stremmel et al. 1985; Stump et al. 1993). A role for FABP\textsubscript{pm} in the transport of long-chain saturated and unsaturated fatty acids has been suggested from the use of antibodies against FABP\textsubscript{pm}, which leads to inhibition of LCFA uptake in various cell types and of transport of LCFA into giant vesicles of skeletal muscle in rats in a dose-dependent manner (Schwierterman et al. 1988; Sorrentino et al. 1988; Stremmel, 1988; Zhou et al. 1992; Bonen et al. 1998b; Turcotte, 1999). However, elucidation of the role of FABP\textsubscript{pm} in LCFA transport was challenged by the finding that FABP\textsubscript{pm} is identical to the mitochondrial isoform of the enzyme aspartate aminotransferase (mAspAT; Stremmel et al. 1985; Berk et al. 1990; Stump et al. 1993; Bradbury & Berk, 2000). A role for mAspAT/FABP\textsubscript{pm} in fatty acid binding was suggested by molecular-modelling studies of the crystal structure of mAspAT that have identified a pocket, within the larger domain of the enzyme, that is of sufficient size to accommodate the typical LCFA (Berk & Stump, 1999). Whether this pocket serves as a fatty acid-binding site remains to be elucidated. Recently, a study using a polyclonal antibody against rat mAspAT in immunogold electron microscopy of rat tissue sections has shown a strong labelling of mitochondria in several cell types (Cechetto et al. 2002). Labelling was also observed in other locations, such as endothelial cell surfaces, and it was concluded from these observations that mAspAT/FABP\textsubscript{pm} is both a mitochondrial enzyme and a plasma membrane protein (Cechetto et al. 2002).

**Influence of diet on plasma membrane fatty acid-binding protein, fatty acid translocase/CD36 and cytosolic fatty acid-binding protein**

As both the membrane-associated (FABP\textsubscript{pm}, FAT/CD36, fatty acid transport protein) and cytoplasmic (FABP\textsubscript{c}, acyl-CoA-binding protein) lipid-binding proteins are involved in the lipid metabolism of the cell, interventions leading to changes in lipid metabolism may also induce altered regulation of these proteins. Dietary manipulation is one such intervention, but there is little information in the literature on the effects of diet and dietary composition on the different lipid-binding proteins. Available information is mainly on the cytoplasmic FABP\textsubscript{c}. Moreover, most data have been derived from rat studies in which animals were fed a fat-rich diet, mainly composed of saturated fatty acids (Coe & Bernlohr, 1998). Collectively, these data have shown that FABP\textsubscript{c} in heart and skeletal muscle does not respond to an increase in dietary fatty acids (Coe & Bernlohr, 1998; Storch & Thumser, 2000). In contrast, a recent study in rat heart and skeletal muscle has shown that ingestion of a diet rich in n-3 fatty acids markedly increases FABP\textsubscript{c} content (Clavel et al. 2002), suggesting that the length and extent of saturation of the C chain of the fatty acids are important for the regulation of FABP\textsubscript{c}. To evaluate the influence of dietary composition in human skeletal muscle a group of healthy non-obese young male subjects aged 30–40 years was studied. The subjects were randomly assigned to two groups, with one group consuming a fat-rich diet and the other group consuming a carbohydrate-rich diet for 4 weeks. During the intervention period the subjects kept their physical activity habits unchanged and all subjects followed well-controlled experimental diets. The nutrient composition (% energy) of the experimental diets was carbohydrate 65, protein 15 and fat 20 for the carbohydrate-rich diet and carbohydrate 21, protein 17 and fat 62 for the fat-rich diet. The fat ingested in the fat-rich diet had a high content of n-3 fatty acids and n-6 fatty acids, as described previously (Helge et al. 1996, 1998). Ingestion of the carbohydrate-rich diet for 4 weeks did not influence the FABP\textsubscript{c} protein content in m. vastus lateralis, whereas ingestion of the fat-rich diet induced a significant increase in FABP\textsubscript{c} content (P<0.05; Fig. 1(c)). It has been suggested that FAT/CD36 and FABP\textsubscript{c} cooperate in the uptake of LCFA in cardiac and skeletal muscle (Luiken et al. 1999). Interestingly, in the dietary intervention study a similar change was observed in FABP\textsubscript{c} and FAT/CD36; an increase in FAT/CD36 protein content in m. vastus lateralis was seen during the fat-rich diet whereas no change was observed during the carbohydrate-rich diet (Fig. 1(b)). Similar findings for the effect of diet on FAT/CD36 have emerged from the study by Cameron-Smith et al. (2003), in which the dietary intervention period was only 5 d. In the present diet study an increase in the content of FABP\textsubscript{pm} was also observed after 4 weeks, but only when the fat-rich diet was consumed. In fact, when the carbohydrate-rich diet was consumed a decrease in FABP\textsubscript{pm} content in m. vastus lateralis was obtained (Fig. 1(a)). There was no response in skeletal muscle FABP\textsubscript{pm} content to 1 week of dietary intervention. This finding is supported by the results of a recent study (Cameron-Smith et al. 2003) in which well-trained male subjects ingested either a carbohydrate-rich diet (70–75% energy as carbohydrate and <15% energy as fat) or a fat-rich diet (>65% energy as fat and <20% energy as carbohydrate) for 5 d; no change in the protein content of FABP\textsubscript{pm} in skeletal muscle was detected. In summary, these data show that FABP\textsubscript{pm} expression requires a long-term (>1 week) dietary change for adaptations to take place in human skeletal muscle and that the adaptations to a fat-rich diet and a carbohydrate-rich diet occur in opposite directions. The data also indicate that the FAT/CD36 protein content of human skeletal muscle is increased by ingestion of a fat-rich diet for 5 d and that the long-term (4 weeks) increase in FAT/CD36 is paralleled by an increase in FABP\textsubscript{c} protein content.

**Influence of exercise training on plasma membrane fatty acid-binding protein**

It is well known that exercise training induces an increased capacity for lipid oxidation in skeletal muscle (Kiens et al. 1993). Only limited information is available on the influence of exercise training on the different lipid-binding proteins in skeletal muscle. Data from rats have revealed a 55% higher FABP\textsubscript{pm} protein content in red muscle of trained rats compared with untrained rats (Turcotte et al. 1999). The higher FABP\textsubscript{pm} protein content was associated with a higher palmitate uptake in the trained rats at rest.
(48%), and during exercise both uptake and oxidation of palmitate were higher (57%) in the trained rats compared with the untrained rats (Turcotte et al. 1999). The authors suggested that the enhanced content of FABP<sub>pm</sub> could partly explain the training-induced increase in LCFA oxidation (Turcotte et al. 1999). Similarly, in healthy non-obese male volunteers 3 weeks of exercise training with knee extensors of one leg resulted in an increase in FABP<sub>pm</sub> protein content whereas no changes were observed in the contralateral untrained leg (Kiens et al. 1997). These findings in human skeletal muscle were supported by a recent cross-sectional study (Kiens, C Roepstorff, JFC Glatz, A Bonen, P Schjerling, J Knudsen and JN Nielsen, unpublished results), which showed that the FABP<sub>pm</sub> protein content of <i>m. vastus lateralis</i> was significantly higher (<i>P</i> < 0.05) in a group of endurance-trained male volunteers who had been exercise training for several years, compared with an untrained male group. In contrast to these findings, the FABP<sub>pm</sub> protein content in <i>m. vastus lateralis</i> measured in female volunteers, who were matched to the male subjects according to peak VO<sub>2</sub>/kg lean body mass and training history, was similar in the untrained and the endurance-trained female groups and not significantly different from that for untrained males (B Kiens, C Roepstorff, JFC Glatz, A Bonen, P Schjerling, J Knudsen and JN Nielsen, unpublished results). Furthermore, no effect of training status was observed in skeletal muscle FAT/CD36 or FABP<sub>c</sub> content in either males or females (B Kiens, C Roepstorff, JFC Glatz, A Bonen, P Schjerling, J Knudsen and JN Nielsen, unpublished results). The fact that training induced up-regulation of FABP<sub>pm</sub> protein content was seen only in male subjects could explain, or could be explained by, the gender-related difference in utilization of the different lipid sources during exercise. Recent findings (C Roepstorff and B Kiens, unpublished results) provide support for such a notion. Untrained males (<i>n</i> 7) and females (<i>n</i> 7) as well as endurance-trained males (<i>n</i> 7) and females (<i>n</i> 7) exercised on a bicycle ergometer at the same relative work load (60% peak VO<sub>2</sub>) for 90 min. [<sup>13</sup>C]palmitate was infused intravenously and arterial blood samples were obtained at rest and during exercise. Quantification of the rate of disappearance and oxidation of systemic plasma fatty acid was performed as described elsewhere (Roepstorff et al. 2002). The data demonstrated that during exercise the rate of disappearance of systemic plasma fatty acid was not significantly different for the untrained (26.6 (SE 4.8) µmol/kg lean body mass per min) and endurance-trained (19.1 (SE 3.1) µmol/kg lean body mass per min) female volunteers (C Roepstorff and B Kiens, unpublished results), which parallels the observation of no difference in FABP<sub>pm</sub> protein content in skeletal muscle between the two groups (B Kiens, C Roepstorff, JFC Glatz, A Bonen, P Schjerling, J Knudsen and JN Nielsen, unpublished results). In contrast, a significantly higher (<i>P</i> < 0.05) rate of disappearance of plasma fatty acid was observed during exercise in the endurance-trained male subjects (21.1 (SE 4.9) µmol/kg lean body mass per min) compared with the untrained male subjects (11.2 (SE 2.9) µmol/kg lean body mass per min) (C Roepstorff and B Kiens, unpublished results). These two groups also differed

Fig. 1. Content of lipid-binding proteins in <i>m. vastus lateralis</i> of human volunteers before, during, and after ingestion of a fat-rich diet (●) or a carbohydrate-rich diet (□) for 4 weeks. (a) Plasma membrane fatty acid-binding protein (FABP<sub>pm</sub>); (b) fatty acid translocase (FAT)/CD36; (c) cytosolic fatty acid-binding protein (FAPB<sub>c</sub>). AU, arbitrary units. Values are means with their standard errors represented by vertical bars. Mean values were significantly different from those at 0 weeks on the corresponding diet: *<i>P</i> < 0.05.
FABPpm content in skeletal muscle, but in males only. A
higher FABPpm (B Kiens, C Roepstorff, JFC Glatz, A Bonen,
P Schjerling, J Knudsen and JN Nielsen, unpublished
results). These data suggest that the greater reliance on
plasma LCFA as an energy substrate during exercise in
endurance-trained males compared with untrained males
stimulates the up-regulation of the FABPpm protein in
skeletal muscle. Alternatively, the higher FABPpm in
endurance-trained males compared with untrained males
may induce the greater reliance during exercise on blood-
borne fatty acids in endurance-trained males than in
untrained males.

Regulation of lipid-binding proteins

It may be hypothesized as to why dietary interventions but
not exercise training can lead to such marked overall
responses in the expression of lipid-binding proteins as
those illustrated earlier. When a fat-rich diet is consumed,
the expression of several lipid-binding proteins is up-
regulated. In addition, consumption of a fat-rich diet also
enhances the β-oxidative enzyme capacity of skeletal
muscle (Helge & Kiens, 1997). Despite an increased
β-oxidative capacity after consumption of a fat-rich diet,
the LCFA taken up by muscle cells are obviously not all
metabolized, as re-esterification to triacylglycerol in the
muscle cell has been reported under such circumstances
(Kiens et al. 1987; Helge et al. 2001). It has been
suggested that the excess delivery and uptake of plasma
LCFA in skeletal muscle as compared with the oxidation
rate of LCFA, which is the case during consumption of a
fat-rich diet, will lead to the accumulation of cellular fatty
acids that are then available to stimulate up-regulation of
transcription of the different lipid-binding proteins. In
contrast, when exercise induces an increase in plasma
LCFA concentration and uptake into muscle, this increase
is paralleled by an increased lipid oxidation rate in the
mitochondria as a result of enhanced energy demand.
Under these circumstances accumulation of cellular fatty
acids may not take place and, hence, no up-regulation of
the lipid-binding proteins will occur. One exception is
FABPpm as exercise training induces an increase in
FABPpm content in skeletal muscle, but in males only. A
possible explanation of this gender-related effect of
exercise training on FABPpm could be the larger
dependence on plasma lipids during exercise in males than
in females. Females, in contrast, rely to some extent on other
lipid sources during exercise, such as intramuscular
triacylglycerols (Roepstorff et al. 2002; Steffensen et al.
2002).

The mechanism associated with the up-regulation of
lipid-binding proteins by training and diet has not been
fully elucidated, particularly in relation to FABPpm, and
certainly not in skeletal muscle. Recent studies in other
tissues have indicated that LCFA act as modulators of gene
expression (Grimaldi et al. 1999), and have suggested that
the effects of LCFA are mediated by activation of the
PPAR. The findings indicate that fatty acids of different
chain length and extent of saturation interact with PPAR
(Xu et al. 1999). After activation by LCFA, the PPAR/
retinoid X receptor heterodimer is able to bind to the
peroxisome proliferator response element found in a large
number of genes encoding for proteins involved in lipid
metabolism, such as FAT/CD36 (Van Bilsen et al. 2002)
and FABPp (Bensnard et al. 2002).

Interestingly, recent data on hepatocytes have shown that
the liver isozyme of FABPp induces the fatty acid
transfer to the nuclear receptors through direct protein–
protein interaction with PPARα and PPARγ, indicating that
FABPp exerts an active role in gene regulation
(Wolfrum et al. 2001). However, studies on the effects of
specific PPAR activators on mRNA levels of FAT/CD36
and mAspAT/FABPpm in liver of mice suggest that
expression of only FAT/CD36 mRNA, but not mAspAT/
FABPpm mRNA, is under the control of PPARα (Motojima
et al. 1998).

Conclusion

FABPpm has not been as well studied in rodent models or
in man as other fatty acid transporters such as FAT/CD36
and FABPc. The available evidence indicates that FABPpm
protein is identical to mAspAT. The protein has been
shown to be located in the mitochondria in several tissues,
including skeletal muscle, and also in other sites, including
the endothelial cell surface. The fatty acid content of the
diet is involved in modulating FABPpm protein expression
in human skeletal muscle, whereas exercise training only
seems to influence the FABPpm protein content in males,
as no effect of training status has been observed in
females.

Acknowledgements

We thank Professors Arend Bonen, Jan F.C. Glatz and
Lorraine Turcotte for kindly donating antibodies. We
acknowledge the skilled technical assistance of Irene Bech
Nielsen. The Danish National Research Foundation Grant
504-12 and The Danish Sports Research Council supported
the studies.

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