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Regulation of fatty acid transport by fatty acid translocase/CD36

Arend Bonen[1]*, Shannon E. Campbell[1,2], Carley R. Benton[2], Adrian Chabowski[1], Susan L. M. Coort[3], Xiao-Xia Han[1], Debby P. Y. Koonen[3], Jan F. C. Glatz[3] and Joost J. F. P. Luiken[3]

[1]*Department of Human Biology and Nutritional Sciences, University of Guelph, Guelph, Ontario N1G 2W1, Canada*
[2]*Department of Kinesiology, University of Waterloo, Waterloo, Ontario N2L 3G1, Canada*
[3]*Department of Molecular Genetics, CARIM, Maastricht University, 6200 MD Maastricht, The Netherlands*

Fatty acid (FA) translocase (FAT)/CD36 is a key protein involved in regulating the uptake of FA across the plasma membrane in heart and skeletal muscle. A null mutation of FAT/CD36 reduces FA uptake rates and metabolism, while its overexpression increases FA uptake rates and metabolism. FA uptake into the myocyte may be regulated (a) by altering the expression of FAT/CD36, thereby increasing the plasmalemmal content of this protein (i.e. streptozotocin-induced diabetes, chronic muscle stimulation), or (b) by relocating this protein to the plasma membrane, without altering its expression (i.e. obese Zucker rats). By repressing FAT/CD36 expression, and thereby lowering the plasmalemmal FAT/CD36 (i.e. leptin-treated animals), the rate of FA transport is reduced. Within minutes of beginning muscle contraction or being exposed to insulin FA transport is increased. This increase is a result of the contraction- and insulin-induced translocation of FAT/CD36 from an intracellular depot to the cell surface. Neither PPARα nor PPARγ activation alter FAT/CD36 expression in muscle, despite the fact that PPARα activation increases FAT/CD36 by 80% in liver. A novel observation is that FAT/CD36 also appears to be involved in mitochondrial FA oxidation, as this protein is located on the mitochondrial membrane and seems to be required to participate in moving FA across the mitochondrial membrane. Clearly, FAT/CD36 has an important role in FA homeostasis in skeletal muscle and the heart.

Cardiac: Myocytes: Skeletal muscle: Giant vesicles

Fatty acids (FA) are important for many biological functions. Thus, it could be argued that the entry of FA into the cell should be highly regulated, to ensure that normal cellular functions are not impeded by the uncontrolled entry of FA. However, there continues to be some disagreement as to whether FA enter the cell via diffusion or via a protein-mediated system at the sarcolemma involving one or more FA-binding proteins (Schaffer, 2002). There is little doubt that there is diffusional uptake of FA into the cell but, in complete contrast to the position that this process is the only one (Hamilton & Kamp, 1999), a number of investigators have shown that protein-mediated FA uptake (termed transport for convenience) does occur. The molecular evidence for such a process is now overwhelming and cannot be ignored (Bonen *et al.* 2002). The present paper reviews key studies that demonstrate that FA transport is a highly regulated process, involving one or more FA-binding proteins. In particular, the review will focus on skeletal muscle and the heart, tissues in which FA are important for FA homeostasis (i.e. skeletal muscle, which comprises 40% of the body mass and has a variable metabolic rate) and represent an important fuel (i.e. heart, which receives approximately 70% of its energy from FA oxidation). Moreover, the FA transport process appears to be disturbed in obesity and type 2 diabetes, and may therefore contribute to the FA-mediated impaired insulin signalling in skeletal muscle. A key protein involved in moving FA across the sarcolemma is FA translocase (FAT)/CD36. In the present overview regulation of this protein and its involvement in FA transport will be examined, although it is recognized that other FA transporters (plasma membrane-associated FA-binding protein and FA transport protein1) should also be considered. However, these transporters are beyond the scope of the present overview.

Fatty acid uptake, diffusion and transport

Within the past decade methods have become available to examine FA uptake in biologically-important mammalian

Abbreviations: FA, fatty acids; FAT, fatty acid translocase.

***Corresponding author:** Dr Arend Bonen, fax +1 519 763 59002, email abonen@uoguelph.ca

tissues. In particular, the use of giant vesicles, which can be prepared from many different tissues, provides a means for examining the uptake of FA across the plasma membrane. This preparation has been fully characterized in skeletal muscle and the heart (Bonen *et al*. 1998; Luiken *et al*. 1999), and has also been used for studies in liver and adipose tissues (Luiken *et al*. 2001, 2002*a*).

While FA uptake occurs in an approximately linear fashion when the total FA concentrations are plotted *v*. FA uptake, it is important to realize that FA are bound to albumin, from which they have to dissociate to be taken up. Thus, it is the unbound FA fraction that needs to be examined in relation to its uptake. Such an investigation appeared to indicate that uptake can reach saturation, and, indeed, it can also be inhibited (Bonen *et al*. 1998; Luiken *et al*. 1999), suggesting involvement of binding proteins at the plasma membrane. When the FA uptake is corrected for this component a fully saturable FA transport curve is observed (Fig. 1).

Fatty acid translocase/CD36 and fatty acid transport

FAT/CD36, when overexpressed in muscle, results in an increased rate of FA uptake and increased rate of FA metabolism (Ibrahimi *et al*. 1999). Conversely, in FAT/CD36 null mice FA uptake is reduced (Febbraio *et al*. 1999) and is reconstituted when FAT/CD36 is re-expressed (Brinkman, 2003). These studies provide molecular evidence that FAT/CD36 is intimately involved in the regulation of FA uptake across the plasma membrane and the subsequent metabolism of this substrate. Thus, understanding how this transport protein is regulated is important given the importance of FA as a fuel for heart and muscle, and that in recent years alterations in FA metabolism have been linked to insulin resistance in skeletal muscle.

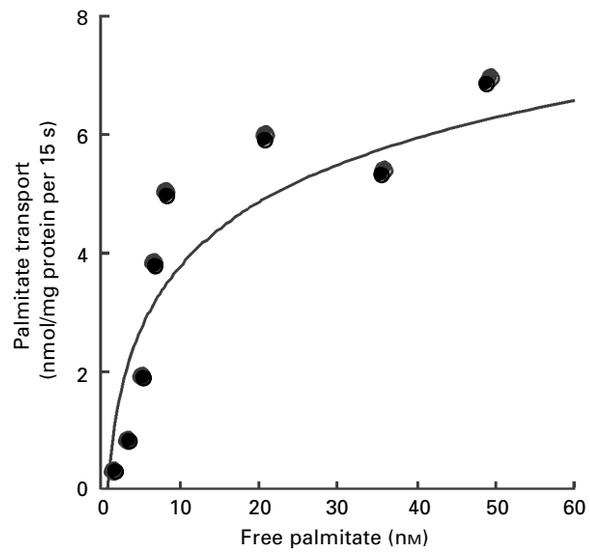

Fig. 1. Palmitate transport into giant sarcolemmal vesicles. The concentration of 'free' (non-protein-bound) palmitate is plotted *v*. the palmitate transport rate. Palmitate transport demonstrates saturation and can be inhibited (not shown). (Redrawn from Bonen *et al.* 1998.)

Long-term alterations in the regulation of FA uptake across the sarcolemma can conceivably occur via (a) an altered expression of FAT/CD36 and (b) possibly a subcellular redistribution of this protein, either in the presence or absence of its altered expression. The latter mechanism can occur because FAT/CD36 is present both at the sarcolemma and in an intracellular depot in skeletal muscle (Bonen *et al*. 2000; Luiken *et al*. 2002*b*) and in the heart (Luiken *et al*. 2002*c*). This distribution is reminiscent of the subcellular compartmentation of GLUT4.

Efforts have been focused on whether FAT/CD36 expression can be regulated, by examining skeletal muscle under conditions when this factor is known to alter its capacity for fatty acid metabolism. Hence, FA transport and FAT/CD36 expression were examined in streptozotocin-induced diabetic rats, obese Zucker rats, rats treated with leptin for 14 d and after chronic muscle stimulation (7 d). With streptozotocin-induced diabetes (Luiken *et al*. 2002*a*) and with chronic muscle contraction (Bonen *et al*. 1999) FAT/CD36 expression was increased, as was the plasmalemmal content of FAT/CD36, although total expression and plasmalemmal content did not necessarily increase in parallel (see streptozotocin-treated group; Fig. 2(a)). Surprisingly, in obese Zucker rats there was an increase in FA transport in muscle, while there was no alteration in the expression of FAT/CD36. However, interestingly, an increase was observed in the plasmalemmal FAT/CD36 (Luiken *et al*. 2001; Fig. 2(d)), which accounted for the increased rate of FA transport.

In a number of studies reduced rates of FA transport have also been observed. Reduced FA transport was first observed in animals treated with leptin for 14 d; FAT/CD36 expression and its presence at the sarcolemma were reduced (Fig. 2(c)). More recently, it was observed that in disused muscle (7 d denervation) FAT/CD36 expression is not altered but plasmalemmal FAT/CD36 is lowered, resulting in a reduction in FA transport (Fig. 2(e); DPY Koonen, CR Benton, Y Arumugam, NN Tandon, J Calles-Escandon, JFC Glatz, JJFP Luiken and A Bonen, unpublished results).

Acute regulation of FA transport by FAT/CD36 (i.e. within minutes) has now also been shown. In contracting muscle (for 30 min) FA transport was increased as a result of the translocation of FAT/CD36 from an intracellular depot to the plasma membrane. Presumably, this mechanism is required to support the increased delivery of FA to the mitochondria to aid in the production of ATP. Surprisingly, insulin also translocates FAT/CD36 from an intracellular depot to the plasma membrane (Luiken *et al*. 2002*b*,*c*). The net effect of this process is an increase in FA esterification, while concomitantly FA oxidation is reduced (Luiken *et al*. 2002*b*). Thus, common physiological stimuli such as muscle contraction not only regulate GLUT4 translocation but also the translocation of FAT/CD36.

The involvement of insulin led to an investigation of whether FA transport is altered in human skeletal muscle. Indeed, it was found that FA transport was markedly up regulated in obese individuals and in those with type 2

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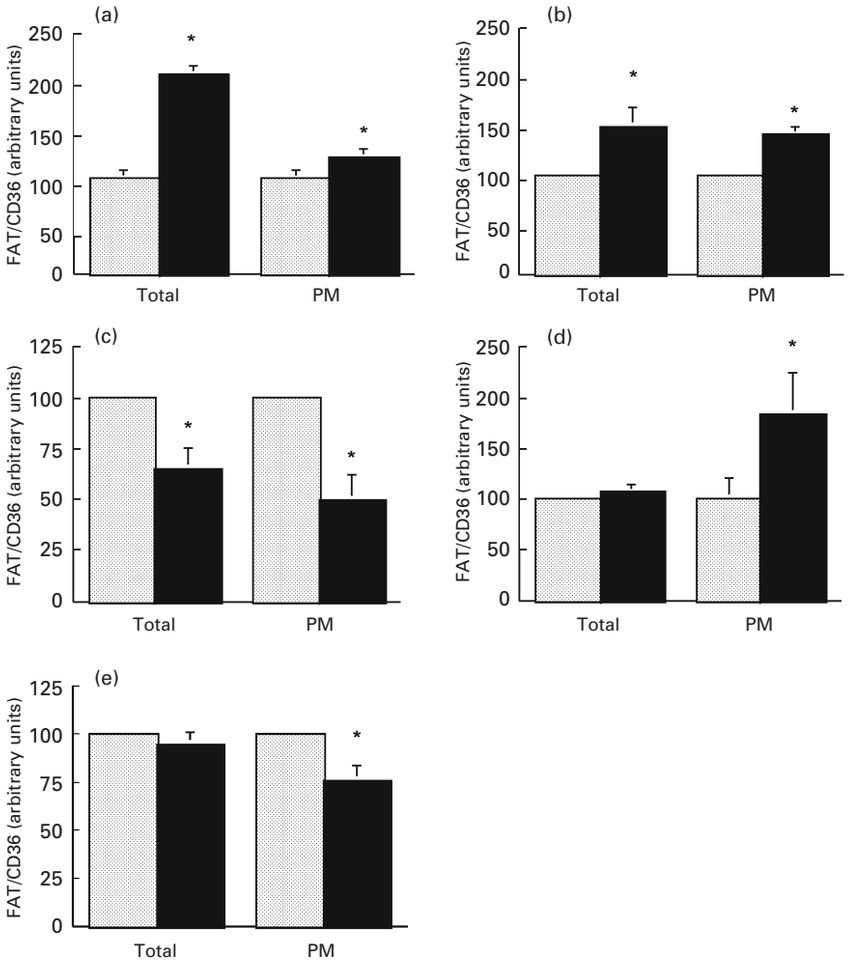

Fig. 2. Fatty acid translocase (FAT)/CD36 in muscle homogenates (total) and at the plasma membrane (PM) in (a) muscle from streptozotocin (STZ)-induced diabetic rats (■), (b) 7 d chronically-stimulated muscle (■), (c) muscle from rats treated with leptin for 14 d (■), (d) obese Zucker rat muscle (■) and (e) 7 d denervated muscle (■). (▨), Control. Values are means with their standard errors represented by vertical bars. Mean values were significantly different from control values: $^*P < 0{\cdot}05$. (a, b), Total FAT/CD36 expression is increased leading to an increase in plasma membrane FAT/CD36; (c), FAT/CD36 expression is decreased, leading to the decrease in plasma membrane FAT/CD36; (d, e), Total FAT/CD36 expression was not altered, but the plasma membrane FAT/CD36 was either increased (d) or decreased (e), indicating that FAT/CD36 can traffic between the plasma membrane and its intracellular depot(s), even when the total FAT/CD36 (plasma membrane + intracellular) is not altered. In all instances when plasma membrane FAT/CD36 was increased palmitate transport was also increased (a, b, d) and, conversely, when plasma membrane FAT/CD36 was decreased palmitate transport was also decreased or increased (c, e; transport data are not shown). (Data are redrawn from Luiken *et al.* 2002*a* (a), Steinberg *et al.* 2002 (c), Luiken *et al.* 2001 (d) and DPY Koonen, CR Benton, Y Arumugam, NN Tandon, J Calles-Escandon, JFC Glatz, JJFP Luiken and A Bonen, unpublished results (b, e).)

diabetes (A Bonen, JFC Glatz and JJFP Luiken, unpublished results). The underlying mechanism appeared not to be an increased expression of FAT/CD36 but an increased sarcolemmal content of this protein. These results parallel closely those observed in obese Zucker rats. Thus, in insulin-resistant states it appears that the signalling machinery involved in the trafficking of FAT/CD36 between the plasma membrane and the intracellular depot is impaired. The specific signalling molecules involved have not been identified.

PPAR do not regulate the expression of fatty acid translocase/CD36

PPAR have been shown to control the expression of many gene products involved in the regulation of FA

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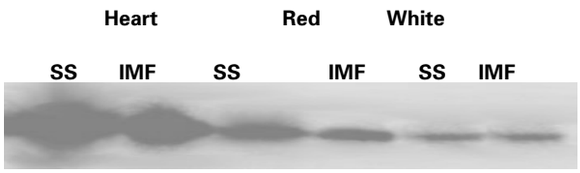

Fig. 3. Representative Western blot demonstrating the presence of fatty acid translocase/CD36 in the subsarcolemmal (SS) and intermyofibrillar mitochondria (IMF) in heart, and red and white muscle (SE Campbell and A Bonen, unpublished results).

metabolism. PPARα governs the expression of genes regulating FA catabolism and seems to be found predominantly in metabolically-active tissues such as the liver, skeletal muscle and heart (Lee *et al*. 2003). PPARγ is found mainly, although not exclusively, in brown adipose tissue and white adipose tissue, and tends to regulate adipogenic processes (Lee *et al*. 2003). PPARβ is expressed ubiquitously and its function remains unclear. A key gene containing a PPAR response element, which can therefore be regulated by PPARα, is FAT/CD36 (Teboul *et al*. 2001). This finding prompted an investigation of whether FAT/CD36 expression is altered by activating PPARα and -γ, using WY 14 643 and rosiglitazone respectively. Even though WY 14 643 markedly increased FAT/CD36 expression in the liver (+ 80 %), PPARα stimulation failed to alter FAT/CD36 expression in muscle. Similarly, activation of PPARγ failed to alter FAT/CD36 expression in muscle (CR Benton, JFC Glatz, JJFP Luiken and A Bonen, unpublished results).

A novel function for fatty acid translocase/CD36?

An apparently novel role for FAT/CD36 has recently been revealed (SE Campbell and A Bonen, unpublished results). This protein is found on the mitochondrial membrane (i.e. heart >> red muscle > white muscle mitochondria; Fig. 3). The reason for this is at present unclear, but it might suggest that FAT/CD36 is also involved in regulating FA oxidation along with the well-known regulator carnitine palmitoyltransferase I.

Summary

It is apparent that FA uptake into skeletal muscle and into the heart is a carefully regulated process. Many studies have now shown that the protein FAT/CD36 is involved in this process. Its absence or presence at the plasma membrane alters the rate of FA transport correspondingly. The fact that FAT/CD36 expression and/or subcellular localization are altered to increase FA transport into the cell identifies FAT/CD36 as a key therapeutic target.

Acknowledgements

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