REVIEW ARTICLE

New insights into long-chain fatty acid uptake by heart muscle: a crucial role for fatty acid translocase/CD36

Joep F. F. BRINKMANN*, Nada A. ABUMRAD†, Azeddine IBRAHIMI†, Ger J. VAN DER VUSSE* and Jan F. C. GLATZ*¹

*Cardiovascular Research Institute Maastricht (CARIM), Department of Physiology, Maastricht University, P.O. Box 616, 6200 MD Maastricht, The Netherlands, and

†Department of Physiology & Biophysics and Institute for Molecular Cardiology, State University of New York at Stony Brook, Stony Brook, NY 11794, U.S.A.

Long-chain fatty acids are an important source of energy for several cell types, in particular for the heart muscle cell. Three different proteins, fatty acid translocase (FAT)/CD36, fatty acid transport protein and plasma membrane fatty acid binding protein, have been identified as possible membrane fatty acid transporters. Much information has been accumulated recently about the fatty acid transporting function of FAT/CD36. Several experimental models to study the influence of altered FAT/CD36 expression on fatty acid homeostasis have been identified or developed, and underscore the importance of FAT/CD36 for adequate fatty acid transport. These models include the FAT/CD36 null mouse, the spontaneously hypertensive rat and FAT/CD36-deficient humans. The fatty acid transporting role of FAT/CD36 is further demonstrated in mice overexpressing muscle-specific FAT/CD36, and in transgenic mice generated

using a genetic-rescue approach. In addition, a wealth of information has been gathered about the mechanisms that regulate FAT/CD36 gene expression and the presence of functional FAT/CD36 on the plasma membrane. Available data also indicate that FAT/CD36 may have an important role in the aetiology of cardiac disease, especially cardiac hypertrophy and diabetic cardiomyopathy. This review discusses our current knowledge of the three candidate fatty acid transporters, the metabolic consequences of alterations in FAT/CD36 levels in different models, and the mechanisms that have been identified for FAT/CD36 regulation.

Key words: cardiac hypertrophy, fatty acid metabolism, lipids, membrane transporter.

INTRODUCTION

Long-chain fatty acids (FA) are important to the cell as a source of energy, and they are essential components for the synthesis of triacylglycerols (TAGs), which can be used for storage of metabolic energy. Moreover, they are building blocks for phospholipids. FA, their CoA derivatives and their metabolites (e.g. prostaglandins, leukotrienes and thromboxanes) are able to regulate gene expression, as well as enzyme, ion channel and membrane receptor activity, directly or indirectly [1–11]. Under normal physiological conditions, approx. 60–90% of the energy that is required for adequate contraction of the heart is generated by mitochondrial oxidation of FA [11,12]. Because cardiac myocytes, like most cells except for hepatocytes and adipocytes, can synthesize or store FA in limited amounts only, the heart relies heavily on the continuous supply of these compounds from the circulatory system.

FA are poorly soluble in aqueous environments, and therefore are supplied to the heart either complexed to plasma albumin or esterified in the TAG core of circulating lipoproteins [13]. Unbound FA, formed by dissociation of the albumin–FA complexes or hydrolysis of TAGs by the action of lipoprotein lipase at the luminal surface of the coronary vascular endothelium, are transferred from the capillary lumen through the capillary endothelium and the interstitial space to the cardiac myocyte. Intracellularly, FA are bound to cytoplasmic fatty acid

binding proteins [FABP_cs; heart-type FABP_c (H-FABP_c) in the cardiomyocyte], which allows transport to their site of action [14,15]. In the cardiomyocyte, FA are converted into fatty acyl-CoAs, which can bind to acyl-CoA binding proteins [16,17]. The bulk of the CoA-activated FA molecules are taken up by mitochondria via a carnitine-mediated process, and are degraded in the β -oxidation pathway followed by the citric acid cycle. An additional site for the β -oxidation of (very) long-chain acyl-CoA is present in peroxisomes. The remaining part of the fatty acyl moiety is incorporated into the esterified lipid pool, of which TAGs and phospholipids are the main components [11].

Tissue energy demands are constantly changing, and cells must match substrate supply with demand [18–20]. Regulation at a rate-limiting membrane transport step is, in general, the most effective way to achieve this [9,21]. Knowledge about the ways by which FA cross cellular membranes is evolving, and will be discussed in detail in the present review. To describe clearly sarcolemmal FA transport in the heart, this review will summarize current knowledge about the common mechanism by which several tissues, not only the heart, transport FA. After describing the proteins initially identified as potential FA transporters, this article will focus on the role of fatty acid translocase (FAT)/CD36 in metabolite homeostasis, and on the mechanisms known to regulate functional FAT/CD36 expression. Several genetic models (both generated and spontaneously occurring models) are now available for FAT/CD36, and these demonstrate that

Abbreviations used: BMIPP, 15-(*p*-iodophenyl)-3(*R,S*)-methyl pentadecanoic acid; FA, long-chain fatty acids; FABP, fatty acid binding protein; FABP_c, cytoplasmic FABP; H-FABP_c, heart-type FABP_c; FABP_{PM}, plasma membrane FABP; FAT, fatty acid translocase; FATP, fatty acid transport protein; PPAR, peroxisome proliferator activator receptor; SHR, spontaneously hypertensive rat; TAG, triacylglycerol; VLACS, very-long-chain fatty acid acyl-CoA synthetase.

¹ To whom correspondence should be addressed (e-mail glatz@fys.unimaas.nl).

impairment of membrane FA transport results in a serious disturbance of metabolic homeostasis.

CANDIDATE FA TRANSPORTERS

It has been clearly shown that FA can diffuse through cellular membranes by a spontaneous diffusional flip-flop process. The use of pyranin fluorescence and both unilamellar vesicles and isolated rat adipocytes demonstrated that uncharged FA move quickly across phospholipid bilayers [22,23]. Cellular studies, however, provided evidence for the presence of an additional, high-affinity, protein-facilitated component in the transport of FA across the cellular membrane in adipose tissue [24,25], liver [26,27], skeletal muscle and heart [28]. This led to the identification of three major potential FA transporting proteins: plasma membrane FA binding protein (FABP_{PM}) [29], FAT/CD36 [30,31] and FA transport protein (FATP) [32]. All three proteins are present in the heart and were found to be associated with the plasmalemma of cardiomyocytes [33,34].

FAT/CD36

FAT/CD36 was identified as a candidate FA transporter based on its binding of both DIDS (4,4'-di-isothiocyanostilbene-2,2'-sulphonate) and reactive sulphydryl-*N*-succinimidyl FA esters [30,31]. These compounds were inhibitors of FA uptake in isolated adipocytes [30]. The corresponding cDNA was isolated from a rat adipose tissue library and called FAT (for FA translocase) [35], the rat homologue of the ubiquitously expressed human platelet CD36 (also known as PASIV, GPIV and GPIIb in the earlier literature [36]). FAT/CD36 is a heavily glycosylated integral membrane protein, with an apparent molecular mass of 88 kDa. The protein was initially isolated from platelet membranes as a thrombospondin receptor [37]. FAT/CD36 is expressed abundantly in tissues active in FA metabolism, such as heart, skeletal muscle, adipose tissue and intestine [34,35,38]. Its expression profile corresponds with a role in FA uptake, and its expression is modulated by conditions that alter lipid metabolism, such as diabetes mellitus and high-fat feeding [34,39,40]. Remarkably, FAT/CD36 expression is absent or low in the liver [34]. Expression of FAT/CD36 in fibroblasts that normally lack the protein resulted in an increase in FA uptake [41]. It has to be mentioned, however, that a similar approach using a rat heart-muscle-derived cell line did not yield a direct correlation between the FAT/CD36 protein and FA uptake [42]. The possible absence of obligatory partner proteins for FA transport in the cells used, with H-FABP_c as a likely candidate, might explain this observation.

FATP

FATP, a 646-amino-acid integral membrane protein with an apparent molecular mass of 63 kDa, was identified as a candidate FA transporter by the use of an expression/cloning strategy. A 3T3-L1 adipocyte cDNA library was brought to expression in COS7 cells, and screening for uptake of fluorescent FA yielded two classes of cDNA clones. One of these encoded the novel protein FATP, and the other coded for an isoform of FA acyl-CoA synthetase [32]. FATP is expressed ubiquitously, and relatively high levels are present in the brain [43,44]. FATP has been shown to have at least one transmembrane and multiple membrane-associated domains [45]. The cloning of the first FATP (FATP1) led to the discovery of a family of evolutionarily conserved proteins, and the presence of at least five different

FATP genes in the mouse. These isoforms, FATP1–FATP5, have different tissue-specific expression/distribution patterns [46]. Analogous to FAT/CD36, expression of FATP1 in fibroblasts, cells that normally lack the protein, resulted in a several-fold increase in the FA uptake rate. The enhancement of FA uptake was similar to the enhancement of uptake of very-long-chain fatty acids in these cells [32]. Other members of the FATP family also increased FA uptake rates in cultured cell systems [46]. In addition, disruption of the FATP1 homologue *FAT1* (encoding Fat1p) in the yeast *Saccharomyces cerevisiae* impairs both FA uptake and growth on media containing FA under conditions where *de novo* FA synthesis is inhibited [47,48]. Complementation of the *FAT1* mutant yeast strains with mouse FATP1 restored FA uptake and reversed the growth deficiencies [48]. Interestingly, FATP1 expression is regulated by both insulin and peroxisome proliferator activator receptors (PPARs) [49–51], and both a functional insulin response sequence and a functional PPAR binding site (peroxisome proliferator responsive element) have been identified in the murine FATP1 promoter [52,53]. Moreover, insulin was shown recently to induce the translocation of FATPs from an intracellular perinuclear compartment to the plasma membrane in adipocytes [54].

All FATP homologues known to date possess a highly conserved domain which contains an AMP binding motif. This domain is commonly present in proteins that bind ATP and form adenylated intermediates, such as very-long-chain fatty acid acyl-CoA synthetases (VLACSs) and members of the luciferase family [46,55–57]. FATP1 mutated in the ATP binding consensus region loses its ability to enhance FA uptake [58,59]. At the protein level, FATP1 has high identity (40% at the amino acid level) with VLACS [57]. Recently, FATP1 and FATP4 were reported to be VLACSs [60,61], and mouse FATP2 turned out to be the mouse homologue of a rat protein identified initially as VLACS [46]. Expression of FATP1 in COS1 cells increased the VLACS activity co-fractionating with the FATP1 protein [61]. Moreover, disruption of *FAT1*, the yeast FATP1 homologue, yields a strain with diminished VLACS activity, increased levels of unbound very-long-chain fatty acids and normal FA acyl-CoA synthetase activity [48,62,63]. These findings suggest that VLACS activity is the main activity of FATP. It has been proposed, however, that FATP might be a bifunctional molecule, with separate transport and esterification activities [48,64]. Understanding the precise function of FATP in FA uptake and metabolism awaits the development of a model in which FA transport can be measured separately from the esterification activity of FATP. Such a model has been obtained recently for the yeast FATP1 homologue Fat1p [65]. Here *FAT1* mutant strains with distinguishable FA transport and VLACS activities were constructed using site-directed mutagenesis. The documented findings imply that Fat1p has separate FA import and very-long-chain fatty acid activation activities [65].

FABP_{PM}

The third of the candidate FA transporters, FABP_{PM}, was identified by its binding of radiolabelled oleate and purified from solubilized rat hepatocyte membranes by oleate-agarose affinity chromatography [29]. This 40 kDa protein is loosely associated with the plasma membrane and was shown to be the membrane-bound form of the mitochondrial isoform of aspartate aminotransferase [27,66]. To date, three lines of indirect evidence support the involvement of FABP_{PM} in FA transport. First, an antibody raised against FABP_{PM} or mitochondrial aspartate aminotransferase could inhibit FA uptake in a variety of cell

Figure 1 Hypothetical model for the involvement of FA transporters in mammalian FA metabolism

After dissociating from their albumin binding sites or liberation from lipoproteins by lipoprotein lipase (not shown), FA could either partition directly into the phospholipid bilayer or bind to one of the proteins suggested to function as FA transporters, i.e. FATP, FABP_{pm} and FAT/CD36. A wealth of evidence, discussed in detail in the present review, suggests that the majority of FA is taken up via a FAT/CD36-mediated mechanism in skeletal and heart muscle and in adipose tissue. A smaller fraction of the FA passes through the cell membrane probably by spontaneous diffusional flip-flop or via facilitation by proteins other than FAT/CD36. FATP might be involved in FA uptake by functioning as a VLACS, or as an alternative transmembrane FA transporter with VLACS activity. FABP_{pm}, as a membrane-bound protein, could facilitate FA transport by trapping extracellular FA and channelling them towards FAT/CD36 and/or FATP. In order to exert its function, it is possible that FAT/CD36 may associate with FABP_{pm} and/or FATP through protein–protein interactions, to form a cluster that is active in FA uptake. At the inner side of the cell membrane, FA bind to FABP_c, and, after their activation into acyl-CoAs, to acyl-CoA binding protein (ACBP).

types, including hepatocytes, cardiomyocytes and adipocytes [38,66,67]. Secondly, expression of FABP_{pm} in *Xenopus laevis* oocytes [68] and in fibroblasts [67] results in increased FA uptake rates. The third line of evidence derives from the finding that a variety of conditions where FA transport is altered show concomitant changes in tissue FABP_{pm} levels. For instance, in parallel with an increase in FA uptake, FABP_{pm} is induced during differentiation of 3T3-L1 fibroblasts into adipocytes [67]. Both FA utilization and FABP_{pm} expression are increased during fasting [69], whereas endurance training, a condition which is associated with an increase in skeletal muscle FA uptake, also increases FABP_{pm} levels [70]. Expression of FABP_{pm} in diabetic and obese Zucker rats is similarly increased in heart, skeletal muscle and adipose tissue, and these changes appear to generally correlate with changes in FA uptake rates [71,72]. Direct evidence for the involvement of FABP_{pm} in FA transport, however, awaits further research.

Synergistic action of the three FA transporters

It has been proposed that FAT/CD36, FABP_{pm} and FATP might act synergistically in FA uptake, and a potential model for the involvement of the three candidate FA transporters based on current knowledge of protein characteristics is shown in Figure 1 [73]. Supporting the synergistic concept is the observation that

while FA uptake by muscle giant sarcolemmal vesicles could be inhibited by both sulpho-*N*-succinimidyl oleate (an inhibitor specific for FAT/CD36) and an anti-FABP_{pm} antiserum, applying both agents together did not result in a further decrease in FA uptake [38].

PHYSIOLOGICAL ROLE OF FAT/CD36 IN FA UPTAKE

Mouse models with altered FAT/CD36 expression

Knowledge about the role of FAT/CD36 in FA transport has accumulated quickly since the generation of transgenic mouse lines (Table 1). The first of these models is a mouse that overexpresses FAT/CD36 by 2–4-fold in heart and skeletal muscle [74]. These mice develop normally, but during the first 6 months of growth their body weight is 13–15% lower compared with their wild-type littermates. One of the major findings was that isolated soleus muscles from these mice show enhanced muscle FA oxidation rates upon stimulated contraction. While stimulated muscles from wild-type mice showed a 1.9-fold rise in FA oxidation compared with the non-stimulated condition, muscles from transgenic mice showed a 5.6-fold increase [74]. The increased ability to oxidize FA was accompanied by significant decreases in plasma TAG and FA levels, combined with a general decrease in body fat. These combined findings are indicative of increased muscle FA uptake and oxidation and decreased FA availability for hepatic TAG synthesis. The decrease in

Table 1 Overview of transgenic mouse models generated for FAT/CD36: effect of FAT/CD36 expression on fasted blood parameters

WT, statistically not different from wild-type levels; ↓, lowered compared with wild-type levels; ↑, increased compared with wild-type levels. Data were obtained from [74,75] and J. F. F. Brinkmann, N. A. Abumrad, W. Jou, J. A. Johnson, G. J. Van der Vusse, J. F. C. Glatz and A. Ibrahim (unpublished work). Mice were fed a standard chow diet *ad libitum*, and plasma parameters were determined after an overnight fast.

Mouse model	Plasma FA	Plasma TAG	Blood glucose
FAT/CD36 overexpression	↓	↓	↑
FAT/CD36 null	↑	↑	↓
FAT/CD36 rescue	WT	WT	WT

plasma total TAG levels was mainly in the very-low-density lipoprotein fraction. Plasma cholesterol levels were decreased in the FAT/CD36-overexpressing mice. Blood glucose levels were increased, most probably due to an FA-induced sparing of heart and muscle glucose utilization. The increase in glucose was accompanied by an increase in plasma insulin levels [74].

A mouse model deficient in FAT/CD36 was generated by targeted gene disruption [75]. Most interestingly, these mice exhibit a phenotype that is opposite to the phenotype of the FAT/CD36-overexpressing mice in several respects. Upon fasting, FAT/CD36 null mice show significantly increased plasma levels of FA, TAG and cholesterol. This is accompanied by a decrease in blood glucose. These findings are indicative of decreased tissue FA utilization and increased tissue glucose utilization, exactly opposite to the findings in the FAT/CD36-overexpressing mice. TAG associated with very-low-density lipoprotein was found to be increased in the FAT/CD36 null mice, while intermediate-density lipoprotein/low-density lipoprotein particles were found to be relatively TAG-poor. The increase in cholesterol was reflected in the high-density lipoprotein fraction, and is most probably an effect caused by the disturbance of peripheral FA utilization [75]. FA uptake in the FAT/CD36 null mice was assessed directly by injecting an iodinated FA analogue, [¹²⁵I]BMIPP [15-(*p*-iodophenyl)-3(*R,S*)-methyl pentadecanoic acid], into a lateral tail vein. This analysis showed that FAT/CD36 deficiency leads to severe decreases in FA uptake by the heart, skeletal muscle and adipose tissue of 40–80%. FAT/CD36 null mice did not show alterations of FA uptake in the liver, an organ with absent or very low FAT/CD36 expression. BMIPP is an FA analogue that is metabolized slowly, and these results were confirmed using the readily oxidized FA analogue 15-(*p*-iodophenyl)pentadecanoic acid [76]. Moreover, *in vitro* analysis showed that isolated adipocytes lack the high-affinity component of FA transport that is present in wild-type cells [75].

Recently, a third mouse model was generated in which FAT/CD36 is re-expressed in heart and skeletal muscle on the FAT/CD36 null background. These mice, developed using the mouse muscle creatine kinase promoter, show rescue of the main abnormalities mentioned above. Remarkably, they showed almost complete normalization of plasma parameters. Fasted plasma FA, TAG and glucose levels were not different from those of the wild type. FA oxidation by isolated soleus muscles, which is prominently reduced in the FAT/CD36 null mouse, was also normalized (J. F. F. Brinkmann, N. A. Abumrad and A. Ibrahim, unpublished work). These results show that the reported metabolic phenotypes of the FAT/CD36 null and FAT/CD36-overexpressing mice are direct and gene-specific effects. The absence of FAT/CD36 thus leads directly to a severe

defect in FA transport in the heart, skeletal muscle and adipose tissue.

Metabolic abnormalities linked to FAT/CD36 deficiency in the spontaneously hypertensive rat (SHR)

Additional information about the consequences of FAT/CD36 deficiency was obtained with a fourth rodent model. The SHR is the most widely studied animal model of essential hypertension, and several disturbances of lipid and carbohydrate metabolism in this rat have been reported [77–82]. Genomic screening for loci linked to insulin and catecholamine action in the SHR identified two quantitative trait loci for defective insulin action. One was localized on chromosome 4 and the other on chromosome 12. Defects in both glucose and FA metabolism mapped to the same locus on chromosome 4 [83]. Congenic mapping and microarray screening identified FAT/CD36 on rat chromosome 4 as a defective SHR gene at the peak of linkage to these quantitative trait loci. The SHR FAT/CD36 cDNA was shown to contain multiple sequence variants, which reflect unequal genomic recombination of a duplicated ancestral gene. The resulting FAT/CD36 deficiency was demonstrated by the fact that the FAT/CD36 protein could not be detected in SHR (SHR/NIH) adipocyte membranes [84]. Surprisingly, an SHR-derived strain that has been maintained in Japan since 1966 (SHR/Izm) was shown not to contain the FAT/CD36 deletion mutation, which also holds true for the stroke-prone SHR (SHRP; both the NIH strain and the Japanese strain SHRP/Izm) [85–87]. These findings are especially remarkable, as both the SHR/NIH and SHR/Izm strains develop insulin resistance. The two strains diverged, however, a long time before they were fully inbred. As a result, they differ throughout their entire genomes, and thus cannot be compared directly [85,88].

To analyse directly the consequences of FAT/CD36 deficiency in the SHR/NIH strain, an SHR–Brown Norway congenic strain was developed. A segment of the Brown Norway chromosome 4 (including FAT/CD36) was transferred on to the SHR background. This rat showed reductions in blood pressure and dietary induced glucose intolerance, hyperinsulinaemia and hyperlipidaemia [87]. More direct was the analysis of a FAT/CD36 transgenic SHR, in which the protein was re-introduced by a genetic-rescue approach. Insulin resistance was improved and plasma FA and insulin levels were lowered in this rat. Hypertension, in contrast, was not ameliorated; although this condition might be linked to FAT/CD36 deficiency, it is likely to be the consequence of multiple influences [88].

As with the FAT/CD36 null mice, the absence of FAT/CD36 in the SHR leads to decreased FA transport in the heart and adipose tissue. Although relatively modest decreases were found using BMIPP, *in vivo* measurements with the glucose tracer 2-[¹⁸F]fluorodeoxyglucose showed an impressive compensatory increase in glucose uptake in heart and diaphragm muscle [89]. Basal glucose uptake is also, but to a lesser extent, increased in adipose tissue and intercostal and hip muscles. The FA transport defect was confirmed using both isolated adipocytes and cardiomyocytes, and glucose uptake is normalized in the congenic FAT/CD36 re-expressing SHR–Brown Norway rat referred to above [89]. That the effects mentioned are caused specifically by the absence of FAT/CD36 is also indicated by the fact that rats fed a mixture of short- and medium-chain fatty acids showed almost complete normalization of heart glucose uptake and plasma insulin levels [89]. Uptake of short- and medium-chain fatty acids is not dependent on FAT/CD36, because the saturable, facilitated component of FA uptake does not recognize, and in addition highly purified FAT/CD36 does not bind, these

fatty acids [24,89]. As might be expected from the characterization of the transgenic FAT/CD36 re-expressing rescued SHR [88], the dietary approach did not have an influence on the high blood pressure present in the SHR [89].

Effects of FAT/CD36 deficiency on cardiac function

Several mutations leading to FAT/CD36 deficiency in humans have been reported. The prevalence of this deficiency varies from 0.3 to 11%, and higher incidences are found in Asian and African populations [90]. Recently, an extensive study by Tanaka et al. [91] analysed 47 patients with a total defect in myocardial BMIPP uptake. All these patients were shown to carry a mutated FAT/CD36 gene, and the FAT/CD36 protein was not detectable on either platelet or monocyte membranes. In total, nine segregated mutations were found [91]. It has been proposed that FAT/CD36 deficiency in the human heart, leading to a severe defect in FA transport as in the FAT/CD36 null mouse, might be the basis for the development of hypertrophic cardiomyopathy. This hypothesis was based mainly on the fact that about 40% of patients with hypertrophic cardiomyopathy carry mutations in FAT/CD36 [92]. The more extensive study mentioned above, however, did not support this hypothesis, and indicated that a less specific relationship might exist between FAT/CD36 deficiency and increased susceptibility to a variety of heart diseases [91].

Remarkably, the development of cardiac hypertrophy in the SHR appears to be mediated by FAT/CD36. A dietary rescue approach by feeding a diet supplemented with short- and medium-chain fatty acids not only lowered hyperinsulinaemia as described above, but also alleviated cardiac hypertrophy in the SHR. As the short-/medium-chain fatty acid-supplemented diet restored FA oxidation, this observation links FA-generated energy to the symptoms of hypertrophy [89]. Also notable is the observation that the hearts of FAT/CD36 null mice develop dilated cardiomyopathy [93].

Additional information on the functional consequences of FAT/CD36 deficiency in the heart was recently obtained by further analysis of the FAT/CD36 null mouse. The tolerance to ischaemia/reperfusion of isolated hearts from FAT/CD36 null mice was shown to be severely compromised compared with that of hearts from their wild-type counterparts. Hearts from mice re-expressing FAT/CD36 showed an ischaemic tolerance comparable with that of wild-type hearts, and it was thus concluded that the decreased tolerance is due to FAT/CD36 deficiency. Furthermore, dietary rescue with short- and medium-chain fatty acids resulted in normalization of the ischaemic tolerance of FAT/CD36 null hearts, and FAT/CD36-overexpressing hearts showed an increased tolerance to ischaemia. This led to the concept that cellular energy derived from FAT/CD36-mediated FA uptake is an important determinant of ischaemic tolerance in the heart. In accordance with this, both heart and skeletal muscle energy contents in FAT/CD36 null mice are significantly reduced compared with those in wild-type tissues (H. Irie, I. Krukenhamp and A. Ibrahim, unpublished work). Not surprisingly, the endurance of FAT/CD36 null mice during forced exercise is significantly decreased. The absence of FAT/CD36 from the heart and skeletal muscle thus impacts on energy production, and the effects become apparent during heightened cellular demands such as ischaemia. Another important finding was that the end-diastolic pressure in hearts from FAT/CD36 null mice is significantly increased in the working heart preparation of Irie and co-workers (H. Irie, I. Krukenhamp and A. Ibrahim, unpublished work). Although the transgenic rescue of FAT/CD36 expression in the heart restored the tolerance to ischaemia, it did

not normalize the end-diastolic pressure. Because the rescue approach used only restored cardiomyocyte and not endothelial cell FAT/CD36 expression, it could be concluded that the lack of endothelial FAT/CD36 might lead to diastolic dysfunction (H. Irie, I. Krukenhamp and A. Ibrahim, unpublished work).

In conclusion, expression of FAT/CD36 has been linked to cardiac hypertrophy, tolerance to ischaemia and diastolic dysfunction in rodent models. These links cannot as yet be established directly in humans, although the effect of FAT/CD36 deficiency on FA transport in murine and human hearts seems to be of a comparable magnitude. A deficiency in FAT/CD36, however, is associated with a diversity of heart diseases in humans, and, accompanied by other unknown factors, cardiac hypertrophy might be involved in the development of these diseases [91]. Further exploration of the different rodent models might yield valuable information about the links between FAT/CD36 expression and heart function and failure.

FAT/CD36 EXPRESSION IS REGULATED BY SEVERAL MECHANISMS

Much effort has been invested to gain insight into the mechanisms by which FAT/CD36 expression and function are regulated. One of the first findings was that FAT/CD36 expression parallels the expression of the intracellular FABPs in muscle tissues, intestine and mammary gland [33,34,94,95]. In muscle tissues, the levels of both H-FABP_c and FAT/CD36 correlate with the capacity for FA oxidation, as their expression is high in the heart, lower in red skeletal muscle and lowest in white skeletal muscle, at both the mRNA and protein levels [34,40]. More interestingly, FAT/CD36 gene expression was shown to be altered under conditions that also modulate FA metabolism. For example, FAT/CD36 expression is induced in murine models of diabetes, during heart development and during the differentiation of pre-adipocytes into adipocytes [34,39,96]. Work with cultured adipocytes as model system showed that FAT/CD36 mRNA is strongly and reversibly induced by both saturated and unsaturated FA. Metabolism is not required for this induction, as shown by applying the non-metabolized FA analogue 2-bromopalmitate, and FAT/CD36 induction parallels the induction by FA of other genes involved in FA metabolism [1,96,97]. This effect is mediated, at least in part, by the lipid-activated nuclear receptors PPARs. Expression of the PPAR δ isoform in fibroblasts unresponsive to FA conferred FA-specific induction of FAT/CD36 [98], and two functional PPAR binding sites are present in the murine FAT/CD36 promoter [99]. These two functional bindings sites were shown to drive strong FAT/CD36 promoter activity in adipose cells [99]. The role of PPARs in the heart is underscored by the observation that the expression of several genes involved in FA catabolism is lowered in the hearts of PPAR α null mice. Also, fasting failed to induce most of these genes in the PPAR α -deficient heart [100]. Moreover, heart-specific overexpression of PPAR α results in a heightened expression of genes, including FAT/CD36, involved in FA uptake and oxidation pathways, combined with increased cardiac FA oxidation rates [101]. A recent study documented a time delay in the PPAR-mediated regulation of FAT/CD36 and suggested that FAT/CD36 activation by PPARs is indirect [102].

An additional mechanism for FAT/CD36 regulation was shown to be present at the level of translation in human monocyte-derived macrophages [103]. When cultivated in the presence of high glucose concentrations, these cells show a concentration-dependent increase in functional FAT/CD36 protein expression at their cell membrane. By using RNase protection assays and by fractionating polysome preparations,

Figure 2 Acute regulation of functional FAT/CD36 expression by protein translocation

In tissues expressing FAT/CD36, acute regulation of cellular FA uptake involves the cellular redistribution of FAT/CD36 to and from the plasma membrane. This redistribution can be triggered by insulin and by contraction (in muscle). Preliminary results indicate that the transductional mechanism for electrical activation is different from the mechanism for activation by insulin, and this Figure reflects the current view that the first is mediated by an as yet unidentified protein kinase (PKX) and the second by phosphatidylinositol 3-kinase (PI 3 kinase). IR, insulin receptor.

it was shown that this effect is due to a greater ribosomal loading of the FAT/CD36 transcript. Reporter gene assays with mutated FAT/CD36 5'-untranslated regions identified the corresponding primary mechanism to be regulation of the efficiency of re-initiation following translation of upstream open reading frames. The FAT/CD36 untranslated region, with a length of more than 200 bp, contains three upstream open reading frames, and the reporter assays indicated that re-initiation following translation of the first of these is responsible for the increase in translational efficiency under hyperglycaemic conditions [103]. Translational regulation in response to glucose has also been described in yeast [104], and glucose is also thought to regulate the translation of several other genes important in glucohomeostasis, such as those encoding proinsulin, glucokinase and fatty acid synthetase [105–107]. This type of FAT/CD36 regulation, presented as a link between diabetes and atherosclerosis, seems to be relevant especially for conditions associated with hyperglycaemia.

A third FAT/CD36 regulational mechanism is present at the protein level. Skeletal muscle, as a key FA oxidizer and a principal site for the removal of FA from the circulation, has a highly variable metabolic rate and should be able to adapt FA transport quickly so as to respond appropriately to changes in energy demand. By comparing resting and *in vivo* electrically

stimulated skeletal muscles, it was shown that contraction is able to induce translocation of FAT/CD36 from an intracellular pool to the plasma membrane [108]. This method of acute regulation of FA transport is analogous to the manner in which glucose transport is regulated by the transporter GLUT4 [109]. The translocational mechanism is substantiated by several observations. Most importantly, FA uptake by muscle giant sarcolemmal vesicles is increased after just 5 min of *in vivo* electrical stimulation. The giant sarcolemmal vesicle preparation is a model representing the plasma membrane, and is suitable for measuring FA uptake in the absence of metabolism. Giant-vesicle palmitate uptake increased further over a 30 min contraction period, and decreased time-dependently after termination of the electrical stimulus. Moreover, the increase in vesicular FA uptake was shown to be proportional to the rate of muscle stimulation, and could be inhibited by sulpho-*N*-succinimidyl oleate, an inhibitor specific for FAT/CD36. By comparison with GLUT4, gradient centrifugation showed that FAT/CD36 in skeletal muscle is present in both an intracellular pool and a membrane-associated pool (Figure 2). Almost equal quantities of FAT/CD36 were found in the two pools under basal conditions; electrical stimulation increased the amount of FAT/CD36 in the sarcolemmal fraction and decreased that in the intracellular pool [108]. Insulin was also shown recently to

induce FAT/CD36 translocation in perfused rat hindlimb muscle [110].

In conclusion, FAT/CD36 is a tightly regulated protein. Regulatory mechanisms are present at the level of mRNA, which is mediated by PPARs, and at the translational level. Moreover, FA transport is acutely regulated by translocation of FAT/CD36 protein. Post-translational modifications of FAT/CD36, namely glycosylation, palmitoylation and phosphorylation, which have all been shown to occur [36,111], might also play a role in the regulation of FAT/CD36 localization, its activity or its interaction with other proteins. An interaction between FAT/CD36 and FABP_c has been shown to occur in the bovine mammary gland [95], and FAT/CD36 was found to be associated with protein-tyrosine kinases in human platelets [112]. The heart was reported to regulate FAT/CD36 transcription in response to an altered availability of FA [39], whereas the role of translational FAT/CD36 regulation in the heart is still to be fully explored. FAT/CD36 translocation has been shown to be altered in the hearts of Zucker rats, wherein the distribution of FAT/CD36 appears to be shifted towards a permanent relocation at the plasma membrane [71]. Interestingly, abnormalities in all three mechanisms are associated with, and might contribute to, the development of pathologies. PPAR α -overexpressing hearts show decreases in both glucose uptake and oxidation rates, which are accompanied by the repression of enzymes involved in glucose transport and oxidation. These hearts show ventricular hypertrophy, activation of gene markers for pathological hypertrophy, and systolic ventricular dysfunction. Taken together with the alterations in FA metabolism, this phenotype typically resembles that of the diabetic heart [101]. Glucose-mediated regulation of FAT/CD36 translation appears to explain the association between diabetes and the development of accelerated atherosclerosis. As a result of hyperglycaemia, the increased FAT/CD36 levels are thought to lead to an increased uptake of oxidized low-density lipoprotein by macrophages, resulting in subsequent foam-cell development [103]. A link between FAT/CD36 translocation and obesity has been established in the Zucker rat [71]. The FA uptake rates of giant vesicles from heart, skeletal muscle and adipose tissue are higher in tissues from obese Zucker rats than in those isolated from lean control rats. Neither FAT/CD36 mRNA nor total tissue FAT/CD36 protein levels show a consistent quantitative relationship with these higher uptake rates. The finding that FA uptake was correlated with plasma membrane FAT/CD36 levels led to the speculation that FAT/CD36 is relocated from an intracellular store to the plasma membrane in obesity. The observation that FAT/CD36 translocation can be induced by insulin also substantiates a role for this transporter in the pathology of diabetes [110]. Further elucidation of the precise regulatory mechanisms and the consequences of the resulting alterations in FAT/CD36 thus seems extremely relevant to the development of and complications associated with pathologies, especially insulin resistance and diabetes.

SUMMARY AND FUTURE DIRECTIONS

Of the three proteins initially identified as candidate membrane FA transporters, i.e. FAT/CD36, FATP and FABP_{PM}, a crucial role for FAT/CD36 in FA transport is now finally established. Although evidence supporting a role for FATP and for FABP_{PM} in FA metabolism has been obtained, it is at present unclear whether these two proteins have a direct role in FA transport. It has been clearly established that FATP possesses VLACS activity. Whether this activity is accompanied by an additional function in FA transport requires further experimentation. Based

on its peripheral localization, FABP_{PM} might have a function in the trapping of FA and their subsequent channelling towards FA transporters.

Several models to study the influence of FAT/CD36 on FA transport have been generated or identified, including a method to study the effects of FAT/CD36 deficiency in the human heart. Both *in vitro* and *in vivo* studies have shown that FAT/CD36-mediated FA transport in the heart accounts for 60–80 % of the FA taken up by the cardiomyocyte. In addition, FAT/CD36 is an important mediator of FA uptake in adipose tissue, intestine [94] and in muscle in general. The molecular mechanism by which FAT/CD36 exerts this action is currently unknown, and several possible models have been proposed by Abumrad et al. [113]. In these models, it is suggested that FAT/CD36 might facilitate FA transport by decreasing the activation energy for the movement of the polar FA carboxy group through the membrane bilayer. This could be achieved through favourable interactions with polar side chains or peptide bond dipole moments, and in this respect FAT/CD36 might form a multimeric pore or channel, or the protein could bind FA at specific sites within the annular lipids directly adjacent to the protein. Alternatively, a hydrophobic segment of FAT/CD36 that extends below the water/lipid interface might mediate the insertion of the carboxy group within the outer leaflet of the membrane [113]. The possibility that interactions between FAT/CD36 and one or more other proteins are essential for the functioning of this transporter in FA uptake still has to be fully explored. Indicative of a possible interaction between FABP_c, which acts as intracellular FA transporter, and FAT/CD36 is the finding that an association between FAT/CD36 and FABP_c has been found in bovine mammary gland [95]. In addition, the phenotype of the mouse null for H-FABP_c is similar to that of the FAT/CD36 null mouse [114].

Alterations in FAT/CD36 levels in rodent models leads to alterations in the levels of plasma metabolites, and FAT/CD36 deficiency is linked directly to the development of insulin resistance and cardiac hypertrophy. Furthermore, in humans an association is present between FAT/CD36 deficiency and increased susceptibility to heart diseases. FAT/CD36 has been shown to be regulated at several levels. Both FAT/CD36 mRNA and protein levels are subject to regulation. Moreover, acute regulation of FA transport is accomplished at the protein level through translocation of FAT/CD36 from an intracellular store to the plasma membrane. All three regulatory mechanisms appear to be relevant to the development of diseases and their associated complications. Elucidation of the molecular mechanism by which FAT/CD36 mediates FA transport, the possible involvement of additional proteins and the interactions between the regulatory mechanisms, is expected to provide valuable information about the interactions between FA metabolism and the metabolism of other major substrates. This information, in turn, should contribute to a better understanding of the aetiology of many life-threatening pathologies, such as cardiac hypertrophy, heart failure and diabetes.

J.F.F.B. was supported by the Netherlands Heart Foundation, grant 97.149.

REFERENCES

- 1 Amri, E. Z., Bertrand, B., Ailhaud, G. and Grimaldi, P. (1991) Regulation of adipose cell differentiation. I. Fatty acids are inducers of the aP2 gene expression. *J. Lipid. Res.* **32**, 1449–1456
- 2 Distel, R. J., Robinson, G. S. and Spiegelman, B. M. (1992) Fatty acid regulation of gene expression. Transcriptional and post-transcriptional mechanisms. *J. Biol. Chem.* **267**, 5937–5941

- 3 Ordway, R. W., Singer, J. J. and Walsh, Jr, J. V. (1991) Direct regulation of ion channels by fatty acids. *Trends Neurosci.* **14**, 96–100
- 4 Anderson, M. P. and Welsh, M. J. (1990) Fatty acids inhibit apical membrane chloride channels in airway epithelia. *Proc. Natl. Acad. Sci. U.S.A.* **87**, 7334–7338
- 5 Mundy, D. I. (1995) Protein palmitoylation in membrane trafficking. *Biochem. Soc. Trans.* **23**, 572–576
- 6 Jackson, C. S., Zlatkine, P., Bano, C., Kabouridis, P., Mehul, B., Parenti, M., Milligan, G., Ley, S. C. and Magee, A. I. (1995) Dynamic protein acylation and the regulation of localization and function of signal-transducing proteins. *Biochem. Soc. Trans.* **23**, 568–571
- 7 Bouvier, M., Loisel, T. P. and Hebert, T. (1995) Dynamic regulation of G-protein coupled receptor palmitoylation: potential role in receptor function. *Biochem. Soc. Trans.* **23**, 577–581
- 8 Yamashita, A., Sugiyama, T. and Waku, K. (1997) Acyltransferases and transacylases involved in fatty acid remodeling of phospholipids and metabolism of bioactive lipids in mammalian cells. *J. Biochem. (Tokyo)* **122**, 1–16
- 9 Galli, C. and Marangoni, F. (1997) Recent advances in the biology of n-6 fatty acids. *Nutrition* **13**, 978–985
- 10 Glatz, J. F. C., Borchers, T., Spener, F. and van der Vusse, G. J. (1995) Fatty acids in cell signalling: modulation by lipid binding proteins. *Prostaglandins Leukotrienes Essent. Fatty Acids* **52**, 121–127
- 11 Van der Vusse, G. J., Glatz, J. F. C., Stam, H. C. G. and Reneman, R. S. (1992) Fatty acid homeostasis in the normoxic and ischemic heart. *Physiol. Rev.* **72**, 881–940
- 12 Neely, J. R., Rovetto, M. J. and Oram, J. F. (1972) Myocardial utilization of carbohydrate and lipids. *Prog. Cardiovasc. Dis.* **15**, 289–329
- 13 Spector, A. A. (1984) Plasma lipid transport. *Clin. Physiol. Biochem.* **2**, 123–134
- 14 Storch, J. and Thumser, A. E. (2000) The fatty acid transport function of fatty acid-binding proteins. *Biochim. Biophys. Acta* **1486**, 28–44
- 15 Schaap, F. G., Binas, B., Danneberg, H., van der Vusse, G. J. and Glatz, J. F. (1999) Impaired long-chain fatty acid utilization by cardiac myocytes isolated from mice lacking the heart-type fatty acid binding protein gene. *Circ. Res.* **85**, 329–337
- 16 Faergeman, N. J. and Knudsen, J. (1997) Role of long-chain fatty acyl-CoA esters in the regulation of metabolism and in cell signalling. *Biochem. J.* **323**, 1–12
- 17 Knudsen, J. (1996) The role of acyl-CoA binding protein (ACBP) and long-chain acyl-CoA esters in cell regulation and function. In *Proceedings of XVI Washington International Spring Symposium* (Vanderhoeck, J. Y., ed.), pp. 73–82, Plenum Press, NY
- 18 Turcotte, L. P., Richter, E. A. and Kiens, B. (1992) Increased plasma FFA uptake and oxidation during prolonged exercise in trained vs. untrained humans. *Am. J. Physiol.* **262**, E791–E799
- 19 Mackie, B. G., Dudley, G. A., Kaciuba-Uscilko, H. and Terjung, R. L. (1980) Uptake of chylomicron triglycerides by contracting skeletal muscle in rats. *J. Appl. Physiol.* **49**, 851–855
- 20 Martin, III, W. H., Dalsky, G. P., Hurley, B. F., Matthews, D. E., Bier, D. M., Hagberg, J. M., Rogers, M. A., King, D. S. and Holloszy, J. O. (1993) Effect of endurance training on plasma free fatty acid turnover and oxidation during exercise. *Am. J. Physiol.* **265**, E708–E714
- 21 Suzuki, K. and Kono, T. (1980) Evidence that insulin causes translocation of glucose transport activity to the plasma membrane from an intracellular storage site. *Proc. Natl. Acad. Sci. U.S.A.* **77**, 2542–2545
- 22 Civelek, V. N., Hamilton, J. A., Tornheim, K., Kelly, K. L. and Corkey, B. E. (1996) Intracellular pH in adipocytes: effects of free fatty acid diffusion across the plasma membrane, lipolytic agonists, and insulin. *Proc. Natl. Acad. Sci. U.S.A.* **93**, 10139–10144
- 23 Kamp, F., Hamilton, J. A. and Westerhoff, H. V. (1993) Movement of fatty acids, fatty acid analogues, and bile acids across phospholipid bilayers. *Biochemistry* **32**, 11074–11086
- 24 Abumrad, N. A., Park, J. H. and Park, C. R. (1984) Permeation of long-chain fatty acid into adipocytes. Kinetics, specificity, and evidence for involvement of a membrane protein. *J. Biol. Chem.* **259**, 8945–8953
- 25 Abumrad, N. A., Perkins, R. C., Park, J. H. and Park, C. R. (1981) Mechanism of long chain fatty acid permeation in the isolated adipocyte. *J. Biol. Chem.* **256**, 9183–9191
- 26 Sorrentino, D., Robinson, R. B., Kiang, C. L. and Berk, P. D. (1989) At physiologic albumin/oleate concentrations oleate uptake by isolated hepatocytes, cardiac myocytes, and adipocytes is a saturable function of the unbound oleate concentration. Uptake kinetics are consistent with the conventional theory. *J. Clin. Invest.* **84**, 1325–1333
- 27 Stump, D. D., Zhou, S. L. and Berk, P. D. (1993) Comparison of plasma membrane FABP and mitochondrial isoform of aspartate aminotransferase from rat liver. *Am. J. Physiol.* **265**, G894–G902
- 28 Luiken, J. J., van Nieuwenhoven, F. A., America, G., van der Vusse, G. J. and Glatz, J. F. (1997) Uptake and metabolism of palmitate by isolated cardiac myocytes from adult rats: involvement of sarcolemmal proteins. *J. Lipid. Res.* **38**, 745–758
- 29 Stremmel, W., Strohmeyer, G., Borchard, F., Kochwa, S. and Berk, P. D. (1985) Isolation and partial characterization of a fatty acid binding protein in rat liver plasma membranes. *Proc. Natl. Acad. Sci. U.S.A.* **82**, 4–8
- 30 Harmon, C. M. and Abumrad, N. A. (1993) Binding of sulfo succinimidyl fatty acids to adipocyte membrane proteins: isolation and amino-terminal sequence of an 88-kD protein implicated in transport of long-chain fatty acids. *J. Membr. Biol.* **133**, 43–49
- 31 Abumrad, N. A., Harmon, C. M. and Ibrahim, A. (1998) Membrane transport of long-chain fatty acids: evidence for a facilitated process. *J. Lipid. Res.* **39**, 2309–2318
- 32 Schaffer, J. E. and Lodish, H. F. (1994) Expression cloning and characterization of a novel adipocyte long chain fatty acid transport protein. *Cell* **79**, 427–436
- 33 Van Nieuwenhoven, F. A., Willemsen, P. H., van der Vusse, G. J. and Glatz, J. F. (1999) Co-expression in rat heart and skeletal muscle of four genes coding for proteins implicated in long-chain fatty acid uptake. *Int. J. Biochem. Cell Biol.* **31**, 489–498
- 34 Van Nieuwenhoven, F. A., Verstijnen, C. P., Abumrad, N. A., Willemsen, P. H., van Eys, G. J., van der Vusse, G. J. and Glatz, J. F. (1995) Putative membrane fatty acid translocase and cytoplasmic fatty acid-binding protein are co-expressed in rat heart and skeletal muscles. *Biochem. Biophys. Res. Commun.* **207**, 747–752
- 35 Abumrad, N. A., el-Maghrabi, M. R., Amri, E. Z., Lopez, E. and Grimaldi, P. A. (1993) Cloning of a rat adipocyte membrane protein implicated in binding or transport of long-chain fatty acids that is induced during preadipocyte differentiation. Homology with human CD36. *J. Biol. Chem.* **268**, 17665–17668
- 36 Greenwalt, D. E., Lipsky, R. H., Ockenhouse, C. F., Ikeda, H., Tandon, N. N. and Jamieson, G. A. (1992) Membrane glycoprotein CD36: a review of its roles in adherence, signal transduction, and transfusion medicine. *Blood* **80**, 1105–1115
- 37 Asch, A. S., Barnwell, J., Silverstein, R. L. and Nachman, R. L. (1987) Isolation of the thrombospondin membrane receptor. *J. Clin. Invest.* **79**, 1054–1061
- 38 Luiken, J. J., Turcotte, L. P. and Bonen, A. (1999) Protein-mediated palmitate uptake and expression of fatty acid transport proteins in heart giant vesicles. *J. Lipid. Res.* **40**, 1007–1016
- 39 Greenwalt, D. E., Scheck, S. H. and Rhinehart-Jones, T. (1995) Heart CD36 expression is increased in murine models of diabetes and in mice fed a high fat diet. *J. Clin. Invest.* **96**, 1382–1388
- 40 Pelsers, M. M., Lutgerink, J. T., van Nieuwenhoven, F. A., Tandon, N. N., van der Vusse, G. J., Arends, J. W., Hoogenboom, H. R. and Glatz, J. F. (1999) A sensitive immunoassay for rat fatty acid translocase (CD36) using phage antibodies selected on cell transfectants: abundant presence of fatty acid translocase/CD36 in cardiac and red skeletal muscle and up-regulation in diabetes. *Biochem. J.* **337**, 407–414
- 41 Ibrahim, A., Steir, Z., Magharaie, H., Amri, E. Z., Grimaldi, P. and Abumrad, N. A. (1996) Expression of the CD36 homolog (FAT) in fibroblast cells: effects on fatty acid transport. *Proc. Natl. Acad. Sci. U.S.A.* **93**, 2646–2651
- 42 Van Nieuwenhoven, F. A., Luiken, J. J., De Jong, Y. F., Grimaldi, P. A., van der Vusse, G. J. and Glatz, J. F. (1998) Stable transfection of fatty acid translocase (CD36) in a rat heart muscle cell line (H9c2). *J. Lipid Res.* **39**, 2039–2047
- 43 Utsunomiya, A., Owada, Y., Yoshimoto, T. and Kondo, H. (1997) Localization of mRNA for fatty acid transport protein in developing and mature brain of rats. *Brain Res. Mol. Brain Res.* **46**, 217–222
- 44 Schaap, F. G., Hamers, L., van der Vusse, G. J. and Glatz, J. F. (1997) Molecular cloning of fatty acid-transport protein cDNA from rat. *Biochim. Biophys. Acta* **1354**, 29–34
- 45 Lewis, S. E., Listenberger, L. L., Ory, D. S. and Schaffer, J. E. (2001) Membrane topology of the murine fatty acid transport protein 1. *J. Biol. Chem.* **276**, 37042–37050
- 46 Hirsch, D., Stahl, A. and Lodish, H. F. (1998) A family of fatty acid transporters conserved from mycobacterium to man. *Proc. Natl. Acad. Sci. U.S.A.* **95**, 8625–8629
- 47 Faergeman, N. J., DiRusso, C. C., Elberger, A., Knudsen, J. and Black, P. N. (1997) Disruption of the *Saccharomyces cerevisiae* homologue to the murine fatty acid transport protein impairs uptake and growth on long-chain fatty acids. *J. Biol. Chem.* **272**, 8531–8538
- 48 DiRusso, C. C., Connell, E. J., Faergeman, N. J., Knudsen, J., Hansen, J. K. and Black, P. N. (2000) Murine FATP alleviates growth and biochemical deficiencies of yeast fat1Delta strains. *Eur. J. Biochem.* **267**, 4422–4433
- 49 Man, M. Z., Hui, T. Y., Schaffer, J. E., Lodish, H. F. and Bernlohr, D. A. (1996) Regulation of the murine adipocyte fatty acid transporter gene by insulin. *Mol. Endocrinol.* **10**, 1021–1028
- 50 Martin, G., Schoonjans, K., Lefebvre, A. M., Staels, B. and Auwerx, J. (1997) Coordinate regulation of the expression of the fatty acid transport protein and acyl-CoA synthetase genes by PPAR α and PPAR γ activators. *J. Biol. Chem.* **272**, 28210–28217

- 51 Motojima, K., Passilly, P., Peters, J. M., Gonzalez, F. J. and Latruffe, N. (1998) Expression of putative fatty acid transporter genes are regulated by peroxisome proliferator-activated receptor α and γ activators in a tissue- and inducer-specific manner. *J. Biol. Chem.* **273**, 16710–16714
- 52 Frohnert, B. I., Hui, T. Y. and Bernlohr, D. A. (1999) Identification of a functional peroxisome proliferator-responsive element in the murine fatty acid transport protein gene. *J. Biol. Chem.* **274**, 3970–3977
- 53 Hui, T. Y., Frohnert, B. I., Smith, A. J., Schaffer, J. E. and Bernlohr, D. A. (1998) Characterization of the murine fatty acid transport protein gene and its insulin response sequence. *J. Biol. Chem.* **273**, 27420–27429
- 54 Stahl, A., Evans, J. G., Pattel, S., Hirsch, D. and Lodish, H. F. (2002) Insulin causes fatty acid transport protein translocation and enhanced fatty acid uptake in adipocytes. *Dev. Cell* **2**, 477–488
- 55 Berger, J., Truppe, C., Neumann, H. and Forss-Petter, S. (1998) A novel relative of the very-long-chain acyl-CoA synthetase and fatty acid transporter protein genes with a distinct expression pattern. *Biochem. Biophys. Res. Commun.* **247**, 255–260
- 56 Berger, J., Truppe, C., Neumann, H. and Forss-Petter, S. (1998) cDNA cloning and mRNA distribution of a mouse very long-chain acyl-CoA synthetase. *FEBS Lett.* **425**, 305–309
- 57 Uchiyama, A., Aoyama, T., Kamijo, K., Uchida, Y., Kondo, N., Orii, T. and Hashimoto, T. (1996) Molecular cloning of cDNA encoding rat very long-chain acyl-CoA synthetase. *J. Biol. Chem.* **271**, 30360–30365
- 58 Stuhlsatz-Krouper, S. M., Bennett, N. E. and Schaffer, J. E. (1998) Substitution of alanine for serine 250 in the murine fatty acid transport protein inhibits long chain fatty acid transport. *J. Biol. Chem.* **273**, 28642–28650
- 59 Stuhlsatz-Krouper, S. M., Bennett, N. E. and Schaffer, J. E. (1999) Molecular aspects of fatty acid transport: mutations in the IYSGTTGXPX motif impair fatty acid transport protein function. *Prostaglandins Leukotrienes Essent. Fatty Acids* **60**, 285–289
- 60 Herrmann, T., Buchkremer, F., Gosch, I., Hall, A. M., Bernlohr, D. A. and Stremmel, W. (2001) Mouse fatty acid transport protein 4 (FATP4): characterization of the gene and functional assessment as a very long chain acyl-CoA synthetase. *Gene* **270**, 31–40
- 61 Coe, N. R., Smith, A. J., Frohnert, B. I., Watkins, P. A. and Bernlohr, D. A. (1999) The fatty acid transport protein (FATP1) is a very long chain acyl-CoA synthetase. *J. Biol. Chem.* **274**, 36300–36304
- 62 Watkins, P. A., Lu, J. F., Steinberg, S. J., Gould, S. J., Smith, K. D. and Braiterman, L. T. (1998) Disruption of the *Saccharomyces cerevisiae* FAT1 gene decreases very long-chain fatty acyl-CoA synthetase activity and elevates intracellular very long-chain fatty acid concentrations. *J. Biol. Chem.* **273**, 18210–18219
- 63 Choi, J. Y. and Martin, C. E. (1999) The *Saccharomyces cerevisiae* FAT1 gene encodes an acyl-CoA synthetase that is required for maintenance of very long chain fatty acid levels. *J. Biol. Chem.* **274**, 4671–4683
- 64 Schaffer, J. E. (2002) Fatty acid transport: the roads taken. *Am. J. Physiol. Endocrinol. Metab.* **282**, E239–E246
- 65 Zou, Z., DiRusso, C. C., Ctrnacta, V. and Black, P. N. (2002) Fatty acid transport in *Saccharomyces cerevisiae*. Directed mutagenesis of FAT1 distinguishes the biochemical activities associated with Fat1p. *J. Biol. Chem.* **277**, 31062–31071
- 66 Berk, P. D., Wada, H., Horio, Y., Potter, B. J., Sorrentino, D., Zhou, S. L., Isola, L. M., Stump, D., Kiang, C. L. and Thung, S. (1990) Plasma membrane fatty acid-binding protein and mitochondrial glutamic-oxaloacetic transaminase of rat liver are related. *Proc. Natl. Acad. Sci. U.S.A.* **87**, 3484–3488
- 67 Zhou, S. L., Stump, D., Sorrentino, D., Potter, B. J. and Berk, P. D. (1992) Adipocyte differentiation of 3T3-L1 cells involves augmented expression of a 43-kDa plasma membrane fatty acid-binding protein. *J. Biol. Chem.* **267**, 14456–14461
- 68 Zhou, S. L., Stump, D., Isola, L. and Berk, P. D. (1994) Constitutive expression of a saturable transport system for non-esterified fatty acids in *Xenopus laevis* oocytes. *Biochem. J.* **297**, 315–319
- 69 Turcotte, L. P., Srivastava, A. K. and Chiasson, J. L. (1997) Fasting increases plasma membrane fatty acid-binding protein [FABP(PM)] in red skeletal muscle. *Mol. Cell. Biochem.* **166**, 153–158
- 70 Kiens, B., Kristiansen, S., Jensen, P., Richter, E. A. and Turcotte, L. P. (1997) Membrane associated fatty acid binding protein (FABPm) in human skeletal muscle is increased by endurance training. *Biochem. Biophys. Res. Commun.* **231**, 463–465
- 71 Luiken, J. J., Arumugam, Y., Dyck, D. J., Bell, R. C., Pelsers, M. M., Turcotte, L. P., Tandon, N. N., Glatz, J. F. and Bonen, A. (2001) Increased rates of fatty acid uptake and plasmalemmal fatty acid transporters in obese Zucker rats. *J. Biol. Chem.* **276**, 40567–40573
- 72 Berk, P. D., Zhou, S. L., Kiang, C. L., Stump, D., Bradbury, M. and Isola, L. M. (1997) Uptake of long chain free fatty acids is selectively up-regulated in adipocytes of Zucker rats with genetic obesity and non-insulin-dependent diabetes mellitus. *J. Biol. Chem.* **272**, 8830–8835
- 73 Glatz, J. F., van Nieuwenhoven, F. A., Luiken, J. J., Schaap, F. G. and van der Vusse, G. J. (1997) Role of membrane-associated and cytoplasmic fatty acid-binding proteins in cellular fatty acid metabolism. *Prostaglandins Leukotrienes Essent. Fatty Acids* **57**, 373–378
- 74 Ibrahim, A., Bonen, A., Blinn, W. D., Hajri, T., Li, X., Zhong, K., Cameron, R. and Abumrad, N. A. (1999) Muscle-specific overexpression of FAT/CD36 enhances fatty acid oxidation by contracting muscle, reduces plasma triglycerides and fatty acids, and increases plasma glucose and insulin. *J. Biol. Chem.* **274**, 26761–26766
- 75 Febbraio, M., Abumrad, N. A., Hajjar, D. P., Sharma, K., Cheng, W., Pearce, S. F. and Silverstein, R. L. (1999) A null mutation in murine CD36 reveals an important role in fatty acid and lipoprotein metabolism. *J. Biol. Chem.* **274**, 19055–19062
- 76 Coburn, C. T., Knapp, Jr, F. F., Febbraio, M., Beets, A. L., Silverstein, R. L. and Abumrad, N. A. (2000) Defective uptake and utilization of long chain fatty acids in muscle and adipose tissues of CD36 knockout mice. *J. Biol. Chem.* **275**, 32523–32529
- 77 Reaven, G. M. and Chang, H. (1991) Relationship between blood pressure, plasma insulin and triglyceride concentration, and insulin action in spontaneous hypertensive and Wistar-Kyoto rats. *Am. J. Hypertens.* **4**, 34–38
- 78 Reaven, G. M. (1991) Insulin resistance, hyperinsulinemia, hypertriglyceridemia, and hypertension. Parallels between human disease and rodent models. *Diabetes Care* **14**, 195–202
- 79 Reaven, G. M., Chang, H., Hoffman, B. B. and Azhar, S. (1989) Resistance to insulin-stimulated glucose uptake in adipocytes isolated from spontaneously hypertensive rats. *Diabetes* **38**, 1155–1160
- 80 Mondon, C. E. and Reaven, G. M. (1988) Evidence of abnormalities of insulin metabolism in rats with spontaneous hypertension. *Metabolism, Clin. Exp.* **37**, 303–305
- 81 Horl, W. H., Schaefer, R. M. and Heidland, A. (1988) Abnormalities of carbohydrate metabolism in spontaneously hypertensive rats. *Klin. Wochenschr.* **66**, 924–927
- 82 Rao, R. H. (1993) Insulin resistance in spontaneously hypertensive rats. Difference in interpretation based on insulin infusion rate or on plasma insulin in glucose clamp studies. *Diabetes* **42**, 1364–1371
- 83 Aitman, T. J., Gotoda, T., Evans, A. L., Imrie, H., Heath, K. E., Trembling, P. M., Truman, H., Wallace, C. A., Rahman, A., Dore, C. et al. (1997) Quantitative trait loci for cellular defects in glucose and fatty acid metabolism in hypertensive rats. *Nat. Genet.* **16**, 197–201
- 84 Aitman, T. J., Glazier, A. M., Wallace, C. A., Cooper, L. D., Norsworthy, P. J., Wahid, F. N., Al-Majali, K. M., Trembling, P. M., Mann, C. J., Shoulders, C. C. et al. (1999) Identification of Cd36 (Fat) as an insulin-resistance gene causing defective fatty acid and glucose metabolism in hypertensive rats. *Nat. Genet.* **21**, 76–83
- 85 Gotoda, T., Lizuka, Y., Kato, N., Osuga, J., Bihoreau, M. T., Murakami, T., Yamori, Y., Shimano, H., Ishibashi, S. and Yamada, N. (1999) Absence of Cd36 mutation in the original spontaneously hypertensive rats with insulin resistance. *Nat. Genet.* **22**, 226–228
- 86 Collison, M., Glazier, A. M., Graham, D., Morton, J. J., Dominiczak, M. H., Aitman, T. J., Connell, J. M., Gould, G. W. and Dominiczak, A. F. (2000) Cd36 and molecular mechanisms of insulin resistance in the stroke-prone spontaneously hypertensive rat. *Diabetes* **49**, 2222–2226
- 87 Pravenec, M., Zidek, V., Simakova, M., Kren, V., Krenova, D., Horky, K., Jachymova, M., Mikova, B., Kazdova, L., Aitman, T. J. et al. (1999) Genetics of Cd36 and the clustering of multiple cardiovascular risk factors in spontaneous hypertension. *J. Clin. Invest.* **103**, 1651–1657
- 88 Pravenec, M., Landa, V., Zidek, V., Musilova, A., Kren, V., Kazdova, L., Aitman, T. J., Glazier, A. M., Ibrahim, A., Abumrad, N. A. et al. (2001) Transgenic rescue of defective Cd36 ameliorates insulin resistance in spontaneously hypertensive rats. *Nat. Genet.* **27**, 156–158
- 89 Hajri, T., Ibrahim, A., Coburn, C. T., Knapp, Jr, F. F., Kurtz, T., Pravenec, M. and Abumrad, N. A. (2001) Defective fatty acid uptake in the spontaneously hypertensive rat is a primary determinant of altered glucose metabolism, hyperinsulinemia, and myocardial hypertrophy. *J. Biol. Chem.* **276**, 23661–23666
- 90 Lee, K., Godeau, B., Fromont, P., Plonquet, A., Debili, N., Bachir, D., Revirion, D., Gourin, J., Fernandez, E., Galacteros, F. and Bierling, P. (1999) CD36 deficiency is frequent and can cause platelet immunization in Africans. *Transfusion* **39**, 873–879
- 91 Tanaka, T., Nakata, T., Oka, T., Ogawa, T., Okamoto, F., Kusaka, Y., Sohmiya, K., Shimamoto, K. and Itakura, K. (2001) Defect in human myocardial long-chain fatty acid uptake is caused by FAT/CD36 mutations. *J. Lipid Res.* **42**, 751–759
- 92 Okamoto, F., Tanaka, T., Sohmiya, K. and Kawamura, K. (1998) CD36 abnormality and impaired myocardial long-chain fatty acid uptake in patients with hypertrophic cardiomyopathy. *Jpn. Circ. J.* **62**, 499–504
- 93 Hahn, R. T., Febbraio, M., Bella, J. N. and Silverstein, R. L. (1998) The role of CD36 in cardiac size and function: a possible murine model of dilated cardiomyopathy. *Circulation (suppl. 1)* **98**, I236

- 94 Poirier, H., Degrace, P., Niot, I., Bernard, A. and Besnard, P. (1996) Localization and regulation of the putative membrane fatty-acid transporter (FAT) in the small intestine. Comparison with fatty acid-binding proteins (FABP). *Eur. J. Biochem.* **238**, 368–373
- 95 Spitsberg, V. L., Matitashvili, E. and Gorewit, R. C. (1995) Association and coexpression of fatty-acid-binding protein and glycoprotein CD36 in the bovine mammary gland. *Eur. J. Biochem.* **230**, 872–878
- 96 Steir, Z., Ibrahimi, A., Amri, E., Grimaldi, P. and Abumrad, N. (1997) Regulation of FAT/CD36 gene expression: further evidence in support of a role of the protein in fatty acid binding/transport. *Prostaglandins Leukotrienes Essent. Fatty Acids* **57**, 17–21
- 97 Amri, E. Z., Ailhaud, G. and Grimaldi, P. (1991) Regulation of adipose cell differentiation. Kinetics of induction of the aP2 gene by fatty acids and modulation by dexamethasone. *J. Lipid Res.* **32**, 1457–1463
- 98 Amri, E. Z., Bonino, F., Ailhaud, G., Abumrad, N. A. and Grimaldi, P. A. (1995) Cloning of a protein that mediates transcriptional effects of fatty acids in preadipocytes. Homology to peroxisome proliferator-activated receptors. *J. Biol. Chem.* **270**, 2367–2371
- 99 Teboul, L., Febbraio, M., Gaillard, D., Amri, E. Z., Silverstein, R. and Grimaldi, P. A. (2001) Structural and functional characterization of the mouse fatty acid translocase promoter: activation during adipose differentiation. *Biochem. J.* **360**, 305–312
- 100 Leone, T. C., Weinheimer, C. J. and Kelly, D. P. (1999) A critical role for the peroxisome proliferator-activated receptor α (PPAR α) in the cellular fasting response: the PPAR α -null mouse as a model of fatty acid oxidation disorders. *Proc. Natl. Acad. Sci. U.S.A.* **96**, 7473–7478
- 101 Finck, B. N., Lehman, J. J., Leone, T. C., Welch, M. J., Bennett, M. J., Kovacs, A., Han, X., Gross, R. W., Kozak, R., Lopaschuk, G. D. and Kelly, D. P. (2002) The cardiac phenotype induced by PPAR α overexpression mimics that caused by diabetes mellitus. *J. Clin. Invest.* **109**, 121–130
- 102 Sato, O., Kuriki, C., Fukui, Y. and Motojima, K. (2002) Dual promoter structure of mouse and human FAT/CD36 genes and unique transcriptional activation by PPAR α and γ ligands. *J. Biol. Chem.* **277**, 15703–15711
- 103 Griffin, E., Re, A., Hamel, N., Fu, C., Bush, H., McCaffrey, T. and Asch, A. S. (2001) A link between diabetes and atherosclerosis: glucose regulates expression of CD36 at the level of translation. *Nat. Med.* **7**, 840–846
- 104 Ashe, M. P., De Long, S. K. and Sachs, A. B. (2000) Glucose depletion rapidly inhibits translation initiation in yeast. *Mol. Biol. Cell* **11**, 833–848
- 105 Skelly, R. H., Schupp, G. T., Ishihara, H., Oka, Y. and Rhodes, C. J. (1996) Glucose-regulated translational control of proinsulin biosynthesis with that of the proinsulin endopeptidases PC2 and PC3 in the insulin-producing MIN6 cell line. *Diabetes* **45**, 37–43
- 106 Iynedjian, P. B., Pilot, P. R., Noursipkel, T., Milburn, J. L., Quaade, C., Hughes, S., Ucla, C. and Newgard, C. B. (1989) Differential expression and regulation of the glucokinase gene in liver and islets of Langerhans. *Proc. Natl. Acad. Sci. U.S.A.* **86**, 7838–7842
- 107 Semenkovich, C. F., Coleman, T. and Fiedorek, Jr, F. T. (1995) Human fatty acid synthase mRNA: tissue distribution, genetic mapping, and kinetics of decay after glucose deprivation. *J. Lipid Res.* **36**, 1507–1521
- 108 Bonen, A., Luiken, J. J., Arumugam, Y., Glatz, J. F. and Tandon, N. N. (2000) Acute regulation of fatty acid uptake involves the cellular redistribution of fatty acid translocase. *J. Biol. Chem.* **275**, 14501–14508
- 109 Goodyear, L. J. and Kahn, B. B. (1998) Exercise, glucose transport, and insulin sensitivity. *Annu. Rev. Med.* **49**, 235–261
- 110 Luiken, J. J., Dyck, D. J., Han, X. X., Tandon, N. N., Arumugam, Y., Glatz, J. F. and Bonen, A. (2002) Insulin induces the translocation of the fatty acid transporter FAT/CD36 to the plasma membrane. *Am. J. Physiol. Endocrinol. Metab.* **282**, E491–E495
- 111 Asch, A. S., Liu, I., Briccetti, F. M., Barnwell, J. W., Kwakye-Berko, F., Dokun, A., Goldberger, J. and Pernambuco, M. (1993) Analysis of CD36 binding domains: ligand specificity controlled by dephosphorylation of an ectodomain. *Science* **262**, 1436–1440
- 112 Huang, M. M., Bolen, J. B., Barnwell, J. W., Shattil, S. J. and Brugge, J. S. (1991) Membrane glycoprotein IV (CD36) is physically associated with the Fyn, Lyn, and Yes protein-tyrosine kinases in human platelets. *Proc. Natl. Acad. Sci. U.S.A.* **88**, 7844–7848
- 113 Abumrad, N. A., Coburn, C. T. and Ibrahimi, A. (1999) Membrane proteins implicated in long-chain fatty acid uptake by mammalian cells: CD36, FATP and FABPm. *Biochim. Biophys. Acta* **1441**, 4–13
- 114 Binas, B., Danneberg, H., McWhir, J., Mullins, L. and Clark, A. J. (1999) Requirement for the heart-type fatty acid binding protein in cardiac fatty acid utilization. *FASEB J.* **13**, 805–812

Received 7 May 2002/24 June 2002; accepted 28 June 2002

Published as BJ Immediate Publication 28 June 2002, DOI 10.1042/BJ20020747