Annu. Rev. Nutr. 2002. 22:383–415
doi: 10.1146/annurev.nutr.22.020402.130846

FATTY ACID TRANSPORT ACROSS MEMBRANES: Relevance to Nutrition and Metabolic Pathology[1]

Tahar Hajri and Nada A. Abumrad
Department of Physiology and Biophysics, State University of New York at Stony Brook, Stony Brook, New York, 11794-8661; e-mail: thajri@dualphy.pnb.sunysb.edu, nadaa@dualphy.pnb.sunysb.edu

Key Words membrane transport, CD36, FATP, FAPBpm, FAT, fatty acid translocase, SHR, CD36 overexpression, CD36 null

■ **Abstract** Long-chain fatty acids are an important constituent of the diet and they contribute to a multitude of cellular pathways and functions. Uptake of long-chain fatty acids across plasma membranes is the first step in fatty acid utilization, and recent evidence supports an important regulatory role for this process. Although uptake of fatty acids involves two components, passive diffusion through the lipid bilayer and protein-facilitated transfer, the latter component appears to play the major role in mediating uptake by key tissues. Identification of several proteins as fatty acid transporters, and emerging evidence from genetically altered animal models for some of these proteins, has contributed significant insight towards understanding the limiting role of transport in the regulation of fatty acid utilization. We are also beginning to better appreciate how disturbances in fatty acid utilization influence general metabolism and contribute to metabolic pathology.

CONTENTS

[1]Abbreviations: LCFAs, long-chain fatty acids; FA, fatty acids; FABPs, fatty acid–binding proteins; FABPpm, membrane fatty acid–binding proteins; FATPs, fatty acid transport proteins; PPAR, peroxisome proliferative-activated receptor; ubFA, unbound fatty acid; SHR, spontaneously hypertensive rats.

INTRODUCTION

Long-chain fatty acids (LCFAs) are taken up by cells and used for a large number of biological functions. In addition to their important role in energy generation and storage, LCFAs contribute to the synthesis of phospholipids, molecules necessary for the structure, integrity, and function of plasma membranes, and are precursors for prostaglandins, which have numerous functions. Fatty acid acylation of proteins regulates their function and recruitment to plasma membranes (70, 80). Fatty acids modulate the function of numerous enzymes and regulate the expression of multiple genes (12, 39), notably those involved in fatty acid metabolism. Fatty acids also play an important role in nerve system function and synaptic transmission (159). As a result of their many actions, fatty acids play a role in many chronic diseases such as atherosclerosis and cancer, and in the response to infection and inflammation (32, 59, 95, 144).

Fat intake in the Western world averages between 30–40% of the total calories consumed. The bulk of dietary fatty acids tend to be of the long-chain variety ($C > 14$) and are delivered in esterified form as triacylglycerols. The free fatty acids generated after hydrolysis by lingual and pancreatic lipases are absorbed by the enterocytes mainly in the jejunum and the ileum, reesterified as triglycerides, and incorporated with other lipids, lipid-soluble vitamins, and apolipoproteins into triglyceride-rich very low-density lipoproteins and chylomicrons. Once in the circulation, triglycerides carried by chylomicrons and very low-density lipoproteins are hydrolyzed by lipoprotein lipase located at the surface of capillaries. The

LCFAs released are bound by circulating albumin and rapidly taken up by tissues where they are used for various cellular pathways.

To get into the cell, fatty acids have to cross the plasma membrane barrier. In the case of most substrates, membrane transfer is an important step for regulating utilization of the substrate by various tissues. Because fatty acids are lipophilic and can easily cross lipid bilayers, it was proposed that their transfer across membranes would be passive and not facilitated by proteins. However, passive transfer is difficult to reconcile with the well-known multiple functions and regulatory roles of LCFAs. It would seem essential for cells to have mechanisms that allow tight regulation of fatty acid metabolism because disturbances in fatty acid utilization could lead to various metabolic aberrations and pathologies. In the past two decades significant evidence has accumulated to indicate that membrane fatty acid transfer is facilitated by proteins and is regulated acutely and chronically. Below, we first briefly review published work related to the mechanism(s) of fatty acid uptake. We then describe progress made with the identification of CD36 as a major facilitator of fatty acid transport in vivo and discuss the insight that this work is beginning to provide into the underlying causes of common metabolic pathologies. Other proteins with potentially important roles in fatty acid uptake and in fatty acid–related diseases are discussed in the last section.

CELLULAR UPTAKE OF LONG-CHAIN FATTY ACIDS: TRANSPORT AND DIFFUSION

Most tissues rely heavily on fatty acids but, except for liver and adipose tissue, have limited or no capacity for fatty acid synthesis. As a result, the needed fatty acids are taken up from the circulation, and cells should be able to regulate fatty acid uptake to adapt to constant changes in energy demands. For example, muscle increases its uptake and oxidation of circulating fatty acids as its energy needs increase during exercise (147). Cells regulate the membrane transport of other major substrates such as glucose (36, 138) or amino acids (31) in accordance with cellular requirements.

It was thought that fatty acids would transfer passively and rapidly through the membrane lipid and uptake would be governed by two factors: the molar ratio of fatty acids to albumin in the circulation and cellular fatty acid metabolism (56, 120). However, studies using biochemical (2) and biophysical approaches (78) have provided evidence that argues for the existence of two distinct processes in cellular uptake of LCFAs: passive diffusion through the lipid bilayer and protein-facilitated transport. The relative contribution of each component depends on the concentrations and molar ratios of fatty acids and albumin in the circulation, as discussed in the following section.

Determinants of Fatty Acid Transport

In the circulation and in the extracellular medium fatty acids are carried quantitatively bound to albumin. Complexes of fatty acids and albumin are also used

in uptake assays in vitro because fatty acid adsorption to assay tubes and pipette walls and fatty acid aggregation can be serious complications. In the absence of albumin or other fatty acid binding protein (FABP), the concentration of LCFA that can be used is very low (153) and is depleted by cells instantaneously, meaning the linear portion of the uptake time course would be too short to measure. When mixtures of fatty acids and albumin are used, uptake follows the unbound fatty acid (ubFA), which is determined by the molar ratio of fatty acids to albumin. This has been documented in numerous studies using a variety of cell types (5, 6, 116, 120) and a wide range of fatty acid:albumin ratios. ubFA is generally computed based on fatty acid:albumin binding constants. Early estimates (48, 119) have been revised based on recent work by Richieri et al. (104), which used a fluorescent fatty acid–binding protein (ADIFAB) included at low concentration in a solution of protein-bound fatty acids. The amount of ubFA present can be evaluated from the shift in ADIFAB absorbance, and current estimates of ubFA levels in the circulation are in the 5–50 nanomolar range (105).

Inside the cell, one or more types of abundant cytosolic fatty acid–binding proteins (FABPs) buffer intracellular fatty acid. An important role for cytosolic FABPs in shuttling fatty acids between the plasma membrane and intracellular membranes or metabolic compartments is supported by a wealth of evidence, notably the demonstration by Storch and colleagues of a collisional mechanism for transfer of the bound fatty acids for most of the FABPs (47).

Fatty Acid Diffusion: Is it Fast Enough to Accommodate Cellular Uptake Rates?

Although rapid fatty acid diffusion through lipid bilayers is a well-documented phenomenon, there is strong evidence that it is not the main mechanism for cellular fatty acid uptake in vivo. First, significant transfer of fatty acids from albumin to bilayer lipid can only be demonstrated at very high and nonphysiological fatty acids to albumin ratios, whereas at the low ratios present in the circulation (0.25–1.0) transfer appears limited by tight fatty acids binding to the high affinity sites on albumin. Second, fatty acid uptake in a variety of cells or membrane vesicles is sensitive to a list of known anion inhibitors, which can often block the major part of the uptake process (2). This suggests that the anionic and not the protonated form of the fatty acids is the one most relevant for uptake. Finally, the interpretation that the fatty acids partitioned in the outer leaflet of the membrane can flip-flop to the inner leaflet at a rate that is fast enough to support cellular metabolism has been questioned by recent work, as discussed in the following section.

Rates of Fatty Acids Flip-Flop Across Lipid Bilayers

All fatty acids regardless of chain length can transfer rapidly across lipid membranes (Figure 1). In the case of long-chain fatty acids, estimates of transfer rates are widely variable (57, 71, 78) and this may reflect dependence of the transfer on fatty acid size and on the composition and organization of the bilayer (78). Recent

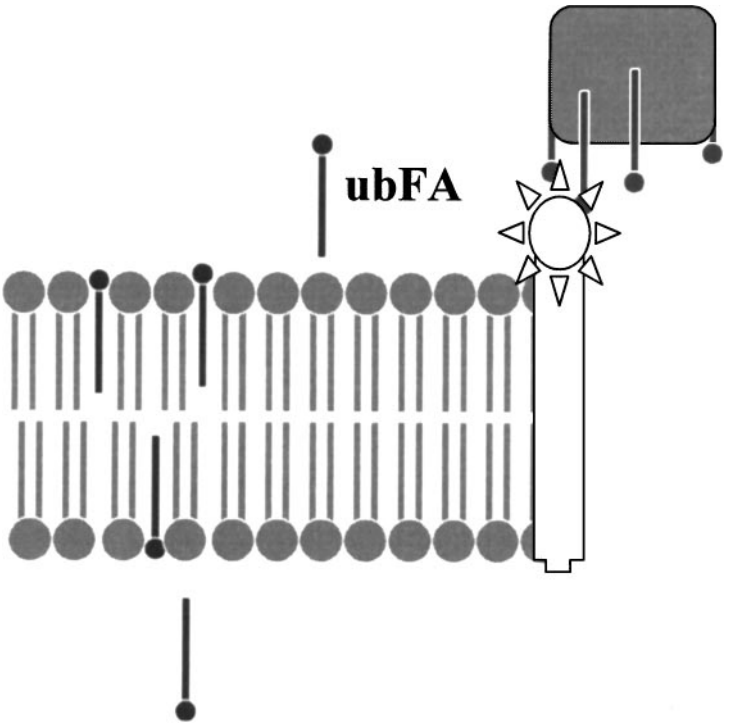

Figure 1 The diffusion and protein-facilitated components of long-chain fatty acid (LCFA) uptake. Uptake of LCFAs involves two components, passive diffusion and protein-facilitated transfer. LCFA can partition into the membrane lipid and flip-flop rapidly to the inside of the cell. However, membrane partitioning is limited by high affinity binding of the fatty acids by plasma albumin. In vivo, the protein-facilitated component appears to mediate a major fraction of fatty acid uptake in key metabolic tissues. Membrane proteins could play important roles by allowing recruitment of fatty acids by a high affinity mechanism. This would be very advantageous for cells active in fatty acid metabolism since the ubFA concentration range in the blood is very low (5–50 nM). Proteins could channel the fatty acids to a metabolic site, preventing fatty acid efflux back into the albumin-containing medium. Most importantly, proteins could provide a way to regulate fatty acid biodistribution so uptake can be modulated to meet changing cellular needs. ubFA, unbound fatty acids in equilibrium with albumin-bound fatty acids.

measurements support the interpretation that flip-flop of long-chain fatty acids rate-limits their membrane transfer, and transfer rate across vesicles approaching cell size and membrane composition is estimated to be in the order of 1–10 per second for palmitate and oleate (78). These findings suggest that lipid phase–mediated transfer of long-chain fatty acids may be adequate for metabolically simple cells such as erythrocytes but would not meet the fatty acid needs of more

complex cells (20, 78). For example, flip-flop would be estimated to be 1.5 to 50 times slower than the uptake needed to support fatty acid metabolism by isolated myocytes.

Biochemical Evidence for Long-Chain Fatty Acid Transport

The first detailed studies of fatty acid transport kinetics under conditions in which membrane fatty acid permeation was the rate-limiting step measured were carried out in isolated rat adipocytes (5, 6). These studies documented that membrane fatty acid permeation exhibited nonlinear saturation kinetics as a function of the concentration of ubFA. When these data are replotted based on the revised (104) ubFA estimates, the transport Km obtained is in the low nanomolar range (7 nM), which is in line with ubFA concentrations in the circulation, currently estimated to be in the range of 7.5 nM $\pm$ 2.5 nM. At physiological ubFA levels the saturable component of fatty acid permeation would account for virtually all cellular uptake. A linear, diffusion-like component becomes more significant as fatty acid:albumin ratios are increased. A recent study by Stump et al. (135) reexamined the kinetics of fatty acid uptake in adipocytes based on the new set of association constants for fatty acid:bovine serum albumin binding and confirmed previous findings of two distinct components: a saturable component that would mediate 90% of uptake at physiological fatty acid:albumin ratios and a nonsaturable process that is quantitatively important only at high ratios that are not commonly achieved in plasma. The diffusion component would be expected to contribute more to uptake under conditions in which blood fatty acids are increased such as with active intravascular lipolysis of lipoprotein triglycerides. This component is likely to become more significant after exposure to lipolytic agonists that induce fatty acid release from hydrolysis of intracellular triglycerides in adipose tissue. It is also conceivable that diffusion could account for a variable fraction of uptake by cells depending on the density of fatty acid receptors or carriers that are present on the cell membrane.

Saturability of fatty acid permeation was demonstrated in many cell types other than adipocytes, including hepatocytes (129), myocytes (84), and intestinal cells (29, 145). Saturable palmitate transport across giant sarcolemmal membrane vesicles prepared from rat hindlimb skeletal muscles and containing fatty acid–binding protein as a fatty acid sink but no mitochondria was also documented (83). The Km for palmitate was estimated to be 6 nM and was similar whether vesicles were prepared from red or white muscles. On the other hand, the Vmax was 1.8-fold greater in red than white vesicles, indicating that the higher capacity of red muscle to metabolize fatty acids may reflect in part a higher transport capacity at the membrane level.

Competition between different fatty acids for uptake, which is another way to demonstrate saturability of the process, was documented in many cell systems. In isolated adipocytes (5) competition was evident in the case of several LCFAs but not

in the case of short-chain fatty acids (fewer than 8 carbons), in line with specificity of the transport component for LCFA. Sorrentino et al. (118) carried out rigorous competition studies using isolated hepatocytes and the perfused liver. The authors estimated the ubFA concentrations of oleate and palmitate present simultaneously on albumin and documented competition between the two fatty acids. In studies with cultured adipocytes, transfer of an ^{18}C fluorescent fatty acid derivative added at low micromolar concentrations in the absence of albumin was inhibited by oleic acid, whereas that of the ^{11}C analog was not affected (126).

A strong argument supporting the existence of carrier-mediated membrane permeation of fatty acids is sensitivity of the process to protein-modifying agents. A detailed list of the inhibitors found to be effective on fatty acid transport and the pertinent references is presented elsewhere (4), so this is only discussed briefly. As pointed out previously, many of the inhibitory compounds are known anion inhibitors, suggesting that the fatty acid may permeate the membrane as an anion rather than in the protonated form. Fatty acid uptake is inhibited reversibly by phloretin, and a stop solution containing phloretin is effective in blocking membrane flux of fatty acids and is used often in uptake studies. Irreversible inhibition of fatty acid uptake is observed by reacting cells with diisothiodisulfonic acid and with reactive sulfosuccinimidyl derivatives of oleate and myristate, SSO and SSM, or with other fatty acid derivatives such as 11-azistearate. Fatty acid transport shows relatively low sensitivity to proteases.

Fatty Acid Transport Rate-Limits Fatty Acid Metabolism in Key Tissues

A transport step is likely to be an important site of regulation if it rate-limits cellular metabolism. For example, in isolated adipocytes, kept in the absence of insulin, intracellular glucose stays at about 20% of extracellular glucose (157), indicating that the activity of glucose metabolism exceeds that of transport. Insulin increases transport capacity about 30-fold, producing equilibration of glucose across the plasma membrane.

In the case of fatty acids it is difficult to examine the relative rates of transport and metabolism. The concentration of ubFA can be estimated for the extracellular medium, as the total amounts of fatty acid and binding protein are known, but it cannot be done for the intracellular space because distribution of intracellular fatty acids between binding proteins and intracellular membranes is not known. However, the following evidence indicates that transport of fatty acids may rate-limit cellular metabolism and may be subject to modulation. First, isotopic equilibrium is not observed in fatty acid uptake studies, suggesting the exogenous fatty acid is metabolized as fast as it enters the cell. Two phases can be distinguished in the initial time course of fatty acid uptake by adipocytes (6). The early one, which would represent approach of intracellular fatty acids to isotopic steady state, is very

short (0.2 min). If all measured cellular fatty acids exchanged with extracellular fatty acids, isotopic equilibrium would require an estimated 5 min. In addition, dilution of exogenous fatty acids inside the cell by unesterified fatty acids is low (as assessed by [^{3}H]oleate/[^{14}C]glucose incorporation ratios) and indicates that the fatty acid exchanges with less than 2% of cell fatty acids (88).

Second, there is evidence for regulation of fatty acid transport. In 1971, Schimmel & Goodman (111) reported that efflux of preformed fatty acids from adipocytes is accelerated by cAMP and may require energy. Our studies documented a stimulatory effect of norepinephrine and cAMP analogs on fatty acid influx and efflux in and out of adipocytes (7). These early studies were carried out in the presence of lipolytic inhibitors to distinguish effects on transport from those on lipolysis. However, localized changes in intracellular free fatty acid concentration could not be ruled out and may have complicated interpretation. More recent studies present more convincing evidence to support regulation of membrane fatty acid transport to bring about optimal rates of fatty acid oxidation or to coordinate lipolysis and fatty acid release.

Turcotte et al. (146) reported that muscle contraction was associated with an increase in uptake and oxidation of palmitate, which was independent of the increase in palmitate delivery as a result of enhanced plasma flow. The authors concluded that the data were consistent with an effect of contraction on membrane fatty acid transport. Such an effect was demonstrated directly by Luiken et al. (83) and by Bonen et al. (24), who documented that muscle contractile activity increased membrane fatty acid transport. In subsequent work electrical stimulation/contraction was shown to produce translocation of the fatty acid transporter CD36 to the plasma membrane, similar to what occurs with insulin stimulation of glucose transport (25). As is discussed in more detail below, work with mice models with deficiency or overexpression of CD36 has generated strong evidence for a rate-limiting role of the transport step in fatty acid metabolism. Mice with muscle overexpression of CD36 have an enhanced ability to oxidize fatty acids in response to contraction (66), whereas CD36-null mice have a marked defect in muscle and adipose fatty acid utilization (33). Thus, there is increasing support for the concept that fatty acid transport rate-limits fatty acid metabolism in some tissues; notably muscle and transport activation is required under conditions in which fatty acid utilization is to be increased.

Several proteins implicated in LCFA transport have been recently identified and characterized. Detailed work related to an outer membrane protein functioning as a receptor for LCFAs in *Escherichia coli* has been described [for a recent review see (37)]. Several proteins have been identified and proposed to function in fatty acid transport in mammalian cells (1, 82), as discussed in the sections below titled CD36: Major Role in FA Transport by Muscle and Adipose Tissues; The Family of FA Transport Proteins (FATPs); and Membrane Fatty Acid Binding Protein (FABPpm). However, the main focus of the review is on one of these proteins, CD36, because significant progress has been generated recently related to its role in fatty acid transport and utilization.

CD36: MAJOR ROLE IN FATTY ACID TRANSPORT BY HEART, SKELETAL MUSCLE, AND ADIPOSE TISSUES

Introduction

Using labeled reactive succinimidylesters, of LCFAs, which inhibited fatty acid transport in adipocytes, Harmon & Abumrad identified and isolated (58) an 88-kDa membrane protein that was designated as FAT for fatty acid translocase. Cloning of the cDNA for FAT from a rat adipose tissue cDNA library (3) revealed a high homology with human platelet CD36 (49), also known as GPIV, GPIIIb, and PAS IV.

CD36 is an integral membrane glycoprotein found on the surface of a variety of cells such as megakaryocytes, platelets, monocytes, dendritic cells, adipocytes, myocytes, retinal and mammary epithelial cells, and endothelial cells of the microvasculature and small intestine. The primary role of CD36 may vary with the cell type on which it is expressed. This review describes recent evidence documenting an important role of CD36 as a facilitator of membrane fatty acid transport by muscle and adipose tissues. However, in other tissues CD36 may primarily function as a cell adhesion molecule or as a class B scavenger receptor. This versatility is a result of the wide variety of ligands for CD36.

The role of CD36 as an adhesion molecule reflects its ability to bind the extracellular matrix proteins thrombospondin-1 and collagens type I and IV. As a result, CD36 plays a role in modulating platelet adhesion and aggregation and in facilitating the cell-cell interactions important to the recruitment and trafficking of monocytes to damaged tissues (42). CD36 is also the adhesion receptor for a *Plasmodium falciparum* protein expressed on the surface of malaria-infected erythrocytes, and this interaction contributes to the virulence of this form of malaria (63). On macrophages, CD36 functions as a scavenger receptor important to recognition and phagocytosis of apoptotic cells and for the binding and internalization of oxidized low density lipoproteins. This reflects its ability to bind anionic phospholipids as well as lipids or proteins modified by lipid peroxidation.

Primary Structure

CD36 is the originating member of a gene family of structurally related glycoproteins expressed both at the cell surface and within lysosomes. Members include, in addition to CD36, the high density lipoprotein receptor SR-B1 (also CLA-1), the drosophila plasma membrane proteins emp and croquemort, the lysosomal membrane protein LIMPII, and the recently identified amoeboid endolysosomal proteins LmpA, LmpB, and LmpC. All family members are thought to share a hairpin membrane topology with two transmembrane domains and with both termini in the cytoplasm (Figure 2). This configuration has been confirmed for CD36 and SR-BI (34, 141).

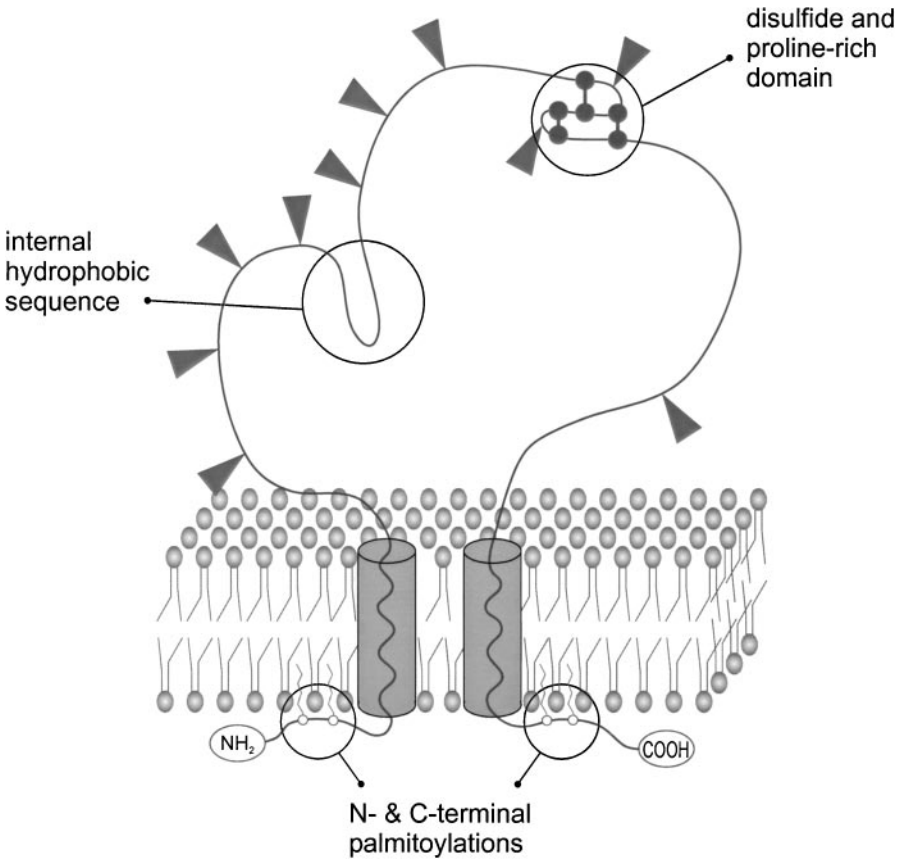

Figure 2 Cartoon showing predicted structure and membrane topology of CD36. The protein is positioned in the membrane in a hairpin configuration with two transmembrane domains and short cytoplasmic segments. A hydrophobic segment that is not long enough to span the membrane is shown looping back towards the bilayer. This segment may form a hydrophobic pocket embedded in the extracellular domain or may partially extend into the membrane. Extensive glycosylation accounts for ~40% of the molecular weight of CD36. Palmitoylation at the carboxyl and amino ends is thought to help recruit CD36 to the detergent-resistant membrane microdomains, known as rafts or caveolae.

The extracellular domain of CD36 has over 10 potential glycosylation sites, and glycosylation increases the apparent mass of the protein from 53 kDa (nonglycosylated) to between 78 (mammary tissue) and 88 kDa (muscle, adipose tissue, platelets). Glycosylation may explain the high resistance of CD36 to protease digestion because treatment with endoglycosidase F yields a deglycosylated protein that is susceptible to a range of proteases.

Membrane Localization

CD36 is associated with the cholesterol- and sphingolipid-rich detergent insoluble membrane domains. These domains, referred to as rafts (or as caveolae when they contain caveolin), are enriched in membrane receptors and signaling molecules and may act as organizational centers for signal transduction (70). Proteins are localized to these domains by one or more caveolin scaffold recognition sequences, which are not present in CD36, or by modification with saturated acyl chains that pack well into the ordered lipid environment of the rafts. The dual palmitoylation sites on each of the cytoplasmic tails of CD36 (Figure 2) might provide a flexible signal to regulate its association with these domains.

Localization of CD36 within membrane rafts suggests that it may work in concert with other membrane-associated receptors to effect functions in cell signaling. Accordingly, collagen binding or antibody-mediated clustering of CD36 activate platelets concomitant with an increase in tyrosine phosphorylation. In line with this, immunoprecipitates of CD36 from platelets contain the Src-family tyrosine kinases Fyn, Lyn, and Yes (26, 64). Binding of thrombospondin-1, which induces dimerization of CD36, has been shown in microvascular endothelial cells to activate the associated tyrosine kinase Fyn. In monocytes, CD36-specific IgG antibodies induce an oxidative burst.

Studies have also documented a physical and/or functional association between CD36 and integrins on the surface of many cell types (92, 107, 142). The integrins associated with CD36 also function as cell-surface receptors for either collagen or thrombospondin-1, which has led to the hypothesis that CD36 together with integrins may complement or stabilize interactions of the cell with these adhesion molecules to enhance their signaling efficiencies (150).

Evidence for CD36 Function in Fatty Acid Uptake

As already mentioned, CD36 was identified by binding reactive LCFA derivatives under conditions in which these reagents inhibited fatty acid transport in rat adipocytes by ~70% (58). The oleate derivative bound specifically to one membrane protein band and was used to isolate the labeled protein (FAT), which was later identified to be homologous to CD36. Thus, binding of these fatty acid derivatives was the first evidence for CD36 function in fatty acid uptake. The protein purified from adipose tissue was later shown to bind native long- but not short-chain fatty acids in vitro (14).

Distribution of CD36 is consistent with its role as a fatty acid transporter, as it favors tissues with a high metabolic capacity for LCFAs (3). It is high in the intestine (29, 81, 100), where it is differentially expressed along the longitudinal and horizontal axes of the tissue, being abundant in proximal segments and in villi enterocytes, where most lipid absorption occurs. Expression is high in adipose tissue (3), where fatty acid is stored as neutral lipids, and in the heart (3, 83), which relies heavily on fatty acid oxidation. In skeletal muscle,

CD36 is highly expressed in muscles with predominance of oxidative fibers, whereas expression is low in muscles with predominance of glycolytic fibers (24).

CD36 is also expressed on macrophages (143), endothelial cells (102), platelets (149), and lung pneumocytes (54), where it has been linked to fatty acid uptake (40, 54, 75), lipid accumulation (154), and binding of oxidized low density lipoproteins (96).

Expression of CD36 in fibroblasts provided more direct evidence for its role in fatty acid transport, as it induced appearance of a saturable, high affinity, phloretin-sensitive component of fatty acid uptake (67). This component mediated most uptake at low ratios of fatty acids to albumin. Its contribution decreased as ratios were increased to 2.0 and as more fatty acids permeated the membrane via the diffusion component constitutively present in the cell. Expression of the antisense CD36 cDNA in preadipocytes reduced fatty acid uptake by these cells (114). In addition, expression interfered with normal differentiation of these cells into adipocytes. These studies suggested that CD36 mediates the high affinity, saturable component of fatty acid uptake, characterized in early studies with isolated adipocytes (5, 6). They also documented the important role of fatty acid uptake and/or of CD36-mediated transcriptional effects of fatty acids on the preadipocyte-adipocyte differentiation program (53).

CD36 Expression: Tissue-Specific Regulation and Alteration in Metabolic Pathologies

In line with its role as a facilitator of fatty acid uptake, CD36 expression is regulated by developmental, metabolic, or nutritional factors. CD36 is strongly induced during differentiation and development. Its expression is a prominent marker of preadipocyte differentiation into adipocytes (3). In the heart, there is coordinated upregulation of the expression of CD36 and of muscle FABP during heart development when fatty acid utilization increases (152).

CD36 expression is regulated by agonists of the nuclear peroxisome proliferator receptors (PPAR) in a tissue-specific manner. The PPARs, alpha, gamma, and delta, regulate transcription of many genes involved in lipid and carbohydrate metabolism. PPAR-α is expressed in tissues exhibiting high rates of fatty acid oxidation such as heart, muscle, liver, kidney, and brown-adipose tissue and regulates expression of proteins involved in fatty acid catabolism. PPAR-γ is highly expressed in adipose tissue and is a key regulator of adipogenesis and insulin sensitivity. PPAR-δ occurs at low levels in most cells in which it may mediate transcriptional effects of fatty acids on genes of lipid metabolism. Synthetic agonists for PPAR-α known as fibrates decrease serum triglyceride levels and are widely prescribed for treating hypertriglyceridemia and compound lipidemia. Synthetic PPAR-γ agonists known as thiazolidinediones, or glitazones, are used as insulin sensitizers for treatment of type II diabetes. A number of LCFAs and naturally occurring fatty acid derivatives have been shown in vitro to stimulate PPAR-mediated

transcription, and as a result, PPARs may act as fatty acid sensors to modulate gene expression according to fatty acid supply.

All three PPAR subtypes transcriptionally regulate CD36 expression. In preadipocytes, CD36 mRNA is strongly induced by glitazones, LCFAs, and by the nonmetabolizable fatty acid analog 2-bromopalmitate (16), an agonist of PPAR-δ (13). When PPAR-δ (also known as the fatty acid-activated receptor) was stably transfected into 3T3-C2 fibroblasts, the cells acquired the capacity to fatty acid–induce expression of CD36 as well as expression of the adipocyte cytosolic fatty acid–binding protein. PPAR-γ agonists also increase CD36 mRNA in adipose tissue (115). This effect may involve recruitment of preadipocytes into adipocytes because CD36 levels in mature adipocytes are not altered by PPAR-γ agonists (52).

PPAR activation of CD36 gene transcription may provide a link to the increased CD36 expression observed with pathologic states characterized by hyperlipidemia. CD36 expression is increased, for example, in animal models of genetic obesity and diabetes and in mice fed a high fat diet (50). In a recent in vivo experiment in humans, elevated plasma concentrations of fatty acids were maintained for 5 h by infusion of a commercial soybean oil emulsion with added heparin. Following the infusion, PPAR-γ and CD36 mRNAs isolated from biopsied subcutaneous fat were increased two- and sevenfold, respectively, from pretreatment levels (97). Similar results were obtained following lipid infusion in lean Zucker rats. In contrast, lipid infusion resulted in a decrease in muscle CD36, and this change was implicated in fatty acid-induced insulin resistance (60). These findings suggest that elevated plasma fatty acids may regulate the partitioning of calories to adipose tissue by inducing genes like CD36 that promote fat cell differentiation, fatty acid uptake, and triglyceride deposition.

Both PPAR-γ (28) and PPAR-δ (154) mediate upregulation of CD36 in human macrophages. In the case of PPAR-δ this is associated with significant lipid deposition. PPAR-δ agonists are also potent regulators of CD36 mRNA in keratinocytes (156).

In muscle, CD36 expression appears to be sensitive to regulation by agonists of PPAR-γ (27) and PPAR-α, and CD36 expression is significantly decreased in the PPAR-α null mouse (94). By comparison, cardiac CD36 expression has only been reported to be responsive to PPAR-α and not to PPAR-γ (151). PPAR-γ (91) and PPAR-α also mediate regulation of CD36 expression in the liver (77).

Also consistent with its role in fatty acid transport, CD36 expression has been shown to be upregulated by LCFAs in isolated cells (52) and by dietary fat in tissues such as adipose tissue (97), intestine (100, 137), mammary gland (121), and heart muscle (50). We observed a marked decrease in heart CD36 protein levels in mice fed diets supplemented with medium- and short-chain fatty acids (unpublished data). A recent study reported that infusion of fatty acids to rats decreased CD36 protein levels by more than 50% (60). However, Greenwalt et al. (50) reported an increase in heart CD36 protein levels in mice maintained on high fat diet. A more systematic approach is needed to determine the effects of dietary fat on CD36 levels in different tissues. It is possible that the acute and long-term

effects of dietary lipid will differ and that these effects will depend on the type of fat used. In addition, mRNA and protein levels may not be regulated in the same way. Posttranscriptional mechanisms may be involved in fat regulation of CD36, as was shown recently in the case of regulation by glucose, which increases the translational efficiency of the CD36 mRNA (51). In summary, in line with CD36 being a nutritionally important gene, there is strong evidence for regulation of its expression by dietary and metabolic factors. This regulation is likely to be important in modulating the adaptive responses of different tissues to environmental challenges.

CD36 and Lipid Metabolism in Muscle and Adipose Tissue

Mice null for CD36 (41) and mice overexpressing CD36 in muscle (66) were generated recently. Coburn and colleagues (33) demonstrated that mice null for CD36 have greater than a 60% reduction in vivo in fatty acid uptake by heart, red skeletal muscle, and adipose tissue. Fatty acid incorporation into lipid was altered in tissues of CD36 null mice with a 20-fold increase in the diglyceride to triglyceride ratio. As shown in Figure 3, this indicated a block in the conversion of diglyceride to triglyceride because the low levels of fatty acids and fatty acid-acyl CoA were below those essential for optimal activity of the enzyme diglyceride acyl-CoA transferase.

Fatty acid oxidation by CD36-null muscles is decreased as a result of the defect in uptake. To compensate and derive the needed metabolic energy, glucose utilization by muscle is increased severalfold (C.T. Coburn & N.A. Abumrad, unpublished observations, and T. Hajri, X. Han, A. Bonen, & N.A. Abumrad, unpublished observations). CD36-null mice have a decreased ability to perform strenuous exercise, whereas mice with CD36 overexpression perform better than wild-type mice (N.A. Abumrad, A. Ibrahimi, & J. Johnson, unpublished data) as a result of the enhanced ability of muscle to oxidize fatty acids in response to contraction (1). Bonen et al. (24, 25) recently documented acute upregulation of fatty acid uptake by muscular activity that is mediated by translocation of CD36 from intracellular stores to the sarcolemma, analogous to the regulation of glucose uptake by membrane recruitment of GLUT-4. Steinberg et al. (125) reported that membrane recruitment of CD36 in muscle is influenced by leptin and so may play a role in the peripheral effects of this hormone.

These data indicate that CD36 expression levels significantly impact muscle function and performance. It will be important to determine if alterations in CD36 levels brought about by genetic or environmental factors can modulate athletic ability in humans.

Fatty Acid Transport Rate-Limits Fatty Acid Metabolism in Key Tissues

Studies of CD36 null mice (33), spontaneously hypertensive rats (SHR) (55), which are CD36-deficient (11), and CD36-deficient humans (45), using the slowly

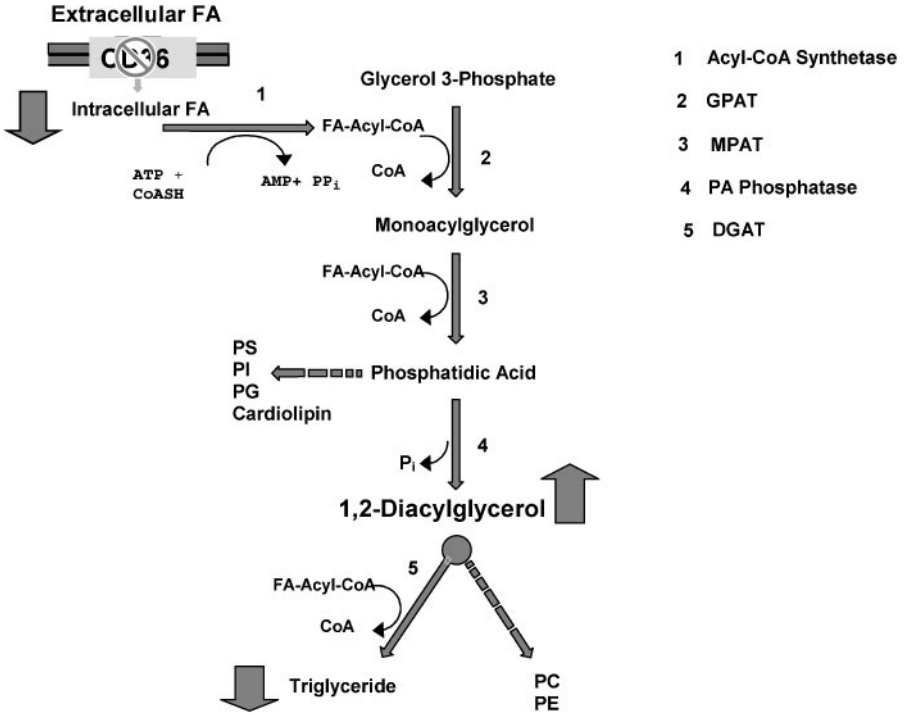

Figure 3 Abnormalities in fatty acid utilization in muscle and adipose cells deficient in CD36. Lack of CD36 results in slow clearance of blood fatty acid by peripheral tissues such as muscle and adipose tissues. As a result, blood fatty acid is increased, as shown by the high extracellular fatty acid in the diagram. However, despite this increase, little fatty acid penetrates the muscle or adipose cell, which is reflected in the low levels of intracellular fatty acid and fatty acid–acyl-CoA. These changes have several important consequences. The first one is not shown on the diagram but is discussed in the text: Fatty acid oxidation is depressed as a result of diminished fatty acid supply, and this is associated with a compensatory increase in glucose utilization. Current work is aimed at understanding how this change impacts glucose responsiveness to insulin and the susceptibility to diet-induced insulin resistance. The second change, depicted in the diagram, involves an increase in the intracellular diglyceride to triglyceride ratio. Low levels of fatty acid–acyl-CoA result in suboptimal activity of the enzyme diacylglycerol transferase and in a block in the conversion of diglycerides to triglycerides, whereas synthesis of phospholipids is not altered. Whether these changes confer some protection against obesity is under investigation. In general, the diagram is meant to highlight the concept that modulation of the membrane uptake step greatly alters fatty acid utilization not only in absolute levels but also in terms of the direction of fatty acid metabolism. Regulation of the fatty acid transport step acutely by translocation of CD36 to the membrane or chronically by changes in CD36 expression have been documented recently with muscle contraction, fatty acid supply, leptin, etc.

oxidized fatty acid analogue 15-(*p*-iodophenyl)-3-(*R,S*)-methylpentadecanoic acid (BMIPP), have identified significant defects in myocardial fatty acid uptake. CD36−/− mice exhibited between 60 and 80% reduction in BMIPP uptake by heart tissue, which was similar to that observed in CD36-deficient humans. Hearts of SHR exhibited a similar but less pronounced defect in fatty acid uptake (55).

CD36−/− mice have heart hypertrophy (96), as do SHR (38). Supplementation of the diet with short-chain fatty acids, which do not require CD36-facilitated transport, eliminated heart hypertrophy in SHR. This indicated that lack of metabolic energy consequent to deficient fatty acid uptake is the primary defect behind heart hypertrophy in SHR (55).

Energy from fatty acid metabolism is also thought to play an important role in ischemic tolerance (20). We recently examined whether alteration in expression of CD36 influenced heart function during normal perfusion conditions and with ischemia/reperfusion, using the isolated working heart preparation. Our studies showed an increase in end diastolic pressure in CD36-null hearts under normal perfusion conditions (H. Irie, I. Krukenkemp, G. Gaudette, N.A. Abumrad, & A. Ibrahimi, unpublished data), possibly suggesting a structural change in myocardial tissue. Tolerance to ischemia was significantly impaired in CD36-null as compared with wild-type mice, and this was reversed by rescuing CD36 expression. Accordingly, CD36 overexpression in the heart appeared to be cardio-protective. This supported the interpretation that because energy from fatty acid oxidation is important for ischemic tolerance, increasing the CD36-facilitated fatty acid supply may improve myocardial survival from ischemic episodes.

CD36 Deficiency in Humans

CD36 deficiency has a relatively high incidence, especially in some subpopulations. Deficiency can be divided into two subgroups. The type I CD36 deficient phenotype is distinguished by a lack of CD36 expression on the surface of both platelets and monocytes. The type II phenotype lacks expression on the surface of platelets, but monocyte expression can be normal, which may reflect deficiencies in intracellular processing or transport of CD36 to the membrane.

CD36 deficiency has a prevalence of 3–10% in Asian populations (72, 74), 5–18.5% in African populations (10, 35), and less than 0.3% in Caucasians. Incidences in the Japanese population of the type I and type II phenotypes are 1.0% and 5.8%, respectively. The most common mutation among the Japanese is a thymine for cytosine substitution at cDNA nucleotide 478, resulting in a proline substitution for serine 90 (73, 139, 158). This mutation has been reported to result in degradation of the immature protein in transfected cells. In sub-Saharan Africa the most common mutation is a substitution of guanine for thymine at nucleotide position 1264, which encodes a truncated protein lacking the C-terminal membrane-spanning domain.

The pathophysiology of CD36 deficiency in humans is currently unclear and has only been studied sporadically. The type I phenotype is characterized by a marked

defect in myocardial uptake of LCFAs (65, 140) as visualized by scintigraphy using iodinated BMIPP. Also, a recent study with a limited number of subjects suggests an association with dyslipidemia and impaired insulin action (93).

There are insufficient data to establish a role for CD36 deficiency in the pathogenesis of cardiomyopathies in humans, although such a link is documented in CD36-null mice and in SHR. The deficiency may underlie some cases of cardiac hypertrophy (73, 139, 140), and incidence of CD36 deficiency in patients with heart disease may exceed that in the general population according to some (65) but not all (69) studies. However, because it has been well documented that humans with CD36 deficiency show markedly reduced myocardial fatty acid uptake (65, 140) and studies with CD36-deficient animals show alterations in heart morphology and tolerance to ischemia, careful studies of humans are warranted and needed. It will be important to examine the impact of CD36 deficiency in humans on heart performance with challenging workloads and on heart recovery from ischemic episodes.

Role of CD36 in Insulin Resistance and Diabetes

High blood fatty acids are a common feature of insulin resistant states (23), and raising the level of plasma fatty acids can induce acute insulin resistance (106). An inverse relationship between plasma fatty acids and insulin sensitivity is usually observed (98), and an even stronger negative correlation is documented with intramuscular triglycerides (79). The latter finding suggests that insulin resistance may be associated with high uptake of circulating fatty acids, which exceeds the muscle capacity for fatty acid oxidation. Based on this, it would be expected that the expression level of a protein that regulates muscle fatty acid uptake would strongly influence insulin responsiveness. The first link between CD36 and insulin resistance was reported with the SHR, a well-studied model of human insulin-resistance syndrome X. Quantitative trait loci for SHR defects in glucose and fatty acid metabolism map to a single locus on rat chromosome 4. The use of cDNA microarrays identified CD36 as a defective gene in SHR at the peak of linkage to these quantitative trait loci. The SHR-CD36 gene contained multiple sequence variants, and the CD36 protein was undetectable in SHR adipocyte plasma membrane (11). A congenic SHchr4 line, in which a piece of chromosome 4 with CD36 was integrated into the SHR genome (11), and later transgenic rescue of CD36 in SHR (101) normalized blood lipids and insulin responsiveness, but hypertension was only marginally improved. These results suggested that CD36 deficiency underlies defective fatty acid metabolism and hypertriglyceridemia in SHR and may be important in the pathogenesis of insulin resistance in this animal model. Accordingly, feeding the SHR a diet supplemented with medium- and short-chain fatty acids reversed the hyperinsulinemia (55) by providing tissues with energy from fatty acid oxidation because uptake of these fatty acids is independent of CD36 (5).

Greenwalt et al. (50) documented several-fold increases in myocardial CD36 in genetically diabetic mice KKAy and NOD. These changes may result to some

extent from the hyperglycemia of diabetes. We found that muscle CD36 protein levels were significantly increased in streptozotocin diabetic rats and that the magnitude of the increase correlated well with the severity of diabetes as assessed by the degree of hyperglycemia. The effect appeared to reflect changes at the post-transcriptional level because CD36 mRNA expression was not altered. As already mentioned, Griffin et al. (51) established that high glucose upregulates CD36 by increasing translation efficiency of the mRNA.

Other evidence for a role of CD36 in insulin responsiveness comes from studies with mice with muscle overexpression of CD36 (66) and from CD36-null mice (T. Hajri, X. Han, A. Bonen, & N.A. Abumrad, unpublished data). These studies indicate that CD36 expression level strongly impacts muscle glucose utilization and insulin sensitivity. For example, transgenic mice overexpressing CD36 (6–8 months) show increases in plasma glucose and insulin levels. On the other hand, CD36-null mice are hypoglycemic (41), hypoinsulinemic, and more insulin sensitive than the wild type, but they show reduced tolerance to fructose-induced insulin resistance (T. Hajri, X. Han, A. Bonen, & N.A. Abumrad, unpublished data). Further studies should help document the role of fatty acid utilization in insulin resistance and allow a better understanding of the molecular mechanisms involved.

Data linking CD36 deficiency and insulin resistance in humans are currently limited and controversial (9, 30, 68, 93, 99). Miyaoka et al. (93) studied a limited number of CD36-deficient patients, using the euglycemic hyperinsulinemic clamp technique and documented in all cases abnormalities of glucose metabolism (9, 93). However, conclusions from this study were not supported by findings from other studies (30, 158), which reported that young CD36-deficient patients showed no sign of insulin resistance. As Chiba et al. suggested (30), the data obtained by Miyaoka et al. might reflect the older age of the CD36-deficient patients used (over 64 years old versus 60 years old for controls), who exhibited other abnormalities such as hypertension, hyperlipidemia, or hyperglycemia. Our data from CD36-null mice are more consistent with the studies of Yanai and colleagues (158). Our current interpretation is that the effect of CD36 deficiency on insulin responsiveness is strongly diet-dependent, and consideration of this interaction could help reconcile some of the divergent effects in humans.

Conclusion

Over the past several years the identification of CD36 as an LCFA transporter has significantly contributed to understanding the regulation of fatty acid uptake and utilization. It will be important to gain a better understanding of the interactions between defects in CD36 expression or function and abnormalities in other proteins important for fatty acid or glucose utilization. Such proteins could include those implicated in fatty acid transport, as discussed below. CD36 interaction with cytosolic FABPs and with enzymes important for activation or esterification of fatty acids are also likely to be important. Most metabolic diseases are not caused by defects in a single gene but are likely to involve a set of interactions

between various genes, and these are in turn modulated by environmental factors. A better knowledge of the metabolic role of CD36 and the tissue-specific alterations in its expression brought about by hormonal or nutritional factors should contribute valuable insight into the set of conditions that result in metabolic pathology. CD36 is likely to be important for a better evaluation of the role played by lipid metabolism in the etiology of diseases such as obesity, insulin resistance, heart hypertrophy, and possibly heart failure.

In addition to CD36, other proteins have been implicated in fatty acid transport and are discussed in the following sections. Although there is little in vivo data documenting a role of these proteins in fatty acid uptake, there is substantial in vitro evidence to support such a role. The discussion is limited to the fatty acid transport protein (FATP) family of proteins and to membrane FABP (FABPpm) because of the amount of work devoted to these proteins and based on the progress generated so far. As shown in Table 1, adipocyte differentiation–related protein (ADRP) and caveolin have also been implicated in fatty acid uptake by a few

TABLE 1 Membrane proteins implicated in FA transport

Protein	Mass (KDa)	Tissue expression[a]	Animal models
FABPpm	40	liver, heart, muscle, fat, intestine	—
CD36/FAT	88[b]	heart, testis, intestine, muscle, fat, spleen, platelets, monocyte/macrophage, endothelium, epidermis, kidney, brain, liver	CD36 null mice (41) transgenic mice with muscle CD36 +++ (66) SHR (11) CD36 rescued SHR (101)
FATP1	71	fat, heart, muscle, brain, kidney, epidermis, lung	—
FATP2		kidney, liver, intestine	—
FATP3		lung, liver, testis	—
FATP4		intestine, kidney, lung, brain, testis, heart, muscle, spleen	—
FATP5		liver	—
FATP6		heart	—
Caveolin 1[c]	21–24	fat, endothelium, lung	null (103)
ADRP	53	fat, liver	—

[a]For CD36 and FATP1, tissues are listed in decreasing order of expression level. FABPpm is ubiquitously expressed.

[b]Glycosylated protein mass. The mass of the nonglycosylated protein is 54. Glucosylation may differ in some tissues, such as the mammary gland, where CD36 molecular weight is about 75 kDa.

[c]Caveolin 1 is a constituent and a defining structural element of caveolae. Caveolin 1 has been linked to the transport of LCFA into the cell.

ADRP: Adipocyte differentiation related protein (46); FABPpm, membrane fatty acid-binding proteins; FATP, fatty acid transport proteins; FAT, fatty acid translocase.

studies (46, 103, 113). These proteins may function in fatty acid uptake, especially in specific tissues, but more extensive documentation of this role is still lacking.

THE FAMILY OF FATTY ACID TRANSPORT PROTEINS

Using expression cloning and fluorescent LCFA derivatives, Schaffer et al. (110) identified a 63-kDa protein as a fatty acid transporter. Screening of COS7 cells for fluorescence following expression of cDNAs from a 3T3-L1 adipocyte cDNA library yielded two types of cDNAs. One of these coded for a novel protein (63 kDa) with four to six possible transmembrane domains and was designated as FATP (later renamed FATP1). The other cDNA coded for a protein with high analogy to liver fatty acyl-CoA synthetase. Evidence of FATP1's role in fatty acid transport was demonstrated in mouse FATP1-transfected cells (110) by showing a significant increase in uptake of LCFAs (myristic, palmitic, and arachidonic acid) compared with a smaller change in uptake of short chain fatty acids.

Hirsch et al. (62) later demonstrated that mouse FATP1 is a member of a larger family of homologous proteins. FATP-related sequences were assembled into five distinct contiguous DNA sequences. One corresponded to the original FATP protein, mouse FATP1. The second, mouse FATP2, was shown to be the murine homologue of the rat very long-chain acyl-CoA synthetase (149). The remaining three proteins were designated mouse FATP3, FATP4, and FATP5 (122). Six homologous proteins were also identified in man (17, 43, 62, 123, 134, 155). Transfection of mouse FATP1, FATP2, or FATP5 into COS cells induced a strong increase in uptake of the fluorescent fatty acid analog, BODIPY 3823 (4,4-difluoro-5-methyl-4-bora-3a,4a-diaza-3-indacne-3-dodecanoic acid) (62).

The function of these proteins in fatty acid uptake has been questioned based on their similarity with long-chain fatty acid–acyl-CoA synthetases. All FATPs have a highly conserved domain related to ATP-binding and hydrolysis. This domain is present in proteins that bind ATP and form adenylated intermediates, which include members of the luciferase family and long-chain fatty acid–acyl-CoA synthases (17, 149). The ATP-binding site is highly conserved and appears essential for fatty acid transport activity, but the mechanism involved remains unclear.

Long-chain fatty acid–acyl-CoA synthetases esterify fatty acids to produce acyl-CoA and might enhance the entry of fatty acids into cells, probably by lowering the concentration of free fatty acids in the cytosol and creating a concentration gradient between extra- and intracellular fatty acid pools. Thus, it is possible that FATPs function as a membrane-associated CoA-synthetase in concert with membrane fatty acid trasnporters or receptors to facilitate fatty acid uptake in a vectorial fashion. There is no in vivo data available yet to document the physiological role of the FATP family of proteins or pathological alterations related to FATP deficiency or polymorphism. However, animal models null for the various FATPs are being generated and should provide important insight into the role of these

proteins in the near future. It is likely that this role will be significant, based on the indirect data discussed below.

Distribution

The different FATPs have interesting tissue-specific distributions (123). FATP1 is mostly expressed in adipose tissue but is also found in the heart and muscle (22). FATP2 is found mainly in liver and kidney cortex (43, 62), and FATP3 is expressed in many tissues, with a notable high expression in the lung. FATP4 is the only FATP found in the intestine and has been shown to facilitate LCFA uptake in isolated intestinal epithelial cells. It is also expressed in kidney, liver, and brain (43). FATP5 is exclusively expressed in liver (62), and murine FATP6 is mostly located in the heart (122).

Regulation

Recent reports have described how FATP expression is regulated by nutrients, hormones and cytokines (89), and PPAR ligands (86, 87, 94). In the mouse, FATP mRNA levels in adipose tissue increase significantly after 48-h fasting and returned to basal levels after feeding (85). This effect has been attributed to changes in insulin levels during the fasting-feeding cycle (21, 85) because downregulation of FATP mRNA by insulin has been documented in cultured adipocytes (85, 110) and was shown to be exerted at the transcriptional level. A surprising finding was that the adipogenic hormone insulin increased the expression of long-chain fatty acid–acyl-CoA synthetases in murine adipocytes under the same conditions that it downregulated that of FATP1 (85).

In man, insulin infusion reduced FATP-1 mRNA levels in skeletal muscle in lean women but not in lean men (22), suggesting a gender-related effect of insulin. In addition, there was no effect of insulin on FATP-1 mRNA in adipose tissue or in skeletal muscle of obese nondiabetic and diabetic subjects, which may imply that FATP1 does not contribute to a large extent to the alterations of fatty acid uptake in obesity and/or diabetes. In agreement with this conclusion, Memon et al. (90) have shown that FATP1 is downregulated in the adipose tissue of ob/ob mice, which have increased adipogenesis.

Like CD36, FATP is regulated by PPARs (44). Several reports have shown a positive regulation of mouse FATP by ligands that activate either PPAR- or PPAR-RXR heterodimers in hepatoma cell lines, the liver, and the intestine (86, 87, 94). Furthermore, a PPAR-binding site was identified in the murine FATP1 promoter. The PPAR-α activator Wy 14,643 and the PPAR-γ activator triglitazone both induced the expression of mouse FATP1, whereas the PPAR-δ agonist did not. Treatment of AML12 liver cells, 3T3-L1 preadipocytes, or adipocytes with the PPAR-α activator fenofibrate, PPAR-γ activators (BRL 49653, Triglitazone), and 9-cis-retinoic acid (44) stimulated fatty acid uptake, suggesting that FATP1 is upregulated by PPAR-α and PPAR-γ.

Regulation of mouse FATP1 expression by inflammatory mediators seems to be tissue specific. FATP1 mRNA in hamster was downregulated by the tumor necrosis

factor-α, interleukin-1, and by endotoxin in adipose tissue, heart, muscle, and spleen, whereas it was increased in liver. These effects were related to a decrease in fatty acid oxidation in peripheral tissues and to an increase of triglycerides and very low-density lipoprotein synthesis in liver (89).

MEMBRANE FATTY ACID BINDING PROTEIN ($FABP_{pm}$)

Berk and collaborators identified and isolated FABPpm from plasma membranes obtained from rat liver and jejunal microvilli (130, 132). Passage on an oleate-agarose affinity column identified a 40-kDa fatty acid-binding protein, named FABPpm to highlight its association with plasma membranes. In contrast to CD36 and FATP, FABPpm is not a transmembrane protein (132). Partial sequencing of FABPpm showed that it has a physical and chemical similarities with mitochondrial aspartate aminotransferase (19). Later studies showed that FABPpm and aspartate aminotransferase have identical molecular weights, similar affinity for fatty acids, and exhibit cross-reactivity with specific antibodies (19). These studies raised concerns about the identity of FABPpm and mitochondrial aspartate aminotransferase and suggested possible contamination of plasma membrane fractions with mitochondrial proteins. Although it was very difficult to completely rule this out, careful studies by Berk and collaborators demonstrated that mitochondrial contamination was unlikely to account for membrane association of FABPpm.

The role of FABPpm in fatty acid uptake was based initially on its ability to bind labeled photoreactive fatty acid analogs and the oleate affinity column (18). Later studies documented direct binding of LCFAs to purified FABPpm (132, 136). Furthermore, an antibody produced against the protein partially inhibited fatty acid uptake in several cell types including adipocytes, hepatocytes, myocytes, and intestinal epithelial cells (112, 127, 128, 131, 133, 160, 161). Inhibition was never complete, which suggested that FABPpm may account for up to 50% of the measured fatty acid uptake rates. However, the magnitude of the inhibitory effect is likely to depend on the particular experimental conditions, such as the fatty acid:albumin ratio and the affinity of the particular antibody used, and cannot be easily used as a quantitative measure. More recent work has provided additional supporting evidence for a role of FABPpm in fatty acid binding/transport. Expression of FABPpm in *Xenopus laevis* oocytes (160) and in 3T3 fibroblasts (161) was associated in each case with an increase in fatty acid uptake rates. The increase in 3T3 fibroblasts reflected the addition of a saturable, high affinity component and could be inhibited by an antibody against FABPpm. Like CD36/Fat, FABPpm is upregulated during adipocyte differentiation, a process associated with a significant increase in fatty acid uptake (161).

Tissue Distribution

FABPpm was shown to be associated with the plasma membrane in many tissues including liver, adipose tissue, cardiac muscle, intestine, and vascular

endothelium (112, 117, 130). Rat showed that rested red skeletal muscle was shown to contain 80% more FABPpm than white skeletal muscle, which is consistent with the higher oxidative capacity in red muscle relative to white (15, 148).

Regulation

Berk and collaborators (21) have shown that FABP mRNA in a rat hepatoma cell line is upregulated after dexamethasone treatment, and this is reversed by cotreatment with insulin. Treatment with the cAMP analog, 8-bromoadensosine 3′,5′-monophosphate upregulated FABPpm mRNA.

In humans subjected to a three-week one-legged intense exercise, there was significant upregulation of FABPpm content (76). Activity of citrate synthase was increased under the same conditions, suggesting an increase of fatty acid uptake and oxidation (147). Fasting increased FABPpm protein content in red muscle, but there was no change in FABPpm in white muscle (148). This finding again documented an association between oxidative capacity of muscle and FABPpm expression. Ethanol treatment of HepG2 cells (162) caused a dose-dependent increase in FABPpm mRNA and protein released into the media. Immunochemical staining revealed that while FABPpm content in the mitochondria was relatively constant, FABPpm signal in the plasma membrane was increased. The authors reported a parallel increase in cellular oleate uptake and triglyceride content in cells.

CONCLUSIONS AND PERSPECTIVES

In the case of LCFAs, as for other nutritional substrates such as glucose or amino acids, balanced whole-body utilization appears to be achieved in large part by acute and chronic regulation of membrane transport. Protein facilitators of fatty acid uptake that are differentially expressed and regulated would allow an adaptive response that is tailored to metabolic needs at both the level of the cell and between various tissues. Acute regulation of transport, as documented recently, is undoubtedly necessary in the course of the normal daily nutritional transitions and with fasting and strenuous exercise. This regulation is an essential part of the body's ability to maintain a consistent and adequate supply of fuel to all organs. In addition, because utilization of fatty acids is closely linked to that of glucose, protein facilitators of fatty acid uptake are very likely integral to the local homeostatic mechanisms that determine the balance of substrate utilization according to substrate availability, hormonal status, and energy demand. As a result, chronic alterations in the expression or function of these proteins is likely to impact susceptibility to a number of common metabolic pathologies.

The metabolic phenotype of CD36 deficiency has provided a first broad overview of the important role of membrane fatty acid transport in fatty acid utilization and in overall metabolism. Future work will attempt to dissect in detail the molecular and regulatory mechanisms involved. Determining the factors that regulate fatty

acid transport acutely and chronically should provide important information that is relevant both from basic and clinical perspectives. Questions related to the links between abnormalities in fatty acid transport and insulin resistance or coronary disease will be complex and are likely to require integration of findings from various research areas. However, with the availability of various animal models with alterations in fatty acid transport, we now have better tools to begin addressing these questions.

A question that is particularly interesting relates to the role played by alterations in CD36 expression in mediating some of the effects of the various PPAR agonists that are used clinically, such as the fibrates and insulin-sensitizing glitazones. The role of CD36 in supplying ligands for activation of the various PPARs would also be interesting to explore. The PPAR-CD36 interaction could promote a positive feedback loop with effect potentiation that could impact the etiology of conditions such as obesity and diabetes type II. The roles of fatty acid transport and CD36 in the heart and in its response to ischemic insult should be investigated carefully because CD36 deficiency is associated with a severe defect in myocardial fatty acid utilization in humans.

Other areas that are being pursued actively relate to the role of CD36 in cell signaling. For example, a better understanding of the interactions between CD36 and other protein residents of rafts and caveolae is likely to generate insight into the role fatty acids play in so many different aspects of cell physiology.

As research with the other proteins implicated in fatty acid transport is extended to the in vivo situation, we should gain important insight into the physiological role of these membrane proteins, and this will undoubtedly open new areas of investigation. For example, animal models with alterations in the different FATPs are currently under development and should provide valuable models for understanding the function of the different FATPs, which have a distinctive and intriguing tissue-specific distribution.

ACKNOWLEDGMENTS

This work was supported by an American Heart Association Fellowship, AHA0020 639T (to T.H.), and National Institutes of Health Grant RO1-DK33301 (to N.A.A.).

The *Annual Review of Nutrition* is online at http://nutr.annualreviews.org

LITERATURE CITED

1. Abumrad N, Coburn CT, 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
2. Abumrad N, Harmon C, Ibrahimi A. 1998. Membrane transport of long-chain fatty acids: evidence for a facilitated process. *J. Lipid Res.* 39:2309–18
3. Abumrad NA, el-Maghrabi MR, Amri EZ, Lopez E, Grimaldi PA. 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.* 268:17665–68

4. Abumrad NA, Harmon CM, Barnela US, Whitesell RR. 1988. Insulin antagonism of catecholamine stimulation of fatty acid transport in the adipocyte. Studies on its mechanism of action. *J. Biol. Chem.* 263:14678–83
5. Abumrad NA, Park JH, Park CR. 1984. Permeation of long-chain fatty acid into adipocytes. Kinetics, specificity, and evidence for involvement of a membrane protein. *J. Biol. Chem.* 259:8945–53
6. Abumrad NA, Perkins RC, Park JH, Park CR. 1981. Mechanism of long chain fatty acid permeation in the isolated adipocyte. *J. Biol. Chem.* 256:9183–91
7. Abumrad NA, Perry PR, Whitesell RR. 1986. Insulin antagonizes epinephrine activation of the membrane transport of fatty acids. Potential site for hormonal suppression of lipid mobilization. *J. Biol. Chem.* 261:2999–3001
8. Abumrad NA, Sfeir Z, Connelly MA, Coburn C. 2000. Lipid transporters: membrane transport systems for cholesterol and fatty acids. *Curr. Opin. Clin. Nutr. Metab. Care* 3:255–62
9. Aitman TJ. 2001. CD36, insulin resistance, and coronary heart disease. *Lancet* 357:651–52
10. Aitman TJ, Cooper LD, Norsworthy PJ, Wahid FN, Gray JK, et al. 2000. Malaria susceptibility and CD36 mutation. *Nature* 405:1015–16
11. Aitman TJ, Glazier AM, Wallace CA, Cooper LD, Norsworthy PJ, et al. 1999. Identification of Cd36 (Fat) as an insulin-resistance gene causing defective fatty acid and glucose metabolism in hypertensive rats [see comments]. *Nat. Genet.* 21:76–83
12. Amri EZ, Bonino F, Ailhaud G, Abumrad NA, Grimaldi PA. 1995. Cloning of a protein that mediates transcriptional effects of fatty acids in preadipocytes. Homology to peroxisome proliferator-activated receptors. *J. Biol. Chem.* 270:2367–71
13. Amri EZ, Teboul L, Vannier C, Grimaldi PA, Ailhaud G. 1996. Fatty acids regulate the expression of lipoprotein lipase gene and activity in preadipose and adipose cells. *Biochem. J.* 314(Pt 2):541–46
14. Baillie AGS, Coburn CT, Abumrad NA. 1996. Reversible binding of long-chain fatty acids to purified FAT, the adipose CD36 homolog. *J. Membr. Biol.* 153:75–81
15. Baldwin KM, Tipton CM. 1972. Work and metabolic patterns of fast and slow twitch skeletal muscle contracting in situ. *Pflugers Arch.* 334:345–56
16. Bastie C, Luquet S, Holst D, Jehl-Pietri C, Grimaldi PA. 2000. Alterations of peroxisome proliferator-activated receptor delta activity affect fatty acid-controlled adipose differentiation. *J. Biol. Chem.* 275:38768–73
17. Berger J, Truppe C, Neumann H, 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.* 247:255–60
18. Berk PD, Bradbury M, Zhou SL, Stump D, Han NI. 1996. Characterization of membrane transport processes: lessons from the study of BSP, bilirubin, and fatty acid uptake. *Semin. Liver Dis.* 16:107–20
19. Berk PD, Potter BJ, Sorrentino D, Stump D, Kiang CL, et al. 1990. Hepatocellular fatty acid uptake is mediated by a plasma membrane fatty acid binding protein closely related to mitochondrial glutamic oxaloacetic transaminase. *Ann. NY Acad. Sci.* 585:379–85
20. Berk PD, Stump DD. 1999. Mechanisms of cellular uptake of long chain free fatty acids. *Mol. Cell. Biochem.* 192:17–31
21. Berk PD, Zhou SL, Kiang CL, Stump D, Bradbury M, Isola LM. 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.* 272:8830–35
22. Binnert C, Koistinen HA, Martin G, Andreelli F, Ebeling P, et al. 2000. Fatty acid transport protein-1 mRNA expression in skeletal muscle and in adipose tissue in humans. *Am. J. Physiol. Endocrinol. Metab.* 279:E1072–E79
23. Boden G, Chen X, Ruiz J, White JV, Rossetti L. 1994. Mechanisms of fatty acid-induced inhibition of glucose uptake. *J. Clin. Invest.* 93:2438–46
24. Bonen A, Dyck DJ, Ibrahimi A, Abumrad NA. 1999. Muscle contractile activity increases fatty acid metabolism and transport and FAT/CD36. *Am. J. Physiol. Endocrinol. Metab.* 276:E642–E49
25. Bonen A, Luiken JJ, Arumugam Y, Glatz JF, Tandon NN. 2000. Acute regulation of fatty acid uptake involves the cellular redistribution of fatty acid translocase. *J. Biol. Chem.* 275:14501–8
26. Bull HA, Brickell PM, Dowd PM. 1994. Src-related protein tyrosine kinases are physically associated with the surface antigen CD36 in human dermal microvascular endothelial cells. *FEBS Lett.* 351:41–44
27. Cha BS, Ciaraldi TP, Carter L, Nikoulina SE, Mudaliar S, et al. 2001. Peroxisome proliferator-activated receptor (PPAR) gamma and retinoid X receptor (RXR) agonists have complementary effects on glucose and lipid metabolism in human skeletal muscle. *Diabetologia* 44:444–52
28. Chawla A, Barak Y, Nagy L, Liao D, Tontonoz P, Evans RM. 2001. PPAR-gamma dependent and independent effects on macrophage-gene expression in lipid metabolism and inflammation. *Nat. Med.* 7:48–52
29. Chen M, Yang Y, Braunstein E, Georgeson KE, Harmon CM. 2001. Gut expression and regulation of FAT/CD36: possible role in fatty acid transport in rat enterocytes. *Am. J. Physiol. Endocrinol. Metab.* 281:E916–E23
30. Chiba H, Yanai H, Fujiwara H, Matsuno K, Kobayashi K. 2001. CD36 deficiency and insulin resistance. *Lancet* 358:243 (discussion)
31. Christie GR, Hyde R, Hundal HS. 2001. Regulation of amino acid transporters by amino acid availability. *Curr. Opin. Clin. Nutr. Metab. Care* 4:425–31
32. Clinton SK, Giovannucci E. 1998. Diet, nutrition, and prostate cancer. *Annu. Rev. Nutr.* 18:413–40
33. Coburn CT, Knapp FF Jr, Febbraio M, Beets AL, Silverstein RL, Abumrad NA. 2000. Defective uptake and utilization of long chain fatty acids in muscle and adipose tissues of CD36 knockout mice. *J. Biol. Chem.* 275:32523–29
34. Connelly MA, de la Llera-Moya M, Monzo P, Yancey PG, Drazul D, et al. 2001. Analysis of chimeric receptors shows that multiple distinct functional activities of scavenger receptor, class B, type I (SR-BI), are localized to the extracellular receptor domain. *Biochemistry* 40:5249–59
35. Curtis BR, Aster RH. 1996. Incidence of the Nak(a)-negative platelet phenotype in African Americans is similar to that of Asians. *Transfusion* 36:331–34
36. Cushman SW, Wardzala LJ. 1980. Potential mechanism of insulin action on glucose transport in the isolated rat adipose cell. Apparent translocation of intracellular transport systems to the plasma membrane. *J. Biol. Chem.* 255:4758–62
37. DiRusso CC, Black PN. 1999. Long-chain fatty acid transport in bacteria and yeast. Paradigms for defining the mechanism underlying this protein-mediated process. *Mol. Cell. Biochem.* 192:41–52
38. Doggrell SA, Brown L. 1998. Rat models of hypertension, cardiac hypertrophy and failure. *Cardiovasc. Res.* 39:89–105
39. Duplus E, Glorian M, Forest C. 2000. Fatty acid regulation of gene transcription. *J. Biol. Chem.* 275:30749–52

40. Dutta-Roy AK. 2000. Cellular uptake of long-chain fatty acids: role of membrane-associated fatty-acid-binding/transport proteins. *Cell. Mol. Life Sci.* 57:1360–72
41. Febbraio M, Abumrad NA, Hajjar DP, Sharma K, Cheng W, et al. 1999. A null mutation in murine CD36 reveals an important role in fatty acid and lipoprotein metabolism. *J. Biol. Chem.* 274:19055–62
42. Febbraio M, Hajjar DP, Silverstein RL. 2001. CD36: a class B scavenger receptor involved in angiogenesis, atherosclerosis, inflammation, and lipid metabolism. *J. Clin. Invest.* 108:785–91
43. Fitscher BA, Riedel HD, Young KC, Stremmel W. 1998. Tissue distribution and cDNA cloning of a human fatty acid transport protein (hsFATP4). *Biochim. Biophys. Acta* 1443:381–85
44. Frohnert BI, Hui TY, Bernlohr DA. 1999. Identification of a functional peroxisome proliferator-responsive element in the murine fatty acid transport protein gene. *J. Biol. Chem.* 274:3970–77
45. Fukuchi K, Nozaki S, Yoshizumi T, Hasegawa S, Uehara T, et al. 1999. Enhanced myocardial glucose use in patients with a deficiency in long-chain fatty acid transport (CD36 deficiency). *J. Nucl. Med.* 40:239–43
46. Gao J, Serrero G. 1999. Adipose differentiation related protein (ADRP) expressed in transfected COS-7 cells selectively stimulates long chain fatty acid uptake. *J. Biol. Chem.* 274:16825–30
47. Glatz JF, Storch J. 2001. Unravelling the significance of cellular fatty acid-binding proteins. *Curr. Opin. Lipidol.* 12:267–74
48. Goodman DS, Shiratori T. 1964. Fatty acid composition of human plasma lipoprotein fractions. *J. Lipid Res.* 5:307–13
49. Greenwalt DE, Lipsky RH, Ockenhouse CF, Ikeda H, Tandon NN, Jamieson GA. 1992. Membrane glycoprotein CD36: a review of its roles in adherence, signal transduction, and transfusion medicine. *Blood* 80:1105–15
50. Greenwalt DE, Scheck SH, 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.* 96:1382–88
51. Griffin E, Re A, Hamel N, Fu C, Bush H, et al. 2001. A link between diabetes and atherosclerosis: Glucose regulates expression of CD36 at the level of translation. *Nat. Med.* 7:840–46
52. Grimaldi PA. 2001. Fatty acid regulation of gene expression. *Curr. Opin. Clin. Nutr. Metab. Care* 4:433–37
53. Grimaldi PA, Teboul L, Gaillard D, Armengod AV, Amri EZ. 1999. Long chain fatty acids as modulators of gene transcription in preadipose cells. *Mol. Cell. Biochem.* 192:63–68
54. Guthmann F, Haupt R, Looman AC, Spener F, Rustow B. 1999. Fatty acid translocase/CD36 mediates the uptake of palmitate by type II pneumocytes. *Am. J. Physiol. Lung Cell Mol. Physiol.* 277:L191–96
55. Hajri T, Ibrahimi A, Coburn CT, Knapp FF Jr, Kurtz T, et al. 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.* 276:23661–66
56. Hamilton JA, Johnson RA, Corkey B, Kamp F. 2001. Fatty acid transport: the diffusion mechanism in model and biological membranes. *J. Mol. Neurosci.* 16:99–108; discussion, pp. 51–57
57. Hamilton JA, Kamp F. 1999. How are free fatty acids transported in membranes? Is it by proteins or by free diffusion through the lipids? *Diabetes* 48:2255–69
58. Harmon CM, Abumrad NA. 1993. Binding of sulfosuccinimidyl 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
59. Heller A, Koch T, Schmeck J, van Ackern

K. 1998. Lipid mediators in inflammatory disorders. *Drugs* 55:487–96

60. Hevener AL, Reichart D, Janez A, Olefsky J. 2001. Thiazolidinedione treatment prevents free fatty acid-induced insulin resistance in male wistar rats. *Diabetes* 50: 2316–22
61. Deleted in proof
62. Hirsch D, Stahl A, Lodish HF. 1998. A family of fatty acid transporters conserved from mycobacterium to man. *Proc. Natl. Acad. Sci. USA* 95:8625–29
63. Ho M, White NJ. 1999. Molecular mechanisms of cytoadherence in malaria. *Am. J. Physiol. Cell Physiol.* 276:C1231–42
64. Huang MM, Bolen JB, Barnwell JW, Shattil SJ, Brugge JS. 1991. Membrane glycoprotein IV (CD36) is physically associated with the Fyn, Lyn, and Yes protein-tyrosine kinases in human platelets. *Proc. Natl. Acad. Sci. USA* 88:7844–48
65. Hwang EH, Taki J, Yasue S, Fujimoto M, Taniguchi M, et al. 1998. Absent myocardial iodine-123-BMIPP uptake and platelet/monocyte CD36 deficiency. *J. Nucl. Med.* 39:1681–84
66. Ibrahimi A, Bonen A, Blinn WD, Hajri T, Li X, et al. 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.* 274:26761–66
67. Ibrahimi A, Sfeir Z, Magharaie H, Amri EZ, Grimaldi P, Abumrad NA. 1996. Expression of the CD36 homolog (FAT) in fibroblast cells: effects on fatty acid transport. *Proc. Natl. Acad. Sci. USA* 93:2646–51
68. Iizuka Y, Gotoda T, Ishibashi S, Yamada N. 2001. CD36 deficiency and insulin resistance. *Lancet* 358:243 (discussion)
69. Ito K, Sugihara H, Tanabe T, Zen K, Hikosaka T, et al. 2001. A patient with type I CD36 deficiency whose myocardium accumulated 123I-BMIPP after 4 years. *Ann. Nucl. Med.* 15:271–76
70. Jump DB. 2001. The biochemistry of N3-polyunsaturated fatty acids. *J. Biol. Chem.* Published Dec. 17, 2001 as 10.1074/jbc.R100062200
71. Kamp F, Hamilton JA, Westerhoff HV. 1993. Movement of fatty acids, fatty acid analogues, and bile acids across phospholipid bilayers. *Biochemistry* 32:11074–86
72. Kashiwagi H, Tomiyama Y, Honda S, Kosugi S, Shiraga M, et al. 1995. Molecular basis of CD36 deficiency. Evidence that a 478C→T substitution (proline90 →serine) in CD36 cDNA accounts for CD36 deficiency. *J. Clin. Invest.* 95:1040–46
73. Kashiwagi H, Tomiyama Y, Nozaki S, Honda S, Kosugi S, et al. 1996. A single nucleotide insertion in codon 317 of the CD36 gene leads to CD36 deficiency. *Arterioscler. Thromb. Vasc. Biol.* 16:1026–32
74. Kashiwagi H, Tomiyama Y, Nozaki S, Kiyoi T, Tadokoro S, et al. 2001. Analyses of genetic abnormalities in type I CD36 deficiency in Japan: identification and cell biological characterization of two novel mutations that cause CD36 deficiency in man. *Hum. Genet.* 108:459–66
75. Kerkhoff C, Sorg C, Tandon NN, Nacken W. 2001. Interaction of S100A8/S100A9-arachidonic acid complexes with the scavenger receptor CD36 may facilitate fatty acid uptake by endothelial cells. *Biochemistry* 40:241–48
76. Kiens B, Kristiansen S, Jensen P, Richter EA, Turcotte LP. 1997. Membrane associated fatty acid binding protein (FABPpm) in human skeletal muscle is increased by endurance training. *Biochem. Biophys. Res. Commun.* 231:463–65
77. Kim JK, Fillmore JJ, Chen Y, Yu C, Moore IK, et al. 2001. Tissue-specific overexpression of lipoprotein lipase causes tissue-specific insulin resistance. *Proc. Natl. Acad. Sci. USA* 98:7522–27
78. Kleinfeld AM. 2000. Lipid phase fatty acid flip-flop: Is it fast enough for cellular transport? *J. Membr. Biol.* 175:79–86

79. Krssak M, Falk Petersen K, Dresner A, DiPietro L, Vogel SM, et al. 1999. Intramyocellular lipid concentrations are correlated with insulin sensitivity in humans: a 1H NMR spectroscopy study. *Diabetologia* 42:113–16
80. Liang X, Nazarian A, Erdjument-Bromage H, Bornmann W, Tempst P, Resh MD. 2001. Heterogeneous fatty acylation of Src family kinases with polyunsaturated fatty acids regulates raft localization and signal transduction. *J. Biol. Chem.* 276:30987–94
81. Lobo MV, Huerta L, Ruiz-Velasco N, Teixeiro E, de la Cueva P, et al. 2001. Localization of the lipid receptors CD36 and CLA-1/SR-BI in the human gastrointestinal tract: towards the identification of receptors mediating the intestinal absorption of dietary lipids. *J. Histochem. Cytochem.* 49:1253–60
82. Luiken JJ, Glatz JF, Bonen A. 2000. Fatty acid transport proteins facilitate fatty acid uptake in skeletal muscle. *Can. J. Appl. Physiol.* 25:333–52
83. Luiken JJ, Schaap FG, van Nieuwenhoven FA, van der Vusse GJ, Bonen A, Glatz JF. 1999. Cellular fatty acid transport in heart and skeletal muscle as facilitated by proteins. *Lipids* 34(Suppl):S169–75
84. Luiken JJ, van Nieuwenhoven FA, America G, van der Vusse GJ, Glatz JF. 1997. Uptake and metabolism of palmitate by isolated cardiac myocytes from adult rats: involvement of sarcolemmal proteins. *J. Lipid Res.* 38:745–58
85. Man MZ, Hui TY, Schaffer JE, Lodish HF, Bernlohr DA. 1996. Regulation of the murine adipocyte fatty acid transporter gene by insulin. *Mol. Endocrinol.* 10:1021–28
86. Martin G, Poirier H, Hennuyer N, Crombie D, Fruchart JC, et al. 2000. Induction of the fatty acid transport protein 1 and acyl-CoA synthase genes by dimer-selective rexinoids suggests that the peroxisome proliferator-activated receptor-retinoid X receptor heterodimer is their molecular target. *J. Biol. Chem.* 275: 12612–18
87. Martin G, Schoonjans K, Lefebvre AM, Staels B, Auwerx J. 1997. Coordinate regulation of the expression of the fatty acid transport protein and acyl-CoA synthetase genes by PPARalpha and PPARgamma activators. *J. Biol. Chem.* 272:28210–17
88. Melki SA, Abumrad NA. 1992. Glycerolipid synthesis in isolated adipocytes: substrate dependence and influence of norepinephrine. *J. Lipid Res.* 33:669–78
89. Memon RA, Feingold KR, Moser AH, Fuller J, Grunfeld C. 1998. Regulation of fatty acid transport protein and fatty acid translocase mRNA levels by endotoxin and cytokines. *Am. J. Physiol. Endocrinol. Metab.* 274:E210–E17
90. Memon RA, Fuller J, Moser AH, Smith PJ, Grunfeld C, Feingold KR. 1999. Regulation of putative fatty acid transporters and Acyl-CoA synthetase in liver and adipose tissue in ob/ob mice. *Diabetes* 48:121–27
91. Memon RA, Tecott LH, Nonogaki K, Beigneux A, Moser AH, et al. 2000. Up-regulation of peroxisome proliferator-activated receptors (PPAR-alpha) and PPAR-gamma messenger ribonucleic acid expression in the liver in murine obesity: troglitazone induces expression of PPAR-gamma-responsive adipose tissue-specific genes in the liver of obese diabetic mice. *Endocrinology* 141:4021–31
92. Miao WM, Vasile E, Lane WS, Lawler J. 2001. CD36 associates with CD9 and integrins on human blood platelets. *Blood* 97:1689–96
93. Miyaoka K, Kuwasako T, Hirano K, Nozaki S, Yamashita S, Matsuzawa Y. 2001. CD36 deficiency associated with insulin resistance. *Lancet* 357:686–87
94. Motojima K, Passilly P, Peters JM, Gonzalez FJ, Latruffe N. 1998. Expression of putative fatty acid transporter genes are regulated by peroxisome proliferator-activated receptor alpha and gamma activators in

a tissue- and inducer-specific manner. *J. Biol. Chem.* 273:16710–14
95. Mutanen M, Freese R. 2001. Fats, lipids and blood coagulation. *Curr. Opin. Lipidol.* 12:25–29
96. Nicholson AC, Febbraio M, Han J, Silverstein RL, Hajjar DP. 2000. CD36 in atherosclerosis. The role of a class B macrophage scavenger receptor. *Ann. NY Acad. Sci.* 902:128–31; discussion, pp. 31–33
97. Nisoli E, Carruba MO, Tonello C, Macor C, Federspil G, Vettor R. 2000. Induction of fatty acid translocase/CD36, peroxisome proliferator-activated receptor-gamma2, leptin, uncoupling proteins 2 and 3, and tumor necrosis factor-alpha gene expression in human subcutaneous fat by lipid infusion. *Diabetes* 49:319–24
98. Perseghin G, Ghosh S, Gerow K, Shulman GI. 1997. Metabolic defects in lean nondiabetic offspring of NIDDM parents: a cross-sectional study. *Diabetes* 46:1001–9
99. Petrie JR, Collison M, Connell JM, Gould GW, Dominiczak AF. 2001. CD36 deficiency and insulin resistance. *Lancet* 358:242–43 (discussion)
100. Poirier H, Degrace P, Niot I, Bernard A, 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.* 238:368–73
101. Pravenec M, Landa V, Zidek V, Musilova A, Kren V, et al. 2001. Transgenic rescue of defective Cd36 ameliorates insulin resistance in spontaneously hypertensive rats. *Nat. Genet.* 27:156–58
102. Rader DJ, Dugi KA. 2000. The endothelium and lipoproteins: insights from recent cell biology and animal studies. *Semin. Thromb. Hemost.* 26:521–28
103. Razani B, Combs TP, Wang XB, Frank PG, Park DS, et al. 2001. Caveolin-1 deficient mice are lean, resistant to diet-induced obesity, and show hyper-triglyceridemia with adipocyte abnormalities. *J. Biol. Chem.* Published Dec. 5, 2001 as 10.1074/jbc.M110970200
104. Richieri GV, Anel A, Kleinfeld AM. 1993. Interactions of long-chain fatty acids and albumin: determination of free fatty acid levels using the fluorescent probe ADIFAB. *Biochemistry* 32:7574–80
105. Richieri GV, Kleinfeld AM. 1995. Unbound free fatty acid levels in human serum. *J. Lipid Res.* 36:229–40
106. Roden M, Price TB, Perseghin G, Petersen KF, Rothman DL, et al. 1996. Mechanism of free fatty acid-induced insulin resistance in humans. *J. Clin. Invest.* 97:2859–65
107. Savill J, Hogg N, Ren Y, Haslett C. 1992. Thrombospondin cooperates with CD36 and the vitronectin receptor in macrophage recognition of neutrophils undergoing apoptosis. *J. Clin. Invest.* 90:1513–22
108. Schaap FG, Hamers L, Van der Vusse GJ, Glatz JF. 1997. Molecular cloning of fatty acid-transport protein cDNA from rat. *Biochim. Biophys. Acta* 1354:29–34
109. Deleted in proof
110. Schaffer JE, Lodish HF. 1994. Expression cloning and characterization of a novel adipocyte long chain fatty acid transport protein [see comments]. *Cell* 79:427–36
111. Schimmel RJ, Goodman HM. 1971. Efflux of free fatty acids from adipose tissue. *Am. J. Physiol.* 221:118–22
112. Schwieterman W, Sorrentino D, Potter BJ, Rand J, Kiang CL, et al. 1988. Uptake of oleate by isolated rat adipocytes is mediated by a 40-kDa plasma membrane fatty acid binding protein closely related to that in liver and gut. *Proc. Natl. Acad. Sci. USA* 85:359–63
113. Serrero G, Frolov A, Schroeder F, Tanaka K, Gelhaar L. 2000. Adipose differentiation related protein: expression, purification of recombinant protein in Escherichia coli and characterization of its fatty acid binding properties. *Biochim. Biophys. Acta* 1488:245–54

114. Sfeir Z, Ibrahimi A, Amri E, Grimaldi P, Abumrad N. 1999. CD36 antisense expression in 3T3-F442A preadipocytes. *Mol. Cell. Biochem.* 192:3–8
115. Singh Ahuja H, Liu S, Crombie DL, Boehm M, Leibowitz MD, et al. 2001. Differential effects of rexinoids and thiazolidinediones on metabolic gene expression in diabetic rodents. *Mol. Pharmacol.* 59:765–73
116. Sorrentino D, Robinson RB, Kiang CL, Berk PD. 1989. At physiologic albumin/-toleate 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.* 84:1325–33
117. Sorrentino D, Stump D, Potter BJ, Robinson RB, White R, et al. 1988. Oleate uptake by cardiac myocytes is carrier mediated and involves a 40-kD plasma membrane fatty acid binding protein similar to that in liver, adipose tissue, and gut. *J. Clin. Invest.* 82:928–35
118. Sorrentino D, Stump DD, Van Ness K, Simard A, Schwab AJ, et al. 1996. Oleate uptake by isolated hepatocytes and the perfused rat liver is competitively inhibited by palmitate. *Am. J. Physiol. Gastrointest. Liver Physiol.* 270:G385–92
119. Spector AA, John K, Fletcher JE. 1969. Binding of long-chain fatty acids to bovine serum albumin. *J. Lipid Res.* 10:56–67
120. Spector AA, Steinberg D. 1965. The utilization of unesterified palmitate by Ehrlich ascites tumor cells. *J. Biol. Chem.* 240: 3747–53
121. Spitsberg VL, Matitashvili E, Gorewit RC. 1995. Association and coexpression of fatty-acid-binding protein and glycoprotein CD36 in the bovine mammary gland. *Eur. J. Biochem.* 230:872–78
122. Stahl A, Gimeno RE, Tartaglia LA, Lodish HF. 2001. Fatty acid transport proteins: a current view of a growing family. *Trends Endocrinol. Metab.* 12:266–73
123. Stahl A, Hirsch DJ, Gimeno RE, Punreddy S, Ge P, et al. 1999. Identification of the major intestinal fatty acid transport protein. *Mol. Cell.* 4:299–308
124. Stals HK, Top W, Declercq PE. 1994. Regulation of triacylglycerol synthesis in permeabilized rat hepatocytes. Role of fatty acid concentration and diacylglycerol acyltransferase. *FEBS Lett.* 343:99–102
125. Steinberg GR, Dyck DJ, Calles-Escandon J, Tandon NN, Luiken JJ, et al. 2001. Chronic leptin administration decreases fatty acid uptake and fatty acid transporters in rat skeletal muscle. *J. Biol. Chem.* Published Nov. 29, 2001 as 10.1074/jbc.M107683200
126. Storch J, Lechene C, Kleinfeld AM. 1991. Direct determination of free fatty acid transport across the adipocyte plasma membrane using quantitative fluorescence microscopy. *J. Biol. Chem.* 266: 13473–76
127. Stremmel W. 1988. Fatty acid uptake by isolated rat heart myocytes represents a carrier-mediated transport process. *J. Clin. Invest.* 81:844–52
128. Stremmel W. 1988. Uptake of fatty acids by jejunal mucosal cells is mediated by a fatty acid binding membrane protein. *J. Clin. Invest.* 82:2001–10
129. Stremmel W, Berk PD. 1986. Hepatocellular influx of [14C]oleate reflects membrane transport rather than intracellular metabolism or binding. *Proc. Natl. Acad. Sci. USA* 83:3086–90
130. Stremmel W, Lotz G, Strohmeyer G, Berk PD. 1985. Identification, isolation, and partial characterization of a fatty acid binding protein from rat jejunal microvillous membranes. *J. Clin. Invest.* 75:1068–76
131. Stremmel W, Strohmeyer G, Berk PD. 1986. Hepatocellular uptake of oleate is energy dependent, sodium linked, and inhibited by an antibody to a hepatocyte plasma membrane fatty acid binding protein. *Proc. Natl. Acad. Sci. USA* 83: 3584–88

132. Stremmel W, Strohmeyer G, Borchard F, Kochwa S, Berk PD. 1985. Isolation and partial characterization of a fatty acid binding protein in rat liver plasma membranes. *Proc. Natl. Acad. Sci. USA* 82:4–8
133. Stremmel W, Theilmann L. 1986. Selective inhibition of long-chain fatty acid uptake in short-term cultured rat hepatocytes by an antibody to the rat liver plasma membrane fatty acid-binding protein. *Biochim. Biophys. Acta* 877:191–97
134. Stuhlsatz-Krouper SM, Bennett NE, Schaffer JE. 1998. Substitution of alanine for serine 250 in the murine fatty acid transport protein inhibits long chain fatty acid transport. *J. Biol. Chem.* 273:28642–50
135. Stump DD, Fan X, Berk PD. 2001. Oleic acid uptake and binding by rat adipocytes define dual pathways for cellular fatty acid uptake. *J. Lipid Res.* 42:509–20
136. Stump DD, Zhou SL, Berk PD. 1993. Comparison of plasma membrane FABP and mitochondrial isoform of aspartate aminotransferase from rat liver. *Am. J. Physiol.* 265:G894–902
137. Sukhotnik I, Gork AS, Chen M, Drongowski RA, Coran AG, Harmon CM. 2001. Effect of low fat diet on lipid absorption and fatty-acid transport following bowel resection. *Pediatr. Surg. Int.* 17:259–64
138. Suzuki K, 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. USA* 77:2542–45
139. Tanaka T, Nakata T, Oka T, Ogawa T, Okamoto F, et al. 2001. Defect in human myocardial long-chain fatty acid uptake is caused by FAT/CD36 mutations. *J. Lipid Res.* 42:751–59
140. Tanaka T, Okamoto F, Sohmiya K, Kawamura K. 1997. Lack of myocardial iodine-123 15-(p-iodiphenyl)-3-R,S-methylpentadecanoic acid (BMIPP) uptake and CD36 abnormality—CD36 deficiency and hypertrophic cardiomyopathy. *Jpn. Circ. J.* 61:724–25
141. Tao N, Wagner SJ, Lublin DM. 1996. CD36 is palmitoylated on both N- and C-terminal cytoplasmic tails. *J. Biol. Chem.* 271:22315–20
142. Thorne RF, Marshall JF, Shafren DR, Gibson PG, Hart IR, Burns GF. 2000. The integrins alpha3beta1 and alpha6beta1 physically and functionally associate with CD36 in human melanoma cells. Requirement for the extracellular domain OF CD36. *J. Biol. Chem.* 275:35264–75
143. Tontonoz P, Nagy L. 1999. Regulation of macrophage gene expression by peroxisome-proliferator-activated receptor gamma: implications for cardiovascular disease. *Curr. Opin. Lipidol.* 10:485–90
144. Torra IP, Chinetti G, Duval C, Fruchart JC, Staels B. 2001. Peroxisome proliferator-activated receptors: from transcriptional control to clinical practice. *Curr. Opin. Lipidol.* 12:245–54
145. Trotter PJ, Ho SY, Storch J. 1996. Fatty acid uptake by Caco-2 human intestinal cells. *J. Lipid Res.* 37:336–46
146. Turcotte LP, Petry C, Kiens B, Richter EA. 1998. Contraction-induced increase in Vmax of palmitate uptake and oxidation in perfused skeletal muscle. *J. Appl. Physiol.* 84:1788–94
147. Turcotte LP, Richter EA, Kiens B. 1992. Increased plasma FFA uptake and oxidation during prolonged exercise in trained vs. untrained humans. *Am. J. Physiol. Endocrinol. Metab.* 262:E791–99
148. Turcotte LP, Srivastava AK, Chiasson JL. 1997. Fasting increases plasma membrane fatty acid-binding protein (FABP(PM)) in red skeletal muscle. *Mol. Cell. Biochem.* 166:153–58
149. Uchiyama A, Aoyama T, Kamijo K, Uchida Y, Kondo N, et al. 1996. Molecular cloning of cDNA encoding rat very long-chain acyl-CoA synthetase. *J. Biol. Chem.* 271:30360–65
150. van der Flier A, Sonnenberg A. 2001.

Function and interactions of integrins. *Cell Tissue Res.* 305:285–98

151. van der Lee KA, Vork MM, De Vries JE, Willemsen PH, Glatz JF, et al. 2000. Long-chain fatty acid-induced changes in gene expression in neonatal cardiac myocytes. *J. Lipid Res.* 41:41–47
152. Van Nieuwenhoven FA, Verstijnen CP, Abumrad NA, Willemsen PH, Van Eys GJ, et al. 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.* 207:747–52
153. Vorum H, Brodersen R, Kragh-Hansen U, Pedersen AO. 1992. Solubility of long-chain fatty acids in phosphate buffer at pH 7.4. *Biochim. Biophys. Acta* 1126:135–42
154. Vosper H, Patel L, Graham TL, Khoudoli GA, Hill A, et al. 2001. The peroxisome proliferator-activated receptor delta promotes lipid accumulation in human macrophages. *J. Biol. Chem.* 276:44258–65
155. Watkins PA, Pevsner J, Steinberg SJ. 1999. Human very long-chain acyl-CoA synthetase and two human homologs: initial characterization and relationship to fatty acid transport protein. *Prostaglandins Leukot. Essent. Fatty Acids* 60: 323–28
156. Westergaard M, Henningsen J, Svendsen ML, Johansen C, Jensen UB, et al. 2001. Modulation of keratinocyte gene expression and differentiation by PPAR-selective ligands and tetradecylthioacetic acid. *J. Invest. Dermatol.* 116:702–12
157. Whitesell RR, Abumrad NA. 1985. Increased affinity predominates in insulin stimulation of glucose transport in the adipocyte. *J. Biol. Chem.* 260:2894–99
158. Yanai H, Chiba H, Fujiwara H, Morimoto M, Abe K, et al. 2000. Phenotype-genotype correlation in CD36 deficiency types I and II. *Thromb. Haemost.* 84:436–41
159. Yehuda S, Rabinovitz S, Mostofsky DI. 1999. Essential fatty acids are mediators of brain biochemistry and cognitive functions. *J. Neurosci. Res.* 56:565–70
160. Zhou SL, Stump D, Isola L, Berk PD. 1994. Constitutive expression of a saturable transport system for non-esterified fatty acids in Xenopus laevis oocytes. *Biochem. J.* 297(Pt 2):315–19
161. Zhou SL, Stump D, Sorrentino D, Potter BJ, Berk PD. 1992. Adipocyte differentiation of 3T3-L1 cells involves augmented expression of a 43-kDa plasma membrane fatty acid-binding protein. *J. Biol. Chem.* 267:14456–61
162. Zhou SL, Gordon RE, Bradbury M, Stump D, Kiang CL, Berk PD. 1998. Ethanol upregulates fatty acid uptake and plasma membrane expression and export of mitochondrial aspartate aminotransferase in HepG2 cells. *Hepatology* 27:1064–74