

Differential Expression of Fatty Acid Transport Proteins in Epidermis and Skin Appendages

Matthias Schmuth,* Angelica M. Ortegon,† Mao-Qiang Man,‡ Peter M. Elias,‡ Kenneth R. Feingold,‡ and Andreas Stahl†

*Department of Dermatology, Innsbruck Medical University, Innsbruck, Austria; †Department of Medicine, Palo Alto Medical Foundation & Stanford University, Palo Alto, California, USA; ‡Department of Veterans Affairs and University of California, San Francisco, California, USA

Epidermis and sebocyte-derived lipids are derived both from *de novo* synthesis and through uptake of fatty acids from the circulation. Plasma membrane proteins can significantly contribute to the latter process. In particular, fatty acid transport proteins (FATP/solute carrier family 27) are integral transmembrane proteins that enhance the uptake of long-chain fatty acids into cells. Using specific antisera against all six mammalian FATP, we found that both human and mouse skin express FATP1, -3, -4, and -6. In adult skin, FATP1 and -3 are expressed predominantly by keratinocytes, whereas FATP4 is strongly expressed by sebaceous glands and FATP6 by hair follicle epithelia. Sustained barrier disruption leads to increases in FATP1 and -6 levels as well as a robust increase in CD36 protein. Notably, expression of FATP1 by embryonic keratinocytes at day 18.5 was lower, and FATP4 increased in comparison with adult epidermis. Together, these findings indicate that FATP are not only expressed by different cell types within the skin, but also that their localization is dynamically regulated during development.

Key words: barrier/CD36/epidermis/FATP/fatty acid transport proteins/hair follicle/lipids/skin
J Invest Dermatol 125:1174–1181, 2005

A critical function of the epidermis is to provide a barrier between the external environment and the organism. To fulfill this function, keratinocytes undergo a complex pathway of differentiation that culminates in keratinocyte cornification and in the formation of extracellular lipid-enriched lamellar membranes in the stratum corneum (SC). These lipid-enriched membranes are primarily responsible for the permeability barrier to water and electrolyte transit. The lipid-enriched extracellular lamellar membranes derive from the exocytosis of lamellar bodies from stratum granulosum (SG) cells. In order for SG cells to generate lamellar bodies, three families of lipids, specifically cholesterol, phospholipids, and glucosylceramides, must be generated at sufficient levels in SG cells (Grubauer *et al*, 1987; Elias and Feingold, 1988; Feingold and Elias, 1999). A further requirement is that these lipids must be generated at appropriately 1:1:1 molar ratios or abnormal lamellar bodies are formed and barrier dysfunction results. Previous studies have demonstrated that the epidermis is a very active site of lipid synthesis (Nicolaidis and Rothman, 1955; Brooks *et al*, 1966; Hsia *et al*, 1966; Wilkinson, 1970; Grubauer *et al*, 1987; Feingold *et al*, 1991), and that inhibition of cholesterol, fatty acid, ceramide, or glucosylceramide synthesis blocks lamellar body formation (Feingold *et al*, 1990; Holleran *et al*, 1991; Man *et al*, 1993; Chujor *et al*, 1998; Schmuth *et al*,

2000). Lipid synthesis also occurs in the keratinocytes of the hair follicle, where it fluctuates in relation to the hair cycle. Anagen is associated with increased cholesterol and triglyceride content, whereas fatty acid content decreases during anagen (Carruthers, 1962; Carruthers and Heining, 1963; Brooks *et al*, 1966).

Epidermal fatty acids derive not only from *de novo* synthesis in the epidermis but fatty acids can also be derived in part from extracutaneous sites (Grubauer *et al*, 1987; Feingold, 1991). First, essential fatty acids are required for normal permeability barrier function but by definition these fatty acids are not synthesized and therefore they must be derived from the diet (Miller *et al*, 1989). For example, linoleate acid must be derived from the circulation and as the epidermis lacks both the delta 5 and delta 6 desaturases (Chapkin and Ziboh, 1984) it consequently cannot convert linoleate to arachidonic acid, which therefore *must also* be obtained from other sites. Additionally, C22:6, n3 (eicosapentaenoic acid) and C20:5, n3 (docosahexaenoic acid) fatty acids derived from the ingestion of dietary fish oil are incorporated into epidermal lipids (Ziboh *et al*, 1986). Second, plant-derived fatty acids accumulate in the epidermis in certain disease states, such as Refsum's disease (Klenk and Kahike, 1963; Foulon *et al*, 2003). Third, our studies have shown that systemically administered ¹⁴C-labeled fatty acids are delivered to the epidermis (Grubauer *et al*, 1987). Fourth, although inhibition of epidermal fatty acid synthesis perturbs permeability barrier recovery following acute disruption, the degree of inhibition is relatively modest (Man *et al*, 1993), suggesting that other sources of fatty acids are available to the epidermis for lamellar body

Abbreviations: FATP, fatty acid transport protein; LCFA, long-chain fatty acids; PBS, phosphate-buffered saline; SC, stratum corneum; SG, stratum granulosum

We dedicate this work to Professor Peter O. Fritsch in honor of his 65th birthday.

synthesis. Fifth, studies have shown the active uptake of fatty acids by keratinocytes. Fatty acid uptake by keratinocytes is temperature sensitive, has saturable kinetics, and can be reduced by prior treatment with trypsin, indicating that plasma membrane proteins mediate fatty acid transport (Schurer *et al*, 1994).

Human sebaceous glands secrete an oily sebum consisting of triglycerides, fatty acids, wax monoesters, squalene, cholesterol, and cholesterol esters. Although keratinocyte-derived lipids form the permeability barrier, the lipid metabolites secreted by sebaceous glands are deposited at the skin surface and are important for SC hydration and water repulsion. Sebaceous glands have been reported to express the enzymes that catalyze cholesterol, fatty acid, and triglyceride biosyntheses, namely 3-hydroxy-3-methylglutaryl-coenzyme A reductase and acetyl-coenzyme (CoA) carboxylase (Smythe *et al*, 1998), fatty acid synthase, fatty acid desaturase, medium chain acyl-CoA dehydrogenase, squalene synthase, farnesyl transferase (Cooper *et al*, 1976; Cassidy *et al*, 1986; Thiboutot *et al*, 2003), and acyl-CoA: diacylglycerol acyltransferase (DGAT1, DGAT2) (Chen *et al*, 2002; Stone *et al*, 2004). Mutations in the stearoyl-CoA desaturase 1 (*scd1*) which catalyzes the biosynthesis of monounsaturated fatty acids, are the underlying cause for sebaceous gland ablation in *asebia* mice (Zheng *et al*, 1999; Lu *et al*, 2004). Furthermore, the fatty acid chain elongation enzyme LOVL3 participates in the formation of neutral lipids in sebaceous glands (Westerberg *et al*, 2004).

Although it is not known if and how fatty acids are transported into hair follicles and sebocytes, previous studies have demonstrated active fatty acid uptake in epidermal keratinocytes and in a variety of other organs, including muscle (Kim *et al*, 2004), heart (Sorrentino *et al*, 1988; Stremmel, 1989a), adipocytes (Stump *et al*, 2001), liver (Stremmel, 1989b), and small intestine (Stremmel, 1988; Gore and Hoinard, 1993). Several proteins that can increase cellular uptake of long-chain fatty acids (LCFA) have been identified by biochemical means and by expression cloning, and include the scavenger receptor fatty acid translocase (CD36), an integral membrane protein well studied for its role in fatty acid uptake by cells (Brinkmann *et al*, 2002; Ibrahim and Abumrad, 2002), mitochondrial aspartate amino transferase (FABPpm) (Berk *et al*, 1990), and fatty acid transport proteins (FATP/solute carrier family 27) (Stahl, 2004). FATP are integral transmembrane proteins (Lewis *et al*, 2001) that enhance the uptake of LCFA and very LCFA into cells (Stahl, 2004). FATP in humans and mice comprise a family of six highly homologous proteins, FATP1–6, which are found in all fatty acid utilizing tissues of the body, including the skin (Stahl, 2004). FATP4 has recently been implicated in skin development and barrier function. Although targeted deletion of FATP4 exons 1 and 4 resulted in embryonic lethality (Gimeno *et al*, 2003), two other reports showed that mouse strains with FATP4 loss of function because of either a spontaneous transposon insertion in exon 3 (Moulson *et al*, 2003), or targeted disruption of exon 3 via homologous recombination (Herrmann *et al*, 2003) are viable at birth; however, both groups reported that FATP4 null mice die within days after birth because of a phenotype reminiscent of restrictive dermatopathy with tight, wrinkle-free skin, and disrupted skin barrier function. How partial abla-

tion of FATP4 causes this skin phenotype and where FATP are expressed in embryonic and adult skin is unknown.

To investigate the principle fatty acid transporters that facilitate the uptake of fatty acids in the skin during periods of barrier maintenance, re-establishment, and initial formation, we determined the localization and expression of FATP1–6 in mouse and human skin in comparison with CD36. We observed constitutive expression FATP1 and -3 in epidermal keratinocytes, FATP4 in sebaceous glands, and FATP6 in the hair follicle. The distribution of FATP expression was altered both during fetal skin development and when the epidermal barrier was exposed to sustained environmental stress, i.e., the removal of SC lipids by acetone, resulting in sustained barrier disruption. Together, these findings may indicate differential lipid requirements in various compartments of the skin that are met by the presence of multiple transporters with selective specificity. If this system is environmentally challenged during fetal development or by environmental stress, FATP distribution is altered, presumably aimed at maintaining the steady state of lipid compartmentalization in the epidermis.

Results

Murine epidermis and appendages express multiple FATP proteins To determine which FATP proteins are present in mouse epidermis, epidermal lysates from two adult mice were separated by SDS PAGE and probed with antibodies specific for FATP1–6. FATP1, -3, and -4 showed robust expression, FATP6 was weakly expressed and FATP2 and -5 were not detectable (Fig 1A). Control experiments with equal amounts of liver lysates showed strong expression of FATP2 and -5 (data not shown), demonstrating that the results reflected a true lack of FATP2 and -5 protein expressions in the epidermis. Using immunofluorescence microscopy, FATP1 was robustly expressed by keratinocytes both within the interfollicular epidermis and in a follicular distribution within the dermis (Fig 1B). FATP3 in mouse skin was heavily expressed by epidermal keratinocytes (Fig 1B). FATP4 was less prominently expressed by epidermal and hair follicle keratinocytes, but strongly associated with sebaceous glands (Fig 1B). This notion was confirmed by our finding that dermal FATP4 staining of sebaceous glands in murine and human (data not shown) skin co-localized with Nile Red positive, neutral lipid-rich structures (Fig 1C). FATP6 in mouse skin was again associated with both interfollicular and follicular keratinocytes (Fig 1B). As expected from our western blot studies no staining for FATP2 or -5 was observed in mouse skin (Fig S1). Surprisingly, CD36, which previously had been reported to be expressed in epidermis (Lisby *et al*, 1990), showed only a very weak signal that was close in intensity and distribution to background (Fig 1B). The results for murine FATP expression within the epidermis are summarized in Table I.

Changes in FATP expression following sustained barrier disruption To determine whether FATP expression is static or whether one or more proteins are affected by environmental and/or developmental changes, we next

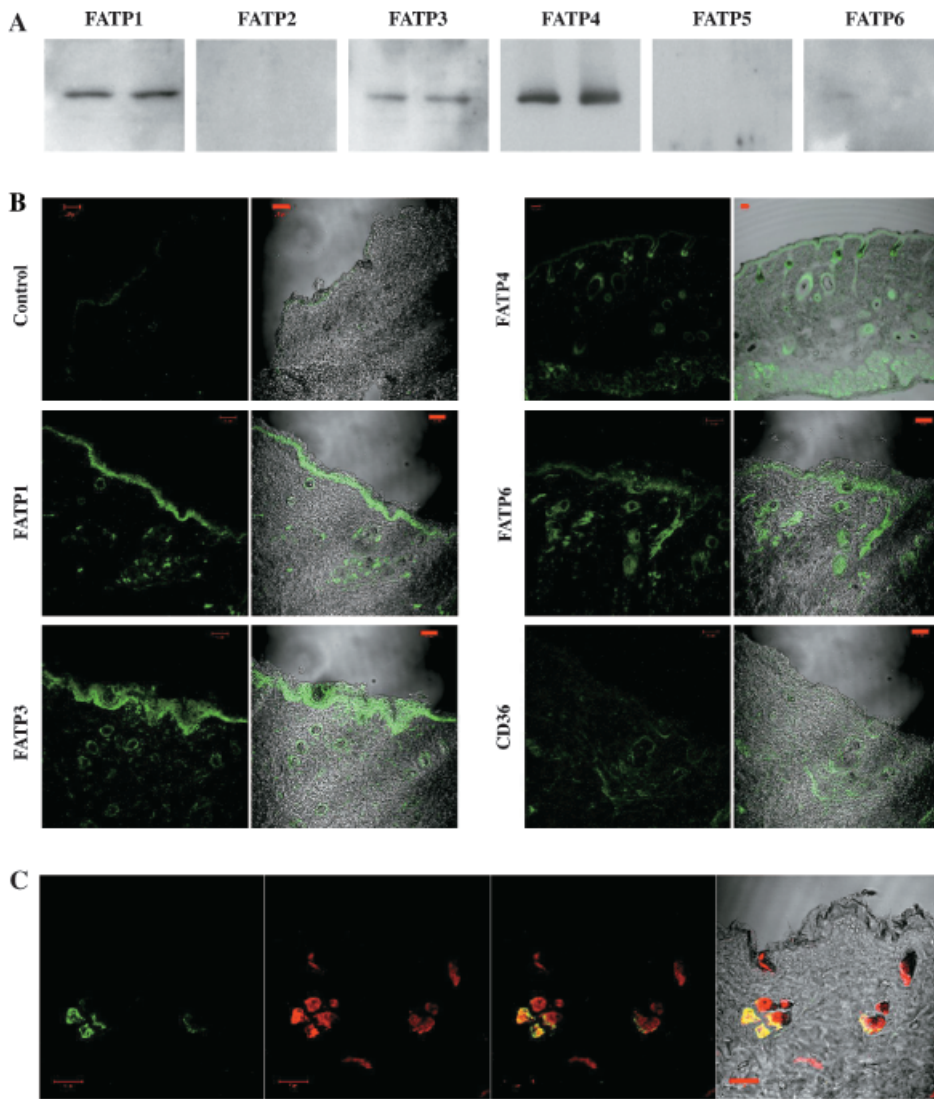


Figure 1
Expression and localization of fatty acid transporter proteins (FATP) in mouse skin. (A) Western blot of two different murine epidermal lysates with antisera against all six FATP family members. (B) Immunofluorescence images of mouse skin with or without phase contrast for pre-immune serum (*control*), FATP1–6 antisera, and for anti-CD36. (C) FATP4 localization (*green*) in mouse skin co-stained for neutral lipids using Nile Red (*red*). Fluorescence for each channel, their overlay, and a phase contrast image are shown. Red size bar equals 50 μ m in all images.

compared the expression patterns of these proteins in normal mouse skin with mouse skin obtained following barrier disruption. Although immunofluorescent studies of mouse skin 3 h after tape stripping (acute barrier disruption) did not reveal any differences *versus* non-tape stripped control animals, sustained barrier disruption by acetone treatment for 3 d demonstrated changes in FATP localization. Although FATP3 and -4 appeared unchanged after barrier disruption, FATP1 showed a more pronounced staining of structures in the dermis (Fig 2A). Acetone treatment did not alter the overall distribution of FATP6 but resulted in an increase in

the amount of FATP6 protein in epidermis *versus* hair follicles (Fig 2A). Most notably, CD36 was expressed only at very low levels in normal mouse skin but was strongly up-regulated in both dermal and epidermal layers after barrier disruption (Fig 2A). This increase in CD36 protein agrees with our previous studies that demonstrated an increase in CD36 mRNA levels following barrier perturbation (Harris *et al*, 1998).

FATP expression changes late in fetal development Fatty acid synthesis and utilization peaks during the later stages

Table I. Summary of murine fatty acid transporter expression in skin

	FATP1	FATP2	FATP3	FATP4	FATP5	FATP6	CD36
Upper epidermis	++	-	+++	+	-	+	-
Lower epidermis	++	-	++	+	-	-	+
Sebaceous glands	-	-	-	+++	-	-	-
Hair follicles	+	-	+	+	-	+++	+
Subcutaneous fat	+++	-	-	+++	-	-	+

Expression, assessed by immunofluorescence staining, in the indicated areas of skin was scored for signal strength. -, undetectable; +, weak expression; ++, robust expression; +++, strong expression; FATP, fatty acid transport proteins.

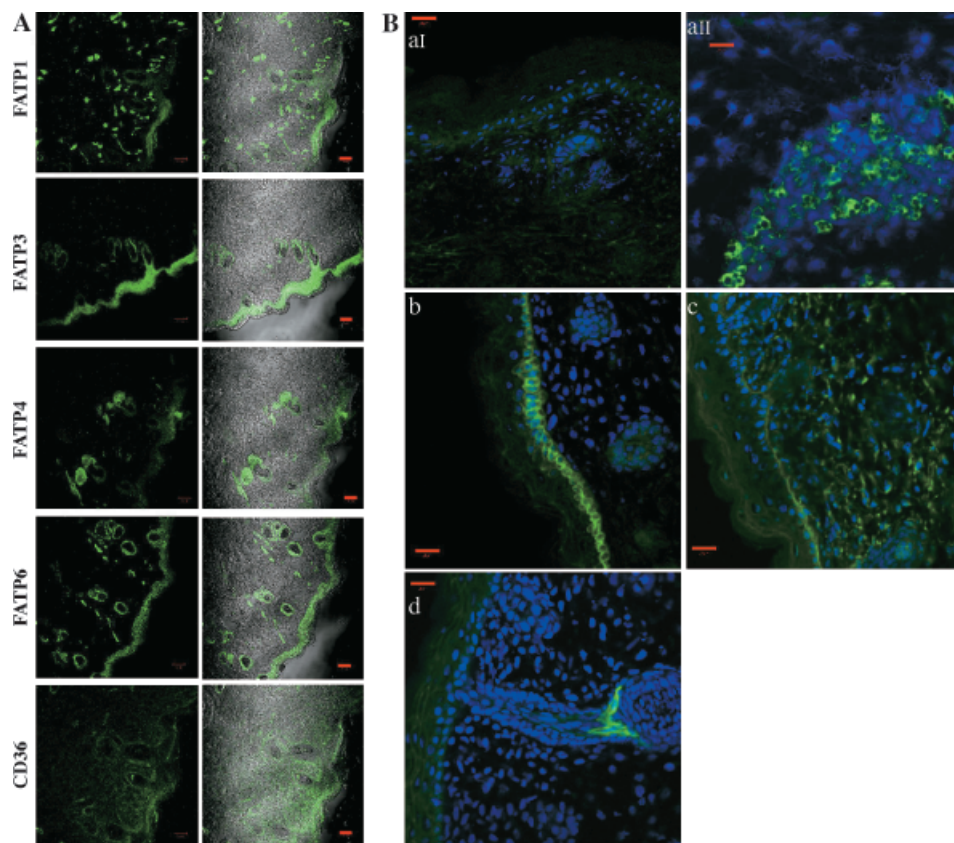


Figure 2
Modulation of fatty acid transporter proteins (FATP) expression following barrier disruption and during development. (A) Immunofluorescence images of mouse skin with or without phase contrast for FATP1–6 and CD36 following sustained barrier disruption by acetone treatment for 3 d. Red size bar equals 50 μ m. (B) FATP localization in day 18.5 embryonic skin. FATP1-specific staining (a) in the epidermis (I) and subcutaneous fat (II) as well as FATP3 (b), FATP4 (c), and FATP6 (d) localization in the skin are shown. DAPI-stained nuclei are shown in blue. Red size bar equals 20 μ m.

of epidermal development (Hurt *et al*, 1995), presumably in preparation for terrestrial life. Therefore, we next compared the adult expression pattern of FATP with their embryonic expression at day 18.5 in mouse embryos. Surprisingly, we found that FATP1 is not expressed by embryonic keratinocytes (Fig 2BaI) but it is robustly expressed by subcutaneous adipocytes (Fig 2BaII). FATP3 was expressed in the epidermis of day 18.5 embryos as it is in adult skin, but there was preferential basal staining (Fig 2Bb). Targeted deletion of exon 3 of FATP4 has been reported to result in neonatal lethality because of skin abnormalities (Herrmann *et al*, 2003) but FATP4 expression in adult skin is predominantly in sebaceous glands (Fig 1B). By contrast, we found that FATP4 in day 18.5 embryonic skin was not only expressed throughout the dermis but also to a lesser extent by the basal layer of epidermal cells adjacent to the basolateral membranes (Fig 2Bc). Finally, FATP6 was expressed by cells within embryonic hair follicles and, less prominently, by epidermal keratinocytes (Fig 2Bd). These findings reveal dynamic changes of FATP expression in embryonic skin that may reflect the generation of lipid compartmentalization during development.

FATP expression in human skin is comparable with that in mice Next, we stained human skin either with a control serum (non-immunized rabbit), sera specific for FATP1, -3, -4, and -6 or with a monoclonal antiserum against CD36. FATP1 and -3 had similar staining patterns showing expression of the transporters by epidermal keratinocytes (Fig 3A). Both FATP1 and -3 appeared to be more prominent on the baso-lateral side of cells. In contrast to mouse skin, there was no FATP1 staining in human dermis. FATP4

stained keratinocytes only moderately but it was robustly expressed by sebaceous glands (Fig 3A), where it again colocalized with Nile Red (data not shown). Human FATP6 showed weak staining of the epidermal layer but, as in mouse skin, it was strongly expressed in hair follicles. CD36 expression approximated the lower detection limit appearing as diffuse staining throughout the dermis (Fig 3A).

In primary cultures of human keratinocytes, the primary expressed FATP was FATP4 (Fig 3B). Upon induction of differentiation by switching the calcium concentration in the culture medium from 0.07 to 1.2 mM, FATP4 expression was downregulated (Fig 3B). Importantly, neither FATP1, -3, nor -6 were expressed in either undifferentiated or differentiated human keratinocytes in marked contrast to human and mouse epidermis. Finally, FATP2 and -5 were absent from both human keratinocytes and skin.

Discussion

The epidermis is a very active site of lipid synthesis, exceeding even the liver, kidneys, and GI epithelia on a per weight basis (Feingold, 1991). Keratinocytes require fatty acids as substrates for the formation of phospho- and sphingolipids, which are key components of the lipid-enriched lamellar bodies. These specialized organelles secrete their content to the extracellular space, thus meeting the special requirements for non-essential fatty acids, derived from the hydrolysis of phospholipids, as key components of the extracellular lamellar membranes. In addition, the epidermis has separate structural requirements for linoleate acid as a critical moiety in unique ceramides (omega-hydroxyl

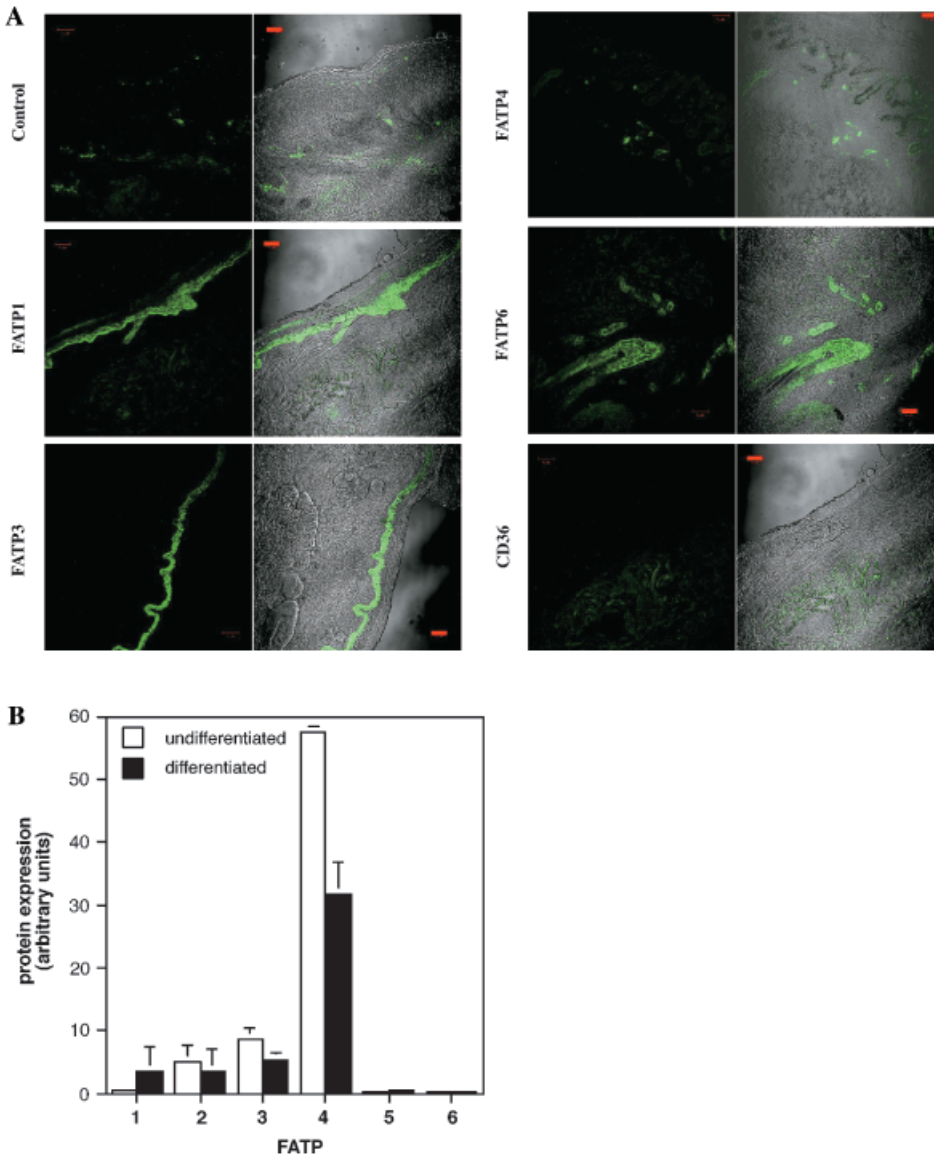


Figure 3
Localization of fatty acid transporter proteins (FATP) in human skin and cultured keratinocytes. (A) Immunofluorescence images of mouse skin with or without phase contrast for pre-immune serum (control), FATP1–6 antisera, and for anti-CD36. Red size bar equals 50 μ m. (B) Densitometric quantification of FATP expression in four independent lysates from undifferentiated (white bars) and differentiated (black bars). Error bars indicated standard deviation of mean.

ceramides, with *o*-acylated linoleate acid), which are essential for linking the bilayers together and providing anchors of the membrane system to the external surface of the cornified envelope of the corneocyte scaffold. Indeed, epidermal lipids comprise considerable quantities of ceramides that contain very long-chain *N*-acylated fatty acids (C24–30). Although keratinocytes exhibit elongase activity, they may also try to import some of the very long-chain species from the blood. In addition to this structural role, epidermal fatty acids also serve as an energy source and as signaling molecules, modulating keratinocyte proliferation and differentiation.

Despite its great synthetic capacity, several lines of evidence indicate that fatty acids are not solely derived from *de novo* synthesis within the epidermis, suggesting that at least a proportion of these lipids must be imported from extra-cutaneous sites. Epidermis lacks not only delta 5, but also delta 6 desaturase, so it must not only import linoleate, but also arachidonate acid. Our knowledge of the pathways of fatty acid transport in keratinocytes is incomplete. It already is clear that fatty acid uptake by keratinocytes does not only occur passively, via a non-energy-dependent plas-

ma membrane flip/flop mechanism, but instead fatty acid transport is carrier mediated. For example, previous studies by our laboratory have reported that FATP, FABP-pm, and CD36/FAT are expressed in epidermis/keratinocytes (Harris *et al*, 1998). Here, we present a comprehensive analysis of FATP expression, localization, and regulation in skin. We report expression of four different FATP, FATP1, -3, -4, and -6 in both human and mouse skin (see Table I). This is to our knowledge the largest number of plasma membrane fatty acid transporters expressed in a single organ and clearly reflects the importance of exogenous LCFA uptake for the skin. FATP1 has previously been shown to be expressed by adipocytes, muscle, and brain (Schaffer and Lodish, 1994; Schaap *et al*, 1997; Hirsch *et al*, 1998; Coe *et al*, 1999; Binnert *et al*, 2000; Stahl, 2004). FATP3 is expressed in a variety of tissues, most notably the lung (Stahl *et al*, 2001), FATP4 is the principle fatty acid transporter in the small intestine and is also present in adipocytes, muscle, and brain (Stahl *et al*, 1999), whereas FATP6 is highly expressed by the heart (Gimeno *et al*, 2003). Why does the skin need such a multitude of FATP? Although no differences in FATP substrate specificity have thus far been reported (Stahl,

2004), one obvious difference lies in the cell types that express the transporters, i.e., interfollicular keratinocytes (FATP1 and -3), hair follicle epithelials (FATP6), and sebaceous glands (FATP4).

In the epidermis, lipids have important structural implications. The extracellular deposition of lamellar membranes in the SC is responsible for the permeability barrier to water and electrolyte transit (Elias and Feingold, 1988). These lamellar membranes are essential for life in an arid, terrestrial environment because they prevent excess evaporation of body fluids and conversely they protect the body from potential outside threats of physical, chemical, or biological nature. Three families of lipids, cholesterol, phospholipids, and glucosylceramides, must be generated at sufficient levels in specialized cell organelles, the lamellar bodies, of SG cells within the epidermis. Our previous studies showed an increase in fatty acyl-CoA synthase mRNA and activity in response to barrier disruption, which suggests a role in restoring normal SC function after external insults (Harris *et al*, 1998). The increase in epidermal FATP6, dermal FATP1, and both epidermal and dermal CD36 after barrier disruption reported here may also reflect the formation of lamellar membranes, aimed at restoring the permeability barrier function. Yet, this increase was only detectable in the setting of sustained barrier disruption (3 consecutive d of repetitive acetone treatment), but not after acute barrier disruption. These findings indicate that the FATP are involved in restoring the steady state of epidermal lipid compartmentalization and metabolism after the cellular stores have been exhausted, rather than being involved in the acute repair response.

The scavenger receptor CD36 is known to be increased in hyperproliferative skin (sustained barrier disruption by acetone treatment for 3 d induces epidermal hyperplasia) as well as in other inflammatory skin disorders such as atopic dermatitis, irritant dermatitis, psoriasis, and following wounding (Harris *et al*, 1998). Weak staining under baseline conditions and inducibility in response to external insults may indicate the involvement of CD36 in epidermal homeostasis. CD36 could be important for both secretion and processing of epidermal lipoproteins. In addition, CD36 appears to be involved in other cellular functions such as cell adhesion, angiogenesis, and immune function. Thus, the increase in CD36 could also reflect an increase in immunosurveillance of barrier disrupted skin because, aside from serving as a receptor for lipid uptake, CD36 is expressed in cutaneous macrophages and in Langerhans, and barrier disruption has been linked to an activation of the immune system both by stimulating the release of inflammatory mediators and by activation of the cellular immune system (Wood *et al*, 1992; Nishijima *et al*, 1997; Jensen *et al*, 1999; Schmuth *et al*, 2002).

Our finding that FATP6 is expressed by hair follicles is interesting and surprising. We have shown previously that out of 16 different tissues (which did not include skin) only the heart showed significant expression of FATP6 (Gimeno *et al*, 2003). Although it is known that the hair follicle is metabolically very active during anagen, it is unclear which requirements could be shared by the heart and the hair follicle, and only little information is available on the role of lipids in hair cycling and development (Carruthers, 1962;

Carruthers and Heining, 1963; Brooks *et al*, 1968). A recent study, however, reported markedly decreased amounts of wax diesters associated with hair loss and atrophy of sebaceous glands in mice deficient in acyl CoA:diacylglycerol acyltransferase (DGAT1) (Chen *et al*, 2002). Wax esters, which are derived from fatty acids, together with triglycerides, comprise the main lipid fraction secreted by sebaceous glands. In this study, FATP4 was strongly expressed in sebaceous glands. In hair follicles, FATP may supply the energy sources as hair has cholesterol, cholesterol sulfate, and LDL receptors. Also, hair has intrinsic lipids, including an unusual fatty acid—18-methyleicosanoic acid (Jones and Rivett, 1997). The reason for the differences in transporters could be that different fatty acids are required in different sites. Substrate specificity of transporters may be the basis for the need for multiple transporters. Another possibility is that each transporter could deliver the fatty acid to a different intracellular location, for example, preferential transport to the mitochondria to be utilized for energy *versus* to ER to be utilized in formation of TG/PL, etc.

Deletion of FATP4 exon 3 results in neonatal lethality from a phenotype reminiscent of restrictive dermatopathy with hyperkeratosis and disturbed epidermal barrier function (Herrmann *et al*, 2003; Moulson *et al*, 2003). The results presented here show that although FATP4 in adult human and mouse skin is predominantly expressed by cells of the sebaceous glands, embryonic expression of FATP4 is high in keratinocytes and throughout the dermis. Using a polyclonal anti FATP4 peptide serum Herrmann *et al* (2003) also reported FATP4 expression by neonatal keratinocytes but failed to detect expression in the dermal layer. This difference could be possibly because of changes between day 18.5 and early postnatal stages or because of the fact that we used saponin to achieve better tissue penetration, whereas the former study was done without detergents. The effect of FATP4 loss on keratinocytes during embryonic development could be further impacted by the absence of the highly homologous transporter FATP1 during development. The fact that FATP1 seems to be expressed only at later stages could also explain why FATP1 null mice show no similar phenotype to FATP4 null mice and have no reported skin abnormalities (Kim *et al*, 2004). Meanwhile expression of FATP4 during early development seems to be vital, its role in adult skin remains to be determined. Although to date no regulatory abnormalities or mutations in FATP have been described in skin disease, these findings broaden our understanding of the mechanisms underlying normal skin function and provide seminary observations for future evaluations of FATP as targets for the diagnosis and therapy of skin disorders.

Materials and Methods

Antibodies Rabbit polyclonal antibodies against FATP1, -4, and -6 were generated as previously described (Stahl *et al*, 1999, 2002; Gimeno *et al*, 2003). Similarly, FATP2, -3, and -5 anti-sera were raised by immunizing rabbits with the last 90 C-terminal amino acids of the respective FATP fused to glutathione S-transferase. Serum was affinity purified over protein A columns and found to have minimal cross-reactivity against other FATP family members. The anti-CD36 monoclonal antibody was a generous gift from Dr Maria Febbraio.

Sources of skin specimens Animals employed in these studies include hairless mice (hr/hr), C57BL/6, and Sv129 (Simonsen Laboratories, Gilroy, California). Adult animals were sacrificed by inhalation anesthesia followed by cervical dislocation at the indicated time points and skin was further processed for western blot or immunofluorescence microscopy. All studies involving animals were approved by the institutional review boards. To determine FATP expression during fetal development, in some experiments pups were delivered by cesarean section on day 18.5 of gestation. Human skin specimens were derived from archival material.

Epidermal permeability barrier function Permeability barrier function was determined by measuring transepidermal water loss (TEWL) with an electronic water analyzer (MEECO, Warrington, Pennsylvania). Acute barrier disruption was induced by sequential tape stripping until TEWL reached 6–8 mg per cm² per h. Sustained barrier disruption was induced in hairless mice by twice daily application of acetone-soaked cotton balls until the TEWL reached 6–8 mg per cm² per h for 3 consecutive days (Komuves *et al*, 2000). Skin specimens were obtained after 3 h and on day 4, respectively, and processed for immunofluorescence microscopy.

Cell culture Second-passage keratinocytes isolated from newborn foreskins were cultured in serum-free keratinocyte growth medium (KGM; Clonetics, San Diego, California). Differentiation was induced in these cells by switching the calcium concentration in the culture medium from 0.07 to 1.2 mM and cells were harvested after 48 h at 70%–100% confluency.

Western blot analysis Subcutaneous fat was removed from the skin specimen, and the epidermis was separated from the dermis after incubation in 10 mM EDTA-phosphate-buffered saline (PBS) for 40–50 min. The tissue was then homogenized in ice cold RIPA buffer (150 mM NaCl, 1% NP-40, 0.5% DOC, 0.1% SDS, 50 mM Tris pH 8.0) and western blots were performed as previously described (Stahl *et al*, 2002).

Immunofluorescence microscopy Unfixed mouse skin was removed by surgical excision, washed in PBS, and embedded in O.C.T. 4583 compound. The material was thick sectioned (15–40 μm). The sections were washed in PBS containing 1% BSA, 10% fetal calf serum, and 1% normal donkey serum (blocking solution) to block non-specific binding. Primary antibodies were diluted in blocking solution incubated for 1 h and washed extensively with blocking solution for 30 min. Incubation and washing steps for secondary antibodies was identical. Finally, sections were rinsed with PBS and mounted in 90% glycerol/PBS containing 1 mg per mL paraphenylenediamine, and examined with a Zeiss LSM510 confocal system. Nile Red staining were performed as previously described (Fowler and Greenspan, 1985).

The authors thank Jon Mulholland and Kitty Lee at the Stanford cell science imaging facility for expert advice and help with confocal microscopy as well as Art Moser and Peggy Lau for help with tissue collection and cell culture and Rosemary Grammer for assistance in the preparation of the manuscript. This work was supported by a grant from the NIDDK (RO1 DK066336) and a Career Development Award from the American Diabetes Association (7-04-CD-14) to A. S., NIH grants to K. R. F. (RO1 AR049932, RO1 AR050629), and a grant by the Austrian Science Fund to M. S.

Supplementary Material

The following material is available online for this article.

Figure S1. Expression and localization of fatty acid transporters 2, 3, and 5 in mouse skin.

Table S1.

Table S2.

DOI: 10.1111/j.0022-202X.2005.23934.x

Manuscript received April 1, 2005; revised May 22, 2005; accepted for publication May 26, 2005

Address correspondence to: Andreas Stahl, PhD, Ames Building, 795 El Camino Real, Palo Alto, California 94301, USA. Email: astahl@stanford.edu

References

- Berk PD, Wada H, Horio Y, *et al*: Plasma membrane fatty acid-binding protein and mitochondrial glutamic-oxaloacetic transaminase of rat liver are related. *Proc Natl Acad Sci USA* 87:3484–3488, 1990
- Binnert C, Koistinen HA, Martin G, *et al*: Fatty acid transport protein-1 mRNA expression in skeletal muscle and in adipose tissue in humans. *Am J Physiol Endocrinol Metab* 279:E1072–E1079, 2000
- Brinkmann JF, Abumrad NA, Ibrahim A, van der Vusse GJ, Glatz JF: New insights into long-chain fatty acid uptake by heart muscle: A crucial role for fatty acid translocase/CD36. *Biochem J* 367:561–570, 2002
- Brooks SC, Godefroi VC, Simpson WL: Specific sites of fatty acid sterol synthesis in isolated skin components. *J Lipid Res* 7:95–102, 1966
- Brooks SC, Langa LK, Godefroi VC: Metabolic studies on skin. 3. Lipid metabolism in mouse skin during the hair growth cycle. *J Invest Dermatol* 50:161–170, 1968
- Carruthers C: Influence of sex and hair growth cycle on lipid composition of mouse epidermis. *Proc Soc Exp Biol Med* 109:390–392, 1962
- Carruthers C, Heining A: Influence of hair growth cycle on the triglyceride fatty acid composition of mouse epidermis. *Proc Soc Exp Biol Med* 112:278–280, 1963
- Cassidy DM, Lee CM, Laker MF, Kealey T: Lipogenesis in isolated human sebaceous glands. *FEBS Lett* 200:173–176, 1986
- Chapkin RS, Ziboh VA: Inability of skin enzyme preparations to biosynthesize arachidonic acid from linoleic acid. *Biochem Biophys Res Commun* 124:784–792, 1984
- Chen HC, Smith SJ, Tow B, Elias PM, Farese RV Jr: Leptin modulates the effects of acyl CoA:Diacylglycerol acyltransferase deficiency on murine fur and sebaceous glands. *J Clin Invest* 109:175–181, 2002
- Chujor CS, Feingold KR, Elias PM, Holleran WM: Glucosylceramide synthase activity in murine epidermis: Quantitation, localization, regulation, and requirement for barrier homeostasis. *J Lipid Res* 39:277–285, 1998
- Coe N, Smith A, Frohnert B, Watkins P, Bernlohr D: The fatty acid transport protein (FATP1) is a very long chain acyl-CoA synthetase. *J Biol Chem* 274:36300–36304, 1999
- Cooper MF, McGrath H, Shuster S: Sebaceous lipogenesis in human skin. Variability with age and with severity of acne. *Br J Dermatol* 94:165–172, 1976
- Elias P, Feingold K: Lipid-related barriers and gradients in the epidermis. *Ann NY Acad Sci* 548:4–13, 1988
- Feingold KR: The regulation and role of epidermal lipid synthesis. *Adv Lipid Res* 24:57–82, 1991
- Feingold KR, Elias PM: The environmental interface: Regulation of permeability barrier homeostasis. In: Loden M, Maibach HI (eds). *Dry Skin and Moisturizers: Chemistry and Function*. Boca Raton, FL: CRC Press, 1999
- Feingold KR, Man MQ, Menon GK, Cho SS, Brown BE, Elias PM: Cholesterol synthesis is required for cutaneous barrier function in mice. *J Clin Invest* 86:1738–1745, 1990
- Feingold KR, Man MQ, Proksch E, Menon GK, Brown BE, Elias PM: The lovastatin-treated rodent: A new model of barrier disruption and epidermal hyperplasia. *J Invest Dermatol* 96:201–209, 1991
- Foulon V, Asselberghs S, Geens W, Mannaerts GP, Casteels M, Van Veldhoven PP: Further studies on the substrate spectrum of phytanoyl-CoA hydroxylase: Implications for Refsum disease? *J Lipid Res* 44:2349–2355, 2003
- Fowler SD, Greenspan P: Application of Nile red, a fluorescent hydrophobic probe, for the detection of neutral lipid deposits in tissue sections: Comparison with oil red O. *J Histochem Cytochem* 33:833–836, 1985
- Gimeno RE, Hirsch DJ, Punreddy S, *et al*: Targeted deletion of fatty acid transport protein-4 results in early embryonic lethality. *J Biol Chem* 278:49512–49516, 2003
- Gimeno RE, Ortegon AM, Patel S, *et al*: Characterization of a heart-specific fatty acid transport protein. *J Biol Chem* 278:16039–16044, 2003
- Gore J, Hoinard C: Linolenic acid transport in hamster intestinal cells is carrier-mediated. *J Nutr* 123:66–73, 1993
- Grubauer G, Feingold KR, Elias PM: Relationship of epidermal lipogenesis to cutaneous barrier function. *J Lipid Res* 28:746–752, 1987
- Harris IR, Farrell AM, Memon RA, Grunfeld C, Elias PM, Feingold KR: Expression and regulation of mRNA for putative fatty acid transport related proteins and fatty acyl CoA synthase in murine epidermis and cultured human keratinocytes. *J Invest Dermatol* 111:722–726, 1998
- Herrmann T, Van Der Hoeven F, Grone HJ, *et al*: Mice with targeted disruption of the fatty acid transport protein 4 (Fatp 4, Slc27a4) gene show features of lethal restrictive dermopathy. *J Cell Biol* 161:1105–1115, 2003
- Hirsch D, Stahl A, Lodish HF: A family of fatty acid transporters conserved from mycobacterium to man. *Proc Natl Acad Sci USA* 95:8625–8629, 1998

- Holleran WM, Man MQ, Gao WN, Menon GK, Elias PM, Feingold KR: Sphingolipids are required for mammalian epidermal barrier function. Inhibition of sphingolipid synthesis delays barrier recovery after acute perturbation. *J Clin Invest* 88:1338–1345, 1991
- Hsia SL, Sofer G, Lane B: Lipid metabolism in human skin. I. Lipogenesis from acetate-1-14C. *J Invest Dermatol* 47:437–442, 1966
- Hurt CM, Hanley K, Williams ML, Feingold KR: Cutaneous lipid synthesis during late fetal development in the rat. *Arch Dermatol Res* 287:754–760, 1995
- Ibrahimi A, Abumrad NA: Role of CD36 in membrane transport of long-chain fatty acids. *Curr Opin Clin Nutr Metab Care* 5:139–145, 2002
- Jensen JM, Schutze S, Forl M, Kronke M, Proksch E: Roles for tumor necrosis factor receptor p55 and sphingomyelinase in repairing the cutaneous permeability barrier. *J Clin Invest* 104:1761–1770, 1999
- Jones LN, Rivett DE: The role of 18-methyleicosanoic acid in the structure and formation of mammalian hair fibres. *Micron* 28:469–485, 1997
- Kim JK, Gimeno RE, Higashimori T, *et al*: Inactivation of fatty acid transport protein 1 prevents fat-induced insulin resistance in skeletal muscle. *J Clin Invest* 113:756–763, 2004
- Klenk E, Kahike W: On the presence of 3,7,11,15-tetramethylhexadecanoic acid (phytanic acid) in the cholesterol esters and other lipid fractions of the organs in a case of a disease of unknown origin (possibly hereditary atactica polyneuritiformis, refsum's syndrome). *Hoppe Seylers Z Physiol Chem* 333:133–139, 1963
- Komuves LG, Hanley K, Man MQ, Elias PM, Williams ML, Feingold KR: Keratinocyte differentiation in hyperproliferative epidermis: Topical application of PPARalpha activators restores tissue homeostasis. *J Invest Dermatol* 115:361–367, 2000
- Lewis SE, Listenberger LL, Ory DS, Schaffer JE: Membrane topology of the murine fatty acid transport protein 1. *J Biol Chem* 276:37042–37050, 2001
- Lisby S, Ralfkiaer E, Hansen ER, Vejlsgaard GL: Keratinocyte and epidermal leukocyte expression of CD36 (OKM5) in benign and malignant skin diseases. *Acta Derm Venereol* 70:18–22, 1990
- Lu Y, Bu L, Zhou S, *et al*: Scd1ab-Xyk: A new asebica allele characterized by a CCC trinucleotide insertion in exon 5 of the stearoyl-CoA desaturase 1 gene in mouse. *Mol Genet Genomics* 272:129–137, 2004
- Man MQ, Feingold KR, Elias PM: Exogenous lipids influence permeability barrier recovery in acetone-treated murine skin. *Arch Dermatol* 129:728–738, 1993
- Miller C, Yamaguchi RY, Ziboh VA: Guinea pig epidermis generates putative anti-inflammatory metabolites from fish oil polyunsaturated fatty acids. *Lipids* 24:998–1003, 1989
- Moulson CL, Martin DR, Lugus JJ, Schaffer JE, Lind AC, Miner JH: Cloning of wrinkle-free, a previously uncharacterized mouse mutation, reveals crucial roles for fatty acid transport protein 4 in skin and hair development. *Proc Natl Acad Sci USA* 100:5274–5279, 2003
- Nicolaidis N, Rothman S: The site of sterol and squalene synthesis in the human skin. *J Invest Dermatol* 24:125–129, 1955
- Nishijima T, Tokura Y, Imokawa G, Seo N, Furukawa F, Takigawa M: Altered permeability and disordered cutaneous immunoregulatory function in mice with acute barrier disruption. *J Invest Dermatol* 109:175–182, 1997
- Schaap F, Hamers L, Van dVG, Glatz J: Molecular cloning of fatty acid-transport protein cDNA from rat. *Biochim Biophys Acta* 1354:29–34, 1997
- Schaffer JE, Lodish HF: Expression cloning and characterization of a novel adipocyte long chain fatty acid transport protein. *Cell* 79:427–436, 1994
- Schmuth M, Man MQ, Weber F, *et al*: Permeability barrier disorder in Niemann-Pick disease: Sphingomyelin-ceramide processing required for normal barrier homeostasis. *J Invest Dermatol* 115:459–466, 2000
- Schmuth M, Neyer S, Rainer C, Grassegger A, Fritsch P, Romani N, Heufler C: Expression of the C-C chemokine MIP-3 alpha/CCL20 in human epidermis with impaired permeability barrier function. *Exp Dermatol* 11:135–142, 2002
- Schurer NY, Stremmel W, Grundmann JU, Schliep V, Kleinert H, Bass NM, Williams ML: Evidence for a novel keratinocyte fatty acid uptake mechanism with preference for linoleic acid: Comparison of oleic and linoleic acid uptake by cultured human keratinocytes, fibroblasts and a human hepatoma cell line. *Biochim Biophys Acta* 1211:51–60, 1994
- Smythe CD, Greenall M, Kealey T: The activity of HMG-CoA reductase and acetyl-CoA carboxylase in human apocrine sweat glands, sebaceous glands, and hair follicles is regulated by phosphorylation and by exogenous cholesterol. *J Invest Dermatol* 111:139–148, 1998
- Sorrentino D, Stump D, Potter BJ, Robinson RB, White R, Kiang CL, Berk PD: 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* 82:928–935, 1988
- Stahl A: A current review of fatty acid transport proteins (SLC27). *Pflügers Arch* 447:722–727, 2004
- Stahl A, Evans JG, Pattel S, Hirsch D, Lodish HF: Insulin causes fatty acid transport protein translocation and enhanced fatty acid uptake in adipocytes. *Dev Cell* 2:477–488, 2002
- Stahl A, Gimeno RE, Tartaglia LA, Lodish HF: Fatty acid transport proteins: A current view of a growing family. *Trends Endocrinol Metab* 12:266–273, 2001
- Stahl A, Hirsch DJ, Gimeno R, *et al*: Identification of a small intestinal fatty acid transport protein. *Mol Cell* 4:299–308, 1999
- Stone SJ, Myers HM, Watkins SM, Brown BE, Feingold KR, Elias PM, Farese RV Jr: Lipopenia and skin barrier abnormalities in DGAT2-deficient mice. *J Biol Chem* 279:11767–11776, 2004
- Stremmel W: Uptake of fatty acids by jejunal mucosal cells is mediated by a fatty acid binding membrane protein. *J Clin Invest* 82:2001–2010, 1988
- Stremmel W: Mechanism of hepatic fatty acid uptake. *J Hepatol* 9:374–382, 1989a
- Stremmel W: Transmembrane transport of fatty acids in the heart. *Mol Cell Biochem* 88:23–29, 1989b
- Stump DD, Fan X, Berk PD: Oleic acid uptake and binding by rat adipocytes define dual pathways for cellular fatty acid uptake. *J Lipid Res* 42:509–520, 2001
- Thiboutot D, Jabara S, McAllister JM, Sivarajah A, Gilliland K, Cong Z, Clawson G: Human skin is a steroidogenic tissue: Steroidogenic enzymes and cofactors are expressed in epidermis, normal sebocytes, and an immortalized sebocyte cell line (SEB-1). *J Invest Dermatol* 120:905–914, 2003
- Westerberg R, Tvrdik P, Uden AB, *et al*: Role for ELOVL3 and fatty acid chain length in development of hair and skin function. *J Biol Chem* 279:5621–5629, 2004
- Wilkinson DI: Incorporation of acetate-1-C14 into fatty acids of isolated epidermal cells. *J Invest Dermatol* 54:132–138, 1970
- Wood LC, Jackson SM, Elias PM, Grunfeld C, Feingold KR: Cutaneous barrier perturbation stimulates cytokine production in the epidermis of mice. *J Clin Invest* 90:482–487, 1992
- Zheng Y, Eilertsen KJ, Ge L, *et al*: Scd1 is expressed in sebaceous glands and is disrupted in the asebica mouse. *Nat Genet* 23:268–270, 1999
- Ziboh VA, Cohen KA, Ellis CN, *et al*: Effects of dietary supplementation of fish oil on neutrophil and epidermal fatty acids. Modulation of clinical course of psoriatic subjects. *Arch Dermatol* 122:1277–1282, 1986