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

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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.

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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 (Nicolaides 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 dietery 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 ^{14}C-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; Ibrahimi 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 dermopathy with tight, wrinkle-free skin, and disrupted skin barrier function. How partial ablation 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 1*A*). 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 1*B*). FATP3 in mouse skin was heavily expressed by epidermal keratinocytes (Fig 1*B*). FATP4 was less prominently expressed by epidermal and hair follicle keratinocytes, but strongly associated with sebaceous glands (Fig 1*B*). 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 1*C*). FATP6 in mouse skin was again associated with both interfollicular and follicular keratinocytes (Fig 1*B*). 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 1*B*). 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

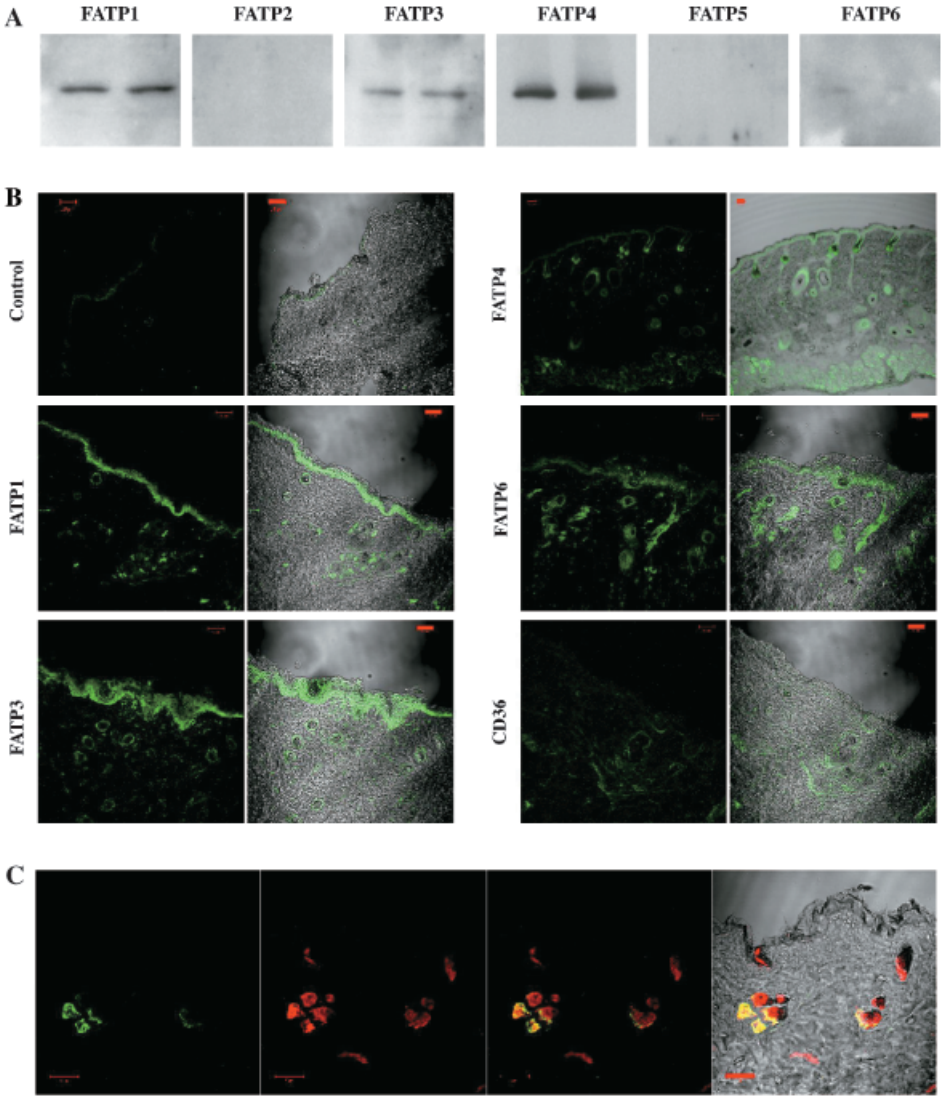

Figure 1
Expression and localization of fatty acid transporter proteins (FATP) in mouse skin. (*A*) Western blot of two different murine epidermal lysates with antisera against all six FATP family members. (*B*) Immunofluorescence images of mouse skin with or without phase contrast for pre-immune serum (*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 μm 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 2*A*). 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 2*A*). Most notably, CD36 was expressed only at very low levels in normal mouse skin but was strongly upregulated in both dermal and epidermal layers after barrier disruption (Fig 2*A*). 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.

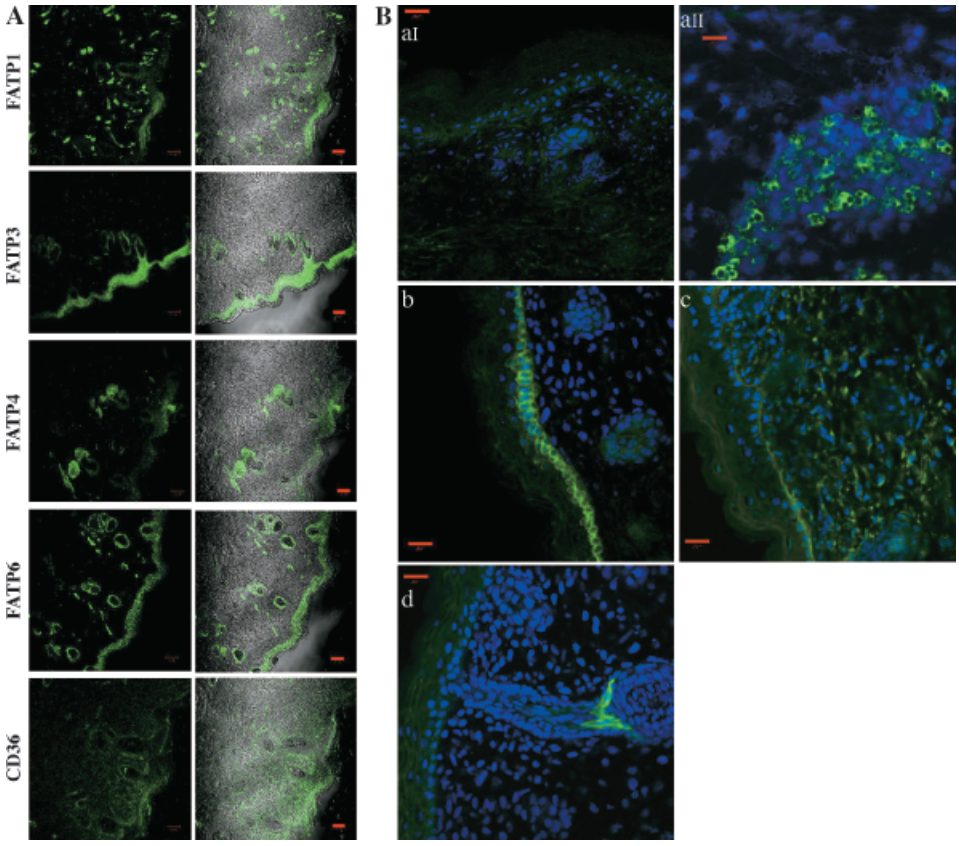

Figure 2
Modulation of fatty acid transporter proteins (FATP) expression following barrier disruption and during development. (*A*) Immunofluorescence images of mouse skin with or without phase contrast for FATP1–6 and CD36 following sustained barrier disruption by acetone treatment for 3 d. Red size bar equals 50 μm. (*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 μm.

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 2*BaI*) but it is robustly expressed by subcutaneous adipocytes (Fig 2*BaII*). FATP3 was expressed in the epidermis of day 18.5 embryos as it is in adult skin, but there was preferential basal staining (Fig 2*Bb*). 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 1*B*). 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 2*Bc*). Finally, FATP6 was expressed by cells within embryonic hair follicles and, less prominently, by epidermal keratinocytes (Fig 2*Bd*). 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 3*A*). 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 3*A*), where it again co-localized 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 3*A*).

In primary cultures of human keratinocytes, the primary expressed FATP was FATP4 (Fig 3*B*). 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 3*B*). 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

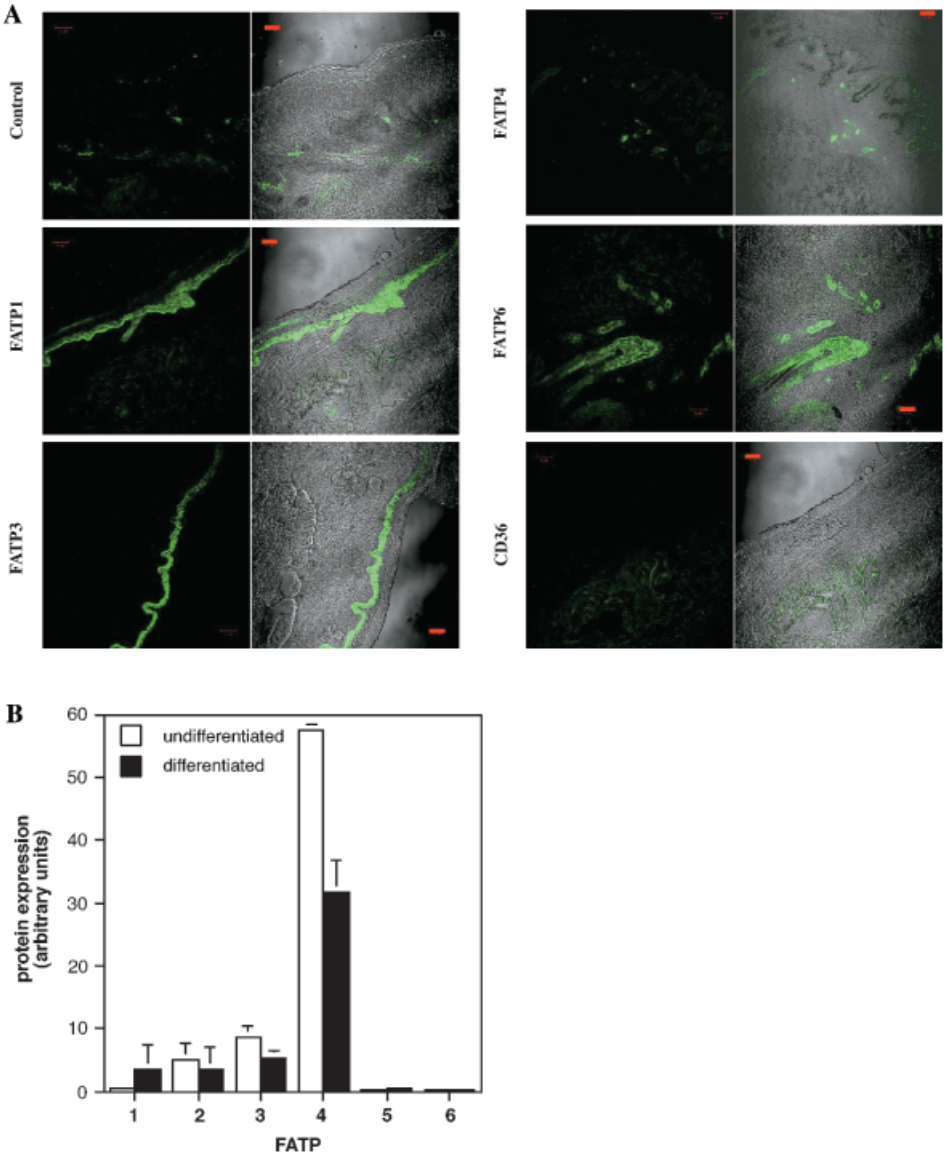

Figure 3
Localization of fatty acid transporter proteins (FATP) in human skin and cultured keratinocytes. (*A*) Immunofluorescence images of mouse skin with or without phase contrast for pre-immune serum (control), FATP1–6 antisera, and for anti-CD36. Red size bar equals 50 μm. (*B*) Densitometric quantification 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 plasma 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 dermopathy 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 cm^2 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 cm^2 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 paraphenylinediamine, and examined with a Zeiss LSM510 confocal system. Nile Red staining were performed as previously described (Fowler and Greenspan, 1985).

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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.

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