Topical Peroxisome Proliferator Activated Receptor Activators Accelerate Postnatal Stratum Corneum Acidification

Joachim W. Fluhr[1,2], Mao-Qiang Man[1], Jean-Pierre Hachem[1], Debra Crumrine[1], Theodora M. Mauro[1,3,4], Peter M. Elias[1,3,4] and Kenneth R. Feingold[1,3,4]

Previous studies have shown that pH declines from between 6 and 7 at birth to adult levels (pH 5.0–5.5) over 5–6 days in neonatal rat stratum corneum (SC). As a result, at birth, neonatal epidermis displays decreased permeability barrier homeostasis and SC integrity, improving days 5–6. We determined here whether peroxisome proliferator-activated receptor (PPAR) activators accelerate postnatal SC acidification. Topical treatment with two different PPARα activators, clofibrate and WY14643, accelerated the postnatal decline in SC surface pH, whereas treatment with PPARγ activators did not and a PPARβ/δ activator had only a modest effect. Treatment with clofibrate significantly accelerated normalization of barrier function. The morphological basis for the improvement in barrier function in PPARα-treated animals includes accelerated secretion of lamellar bodies and enhanced, postsecretory processing of secreted lamellar body contents into mature lamellar membranes. Activity of β-glucocerebrosidase increased after PPARα-activator treatment. PPARα activator also improved SC integrity, which correlated with an increase in corneodesmosome density and increased desmoglein-1 content, with a decline in serine protease activity. Topical treatment of newborn animals with a PPARα activator increased secretory phospholipase A2 activity, which likely accounts for accelerated SC acidification. Thus, PPARα activators accelerate neonatal SC acidification, in parallel with improved permeability homeostasis and SC integrity/cohesion. Hence, PPARα activators might be useful to prevent or treat certain common neonatal dermatoses.

Journal of Investigative Dermatology (2009) **129,** 365–374; doi:10.1038/jid.2008.218; published online 14 August 2008

INTRODUCTION

It is well known that the surface pH of the human adult skin is acidic with a pH between 5 and 5.5 (Heuss, 1892; Schade, 1928; Marchionini and Hausknecht, 1938; Blank, 1939; Bernstein and Hermann, 1942; Draize, 1942; Arbenz, 1952; Behrendt and Green, 1958; Beare *et al.*, 1960; Jolly, 1961; Baden and Pathak, 1967; Tippelt, 1969; Braun-Falco and Korting, 1986; Zlotogorski, 1987; Korting *et al.*, 1990; Seidenari and Giusti, 1995; Berardesca *et al.*, 1998; Yosipovitch *et al.*, 1998; Eberlein-Konig *et al.*, 2000; Fluhr *et al.*, 2000), but the mechanisms that account for this acidification remain only partially defined. It has been postulated that exogenous mechanisms (that is, originating outside the epidermis), such as free fatty acids of pilosebaceous origin (Puhvel *et al.*, 1975; Bibel *et al.*, 1989), microbial metabolites (Di Marzio *et al.*, 1999), and eccrine gland-derived products, such as lactic acid (Ament *et al.*, 1997; Thueson *et al.*, 1998), contribute to stratum corneum (SC) acidification. But recent studies have shown that endogenous pathways also contribute to SC acidification (Fluhr *et al.*, 2001a, 2004a; Behne *et al.*, 2002a). Free fatty acid generation from phospholipid hydrolysis catalyzed by secretory phospholipase A2 (sPLA2, Mao-Qiang *et al.*, 1996; Fluhr *et al.*, 2001a, 2004a), generation of acidic metabolites of filaggrin metabolism, such as *cis*-urocanic acid from histidine (Schwarz *et al.*, 1986; Krien and Kermici, 2000), and a sodium/proton pump antiporter, sodium/hydrogen antiporter-1 (NHE-1; Behne *et al.*, 2002b, 2003; Fluhr *et al.*, 2004a), appears to contribute to SC acidification.

Yet, the skin surface is not fully acidified in newborn rodents and humans (Behrendt and Green, 1958; Visscher *et al.*, 2000; Yosipovitch *et al.*, 2000; Fluhr *et al.*, 2004a). While in humans it takes several weeks to months before the SC is fully acidified (Behrendt and Green, 1958; Visscher *et al.*, 2000; Yosipovitch *et al.*, 2000; Giusti *et al.*, 2001) in

[1]Dermatology and Medical Service, Veterans Affairs Medical Center, San Francisco, California, USA; [2]Bioskin, Berlin, Germany; [3]Department of Dermatology, University of California, San Francisco, California, USA and [4]Department of Medicine, University of California, San Francisco, California, USA

Correspondence: Dr Joachim W. Fluhr, Dermatology and Medical Service (190), Veterans Affairs Medical Center, 4150 Clement Street, San Francisco, California 94121, USA. E-mail: joachim.fluhr@gmx.Net

Abbreviations: BPB, bromphenacylbromide; CD, corneodesmosome; DSG1, desmoglein 1; β-GlucCer'ase, β-glucocerebrosidase; NHE-1, sodium/hydrogen antiporter-1; PPAR, peroxisome proliferator-activated receptor; SC, stratum corneum; SG, stratum granulosum; sPLA2, secretory phospholipase A2; TEWL, transepidermal water loss

Received 24 May 2007; revised 29 April 2008; accepted 30 April 2008; published online 14 August 2008

© 2009 The Society for Investigative Dermatology

the neonatal rat, the SC attains adult pH levels over the first postnatal week (Fluhr *et al.*, 2004a). Inhibition of either sPLA2 or NHE-1 delays postnatal SC acidification, suggesting that these two endogenous pathways of acidification contribute to the postnatal acidification (Fluhr *et al.*, 2004a) at a time when neither the normal microbial flora nor filaggrin proteolysis appear to be contributory. Moreover, murine epidermis is devoid of eccrine glands, whose products therefore are not available to acidify the SC.

While the major function of the low surface pH has been assumed to be antimicrobial (Aly *et al.*, 1975; Hartmann, 1983; Bibel *et al.*, 1989), recent studies have suggested that the acidic pH of the SC is also essential for normal permeability barrier homeostasis and SC integrity and cohesion (the converse of desquamation). Specifically, in newborn rodents the recovery of permeability barrier function following acute barrier disruption is delayed because of incomplete maturation of lamellar membranes in the SC (Fluhr *et al.*, 2004b), an abnormality that has been attributed in turn to decreased activity of at least one key enzyme for lipid processing; β-glucocerebrosidase (β-GlucCer'ase), which exhibits optimal activity at an acidic pH. Pertinently, both permeability barrier homeostasis and the maturation of SC lamellar membranes improve with topical treatment of the newborn animals with an acidic buffer (Fluhr *et al.*, 2004b). Similarly, abnormal SC integrity and cohesion are corrected by topical acidic buffers (Fluhr *et al.*, 2004b); corneodesmosomes (CDs) density increases with acidic buffer treatment, and desmoglein 1 (DSG1) and corneodesmosin protein content increase in parallel. An increase in serine protease activity in the SC in newborn animals, because of the higher pH that optimizes catalytic activity of this family of enzymes, appears to account for increased degradation of CD in neonatal SC. Notably, the increased serine protease activity and the reduced density of CD are corrected by topical treatment with an acidic buffer linking the structural abnormalities in CD with the alterations in SC integrity (Fluhr *et al.*, 2004b). Together, these experiments demonstrate that a decrease in pH in neonatal animals improves both permeability barrier homeostasis and SC integrity indicating an important role for pH in regulating these functions during the perinatal period. Conversely, the elevated surface pH in neonates could contribute to their common clinical abnormalities.

Recent studies have shown that topical treatment of newborn animals with activators of LXR, a nuclear hormone receptor that forms heterodimers with RXR followed by activation of gene transcription, can accelerate SC acidification, at least in part because of an increase in sPLA2 activity (Fluhr *et al.*, 2005). This LXR-mediated acceleration of acidification was associated with an improvement in both permeability barrier homeostasis and SC integrity. Additionally, the structural abnormalities in both SC lamellar membranes and CD density in newborn animals were ameliorated by treatment with LXR activators, accounting for the improvement in these functions (Fluhr *et al.*, 2005).

Peroxisome proliferator-activated receptors (PPARs) are members of the same subfamily of nuclear hormone receptors as LXR. Three isotypes have been described: PPARα (NR1C1), PPARβ/δ (NR1C2), and PPARγ (NR1C3), which all are expressed in epidermis, and most specifically in human keratinocytes (Rivier *et al.*, 1998; Kuenzli and Saurat, 2004). Endogenous fatty acids and their metabolites activate PPARs, as do specific drugs (PPARα, clofibrates and WY14643; PPARβ/δ, GW1514; PPARγ, thiazolidinediones, such as ciglitazone and troglitazone; Kliewer *et al.*, 1999). In adult animals, topical treatment with activators of these PPAR isoforms, and the addition of activators to human keratinocyte cultures stimulates keratinocyte differentiation (Hanley *et al.*, 1998; Komuves *et al.*, 2000; Mao-Qiang *et al.*, 2004; Schmuth *et al.*, 2004). In addition, topical treatment of normal adult animals with PPAR activators improves permeability barrier homeostasis following acute barrier disruption (Man *et al.*, 2006). Finally, PPARα and PPARβ/δ activators accelerate the formation of the epidermal permeability barrier and stimulate differentiation during fetal development, whereas PPARγ displays no effects (Komuves *et al.*, 1998; Hanley *et al.*, 1999; Michalik *et al.*, 2002; Schmuth *et al.*, 2002a). Given the beneficial effect of LXR activation on SC pH and cutaneous structure and function (Fluhr *et al.*, 2005), we hypothesized that treatment with one or more PPAR activators could also accelerate neonatal acidification, resulting in normalization of the abnormalities in cutaneous function that occur in neonatal epidermis.

RESULTS

Topical PPARα activators accelerate postnatal acidification

Our initial experiments examined whether topical treatment of newborn mice with PPAR activators would accelerate the acidification of the SC. Skin surface pH varies from approximately 6.0 to 7.0 between different litters of newborn rats. The explanation for this variation in surface pH is unknown but because of this variation we only compare measurements in the same group of newborn animals studied simultaneously under identical conditions. As shown in Figure 1, treatment with two different PPARα activators, clofibrate and WY14643, resulted in a decrease in SC surface pH, whereas treatment with GW1514, a PPARβ/δ activator, resulted in lesser changes than that nevertheless achieved statistical significance. In contrast, treatment with the PPARγ activator, ciglitazone, had no effect on SC surface pH, whereas the PPARγ activator, troglitazone, actually increased surface pH. Topical treatment with PPAR activators did not alter either basal transepidermal water loss (TEWL) or SC hydration (data not shown).

Accelerated postnatal acidification by PPARα activators improves permeability barrier homeostasis in neonates

We next determined whether PPARα and PPARβ/δ activators, which both accelerated postnatal acidification, would improve permeability barrier homeostasis in neonates. As shown in Figure 2, treatment with the PPARα activator, clofibrate, significantly accelerated the kinetics of barrier recovery following acute disruption by tape stripping. The PPARβ/δ activator, GW1514, displayed only modest effects on barrier recovery that did not achieve statistical significance, consistent with the modest changes in SC pH.

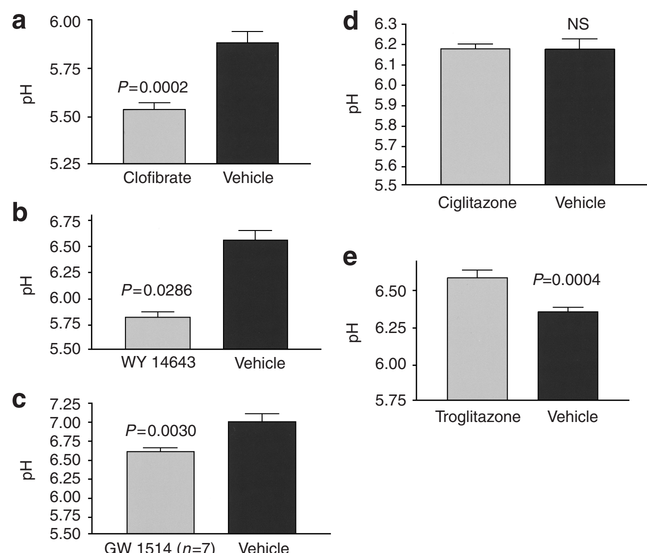

Figure 1. Topical treatment with PPARα ligand accelerates acidification of the stratum corneum. Newborn animals were treated topically with two PPARα activators, clofibrate and WY14643 (**a**, **b**), a PPARβ/δ activator GW1514(**c**), and two PPARγ activators, ciglitazone and troglitazone (**d**, **e**). The compounds were dissolved in acetone at a concentration of 10 mM. The newborn rats were treated on each flank 2 × per day over 3 days with 20 μl of the freshly prepared solutions. To prevent the mothers from licking the applied substances off the newborn rats, we placed the pups in a plastic container in a 37 °C incubator for 2–3 hours. The pH measurements were performed after 3 days of treatment. ($N=7$–13; mean ± SEM).

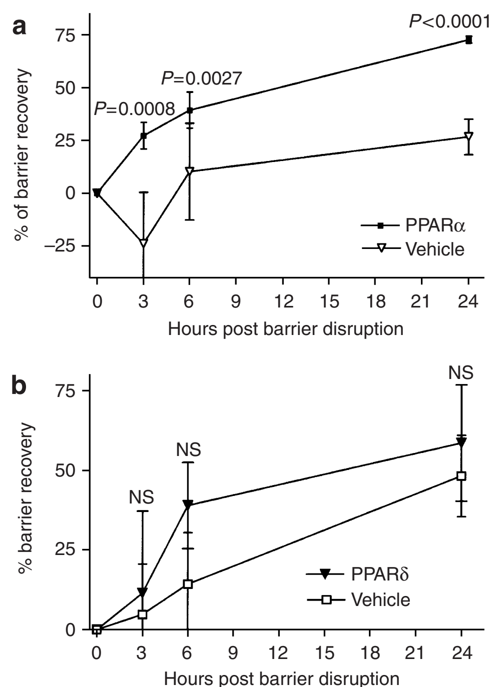

Figure 2. Permeability barrier homeostasis is improved by topical treatment with PPARα ligands. Treatment with the PPARα activator, clofibrate (**a**) and the PPARβ/δ activator, GW1514 (**b**), was carried out as described in Figure 1. To quantify epidermal permeability barrier function and barrier homeostasis, we measured TEWL with an electrolytic water analyzer (MEECO). After acute barrier disruption by tape stripping with a TEWL value of approximately 10–15 × over baseline, barrier recovery rates were calculated with the following formula: 1−(TEWL immediately after stripping−TEWL at different time points)/(TEWL immediately after stripping−baseline TEWL) × 100%. ($N=4$–8; mean ± SEM).

Accelerated lamellar body secretion and postsecretory lipid processing account for improved barrier function

The morphological basis for the PPARα-activator-induced improvement in permeability homeostasis following acute barrier disruption is shown in electron micrographs in Figure 3. First, accelerated secretion of lamellar bodies occurs in clofibrate-treated animals (Figure 3c and c insert). The increase in secretion can be readily detected at the SC: stratum granulosum (SG) interface and within the lower layers of the SG (premature secretion; Figure 3c). In contrast, secretion of lamellar bodies is not observed below the SC: SG interface in vehicle-treated or -untreated animals (Figure 3b). The secretion of lamellar bodies below the SC: SG interface is not usually observed (Figure 3c and c insert, arrows). Second, there are fully processed, mature lamellar membranes within the first and second interspace of the SC in the PPARα-activator-treated animals (Figure 3a, arrows) whereas in vehicle-treated control animals, the lamellar material is largely unprocessed (Figure 3b, asterisks). β-GlucCer'ase is a key enzyme required for processing of secreted lamellar body lipids into mature membranes. As shown in Figure S1, the activity of β-GlucCer'ase, measured by *in situ* zymography, increases following PPARα-activator treatment of neonatal rat skin. Together, these changes likely account for the improvement in permeability barrier homeostasis in PPARα-treated newborn animals.

PPARα activators improve SC integrity

The effects of topical treatment with either PPARα or PPARβ/δ activator on SC integrity are shown in Figure 4. SC integrity was assessed as the change in TEWL after three tape strippings with D-Squame tape. The PPARα activator, clofibrate, markedly reduced the increase in TEWL after tape stripping indicating an improved SC integrity, whereas GW1514, a PPARβ/δ activator, displayed only modest effects, which did not achieve statistical significance. The variation in TEWL after three tape strippings in these experiments was similar to that seen in previous studies. Furthermore, in the basal state there were 17–18 cell layers in PPARα-treated neonates compared to 10–15 cell layers in vehicle-treated neonates suggesting an increase in SC thickness. The morphological basis for the improvement in SC integrity with PPARα treatment is shown in Figure 5. The density of CDs appears to increase markedly in PPARα-treated animals in comparison to vehicle-treated neonates, and CD persists to higher levels of SC (Figure 5a and b; arrows). In vehicle-treated neonates, CD degradation (Figure 5c, open arrows) begins immediately above the SG–SC interface, and few CD persist into mid-SC.

Quantitative comparison of CD density per unit length cornified envelope (CE) in PPARα vs vehicle-treated neonates demonstrated a more than twofold increase in CD density in clofibrate-treated samples (Figure 6; $P<0.001$). Additionally, staining for DSG1, a key protein constituent of CDs, increases in PPARα-treated animals as shown in Figure S2. Although

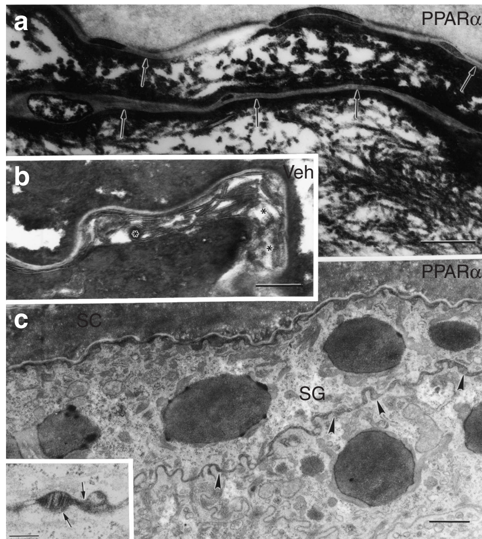

Figure 3. PPARα ligands accelerate the secretion and maturation of extracellular lamellar membranes in neonatal rat SC. The morphological basis for the improvement in permeability homeostasis following acute barrier disruption with PPARα activator treatment is shown in electron micrographs. At 3 hours after barrier disruption by tape stripping, samples were obtained and prepared for EM analysis as described in the MATERIALS and METHODS. First, there is accelerated secretion of lamellar bodies in clofibrate-treated animals (Figure 3c and c insert). The increase in secretion occurs both at the SC: SG interface and in lower layers of the SG (premature secretion, **c**). The secretion of lamellar bodies below the SC: SG interface is not usually observed (**c** and c insert, arrows). Second, there are fully processed, mature lamellar membranes within the first and second interspace of the SC in the PPARα-activator-treated animals (**a**, arrows) whereas in vehicle (Veh)-treated control animals, the lamellar material is largely unprocessed (**b**, asterisks). (**a**–**c** and c insert), ruthenium tetroxide postfixation. Magnification bars = 0.25 μm.

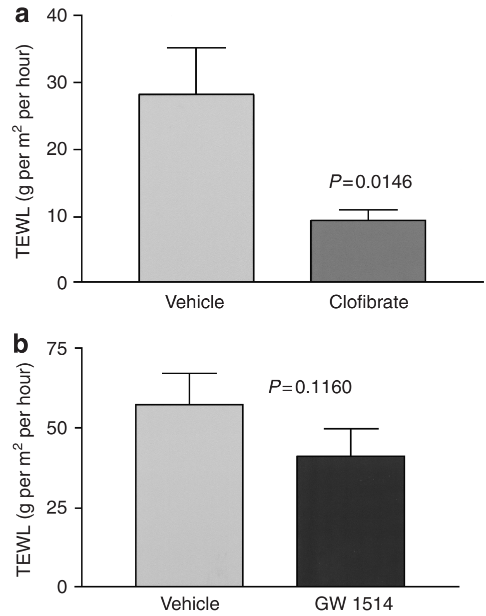

Figure 4. Topical treatment with PPARα activators normalize SC integrity. Newborn animals were treated topically twice per day for 3 days with clofibrate (**a**) or GW1514l (**b**). At 12 hours after the last treatment SC integrity was assessed as the change in TEWL value after a predefined number of D-Squame tape strippings (3 ×). The increase in TEWL was markedly reduced by treatment with the PPARα ligand. Results are presented as mean ± SEM ($n = 5$–6). These animals are a separate group of animals from those studied in Figures 1 and 2. ($N = 5$–6).

the mechanism for the decrease in CD in newborn animals is incompletely understood, in adult epidermis, CD are degraded primarily by serine proteases in the SC (Brattsand *et al.*, 2005). As shown in Figure S3, serine protease activity declines in the epidermis of neonates treated with clofibrate.

PPARα activator increases sPLA2 activity, which is important for acidification

A number of different mechanisms could contribute to the PPARα-induced acceleration of postnatal acidification. Previous studies have shown that sPLA2 plays an important role in the postnatal acidification of newborn animals (Fluhr *et al.*, 2004a), and LXR-activator-induced postnatal acceleration of acidification correlated with an increase in $sPLA_2$ activity. As shown in Figure 7, topical treatment of newborn animals with clofibrate, a PPARα activator, increases sPLA2 activity, whereas in contrast, immunostaining for NHE1 remains similar in vehicle-control and clofibrate-treated newborn animals (data not shown). Finally, that the postnatal increase in sPLA2 activity is important for postnatal acidification is shown in Figure 8. Simultaneous treatment with the PPARα activator and bromphenacylbromide (BPB), a broad inhibitor of sPLA2 activity, blocked the ability of the PPARα activator to acidify the SC of newborn animals.

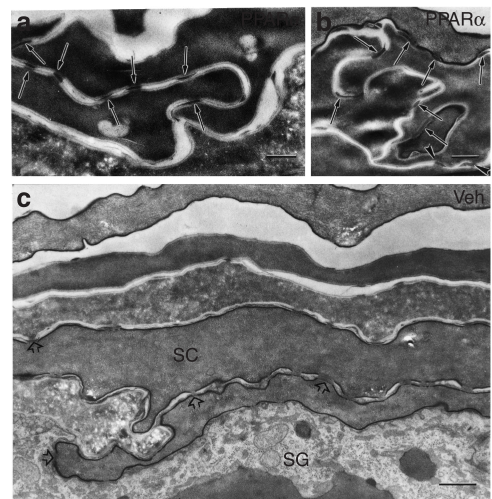

Figure 5. PPARα activator increases corneodesmosome density. Increased density and persistence of corneodesmosomes (CD) in PPARα (clofibrate-treated) neonates (**a**, **b**; arrows). In vehicle (Veh)-treated neonates, DC degradation ((**c**) open arrows) begins immediately above stratum granulosum (SG)–SC interface, and few CD persist into mid-SC. (**a**, **b**) Ruthenium tetroxide postfixation; (**c**) osmium tetroxide postfixation. Magnification bars = 0.5 μm.

DISCUSSION

In both humans and in various animal models skin surface pH is less acidic at birth and in humans it takes several weeks to achieve adult levels of acidification whereas in rodents, acidification occurs rapidly and adult levels are achieved

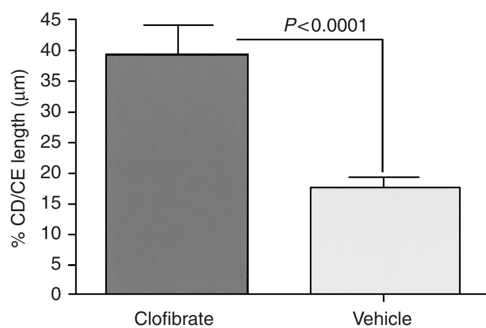

Figure 6. PPARα activator clofibrate increases corneodesmosome density significantly. Quantitation of corneodesmosome (CD) density was performed in 25–30 randomly obtained micrographs, taken at 25,000 magnification, using a planimeter (Plan Wheel, Scalex Corp., Carlsbad, CA), and Scale-Link version 3.0 software (Scalex). CD density was calculated as unit length of CD/total length of cornified envelopes (CE), and means ± SEM for each group (*N* = 20 clofibrate; *N* = 24 vehicle).

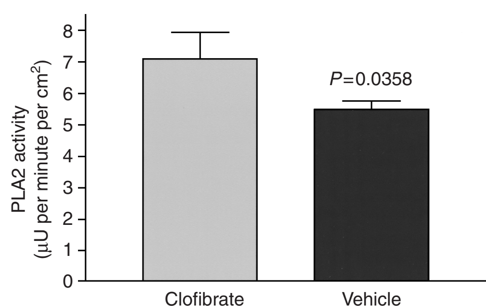

Figure 7. Secretory phospholipase A_2 activity is increased by topical treatment with PPARα ligand. Newborn animals were treated topically twice per day for 3 days with clofibrate or vehicle (control). At 12 hours after the last treatment skin was obtained. Secretory phospholipase A2 ($sPLA_2$) activity was assessed in pooled, sequential SC tape strips with a fluorometric assay after 3 days of treatment. To ensure uniform removal, the D-Squame tapes were placed on the skin surface with forceps, and the tape surface was gently rubbed three times over its entire surface. Total $sPLA_2$ activity in SC was quantified as described in the MATERIALS and METHODS. The activity of $sPLA_2$ was calculated per cm^2 of total SC (*N* = 7–8, mean ± SEM).

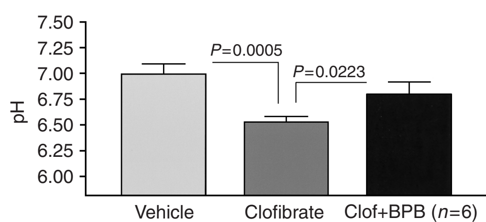

Figure 8. Co-applications of BPB inhibited the SC acidification induced by treatment with PPARα ligands. Newborn animals were treated topically with BPB alone, clofibrate alone or both BPB and clofibrate twice a day for 3 days. On day 3, SC surface pH was measured. (*N* = 6–8; mean ± SEM).

within several days (Behrendt and Green, 1958; Visscher *et al.*, 2000; Yosipovitch *et al.*, 2000; Fluhr *et al.*, 2004a). Thus, in rodents, the acidification of the SC post-birth is compressed into a relatively short time period, which facilitates studies. In rodents, the less acidic skin surface pH that is present at birth results in alterations in permeability barrier homeostasis (normal barrier function at baseline but a delay in barrier repair after acute barrier disruption) and a decrease in SC integrity and cohesion both of which are corrected by acidification of the SC (Fluhr *et al.*, 2004a, b). The combination of decreases in both permeability barrier homeostasis and the ability of the SC to withstand mechanical injury (decreased SC integrity and cohesion) would increase the susceptibility of the skin to cutaneous disorders. Numerous studies and clinical observations have reported that infants' skin is more susceptible to injury than adult skin (Lund *et al.*, 1997). Moreover, it is well recognized that the growth of skin pathogens, such as *Staphylococcus aureus* and *Streptococcus pyogenes*, is inhibited by an acidic skin pH, whereas the growth of normal skin flora flourishes at an acidic pH (Puhvel *et al.*, 1975; Korting *et al.*, 1990, 1992). Therefore, the increased risk of cutaneous infections in newborns could be due in part to an elevated skin surface pH (Yosipovitch *et al.*, 2000) that can persist until at least 3 months (Hoeger and Enzmann, 2002), and may not become completely normal until 2 years of age (Giusti *et al.*, 2001). Thus the less acidic surface pH in the newborn period could have harmful effects. Given the potential harmful consequences of a less acidic skin surface pH in newborns, we have utilized the rodent model to begin to assess several different therapeutic approaches to accelerate the acidification of the SC in the newborn period. A recent study by our laboratory has shown that topical treatment with activators of LXR, a nuclear hormone receptor that is activated by oxysterols, accelerates postnatal acidification of the SC (Fluhr *et al.*, 2005). This accelerated acidification was due in part to an increase in sPLA2 activity. Moreover, this accelerated acidification of the SC was associated with an improvement in both permeability barrier homeostasis and in SC integrity/cohesion. The improvement in barrier function was associated with an increased maturation of lamellar membranes attributable to an increase in the activity of β-GlucCer'ase, an enzyme that is most active at pH 5.5. The improvement of SC integrity/cohesion was associated with an increase in CD and DSG1 expression (Fluhr *et al.*, 2005).

In the present study, we explored the ability of PPAR activators to accelerate SC acidification. There are three PPAR isoforms, α, δ, and γ, and all three isoforms are present in the epidermis (Kuenzli and Saurat, 2003). These receptors heterodimerize with RXR and are activated by fatty acids, prostaglandins, eicosanoids, and other lipid metabolites (Kliewer *et al.*, 1999). Studies by our laboratory and others have shown that activation of PPARα, δ, and γ have major effects on epidermal/keratinocyte function. First, the addition of PPAR ligands to cultured human keratinocytes and topical application of PPAR ligands to mouse skin stimulates the increased expression of differentiation related proteins such as involucrin, loricrin, profilaggrin, and transglutaminase 1 (Hanley *et al.*, 1997; Komuves *et al.*, 2000; Westergaard *et al.*, 2001; Fluhr *et al.*, 2004a; Kuenzli and Saurat, 2004; Mao-Qiang *et al.*, 2004; Burdick *et al.*, 2006). Second, topical treatment of murine skin with PPAR ligands improves permeability barrier homeostasis resulting in an acceleration

of barrier recovery following acute disruption (Man *et al.*, 2006). Associated with the improved permeability barrier homeostasis was an increase in (1) epidermal cholesterol, fatty acid, and sphingolipid synthesis, (2) lamellar body secretion, and (3) β-GlucCer'ase activity, all of which could contribute to the enhanced barrier homeostasis. Finally, PPARα and PPARδ but not PPARγ activators accelerate the formation of the SC during fetal development, resulting in the earlier appearance of a mature permeability barrier (Hanley *et al.*, 1997, 1999; Komuves *et al.*, 1998; Schmuth *et al.*, 2002b).

We demonstrate in the present paper that topical treatment of newborn rat skin with two different PPARα activators accelerates the acidification of newborn SC, whereas a PPARδ activator had only a modest effect. In contrast, treatment with a PPARγ activator, ciglitazone had no effect on SC acidification, whereas the PPARγ activator, troglitazone increased surface pH. The basis for this difference between two different PPARγ activators is unknown but other studies have shown that the spectrum of genes activated by different PPARγ ligands can vary considerably (Camp *et al.*, 2000). In humans it is well recognized that pioglitazone decreases serum triglyceride levels in patients with diabetes whereas rosiglitazone either has no effect or increases serum triglycerides (Goldberg *et al.*, 2005). In this paper, we measured surface pH but previous studies in both adults and neonates have shown that changes in surface pH reflect the changes in pH that occur throughout the SC measured by fluorescence lifetime imaging (FLIM; Behne *et al.*, 2002a, 2003). Thus, the decrease in surface pH reported in this paper indicates that PPAR activators are acidifying the SC, which as discussed below will have an impact on the catalytic activity of the enzymes that reside in the SC. In some cases, an acidic milieu will increase enzyme activity (for example, β-GlucCer'ase) and in other instances will decrease catalytic activity (for example serine proteases).

Treatment with PPARα activators improved permeability barrier homeostasis and SC integrity in newborn animals whereas PPARδ activators had only a modest effect on the functional defects consistent with its limited effect on SC acidification. The improvement in SC integrity/cohesion in PPARα-activator-treated skin was associated with an increase in the size and density of CDs and an increase in staining for DSG1, a major protein of CDs. In adult animals, one of the key factors regulating CD degradation is the activity of serine proteases in the SC and we have shown that increasing the pH in SC of adult animals increases serine protease catalytic activity, that is, serine protease catalytic activity is inhibited by an acidic pH and activated by a neutral pH. In previous studies, we have shown that serine protease activity is increased in the SC of newborn animals and can be reduced by acidifying the SC (Fluhr *et al.*, 2004b). In the present paper, we demonstrate that treatment of newborn skin with PPARα activators, which decreases SC pH, also decreases serine protease activity and we would hypothesize that this reduction in serine protease activity accounts, at least in part, for the increase in CD size and density leading to an improvement in SC integrity and cohesion.

The improvement in permeability barrier homeostasis in the PPARα-treated newborn animals was associated with an increase in β-GlucCer'ase activity in the SC. β-GlucCer'ase is a key enzyme required for the extracellular conversion of glucosylceramidase to ceramides, which allows for the formation of mature lamellar membranes that inhibit water movement (Holleran *et al.*, 1993). Inhibition of β-GlucCer'ase activity or the absence of the enzyme in genetic disorders (Gaucher's disease) or knockout mice results in immature lamellar membranes and impaired permeability barrier homeostasis (Holleran *et al.*, 1994). It is well known that the activity of β-GlucCer'ase is optimal at an acidic pH and that a neutral pH inhibits catalytic activity (Holleran *et al.*, 1992). Thus, the acidification of the SC induced by PPARα activators not unexpectedly increased β-GlucCer'ase activity thereby resulting in the appearance of more mature lamellar membranes in the extracellular spaces of the SC. The more mature lamellar membranes in newborn animals treated with PPARα activators could explain the improvement in permeability barrier homeostasis.

In addition to lowering SC pH, PPARα activators have many other effects on the epidermis that could have a beneficial effect on SC structure and function. Specifically, previous studies in adult animals have shown that PPARα activators improve permeability barrier homeostasis independent of effects on SC pH by increasing epidermal lipid synthesis, stimulating lamellar body secretion, and increasing β-GlucCer'ase activity (Man *et al.*, 2006) Additionally, PPARα activators increase the expression of proteins required for differentiation such as loricrin, involucrin, filaggrin, and transglutaminase 1 (Hanley *et al.*, 1999; Komuves *et al.*, 2000; Westergaard *et al.*, 2001; Kuenzli and Saurat, 2003; Schmuth *et al.*, 2004; Burdick *et al.*, 2006; Kim *et al.*, 2006). Thus, it is likely that while the decrease in SC pH induced by PPARα activators is important in the improved function of neonatal skin other factors are also likely to contribute to the beneficial effects.

There are numerous potential mechanisms by which SC pH could decrease in newborn animals. In previous studies, we demonstrated that both NHE1 and sPLA2 play a role in SC acidification during the neonatal period (Fluhr and Elias, 2002; Behne *et al.*, 2003). In the present study, we demonstrate that sPLA2 activity in the SC is increased by topical treatment with PPARα activators during the neonatal period. Additionally, inhibiting sPLA2 with BPB prevented the acidification of the SC induced by PPARα activators, further supporting a role for sPLA2. It should be recognized that BPB has effects other than inhibiting sPLA2 activity (for example, BPB induces calcium influx in fibroblasts (Ogata *et al.*, 2002) and therefore it is possible that the prevention of SC acidification induced by PPARα activators is because of effects other than inhibition of sPLA2 activity. In contrast, treatment with PPARα activators did not increase staining for NHE1, suggesting that increases in NHE1 were not accounting for the accelerated reduction in SC pH induced by PPARα activators. It should be recognized that many pathways contribute to SC acidification and it is likely that PPAR activators could be activating multiple pathways.

In summary, the present study demonstrates that treatment of newborn skin with PPARα activators accelerates SC acidification during the neonatal period. In contrast, treatment with PPARγ activators had no effect whereas PPARδ activators had only a modest effect. In addition, treatment with PPARα activators also improved permeability homeostasis and SC integrity/cohesion in newborn animals, an effect that could be at least partially attributed to the acidification of the SC. It should be recognized that there are a number of differences between rodent and human skin and therefore further studies are required to determine if the acceleration of stratum corneum acidification and the beneficial effects induced by PPARα activators shown here in newborn rodents also occurs in human newborns. These studies suggest that PPARα activators might be of clinical benefit in the prevention and/or treatment of cutaneous disorders that occur during the neonatal period.

MATERIALS AND METHODS

Materials, animals, and experimental procedures

Timed-pregnant Sprague–Dawley rats were obtained from Simonson Laboratories (Gilroy, California) and fed Purina mouse diet and water *ad libitum*. The PPAR ligands were obtained as follows: PPARα, clofibrate and WY14643 were obtained from Sigma-Aldrich (St Louis, MO); PPARβ/δ, GW1514 was a gift from Dr Willson (GlaxoSmithKline); PPARγ, ciglitazone and troglitazone were obtained from Cayman Chemical Laboratories (Ann Arbor, MI). Animal protocols were approved by the Animal Care Subcommittee (Veterans Affairs Medical Center, San Francisco). The compounds were dissolved in acetone at a concentration of 10 mM. The pHs of the solutions containing PPAR activators or vehicle alone were similar (pH 6.5–7.0). The newborn rats were treated on each flank 2 × per day over 3 days with 20 μl of the freshly prepared solutions. To prevent the mothers from licking the applied substances off the newborn rats, we placed the pups in a plastic container in a 37 °C incubator for 2–3 hours. The pups were placed in groups (vehicle treated separated from activator treated). The measurements were performed after 3 days of treatment.

Permeability barrier homeostasis. Barrier function was determined by measurement of TEWL with an electrolytic water analyzer (MEECO, Warrington, PA). Stratum corneum hydration was measured with a corneometer (Corneometer CM 820, Courage and Khazaka, Cologne, Germany). Surface pH was measured with a flat, glass surface electrode from Mettler-Toledo (Giessen, Germany), attached to a pH meter (Skin pH Meter PH 900, Courage and Khazaka). Propylene glycol and ethanol were from Fisher Scientific (Fairlane, NJ), whereas BPB was from Sigma Chemical Co. (St Louis, MO). EnzCheck Protease Assay Kit was purchased from Molecular Probes (Eugene, OR). The strippings were performed with 22 mm D-Squame 100 tapes, purchased from CuDerm (Dallas, TX). To assess the importance of $sPLA_2$, neonatal animals were treated topically twice daily for 3 days with BPB (4 mg/ml) in propylene glycol:ethanol (7:3, v/v) vehicle or the vehicle alone applied to the backs and flanks of the newborn rats ($\approx 10\,\mu l/cm^2$) as described previously for hairless mice (Mao-Qiang *et al.*, 1996; Fluhr *et al.*, 2001b). The inhibitor doses that we employed have been shown previously to inhibit secretory PLA_2 activity selectively in different tissues and cell types (Jain *et al.*, 1991; Hornfelt *et al.*, 1999; Longo *et al.*, 1999; Okamoto *et al.*, 2007) and to be nontoxic to murine skin, without evidence of inhibition of other synthetic activities (Mao-Qiang *et al.*, 1995, 1996). Most importantly, previous studies have shown that topical treatment with BPB inhibits the metabolism of phospholipids to free fatty acids in the SC (Mao-Qiang *et al.*, 1995, 1996). The animal procedures were approved by the Animal Studies Committee of the San Francisco Veterans Affairs Medical Center and were performed in accordance with the guidelines.

SC integrity and barrier homeostasis. SC integrity was assessed as the change in TEWL value after a predefined number of D-Squame tape strippings. After acute barrier disruption by tape stripping with a TEWL value of about 10–15 × over baseline, barrier recovery rates were calculated with the following formula: (100−(TEWL immediately after stripping−TEWL at different time points)/(TEWL immediately after stripping−baseline TEWL) × 100%).

Electron microscopy

Samples for electron microscopy were minced to $1\,mm^3$ cubes, placed in modified Karnovsky's fixative, and postfixed in either reduced 1% osmium or buffered 0.2% ruthenium tetroxide with 0.5% ferrocyanide (Hou *et al.*, 1991). Samples were examined in a Zeiss 10A electron microscope (Carl Zeiss, Thornwood, NY) operated at 60 kV. Dr Peter Elias reviewed and interpreted the electron micrographs without knowledge of the treatment group. Micrographs shown for electron microscopy are representative of the changes observed in three or more samples obtained from at least two different litters for each treatment. Quantitation of CD density was performed in 25–30 randomly obtained micrographs, all taken at 25,000 magnification, using a planimeter (Plan Wheel, Scalex Corp., Carlsbad, CA), and Scale-Link version 3.0 software (Scalex). CD density was calculated as unit length of CD/total length of cornified envelopes, and means ± SEM for each group was compared statistically (Figure 6).

Assessment of lipid processing enzyme activities $sPLA_2$ activity. $sPLA_2$ activity was assessed in pooled, sequential SC tape strips (D-Squame) with a fluorometric assay, as described by (Radvanyi *et al.*, 1989; Mazereeuw-Hautier *et al.*, 2000). Briefly, in newborn rats, the SC was stripped down to the glistening layer after 3 days of treatment twice daily with PPAR activators or vehicle, and assayed as described previously (Fluhr *et al.*, 2004b).

Zymographic assessment of β-glucocerebrosidase activity. Enzyme activity was measured using a modification of a previously described method (Takagi *et al.*, 1999) that has been previously employed in neonatal rat studies (Hachem *et al.*, 2003; Fluhr *et al.*, 2004a). Briefly, enzyme activity was measured by applying 20 μl of 10 mM resorufin β-D-glucopyranoside dissolved in dimethyl sulfoxide to the backs of the newborn rats. The resorufin dye becomes fluorescent once the substrate is enzymatically cleaved. Following topical application, neonatal pups were placed for 2–3 hours in a plastic container at 37–38 °C. At the end of these *in vivo* incubations, 4-mm punch-out biopsies were taken from treated and control sites. The biopsies were placed on a plastic coverslip with a punched out center, inverted onto a microscopic slide, and covered with a second, nonpunched coverslip. The cleaved compound was

visualized with a confocal microscope at an excitation wavelength of 568 nm and an emission wavelength of 580 nm. Resorufin alone served as an additional negative control and as a further check for specificity (data not shown). β-GlucCer'ase enzyme activity was blocked when the fluorogenic substrate was co-applied with 10 mM conduritol B epoxide (data not shown).

Zymographic assessment of protease activity. Enzyme activity was assessed as previously described (Hachem *et al.*, 2003; Fluhr *et al.*, 2004a) with skin from treated neonatal rats. To obtain en face views harvested skin was placed in a chamber slide system with the SC facing toward the beam and visualized directly in a confocal microscope (Leica TCS SP, Heidelberg, Germany) at an excitation wavelength of 485 nm and an emission wavelength of 530 nm.

Immunofluorescence

Desmoglein 1 expression. DSG 1 expression was visualized as described previously (Fluhr *et al.*, 2001a).

NHE1 expression. Rabbit anti-mouse NHE1 antibody was purchased from Chemicom (Temecula, CA) and FITC-labeled goat anti-rabbit antibody was purchased from Jackson Immunoresearch Laboratories, INC (West Grove, PA). Skin biopsies were taken after 3 days of vehicle or activator treatment. sections (10 μm) were incubated with NHE1 antibody at a concentration of 15 μg/ml for 2 hours at room temperature. After washing three times with PBS a 1:200 dilution of FITC-labeled secondary was applied to sections and incubated for 30 minutes at room temperature.

Statistical analyses

Statistical analyses were performed using Prism 3 (GraphPad Software Inc., San Diego, CA). Normal distribution was tested before calculating the comparison with an unpaired *t*-test. In experiments with more than three groups, an analysis of variance was calculated followed by an α-corrected *post hoc* test (Bonferroni). Values are expressed as mean ± SEM. The significance level was set at $P < 0.05$.

CONFLICT OF INTEREST

The authors state no conflict of interest.

ACKNOWLEDGMENTS

Joan Wakefield provided excellent secretarial assistance, and Dr Roshan Gunathilake assisted with the statistical analysis of corneodesmosome density. This study was supported by NIH grants AR 39448 (PP), AR050629, HD29706, and the Medical Research Service, Department of Veterans Affairs.

SUPPLEMENTARY MATERIAL

Figure S1. The activity of β-glucocerebrosidase in neonatal rat stratum corneum is increased by treatment with PPARα ligands.

Figure S2. Desmoglein 1 Expression is increased by topical treatment with PPARα ligands.

Figure S3. Protease activity is decreased by PPARα ligands.

REFERENCES

Aly R, Maibach HI, Rahman R, Shinefield HR, Mandel AD (1975) Correlation of human in vivo and *in vitro* cutaneous antimicrobial factors. *J Infect Dis* 131:579–83

Ament W, Huizenga JR, Mook GA, Gips CH, Verkerke GJ (1997) Lactate and ammonia concentration in blood and sweat during incremental cycle ergometer exercise. *Int J Sports Med* 18:35–9

Arbenz H (1952) Untersuchungen über die pH-Werte der normalen Hautoberfläche. *Dermatologica* 105:333–54

Baden HP, Pathak MA (1967) The metabolism and function of urocanic acid in skin. *J Invest Dermatol* 48:11–7

Beare J, Cheeseman E, Gailey A, Neill D, Merrett J (1960) The effect of age on the pH of the skin surface in the first week of life. *Br J Dermatol* 72:62–6

Behne MJ, Barry NP, Hanson KM, Aronchik I, Clegg RW, Gratton E *et al.* (2003) Neonatal development of the stratum corneum pH gradient: localization and mechanisms leading to emergence of optimal barrier function. *J Invest Dermatol* 120:998–1006

Behne MJ, Meyer J, Hanson KM, Barry NP, Murata S, Crumrine D *et al.* (2002a) NHE1 regulates the stratum corneum permeability barrier homeostasis: microenvironment acidification assessed with FLIM. *J Biol Chem* 277:47399–406

Behne MJ, Meyer JW, Hanson KM, Barry NP, Murata S, Crumrine D *et al.* (2002b) NHE1 regulates the stratum corneum permeability barrier homeostasis. Microenvironment acidification assessed with fluorescence lifetime imaging. *J Biol Chem* 277:47399–406

Behrendt H, Green M (1958) Skin pH pattern in the newborn infant. *J Dis Child* 95:35–41

Berardesca E, Pirot F, Singh M, Maibach H (1998) Differences in stratum corneum pH gradient when comparing white Caucasian and black African-American skin. *Br J Dermatol* 139:855–7

Bernstein ET, Hermann F (1942) The acidity on the surface of the skin. *NY State J Med* 42:436–42

Bibel DJ, Miller SJ, Brown BE, Pandey BB, Elias PM, Shinefield HR *et al.* (1989) Antimicrobial activity of stratum corneum lipids from normal and essential fatty acid-deficient mice. *J Invest Dermatol* 92:632–8

Blank IA (1939) Measurement of pH of the skin surface. II. pH of the exposed surfaces of adults with no apparent skin lesions. *J Invest Dermatol* 2:75–9

Brattsand M, Stefansson K, Lundh C, Haasum Y, Egelrud T (2005) A proteolytic cascade of kallikreins in the stratum corneum. *J Invest Dermatol* 124:198–203

Braun-Falco O, Korting HC (1986) [Normal pH value of human skin]. *Hautarzt* 37:126–9

Burdick AD, Kim DJ, Peraza MA, Gonzalez FJ, Peters JM (2006) The role of peroxisome proliferator-activated receptor-beta/delta in epithelial cell growth and differentiation. *Cell Signal* 18:9–20

Camp HS, Li O, Wise SC, Hong YH, Frankowski CL, Shen X *et al.* (2000) Differential activation of peroxisome proliferator-activated receptor-gamma by troglitazone and rosiglitazone. *Diabetes* 49:539–47

Di Marzio L, Cinque B, De Simone C, Cifone MG (1999) Effect of the lactic acid bacterium *Streptococcus thermophilus* on ceramide levels in human keratinocytes *in vitro* and stratum corneum *in vivo*. *J Invest Dermatol* 113:98–106

Draize JH (1942) The determination of the pH of the skin of man and common laboratory animals. *J Invest Dermatol* 5:77–85

Eberlein-Konig B, Schafer T, Huss-Marp J, Darsow U, Mohrenschlager M, Herbert O *et al.* (2000) Skin surface pH, stratum corneum hydration, trans-epidermal water loss and skin roughness related to atopic eczema and skin dryness in a population of primary school children. *Acta Derm Venereol* 80:188–91

Fluhr JW, Behne MJ, Brown BE, Moskowitz DG, Selden C, Mao-Qiang M *et al.* (2004a) Stratum corneum acidification in neonatal skin: secretory phospholipase A2 and the sodium/hydrogen antiporter-1 acidify neonatal rat stratum corneum. *J Invest Dermatol* 122:320–9

Fluhr JW, Crumrine D, Mao-Qiang M, Moskowitz DG, Elias PM, Feingold KR (2005) Topical liver x receptor activators accelerate postnatal acidification of stratum corneum and improve function in the neonate. *J Invest Dermatol* 125:1206–14

Fluhr JW, Elias PM (2002) Stratum corneum (SC) pH: formation and function of the ''Acid Mantle''. *Exog Dermatol* 1:163–75

Fluhr JW, Kao J, Jain M, Ahn SK, Feingold KR, Elias PM (2001a) Generation of free fatty acids from phospholipids regulates stratum corneum acidification and integrity. *J Invest Dermatol* 117:44–51

Fluhr JW, Mao-Qiang M, Brown BE, Hachem JP, Moskowitz DG, Demerjian M *et al.* (2004b) Functional consequences of a neutral pH in neonatal rat stratum corneum. *J Invest Dermatol* 123:140–51

Fluhr JW, Pelosi A, Lazzerini S, Dikstein S, Berardesca E (2001b) Differences in corneocyte surface area in pre- and post-menopausal women. Assessment with the noninvasive videomicroscopic imaging of corneocytes method (VIC) under basal conditions. *Skin Pharmacol Appl Skin Physiol* 14:10–6

Fluhr JW, Pfisterer S, Gloor M (2000) Direct comparison of skin physiology in children and adults with bioengineering methods. *Pediatr Dermatol* 17:436–9

Giusti F, Martella A, Bertoni L, Seidenari S (2001) Skin barrier, hydration, and pH of the skin of infants under 2 years of age. *Pediatr Dermatol* 18:93–6

Goldberg RB, Kendall DM, Deeg MA, Buse JB, Zagar AJ, Pinaire JA *et al.* (2005) A comparison of lipid and glycemic effects of pioglitazone and rosiglitazone in patients with type 2 diabetes and dyslipidemia. *Diabetes Care* 28:1547–54

Hachem JP, Crumrine D, Fluhr J, Brown BE, Feingold KR, Elias PM (2003) pH directly regulates epidermal permeability barrier homeostasis, and stratum corneum integrity/cohesion. *J Invest Dermatol* 121:345–53

Hanley K, Jiang Y, Crumrine D, Bass NM, Appel R, Elias PM *et al.* (1997) Activators of the nuclear hormone receptors PPARalpha and FXR accelerate the development of the fetal epidermal permeability barrier. *J Clin Invest* 100:705–12

Hanley K, Jiang Y, He SS, Friedman M, Elias PM, Bikle DD *et al.* (1998) Keratinocyte differentiation is stimulated by activators of the nuclear hormone receptor PPARalpha. *J Invest Dermatol* 110:368–75

Hanley K, Komuves LG, Bass NM, He SS, Jiang Y, Crumrine D *et al.* (1999) Fetal epidermal differentiation and barrier development *In vivo* is accelerated by nuclear hormone receptor activators. *J Invest Dermatol* 113:788–95

Hartmann AA (1983) Effect of occlusion on resident flora, skin-moisture and skin-pH. *Arch Dermatol Res* 275:251–4

Heuss E (1892) Die Reaktion des Schweisses beim gesunden Menschen. *Monatschr Prakt Dermatol* 14:343–59

Hoeger PH, Enzmann CC (2002) Skin physiology of the neonate and young infant: a prospective study of functional skin parameters during early infancy. *Pediatr Dermatol* 19:256–62

Holleran WM, Ginns EI, Menon GK, Grundmann JU, Fartasch M, McKinney CE *et al.* (1994) Consequences of beta-glucocerebrosidase deficiency in epidermis. Ultrastructure and permeability barrier alterations in Gaucher disease. *J Clin Invest* 93:1756–64

Holleran WM, Takagi Y, Imokawa G, Jackson S, Lee JM, Elias PM (1992) Beta-glucocerebrosidase activity in murine epidermis: characterization and localization in relation to differentiation. *J Lipid Res* 33:1201–9

Holleran WM, Takagi Y, Menon GK, Legler G, Feingold KR, Elias PM (1993) Processing of epidermal glucosylceramides is required for optimal mammalian cutaneous permeability barrier function. *J Clin Invest* 91:1656–64

Hornfelt M, Ekstrom PA, Edstrom A (1999) Involvement of axonal phospholipase A2 activity in the outgrowth of adult mouse sensory axons *in vitro*. *Neuroscience* 91:1539–47

Hou SY, Mitra AK, White SH, Menon GK, Ghadially R, Elias PM (1991) Membrane structures in normal and essential fatty acid-deficient stratum corneum: characterization by ruthenium tetroxide staining and x-ray diffraction. *J Invest Dermatol* 96:215–23

Jain MK, Tao WJ, Rogers J, Arenson C, Eibl H, Yu BZ (1991) Active-site-directed specific competitive inhibitors of phospholipase A2: novel transition-state analogues. *Biochemistry* 30:10256–68

Jolly JHW Jr, Hailey CW, Netick J (1961) pH determinations of the skin: readings under normal and abnormal conditions. *J Invest Dermatol* 36:305–8

Kim DJ, Bility MT, Billin AN, Willson TM, Gonzalez FJ, Peters JM (2006) PPARbeta/delta selectively induces differentiation and inhibits cell proliferation. *Cell Death Differ* 13:53–60

Kliewer SA, Lehmann JM, Milburn MV, Willson TM (1999) The PPARs and PXRs: nuclear xenobiotic receptors that define novel hormone signaling pathways. *Recent Prog Horm Res* 54:345–67

Komuves LG, Hanley K, Jiang Y, Elias PM, Williams ML, Feingold KR (1998) Ligands and activators of nuclear hormone receptors regulate epidermal differentiation during fetal rat skin development. *J Invest Dermatol* 111:429–33

Komuves LG, Hanley K, Lefebvre AM, Man MQ, Ng DC, Bikle DD *et al.* (2000) Stimulation of PPARalpha promotes epidermal keratinocyte differentiation *in vivo*. *J Invest Dermatol* 115:353–60

Korting HC, Hubner K, Greiner K, Hamm G, Braun-Falco O (1990) Differences in the skin surface pH and bacterial microflora due to the long-term application of synthetic detergent preparations of pH 5.5 and pH 7.0. Results of a crossover trial in healthy volunteers. *Acta Derm Venereol* 70:429–31

Korting HC, Lukacs A, Vogt N, Urban J, Ehret W, Ruckdeschel G (1992) Influence of the pH-value on the growth of Staphylococcus epidermidis, *Staphylococcus aureus* and *Propionibacterium acnes* in continuous culture. *Zentralbl Hyg Umweltmed* 193:78–90

Krien PM, Kermici M (2000) Evidence for the existence of a self-regulated enzymatic process within the human stratum corneum—an unexpected role for urocanic acid. *J Invest Dermatol* 115:414–20

Kuenzli S, Saurat JH (2003) Peroxisome proliferator-activated receptors in cutaneous biology. *Br J Dermatol* 149:229–36

Kuenzli S, Saurat JH (2004) Peroxisome proliferator-activated receptors as new molecular targets in psoriasis. *Curr Drug Targets Inflamm Allergy* 3:205–11

Longo WE, Grossmann EM, Erickson B, Panesar N, Mazuski JE, Kaminski DL (1999) The effect of phospholipase A2 inhibitors on proliferation and apoptosis of murine intestinal cells. *J Surg Res* 84:51–6

Lund CH, Nonato LB, Kuller JM, Franck LS, Cullander C, Durand DJ (1997) Disruption of barrier function in neonatal skin associated with adhesive removal. *J Pediatr* 131:367–72

Man MQ, Choi EH, Schmuth M, Crumrine D, Uchida Y, Elias PM *et al.* (2006) Basis for improved permeability barrier homeostasis induced by PPAR and LXR activators: liposensors stimulate lipid synthesis, lamellar body secretion, and post-secretory lipid processing. *J Invest Dermatol* 126:386–92

Mao-Qiang M, Feingold KR, Jain M, Elias PM (1995) Extracellular processing of phospholipids is required for permeability barrier homeostasis. *J Lipid Res* 36:1925–35

Mao-Qiang M, Fowler AJ, Schmuth M, Lau P, Chang S, Brown BE *et al.* (2004) Peroxisome-proliferator-activated receptor (PPAR)-gamma activation stimulates keratinocyte differentiation. *J Invest Dermatol* 123:305–12

Mao-Qiang M, Jain M, Feingold KR, Elias PM (1996) Secretory phospholipase A2 activity is required for permeability barrier homeostasis. *J Invest Dermatol* 106:57–63

Marchionini A, Hausknecht W (1938) Säuremantel der Haut und Bakterienabwehr. *Klin Wochenschr* 17:663–6

Mazereeuw-Hautier J, Redoules D, Tarroux R, Charveron M, Salles JP, Simon MF *et al.* (2000) Identification of pancreatic type I secreted phospholipase A2 in human epidermis and its determination by tape stripping. *Br J Dermatol* 142:424–31

Michalik L, Desvergne B, Dreyer C, Gavillet M, Laurini RN, Wahli W (2002) PPAR expression and function during vertebrate development. *Int J Dev Biol* 46:105–14

Ogata Y, Sakurai T, Nakao S, Kuboyama N, Moriwaki K, Furuyama S *et al.* (2002) 4-Bromophenacyl bromide induces Ca2+ influx in human gingival fibroblasts. *Comp Biochem Physiol C Toxicol Pharmacol* 131:315–22

Okamoto K, Kim JS, Rubin BK (2007) Secretory phospholipases A2 stimulate mucus secretion, induce airway inflammation, and produce secretory hyperresponsiveness to neutrophil elastase in ferret trachea. *Am J Physiol Lung Cell Mol Physiol* 292:L62–7

Puhvel SM, Reisner RM, Sakamoto M (1975) Analysis of lipid composition of isolated human sebaceous gland homogenates after incubation with cutaneous bacteria. Thin-layer chromatography. *J Invest Dermatol* 64:406–11

Radvanyi F, Jordan L, Russo-Marie F, Bon C (1989) A sensitive and continuous fluorometric assay for phospholipase A2 using pyrene-labeled phospholipids in the presence of serum albumin. *Anal Biochem* 177:103–9

Rivier M, Safonova I, Lebrun P, Griffiths CE, Ailhaud G, Michel S (1998) Differential expression of peroxisome proliferator-activated receptor subtypes during the differentiation of human keratinocytes. *J Invest Dermatol* 111:1116–21

Schade HMA (1928) Zur physikalischen Chemie der Hautoberfläche. *Arch Dermatol Syphil* 154:690–716

Schmuth M, Haqq CM, Cairns WJ, Holder JC, Dorsam S, Chang S *et al.* (2004) Peroxisome proliferator-activated receptor (PPAR)-beta/delta stimulates differentiation and lipid accumulation in keratinocytes. *J Invest Dermatol* 122:971–83

Schmuth M, Schoonjans K, Yu QC, Fluhr JW, Crumrine D, Hachem JP *et al.* (2002a) Role of peroxisome proliferator-activated receptor alpha in epidermal development in utero. *J Invest Dermatol* 119:1298–303

Schmuth M, Wimmer MA, Hofer S, Sztankay A, Weinlich G, Linder DM *et al.* (2002b) Topical corticosteroid therapy for acute radiation dermatitis: a prospective, randomized, double-blind study. *Br J Dermatol* 146:983–91

Schwarz W, Langer K, Schell H, Schonberger A (1986) Distribution of urocanic acid in human stratum corneum. *Photodermatol* 3:239–40

Seidenari S, Giusti G (1995) Objective assessment of the skin of children affected by atopic dermatitis: a study of pH, capacitance and TEWL in eczematous and clinically uninvolved skin. *Acta Derm Venereol* 75:429–33

Takagi Y, Kriehuber E, Imokawa G, Elias PM, Holleran WM (1999) Beta-glucocerebrosidase activity in mammalian stratum corneum. *J Lipid Res* 40:861–9

Thueson DO, Chan EK, Oechsli LM, Hahn GS (1998) The roles of pH and concentration in lactic acid-induced stimulation of epidermal turnover. *Dermatol Surg* 24:641–5

Tippelt H (1969) Zur Hautoberflächen-pH-Mesung und Alkaliresistenzprobe bei Hautgesunden und Ichthyosis vulgaris-Kranken. *Dermatologica* 139:201–10

Visscher MO, Chatterjee R, Munson KA, Pickens WL, Hoath SB (2000) Changes in diapered and nondiapered infant skin over the first month of life. *Pediatr Dermatol* 17:45–51

Westergaard M, Henningsen J, Svendsen ML, Johansen C, Jensen UB, Schroder HD *et al.* (2001) Modulation of keratinocyte gene expression and differentiation by PPAR-selective ligands and tetradecylthioacetic acid. *J Invest Dermatol* 116:702–12

Yosipovitch G, Maayan-Metzger A, Merlob P, Sirota L (2000) Skin barrier properties in different body areas in neonates. *Pediatrics* 106: 105–8

Yosipovitch G, Xiong GL, Haus E, Sackett-Lundeen L, Ashkenazi I, Maibach HI (1998) Time-dependent variations of the skin barrier function in humans: transepidermal water loss, stratum corneum hydration, skin surface pH, and skin temperature. *J Invest Dermatol* 110:20–3

Zlotogorski A (1987) Distribution of skin surface pH on the forehead and cheek of adults. *Arch Dermatol Res* 279:398–401