esis, including the Δ-7-sterol reductase (Wassif *et al.*, 2005).

In summary, the data of Bogh *et al.* (2010) extend earlier observations of the relationship between LDL to HDL ratios and 25-(OH)-D (Carbone *et al.*, 2008). This points to a relationship between systemic HDL and LDL cholesterol levels and DHCR7 activity in keratinocytes, perhaps via the sterol-sensing domain of the enzyme.

CONFLICT OF INTEREST

The author states no conflict of interest.

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Is the Filaggrin–Histidine–Urocanic Acid Pathway Essential for Stratum Corneum Acidification?

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TO THE EDITOR

Acidification of the surface of the stratum corneum (SC), the acid mantle, was initially thought to be important in the defense against infection. The growth of pathogenic microorganisms, such as *Staphylococcus aureus* and *Streptococcus pyogenes*, is inhibited by an acidic skin pH whereas the growth of resident (normal) skin flora is stimulated (Puhvel *et al.*, 1975; Korting *et al.*, 1990, 1992). However, recent studies have shown that acidification of the SC has additional functions, including regulating several key SC functions. A major function of the skin is to form a permeability barrier between the dry external environment and the moist interior of the body (Elias, 2007). This permeability barrier resides in the extracellular lipid membranes of the SC, and studies have shown that an acidified SC is required for the formation of a functionally competent permeability barrier (Mauro *et al.*, 1998; Fluhr *et al.*, 2001; Hachem *et al.*, 2003). Specifically, in the SC β-glucocerebrosidase and acid sphingomyelinase metabolize glucosylceramides and sphingomyelin, respectively, to ceramides, which is the major family of lipids in the extracellular membranes that mediate permeability barrier function (Feingold, 2007). Both the enzymes require an acidic milieu for optimal enzymatic activity; hence, when the pH of the SC increases, the metabolism of glucosylceramides and sphingomyelin to ceramides is impaired, resulting in abnormal permeability barrier homeostasis (Holleran *et al.*, 1992, 1993; Feingold, 2007). In addition, an acidic SC pH inhibits the activity of serine proteases thereby maintaining the cohesiveness and integrity of the SC (Hachem *et al.*, 2005). With an increase in SC pH, the activities of these serine proteases are stimulated resulting in the degradation of corneodesmosomes and a decrease in SC integrity and cohesion (Fluhr *et al.*, 2004b; Hachem *et al.*, 2005). Thus, an acidic SC is important in regulating the metabolism and function of the SC, and alterations in SC pH could have numerous adverse effects.

A variety of different pathways are postulated to contribute to the acid mantle

Abbreviation: SC, stratum corneum

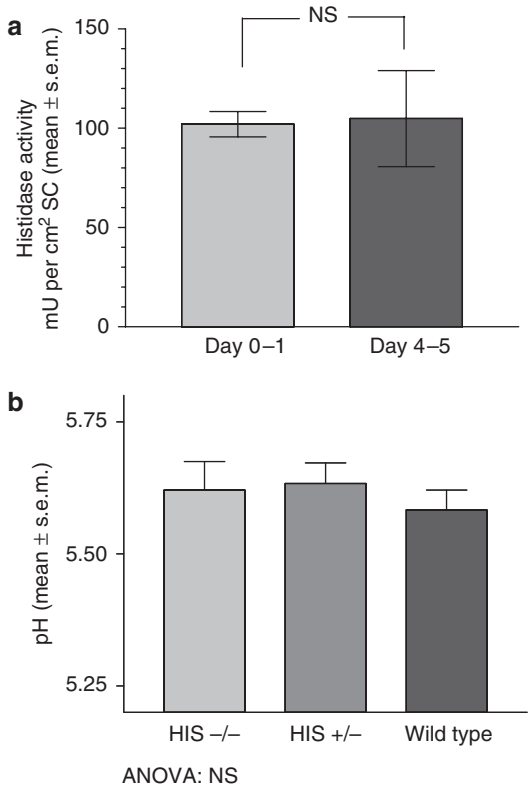

Figure 1. Role of histidase in SC acidification. (**a**) Histidase activity in the stratum corneum (SC) was measured in newborn rats and rats at age 4–5 days ($n=6$). (**b**) The SC surface pH in Peruvian and matched controls was measured with a flat, glass surface electrode (Mettler-Toledo, Giessen, Germany), attached to a pH meter (Skin pH Meter PH 900; Courage & Khazaka, Cologne, Germany). HIS−/−, $n=8$; HIS+/−, $n=14$; wild type, $n=6$.

of the skin. Exogenous mechanisms, 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), are thought to decrease SC pH. Recent studies have shown that endogenous mechanisms also contribute to SC acidification (Fluhr *et al.*, 2001, 2004a; Behne *et al.*, 2002). Both free fatty acid generation from phospholipid hydrolysis catalyzed by one or more isoforms of $sPLA_2$ and a sodium/proton pump antiporter, the sodium/hydrogen antiporter-1 (Behne *et al.*, 2002, 2003; Fluhr *et al.*, 2004a), lower SC pH. A third endogenous mechanism, urocanic acid generation from histidine by the deiminating enzyme, histidase, has been shown to acidify SC *in vitro* (Krien and Kermici, 2000), but its impact on SC acidification and function *in vivo* remains uncertain. The histidase pathway of acidification is dependent upon previous proteolysis of filaggrin to histidine, a reaction triggered by a reduction in external humidity (Rawlings and Matts, 2005).

We therefore hypothesized that if the filaggrin–histidine–urocanic acid cascade is crucial in regulating SC pH *in vivo*, then SC pH should increase with either a reduction in substrate (filaggrin) and/or a decrease in histidase activity. Moreover, as SC acidification occurs over the first few days after birth in parallel with activation of filaggrin proteolysis (Fluhr *et al.*, 2004a), we reasoned that histidase activity should increase simultaneously with SC acidification.

Our initial studies examined histidase activity in newborn albino rats immediately after birth and at 4–5 days after birth (details regarding animals and methods are provided in Supplementary Material online). As reported previously, during this period of time the pH of the SC decreases markedly (Fluhr *et al.*, 2004a). If the filaggrin–histidine–urocanic acid cascade makes a major contribution to this reduction in SC pH, then the activity of histidase in the SC should increase in parallel. As shown in Figure 1a, there was no difference in SC histidase activity at day 0–1 after birth compared to 4–5 days after birth.

To determine the role of histidase in SC acidification more definitively, we next studied mice that were deficient in histidase activity, the Peruvian mouse (Selden *et al.*, 1995). As shown in Figure 1b, SC pH was similar in animals deficient in histidase activity, suggesting again that the histidase pathway is not essential for SC acidification.

We next studied animals deficient in filaggrin production due to impaired proteolytic processing of profilaggrin to filaggrin, the flaky tail (ft/ft) mouse (Presland *et al.*, 2000). As shown in Figure 2a, SC pH was actually slightly decreased, not increased, in flaky tail mice, indicating that filaggrin is not essential for SC acidification. In addition, SC hydration (Figure 2b) is also not altered in the flaky tail mouse, suggesting that this additional function also does not absolutely require filaggrin.

We next asked whether other acidifying mechanisms are upregulated in ft/ft mice to compensate for reduced urocanic acid generation. As shown in Supplementary Figure S1 (Supplementary Material), the immunostainable protein content of both NHE1 and sPLA2A is increased in ft/ft mice whereas two other isoforms of sPLA2, sPLA2F and sPLA2X1, do not change (data not shown). These results suggest that compensatory upregulation of these alternate acidifying pathways could account for the normal to decreased SC pH in ft/ft mice.

Taken together, the above results do not provide support for the hypothesis that the filaggrin–histidine–urocanic acid cascade is essential for SC acidification. Increases in sPLA2 activity and/or NHE1 levels, or other yet to be elucidated mechanisms, appear to acidify the SC in the absence of the filaggrin–histidine–urocanic acid cascade. Yet, when either the sPLA2 or NHE1 pathways of acidification are compromised, the bulk pH of SC rises

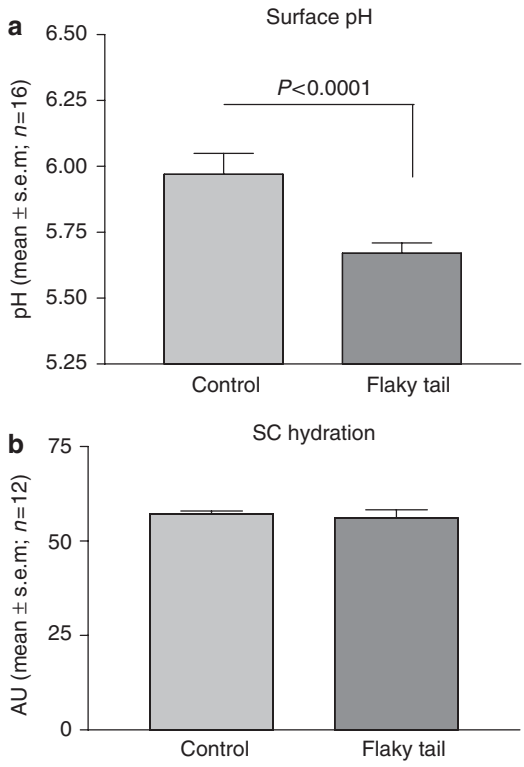

Figure 2. Effect of filaggrin deficiency on SC function. (**a**) In flaky tail (ft/ft) mice, surface pH was measured with a flat, glass surface electrode as described in Figure 1 legend ($n=16$; $P<0.0001$). (**b**) Stratum corneum (SC) hydration was measured with corneometer (Corneometer CM 820; Courage & Khazaka); $n=12$; *t*-test, NS.

(Fluhr *et al.*, 2001, 2004a; Behne *et al.*, 2002), indicating that other acidifying mechanisms cannot compensate for them. One can easily envision that because of the importance of an acid mantle in SC function (Fluhr *et al.*, 2001, 2004b; Schmid-Wendtner and Korting, 2006) that numerous pathways contribute to its formation, and that the absence of any particular pathway will result in minor or no changes in SC pH. Thus, although both sPLA2 and NHE1 activities are required for the formation of the acid mantle (Fluhr *et al.*, 2001, 2004a; Behne *et al.*, 2002), the filaggrin–histidine–urocanic acid cascade is not essential for SC acidification.

CONFLICT OF INTEREST

The authors state no conflict interest in relation to this study except JS (who has a research contract with Procter and Gamble on an unrelated project).

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SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/jid

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Oncogenic B-RAF^{V600E} Promotes Anchorage-Independent Survival of Human Melanocytes

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TO THE EDITOR

Activation of the extracellular signal-regulated kinase (ERK) pathway has an undisputed role in melanoma development. The majority of human melanomas harbor activating mutations in N-RAS (4–50% of primary melanoma) or B-RAF (20–80% of primary melanoma) (reviewed by Platz *et al.*, 2008). These mutations are mutually exclusive, appear early, and are detectable in up to 80% of benign nevi (reviewed by Reifenberger *et al.*, 2002; Thomas, 2006). Thus, aberrant ERK signaling is not sufficient to induce melanoma but triggers an initial cycle of melanocyte proliferation that is subsequently limited by the onset of cellular senescence (Michaloglou *et al.*, 2005).

There is also accumulating evidence that oncogenic activity differences may exist between activated N-RAS and B-RAF, as demonstrated by the unique transcriptional signatures resulting from mutations in these oncogenes (Pavey *et al.*, 2004) and the distinct histology of B-RAF versus N-RAS mutation-positive melanomas (Viros *et al.*, 2008). To evaluate the oncogenic activity of activated N-RAS and B-RAF, the melanoma-associated N-RAS^{Q61K} or B-RAF^{V600E} mutants were transduced into two primary human melanocytes (HEM1455 and HEM1259). As expected, the accumulation of wild-type B-RAF or the coexpressed copepod green fluorescent protein (copGFP) did not induce ERK activation, whereas N-RAS^{Q61K} and B-RAF^{V600E} induced increased levels of phosphorylated ERK (Figure 1a). Importantly, the level of ERK phosphorylation in oncogene-transduced melanocytes was comparable with endogenous ERK phosphorylation in melanoma cells with activated mitogen-activated protein kinase signaling (Supplementary Figure S1 online).

The accumulation of B-RAF^{V600E}, but not wild-type B-RAF, oncogenic N-RAS, or copepod green fluorescent protein, also induced dramatic morphological changes; melanocytes displayed fewer dendrites, appeared rounded, and the majority detached from the culture flask (Figure 1b and c). Importantly, V600E-induced loss of substrate adhesion continued for the length of our experiments, up to 7 days post-transduction (data not shown). The resulting suspension melanocytes appeared rounded, viable, formed loose clusters, and continued to express B-RAF^{V600E} and activated ERK (Figure 1a and b). Trypan blue exclusion assays demonstrated that approximately 90% of B-RAF^{V600E} expressing suspension melanocytes remained viable, whereas a substantial proportion of the suspension melanocytes expressing wild-type B-RAF, N-RAS^{Q61K}, or copepod green fluorescent protein (suspension cells accounted for only 2–10% of these populations) were not viable (Figure 1c).

To determine whether the impact of oncogenic B-RAF on cell adhesion was specific to melanocytes, we repeated the transduction experiments in two primary human dermal fibroblasts (HDF1314 and WS-1). Oncogenic B-RAF and N-RAS activated the ERK pathway. Occasionally, wild-type B-RAF also induced ERK phosphorylation in these cells (Figure 1a), and although this has been observed previously (Gray-Schopfer *et al.*, 2006) it was not a consistent finding in our studies. Expression of oncogenic B-RAF also altered the morphology of fibroblasts (Figure 1d), but these cells remained adhered (Figure 1e). Moreover, the small proportion of B-RAF^{V600E} fibro-

Abbreviation: ERK, extracellular signal-regulated kinase