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Topical Hesperidin Enhances Epidermal Function in an Aged Murine Model

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TO THE EDITOR

As skin ages, the epidermis is thinner with reduced epidermal proliferation, abnormal differentiation, impaired lipid synthesis, and elevated skin surface pH. These alterations have profound consequences for barrier function, skin cohesion, antimicrobial defense, inflammatory threshold, and cutaneous wound healing (Ghadially *et al.*, 1995; Mauro *et al.*, 1998; Choi *et al.*, 2007; Rodriguez-Martin *et al.*, 2011). These abnormalities have been linked, in part, to reduced epidermal IL-1α expression (Ye *et al.*, 2002), reduced epidermal expression of CD44 and its ligand, hyaluronic acid (Bourguignon *et al.*, 2013), and reduced epidermal lipid synthesis.

Among these many changes, much attention has been paid to the epidermal permeability barrier, because of its dominant role in regulating cutaneous homeostasis. Studies have demonstrated that epidermal permeability barrier regulates epidermal proliferation, differentiation, lipid production, and innate immunity. Therefore, strategies that enhance epidermal proliferation, differentiation, and/or lipid production, while also reducing stratum corneum (SC) pH, could prove to be useful for preventing and/or treating the functional abnormalities, including permeability barrier homeostasis, in aged skin. Our previous studies demonstrated that topical applications of a readily available herbal ingredient, hesperidin, improve epidermal permeability barrier function in young mice by stimulating epidermal proliferation, differentiation, and lamellar body formation/secretion (Hou *et al.*, 2012), all of which are likely independent of the antioxidant properties of hesperidin. Here, we show that topical applications of hesperidin improve multiple key epidermal functions in aged mouse skin. After 9 days of treatment, the gross appearance of mouse skin treated with vehicle and hesperidin appeared similar. Histological analysis showed that aged epidermis was thinner than young epidermis; whereas proliferating cell nuclear antigen (PCNA) staining indicated that aged epidermis displayed less robust proliferative activity as compared with young epidermis; hesperidin treatment did not stimulate epidermal proliferation in aged skin, as indicated by PCNA-positive cells per cm epidermal length (2.70 ± 0.10 vs. 2.45 ± 0.13 for vehicle-treated vs. hesperidin-treated skin, NS; 3.46 ± 0.17 for young skin; young vs. vehicle- or hesperidin-treated aged skin, $P<0.001$). These results indicate that topical hesperidin does not stimulate epidermal proliferation in aged mice.

After 9 days of topical hesperidin treatment, baseline SC hydration in hesperidin-treated mice also was no

Abbreviations: ABCA12, ATP-binding cassette transporter 12; FAS, fatty acid synthase; hBD2, human beta-defensin 2; HMGCoA, 3-hydroxy-3-methyl-glutaryl-CoA reductase; mBD3, mouse beta-defensin 3; NHE1, sodium/hydrogen exchanger 1; Nrf2, nuclear factor (erythroid-derived 2)–like 2; PCNA, proliferating cell nuclear antigen; Q-PCR, quantitative reverse transcriptase in real time; SC, stratum corneum; sPLA2, secretory phospholipase A2; SPT, serine palmitoyltransferase 1

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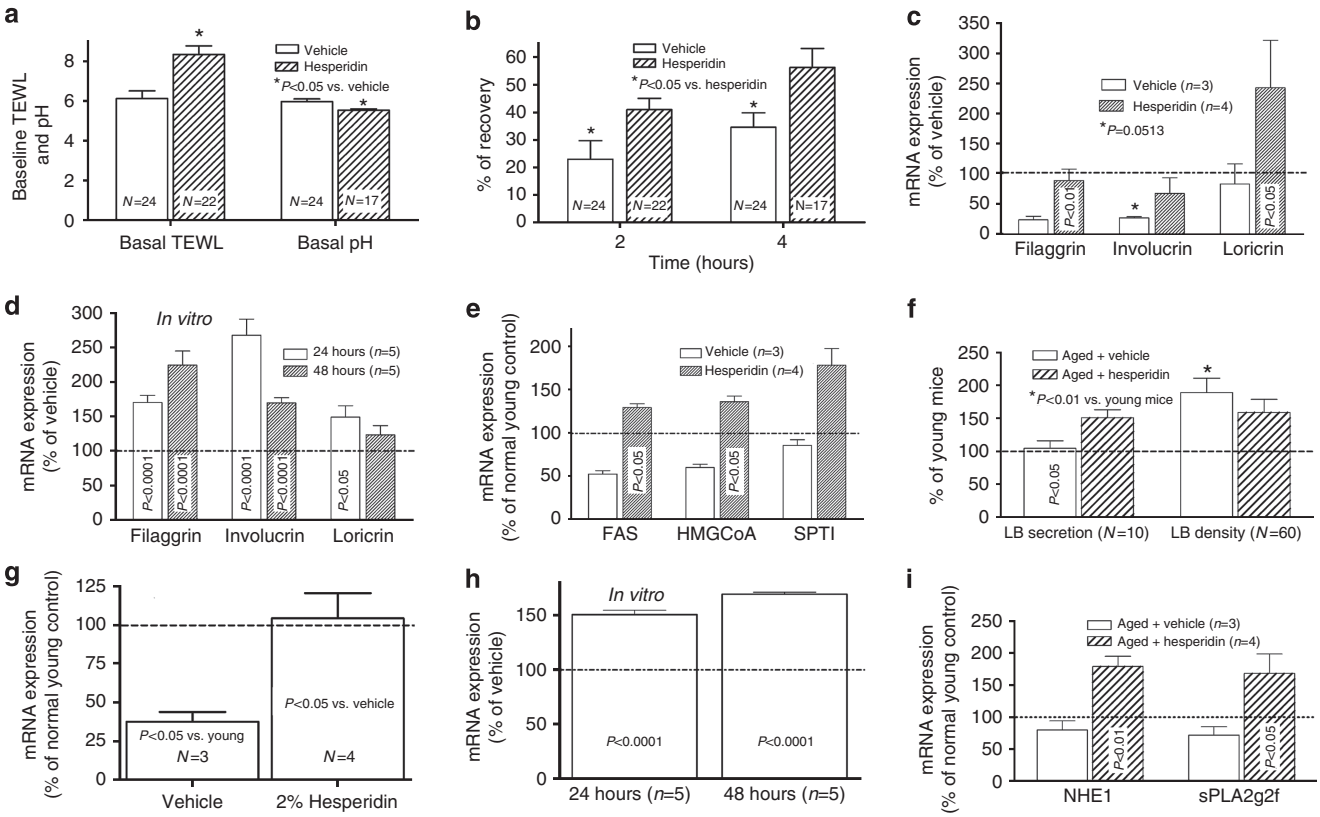

Figure 1. Topical hesperidin improves epidermal permeability barrier homeostasis in aged murine skin. The values present the data *in vivo* in mice unless otherwise specified. (**a**) Displays basal transepidermal water loss (TEWL) and skin surface pH in mice; (**b**) shows barrier recovery in mice; (**c**) shows the levels of epidermal mRNA in mice; (**d**) exhibits the levels of mRNA expression *in vitro* in human keratinocyte cultures, expressed as the % of vehicle-treated samples setting the levels of vehicle treated as 100% (dotted line); (**e**) shows epidermal mRNA levels in mice, expressed as the % of normal young mice setting the levels of young mice as 100% (dotted line); (**f**) shows the results of quantitative analysis of lamellar body density and secretion in mice; (**g** and **h**) present the expression levels of ATP-binding cassette transporter 12 (ABCA12) in mice and *in vitro* in human keratinocyte cultures, respectively; (**i**) shows the expression levels of epidermal sodium/hydrogen exchanger 1 (NHE1) and secretory phospholipase A2 (sPLA2) in mice. Significances and numbers of samples are indicated in the figures.

different from that in vehicle-treated mice (60.77 ± 1.32 for vehicle-treated vs. 58.80 ± 2.27 for hesperidin-treated). However, skin surface pH significantly declined in hesperidin-treated skin compared with vehicle-treated skin (Figure 1a). Although basal transepidermal water loss rates increased slightly in hesperidin-treated skin as compared with vehicle-treated skin (Figure 1a), these levels still fell well within the normal range of young skin. Consistent with previous findings in young mice (Hou *et al.*, 2012), topical hesperidin significantly accelerated barrier recovery at both 2 and 4 hours after acute barrier disruption of aged skin (Figure 1b). These results demonstrate that topical hesperidin improves epidermal permeability barrier homeostasis, while also lowering skin surface pH in aged murine skin.

We next examined the basis for improved barrier function and acidification in aged epidermis. Our previous studies demonstrated that topical hesperidin stimulates epidermal differentiation, accounting in part for improved epidermal permeability barrier homeostasis in young mice. Hence, we next assessed whether topical hesperidin also stimulates epidermal differentiation in aged epidermis. As shown in Figure 1c, topical hesperidin significantly increased the mRNA levels of filaggrin and loricrin in aged mouse epidermis, consistent with the results of immunostaining (Supplementary Figure S1 online). Consistently, hesperidin also increased the mRNA levels of filaggrin, involucrin, and loricrin in adult keratinocyte cultures (Figure 1d). These results indicate that hesperidin stimulates epidermal differentiation, providing one potential mechanism whereby hesperidin improves barrier function in aged skin.

Epidermal lipid synthesis is required for the formation and maintenance of the epidermal permeability barrier. Synthesis of three key barrier-related lipids, cholesterol ceramides, and fatty acids requires their respective rate-limiting enzymes 3-hydroxy-3-methyl-glutaryl-CoA reductase (HMGCoA), serine palmitoyltransferase 1 (SPT1), and fatty acid synthase (FAS). Basal mRNA levels for all three key lipid synthetic enzymes were lower in aged as compared with young epidermis (demonstrated by the dotted line in Figure 1e), consistent with the concept that the lower lipid synthesis rates in aged epidermis could reflect the reduced expression of their synthetic enzymes. Topical hesperidin treatment significantly increased the mRNA levels of HMGCoA, SPT1, and FAS in aged mouse epidermis,