hBD-2 in the sebocytes from T-zone enhanced antimicrobial activity against *P. acnes* and inhibited the expression of proinflammatory cytokines, such as IL-8 and tumor necrosis factor-α.

Our data showed that the T-zone had a low incidence of inflammatory acne lesions compare with U-zone. These data support the previous findings that hBD-2 levels indicate the presence of antiinflammatory lipids, which inhibit inflammation, therefore, only a few clinically inflammatory lesions occur (Georgel *et al.*, 2005; Zouboulis *et al.*, 2010). Previous studies demonstrated that FFAs enhance the innate antibacterial defenses of the skin by inducing the expression of AMP (Lee *et al.*, 2008; Nakatsuji *et al.*, 2010). Our findings also showed that considerable FFAs in the high-sebum-secreting area (T-zone) induce more abundant hBD-2 expression, and enhanced antimicrobial activity against *P. acnes* and inhibited the expression of proinflammatory cytokines. However, it needs to further evaluation for the expression of other AMP in each area, because other AMP may also have a role in regional difference of inflammatory acne lesions (Lee *et al.*, 2008, 2009). There was a previous study that hBD-2 strikingly killed *P. acnes* at concentrations higher than 10 mM (Nakatsuji *et al.*, 2010). Although we showed which amount of hBD-2 in sebocytes from T-zones able to influence the growth of *P. acnes* *in vitro*, it needs further *in vivo* study especially in acne lesions.

In conclusion, we suggest that increased hBD-2 in the T-zone prevents the progress of comedones to papulopustular lesions via either an upregulation of antiinflammatory sebaceous lipid synthesis and/or an inhibitory activity of *P. acnes* proliferation. These data suggest a functional link between topographical variation of inflammatory acne lesions and innate immunity in acne pathogenesis. Further studies of the relationship between hBD-2 expression and *P. acnes* in acne lesion are warranted to gain the confirmation of the effects of hBD-2 on regional difference of inflammatory acne lesions.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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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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Mobility of Water Molecules in the Stratum Corneum: Effects of Age and Chronic Exposure to the Environment

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TO THE EDITOR

It is well established that water in the stratum corneum (SC) has a critical role in skin physiology (Rawlings and Matts, 2005). Depending on their mobility, determined by the strength of hydrogen bonds and space limitations, SC water molecules can be grouped into three categories (Bulgin and Vinson, 1967; Walkley, 1972; Takenouchi *et al.*, 1986; Walling and Dabney, 1989; Gilard *et al.*, 1998; Gniadecka *et al.*, 1998; Kasting *et al.*, 2003; Pieper *et al.*, 2003; Visscher *et al.*, 2003; Yadav *et al.*, 2007; Nakagawa *et al.*, 2011; Vyumvuhore *et al.*, 2013): (a) ''bound''

Abbreviations: NMF, natural moisturization factor; SC, stratum corneum

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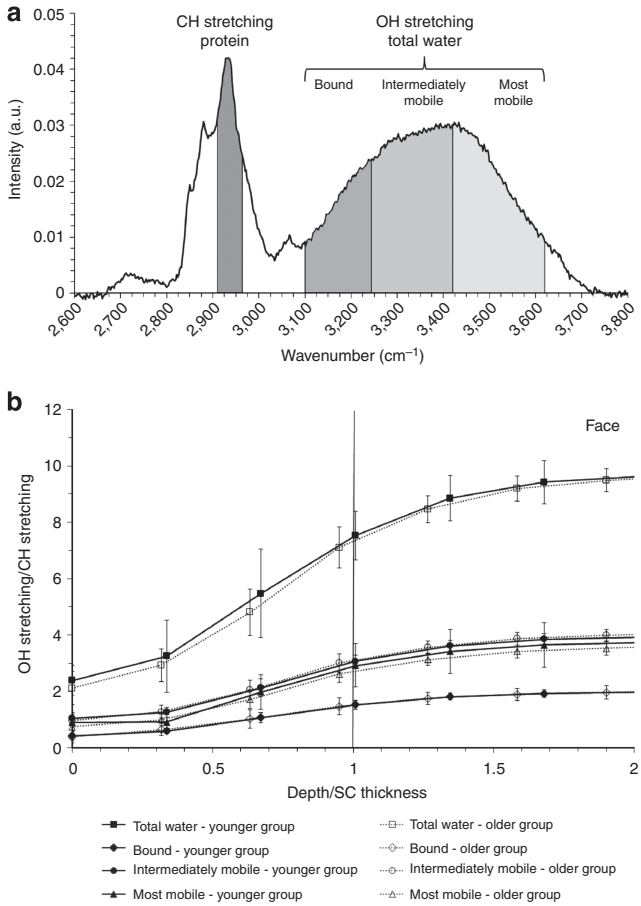

Figure 1. The mobility of water molecules in the stratum corneum (SC) can be studied *in vivo*. (**a**) Raman confocal spectra in the high wavenumber region highlighting the bands of CH stretching and OH stretching that can be used for the calculation of water content and that of the water molecules in the three states based on their mobility. (**b**) The depth profiles of the three states of water according to molecular mobility. The OH stretching bands have been normalized to the CH stretching bands. There are minor differences between the two age groups studied. Data are presented as mean ± 1 SD. a.u., arbitrary units.

(least mobile), molecules that are directly hydrogen-bonded to SC structural molecules; (b) intermediately mobile, molecules that form hydrogen bonds with ''bound'' water molecules and can be secondary, tertiary, or higher order, forming a ''loose cloud'' around the binding site; and (c) most mobile, molecules that can diffuse more freely, continuously forming and breaking hydrogen bonds with their surrounding water molecules. These ''states'' are weakly defined, and in reality there is a continuum of bound states. However, it can be useful to use this analysis as means of providing some perspective to the mobility of water in the SC.

Confocal Raman microspectroscopy is the only *in vivo* method to date that can generate concentration profiles of SC components as a function of distance from the skin surface (Caspers *et al.*, 2001). Water concentration profiles (% mg water/mg protein) can be constructed following a calibration procedure, using the spectral bands 3,350–3,550 cm^{-1} (OH stretching) and 2,910–2,965 cm^{-1} (CH stretching). Water content is low at the skin surface, and it gradually increases to a plateau at the junction between the SC and the stratum granulosum. However, the range 3,350–3,550 cm^{-1} is limited to the spectral contributions of the more mobile water molecules (Vyumvuhore *et al.*, 2013). These authors have defined the band ranges (subbands of the OH stretching mode) that correspond to the three mobility states described above.

The objective of the current study was to examine whether age and chronic environmental exposure affect the relative distribution profiles of these three states in the SC. The production of natural moisturization factors (NMFs) that may influence these distribution profiles and their dependence on age and exposure to the environment are also examined.

The study was conducted according to the principles of the Declaration of Helsinki. Healthy female volunteers participated in the study following written informed consent, and they were divided into two age groups of 10 volunteers each: 20–30 and 50+ years of age. Raman data (Skin Analyzer 3510, River Diagnostics, Rotterdam, The Netherlands) were acquired on three skin sites: face (cheek), relatively exposed arm site (dorsal forearm), and relatively protected arm site (upper inner arm), following 15 min acclimatization in an environmentally controlled room (20 °C, 40% relative humidity). The concentration profiles of water and total NMF, as well as the areas under the curve for the spectral bands defined by Vyumvuhore *et al.* (2013) (Figure 1a), were calculated from the Raman spectra using the Skin Tools software (River Diagnostics). To account for interpersonal differences in the SC thickness, the distance from the SC surface was normalized to the SC thickness measured from the Raman data (Bielfeldt *et al.*, 2009). Data are presented as mean ± 1 SD. For group comparisons, a Student's *t*-test was performed following confirmation of normality (Anderson-Darling test), and statistical significance was accepted at the level of $\alpha = 0.05$.

The water concentration profiles for the three water states show a qualitatively similar pattern with that of total water: a low value close to the skin surface that monotonically increases to a plateau at the base of the SC (Figure 1b). There are minor differences between the two age groups, but distinct differences between body sites: the SC of the face has higher total water content compared with the arm sites.

By normalizing the profiles of the three water states to the total water, we obtain a different pattern that appears to be highly conserved between the two age groups and the three body sites

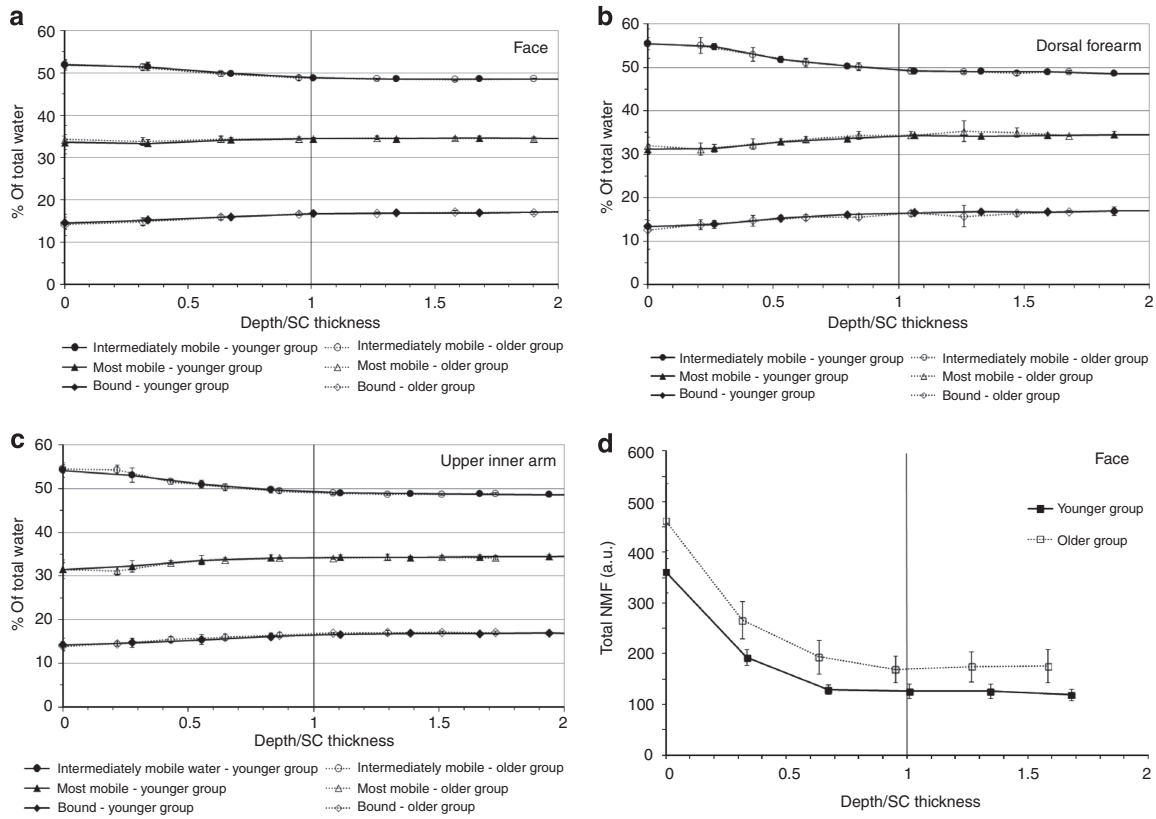

Figure 2. The distributions of the three states of water according to molecular mobility are independent of age and skin site. Data for the two age groups are shown for (**a**) the face, (**b**) the dorsal forearm, and (**c**) the upper inner arm site. (**d**) The total natural moisturization factor (NMF) profile expressed as the sum of the NMF components as a function of depth from the surface. Note the increased NMF toward the skin surface following protein (e.g., filaggrin) break-down processes. All data are presented as mean ± 1 SD. a.u., arbitrary units.

tested (Figure 2a–c). Although the normalized profiles of the bound and most mobile water molecules decrease toward the skin surface, that of the intermediate group increases. The changes are subtle (<6% difference) but statistically significant and with low interpersonal variability (<7%). Interestingly, in the viable epidermis, the relative percentage of the three groups appears to be constant, independent of age or body site. The molecular ratios of bound: intermediate: most mobile water are 17:49:34.

Throughout the SC, corneocytes migrate and mature toward the surface (Harding *et al.*, 2003), whereas proteins of the S100 family, including filaggrin, filaggrin-2, and hornerin, are broken down to give rise to an assembly of amino acids and other products, known as NMF (Rawlings, 2010). The sum of the profiles of these molecules is used to calculate the total NMF concentration profile (Figure 2d). The gradual increase of the total NMF concentration toward the SC surface is indicative of the proteolytic processes along the SC-exposing pockets of bound water molecules, increasing their mobility. This may explain the decrease in the relative amount of bound water and the concurrent increase in the amount of intermediately mobile water toward the SC surface. Another possible explanation is that part of the bound water is linked to lipid head-groups (Yadav *et al.*, 2007), and the relative amount of this state is decreased as the lipid content in the outer SC is depleted. SC equilibrated to ambient conditions of 20–40% relative humidity is expected to have lower water content as we move toward the skin surface, and this water is expected to be more tightly bound pointing to the humectant role of the NMF. This could explain the shift from the most mobile water molecules toward the intermediately bound water molecules.

These observations provide important baseline information for the mobility state of water in the SC in healthy skin. This can be of interest in the study of water diffusion through the SC and trans-cutaneous drug delivery. The surprising consistency of the profiles between age groups and body sites implies that there is a tightly controlled mechanism that defines them. Such mechanisms are expected to be affected by the environmental temperature and humidity conditions (Vyumvuhore *et al.*, 2013). Further studies will shed light on whether these patterns are conserved or differ in the case of pathological skin conditions, including barrier-related disorders, such as psoriasis, atopic dermatitis, icthyosis, senile xerosis, diabetic-related dry skin, and so on.

In conclusion, chronological aging and chronic exposure to environmental factors do not affect the normalized concentration profiles of the three mobility states of water in the SC.

Although the profiles are flat in the viable epidermis, they demonstrate slight changes toward the SC surface. These changes are consistent between the age groups and body sites tested, and they can be explained by the enzymatic proteolysis of filaggrin and similar molecules.

CONFLICT OF INTEREST

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Replication of Associations between GWAS SNPs and Melanoma Risk in the Population Architecture Using Genomics and Epidemiology (PAGE) Study

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TO THE EDITOR

Melanoma is a considerable public health burden, with an estimated 76,690 new diagnoses and 9,480 deaths from melanoma in the United States in 2013 alone (Howlader *et al.*, 2013). Multiplex families have pointed to important genetic factors for melanoma, including high-penetrance risk loci such as *CDKN2A* or *CDK4* (Gruber and Armstrong, 2006). In sporadic disease, genome-wide association studies (GWAS) have also successfully identified at least eight single nucleotide polymorphisms (SNPs) associated with melanoma (Gerstenblith *et al.*, 2010). Our study aimed to replicate these existing GWAS findings within the large Population Architecture using Genomics and Epidemiology (PAGE) study in order to further evaluate their association with melanoma.

In addition to genetic factors, other risk factors for melanoma include exposure to natural and artificial UVR, larger numbers of nevi, pigmentation traits (light versus dark hair, eye, and skin color), race/ethnicity (European versus non-European ancestry), skin response to UV exposure (burn versus tan), older age, and male sex (Gruber and Armstrong, 2006). Anatomic location of melanoma also tends to vary by sex, arising most commonly on the back, abdomen, and chest in males, and on the lower leg, hip, and thigh in females (Gruber and Armstrong, 2006). Females also appear to have lower risk of metastases and longer melanoma-specific survival than males (Joosse *et al.*, 2011).

Abbreviations: EAGLE-BioVU, Epidemiologic Architecture of Genes Linked to Environment, accessing BioVU, the Biorepository of Vanderbilt University; GWAS, genome-wide association study; HPFS, Health Professionals Follow-up Study; MEC, Multiethnic Cohort Study; NHS, Nurses' Health Study; PAGE, Population Architecture Using Genomics and Epidemiology; SNP, single nucleotide polymorphism; WHI, Women's Health Initiative

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