systemic levels and lesional expression of chemerin in psoriasis is unresolved. We assume that systemic production of chemerin was increased so that it could counteract the aggravation of psoriasis by inhibiting recruitment of immune cells to the skin.

As already described by Lehrke et al., chemerin shows a positive correlation with BMI, triglycerides, and leptin [7]. In this study, elevated chemerin significantly correlated with hypercholesterolemia and hypertriglyceridemia, but we did not find any correlation of BMI and serum levels of leptin with chemerin. The reason for this discrepancy is unclear.

Elevated systemic levels of chemerin and leptin in psoriasis seem to be associated not only with metabolic syndrome risk factors but also with psoriasis.

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Letter to the Editor

Relationship between cutaneous barrier function and ceramide species in human stratum corneum

Ceramides constitute a major lipid class in stratum corneum (SC), playing important roles in cutaneous barrier function (CBF) and pathophysiology of skin disorders with barrier disruption [1–3]. Ceramide is a family of lipid molecules composed of a sphingoid base: dihydrosphingosine, sphingosine, phytosphingosine or 6-hydroxysphingosine is amide-linked to nonhydroxy fatty acid, α-hydroxy fatty acid or ester-linked ω-hydroxy fatty acid. The combination of 4 sphingoid bases and 3 fatty acid species can generate 12 possible ceramide classes and more diverse species due to their variable fatty acid chain length. Recently, Masukawa et al. reported that 342 species, 10 classes ceramide exist in human SC [4,5], contributing towards the lipidomics in skin biology research.

CER[NS], the combination of nonhydroxy fatty acids and sphingosine, is extensively studied as a model ceramide, owing to its simple structure and commercial availability of authentic standards [6]. More importantly, it represents a major ceramide class in SC, accounting for 20.5% of total ceramides [7]. Here, we developed a rapid and sensitive quantification method for CER[NS] species with ultra-performance LC/MSMS-ESI based on the previously described method [8]. With this method, CER[NS] species in SC samples from 34(20 + 14) healthy human volunteers were measured and its relationship with CBF was examined.

With commercially available authentic CER[NS] standards (Avanti Polar Lipids, Alabaster, AL), C16Cer(CER[N(16)S(18)]), C18Cer(CER[N(18)S(18)]), C18:1Cer(CER[N(18:1)S(18)]), C20Cer(-CER[N(20)S(18)]), C24Cer(CER[N(24)S(18)]), C24:1Cer(CER[N(24:1)S(18)]) and C16SM([N(16)-sphing-4-enine-1-phosphocholine]) in SC samples from inner forearm regions of 20 healthy volunteers were quantified using C12Cer(CER[N(12)S(18)]) as an internal standard. Chromatographic separation was carried out using ACQUITY UPLC system (Waters Co., Milford, MA) with ACQUITY UPLC BEH C8 column (1.7 μm, 2.1 mm × 50 mm). Column and sample tray were maintained at 50 and 4 °C, respectively. Mobile phase consisted of 5 mM ammonium formate/methanol (3/7, 0.1% formic acid, solvent A) and 5 mM ammonium formate in methanol/isopropanol (7/3, 0.1% formic acid, solvent B). Gradient elution was as follows: isocratic elution with 80% B for 0.5 min, followed by a 2.5 min gradient to 100% B, then isocratic elution with 100% B for 4.5 min and then returned to 80% B in 6 min. Flow rate was 0.35 mL/min and injection volume was 5 μL. MS analysis was performed using Waters Quattro Premier XE. MS spectrometer was operated in the positive ESI mode with capillary voltage, 3.2 kV; ion source temperature, 120 °C; desolvation temperature, 400 °C; desolvation gas flow rate, 800 L/h; cone gas flow rate, 100 L/h. MS chromatogram for the ceramide authentic standards obtained with this method (Fig. 1A) demonstrated a rapid, sensitive and simultaneous quantification of CER[NS] species which made possible high-throughput ceramide

☆ The protocol was approved by the Ethical Committee of the AMOREPACIFIC CO based on the Recommendations from the Declaration of Helsinki.

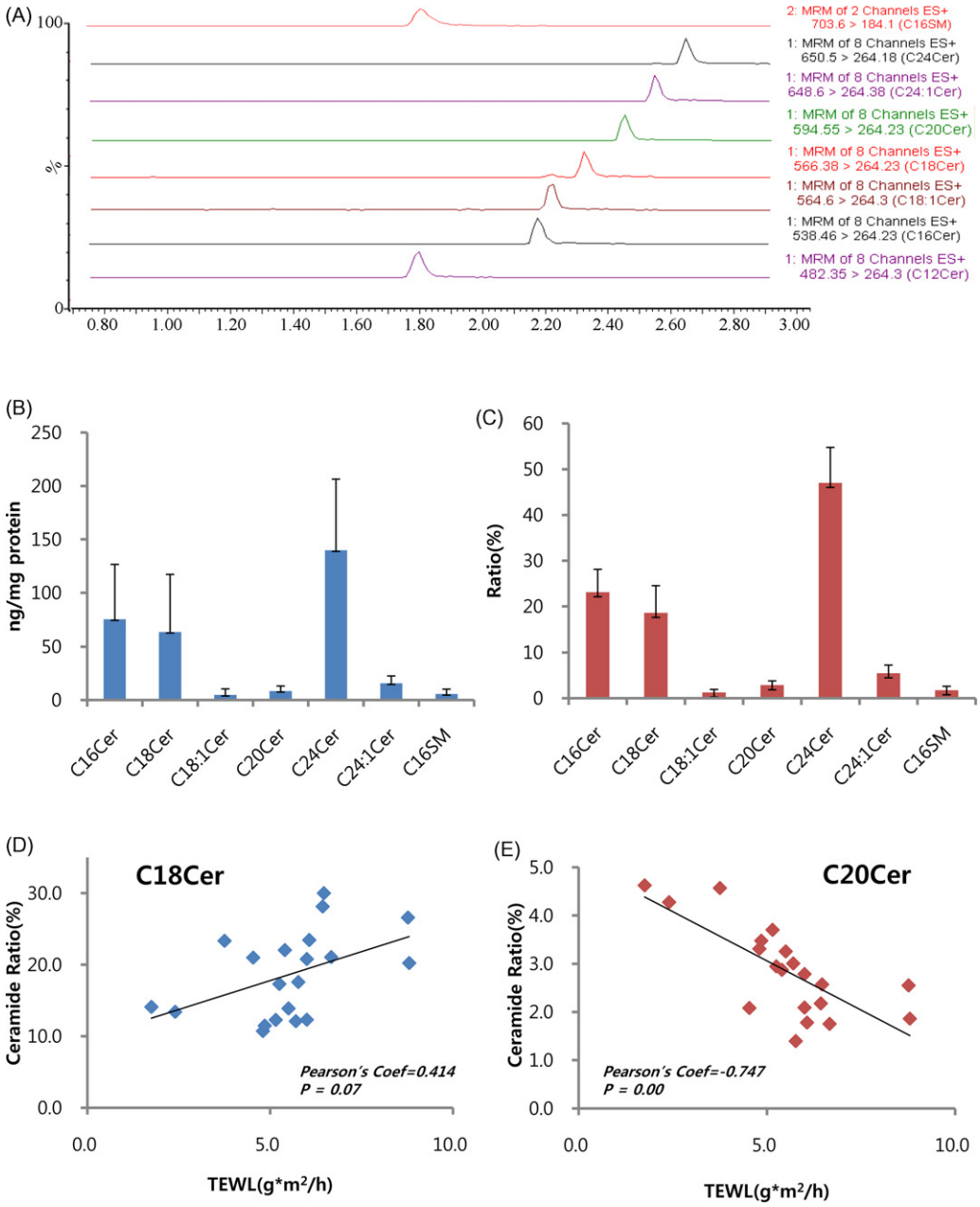

Fig. 1. Analysis of ceramide nonhydroxy fatty acid sphingosine (CER[NS]) species in human stratum corneum. (A) MS chromatogram for CER[NS] species, (B) amount and (C) ratio of CER[NS] species in human SC. To minimize the effect from temperature and humidity on TEWL, the subjects went through a standardized washing procedure (soap washing), and were retained in humidity- and temperature-controlled room (24 ± 2 °C, 40 ± 2%) for 30 min before measuring TEWL and collecting SC samples. After measuring TEWL with VapoMeter (Delfin, Finland) at inner forearm, SC specimens were obtained with a D-squame standard tape (Cuderm, Dallas, TX, diameter, 2.2 cm). Ten consecutive tapes were collected from each volunteer (27–40-years old, seven males and thirteen females, Asian) and stripped tapes were cut into 2 equal parts. To extract ceramides, 5 tapes (10 halves) were immersed into 5 mL of methanol/chloroform (2:1, v/v) with 60 min of sonication. The upper organic layer was collected and evaporated to the dryness using speedvac (EZ-2 Plus, Genevac, Swiss) at 40 °C. The residues were dissolved in 200 μL of methanol/chloroform (2:1, v/v) and vortexed for 5 min, followed by centrifugation at 14,000 rpm, 4 °C for 5 min. For protein quantification, 5 tapes were immersed into 0.1% (w/v) sodium dodecyl sulfate/2% (w/v) propylene glycol in PBS buffer solution and then sonicated for 1 h to obtain soluble proteins. The concentration of soluble protein was assayed with a commercial protein assay kit (Pierce, Rockford, IL). Values are presented as a mean ± SD, $N = 20$. (D) Positive correlation between the ratio of C18Cer and TEWL. (E) Negative correlation between the ratio of C20Cer and TEWL.

speciation in one chromatographic run. Limit of quantification was determined to be from 0.2 to 0.5 ng/mL.

Through this method, CER[NS] species were determined to be abundant in SC of human volunteers, with the order of C24Cer > C16Cer > C18Cer > C24:1Cer > C20-C20Cer > C18:1 ≅ C16SM (Fig. 1B). Conspicuously, the CER[NS] contents in each individual were highly variable; for example, C24Cer was measured to be 140 ± 67 (53 ~ 336) ng/mg protein with a large coefficient of variation (COV) of 48%. This large variation was considered to be from the inter-individual variation in total amount of CER[NS] (313 ± 179 (125 ~ 817) ng/mg protein, COV = 57%). In contrast, the ratios of ceramide species, normalized with total CER[NS] (Fig. 1C), exhibited significantly reduced inter-individual variation (e.g. C24Cer, 47 ± 8%, COV = 17%).

The relationship between the ratios of CER[NS] species and trans-epidermal water loss (TEWL), an index for CBF, was examined using Pearson's correlation analysis which demonstrated statistically significant correlations of C18Cer and C20Cer with