

## **In Vivo Elongation of Topically Applied Fatty Acids From a Range of Different Topical Skincare Formulations**

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### **Synopsis**

The lipid matrix of the *stratum corneum* (SC) is essential to skin barrier function, and detrimental structural changes may occur in response to human behaviour, disease, and external factors. The application of topical formulations is a common approach to repairing the SC and has been investigated *ex vivo* and *in vitro*. We aim to demonstrate the first clinical evidence of deuterated palmitic acid (d31-PA) deposition from topical formulations into the SC of human skin *in vivo* and its conversion into longer-chain free fatty acids (FFAs) and ceramides. Healthy female participants aged 30 to 50 years old participated in two separate product application studies. Participants were randomized to an application of a leave-on lotion or a syndet bar containing d31-PA in the first study, with the second study having an option of a body wash containing different levels of d31-PA. Skin tapes and solvent extracts were collected at baseline and Day 28. Lipid composition and the presence of deuterated sphingosine were analyzed by liquid chromatography tandem mass spectrometry (LC/MS/MS). After four weeks, d31-PA was sufficiently delivered into the SC by all topical formulations. LC/MS/MS data show that FFAs are elongated up to C26:0 and used in the formation of ceramides and free sphingosine. These data provide the first *in vivo* evidence that FFAs deposited from a range of different topical formulations are elongated in the SC and provide the building blocks for ceramide synthesis. Together, the evidence shows that commercially available topical formulations can nourish the skin which may contribute to maintaining a healthy skin barrier.

### **INTRODUCTION**

The skin is the largest organ of the human body, with a surface area of 1.5 m<sup>2</sup> in adults, and acts as a barrier between the body's interior and external environment.<sup>1</sup> The uppermost epidermal layer of the skin, the *stratum corneum* (SC), comprises corneocyte layers embedded in a lipid matrix, which acts as the main pathway for the diffusion of substances through

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the skin.<sup>2</sup> This is essential to the skin barrier function. The lipid classes that dominate the human SC are ceramides, cholesterol and free fatty acids (FFAs), which are organized in lamellae.<sup>3–5</sup> These are integral to skin barrier function as they maintain skin moisture, limit the movement of material through the skin and prevent microbes and allergens from entering the tissues.<sup>6</sup>

In healthy adults, most SC lipids adopt a dense orthorhombic packing.<sup>7,8</sup> They can also adopt a less dense, hexagonal packing that has been shown to increase permeability in lipid model systems mimicking the human SC. Hexagonal packing is more abundant in the skin of patients with atopic dermatitis (AD) than in healthy adults.<sup>9</sup> In patients with AD, SC FFA chain lengths are reduced compared with healthy adults in both non-lesional and lesional skin, which is thought to contribute to reduced repeat distance of the lamellar phases, reduced ceramide chain length, less dense lipid organization and decreased barrier function.<sup>10–12</sup> These findings indicate that both lipid organization and composition are integral to the function of the skin barrier.

The composition and organization of SC lipids are not only affected by disease, but also by external factors and behaviours. During the winter season, levels of SC lipids are dramatically depleted compared with spring and summer.<sup>13,14</sup> In particular, the ceramide profile during winter season strongly correlate with indicators of dry skin condition.<sup>15</sup> This is likely to disrupt skin barrier function, influencing the activity of SC proteases involved in desquamation and interfering with the production of natural moisturizing factors, leaving the skin more prone to dryness.<sup>13</sup> Likewise, regular cleansing with surfactant-containing products is associated with symptoms of dryness, irritation, erythema, and post-wash tightness.<sup>16</sup> Such effects may be a result of the removal of SC lipids, exposing corneocytes to the stresses of cleansing.<sup>17</sup>

Restoring SC lipid composition and organization, and therefore skin barrier function, may be achieved through the application of topical formulations advancing the range of benefits offered to the skin.<sup>4</sup> Syndet bars and liquid cleanser formulations have evolved from simply cleansing the skin to delivering moisturizing benefits, along with employing milder surfactants helping to minimize skin damage.<sup>16</sup> Moisturizers containing FFAs and ceramides are also increasingly used for promoting barrier restoration and reducing water loss.<sup>16,17</sup> However, *ex vivo* evidence reveals that topically applied ceramides remain predominantly on the SC surface, rather than penetrating deeper into the skin.<sup>18</sup> In previous work, we have shown significant increases in ceramides, FFAs, and cholesterol *in vivo* following application of an FFA-containing leave-on lotion to cosmetically dry skin correlated with improvements in the skin dryness.<sup>19</sup> Levels of elongated FFAs were also increased, suggesting increased synthesis and elongation from shorter-chain FFA precursors.<sup>19</sup> However, further investigation into the specific FFAs responsible for influencing these beneficial changes is still needed. It is long established that FFAs are deposited from lotions and cleansers to replenish the endogenous fatty acids lost during the cleansing process.<sup>20</sup> Investigation into the fate of FFAs from topically applied products has been conducted in an *ex vivo* model of skin barrier repair to predict the *in vivo* response.<sup>4</sup> The topical application of both d31-PA and deuterated stearic acid resulted in the elongation of fatty acids *ex vivo*, which were abundant up to C26:0.<sup>4</sup> These findings have been used as the foundation for *in vitro* studies using 3D human living skin equivalent models.<sup>21</sup> We hypothesize that the fatty acid elongation and incorporation into ceramides demonstrated *in vitro* *ex vivo* can also take place *in vivo* from shorter-chain fatty acids provided via topical formulations.

Here, we demonstrate the first clinical evidence of the deposition of d31-PA into the SC of human skin *in vivo* from a body wash, leave-on lotion, and a syndet bar formulation, and its direct conversion into longer-chain FFAs and ceramides.

## METHODS

### STUDY DESIGN

We conducted two separate Institutional Review Board (IRB)-approved, randomized, double-blind product application studies. The first study assessed a syndet bar formulation and a leave-on lotion (Study 1), while the second study assessed body wash formulations (Study 2). The data from each study were analyzed separately and conducted at Hill Top Research, Winnipeg, Canada. Ethical approval was granted by IRB Services (Study 1: MOD00194725, approval granted 29<sup>th</sup> January 2017; Study 2: MOD00244849, approval granted 12<sup>th</sup> January 2018), and complied with the requirements defined in Health Canada regulations (21 Code of Federal Regulations [CFR] parts 56 and 312.3 and US Department of Health and Human Services [DHHS] regulations 45 CFR part 46). The IRB adheres to good clinical practice (GCP) guidelines (e.g., International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use GCP Guidelines), Health Canada regulations, and is in compliance with 21 CFR parts 50 and 56, DHHS 45 CFR part 46 and the Tri-Council Policy Statement for Ethical Conduct of Research Involving Humans as appropriate to the research. Prior to inclusion, all participants gave their informed consent to participate.

Skin dryness was graded by an expert assessor in both studies using a 0–6 scale of visual dryness (“0 = none” to “6 = most severe”) with ½ point increments allowed. Study 1 included 12 healthy female participants aged between 35 to 50 years of age (mean age:  $43.5 \pm 5.1$  years) with no-to-minimal dry skin on their forearms (Grade 0–1). Study 2 included 11 healthy female participants aged between 32 to 50 years of age (mean age:  $41.7 \pm 7.2$  years) with no-to-minimal dry skin on their forearms (Grade 0–1) and moderate dry skin on their lower legs (Grade 2–3).

Both studies included twice-daily controlled product application by trained researchers. Applications were approximately three to four hours apart. Participants were instructed not to use other cleansers during daily bathing or apply lotion to the test sites for the duration of the studies.

In Study 1, after 3 days of conditioning with a mild syndet cleansing bar, one of the following fully formulated products was applied to participants' volar forearms twice daily under controlled conditions:

- A mild syndet bar formulation containing FFAs and isethionate-based surfactant, in which FFAs were partially replaced by d31-PA C16:0 (14% final). This bar was dissolved in a 1:4 dilution with distilled water and 0.3 mL was dispensed onto the designated pre-wet volar forearm. The solution was rubbed on the whole volar forearm for 10 seconds, left for 30 seconds, and then rinsed off with tap water for 30 seconds.
- A leave-on lotion (0.3 mL) containing glycerine, petroleum jelly and d31-PA C16:0 (3.65% final). This was applied to the entire volar forearm and rubbed-in. Solvent extracts and tape strips were collected at baseline and Day 28.

In Study 2, after 5 days conditioning with a soap bar the body wash formulations were applied to marked 5 cm × 5 cm test sites (three on each forearm and leg) twice daily under controlled conditions. The cleansers were fully formulated body wash formulations containing FFAs and glycinate-based surfactants, in which FFAs were replaced with different concentrations of d31-PA C16:0 (0.05%, 0.25%, 0.75% and 2%). Masslinn towels were prepared with 0.15 mL of body wash before washing the pre-wetted test sites for 10 seconds. After 90 seconds, the test sites were rinsed under running water for 15 seconds.<sup>22</sup> Tape strips were collected at baseline and Day 28.

#### LIPID COMPOSITION ANALYSIS

In Study 1, solvent extracts were collected from designated solvent collection sites by three consecutive applications of 3 mL 80:20 methanol/ethyl acetate to a 3 cm diameter skin circle, enclosed with a glass ring. The solvent extracts were further re-extracted using a modified Bligh et al. method.<sup>23</sup> Twenty sequential tape strips were collected. Extraction of the lipids from tape strips was done with 10 mL per tape of 80:20 methanol/ethyl acetate on a roller at room temperature for three hours.

The resulting extracts were subjected to solid-phase extraction (SPE) on amino-propyl columns to remove interfering matrix material and partition the samples into ceramide and FFA fractions. Following SPE column conditioning with organic solvents to remove residual lipids, ceramide fraction was eluted with 66:33 chloroform: isopropanol; FFA fraction was eluted with methanol + 2% acetic acid. Tape strips collected from the body wash application sites in Study 2 followed the same methods of lipid extraction and fractionation using SPE amino-propyl columns.

Obtained lipid fractions were detected and semi-quantitated by liquid chromatography tandem mass spectrometry (LC/MS/MS) using area under the curve values. Deuterated FFA analysis and deuterated sphingosine (a surrogate marker for ceramides) analyzes were performed on an ACQUITY UPLC I-Class connected to a Xevo TQ-S mass-spectrometer.

For FFA analysis, samples were separated on a reversed-phase Halo C18 2.1 × 150 mm, particle size 2.7 µm analytical column, applying mobile phase A (75:25 methanol/water with 0.1% ammonium acetate) and mobile phase B (99:1 isopropanol/methanol with 0.1% ammonium acetate) in a gradient elution method. MS/MS measurements were performed using negative electrospray ionization mode (ESI), utilizing multiple-reaction monitoring (MRM) to selectively detect and semi-quantitate the following deuterated FFA molecules: palmitic acid (C16:0), stearic acid (C18:0), arachidic acid (C20:0), behenic acid (C22:0), lignoceric acid (C24:0) and cerotic acid (C26:0).

For sphingosine analysis, samples were separated on a reversed-phase Phenomenex Kinetex EVO C18, 2.1 × 150 mm, particle size 1.7 µm analytical column, applying mobile phase A (0.1% formic acid in water) and mobile phase B (0.1% formic acid in acetonitrile) in a gradient elution method. MS/MS measurements were performed using positive ESI, utilizing MRM to selectively detect and semi-quantitate deuterated sphingosine released by acid hydrolysis of the ceramide fraction. MRM methodology is a gold standard in mass spectrometry, based on the measurement of peak areas for specific parent-to-daughter ion transitions. This approach allows for reduction of noise and increased accuracy of detection.

Acid hydrolysis of the ceramides was performed using a modification of the Naoi et al. method.<sup>24</sup> Briefly, the ceramide fraction from the SPE column separation was split

equally into two aliquots after dissolving in chloroform: methanol (2:1); the solvent was evaporated at 50°C under a stream of nitrogen gas. The two ceramide aliquots for each sample were dissolved in methanol. The hydrolysis aliquot was acidified with concentrated HCl (12.1 mL). The samples were incubated at 60°C for 20 hours, dried and submitted for analysis.

## RESULTS

### FFA ELONGATION FROM LEAVE-ON FORMULATION

After four weeks application of the leave-on formulation, elongation of d31-PA was detected in solvent extracts from all participants. Higher levels of three elongated FFAs (C18:0, C20:0, and C22:0) were observed versus baseline samples. A consistent trend in the levels of elongated FFAs was observed across the study population: at Day 28, we detected higher levels of C22:0 than C20:0 and C18:0, and higher levels of C20:0 than C18:0. Although all participants showed detectable levels of elongated FFAs, there was variability in the extent of FFA elongation between participants (Figure 1a). LC/MS/MS analysis of tape strips showed elongation up to C26:0 in a subset of three participants (Figure 1b).

### FFA ELONGATION FROM SYNDET BAR FORMULATION

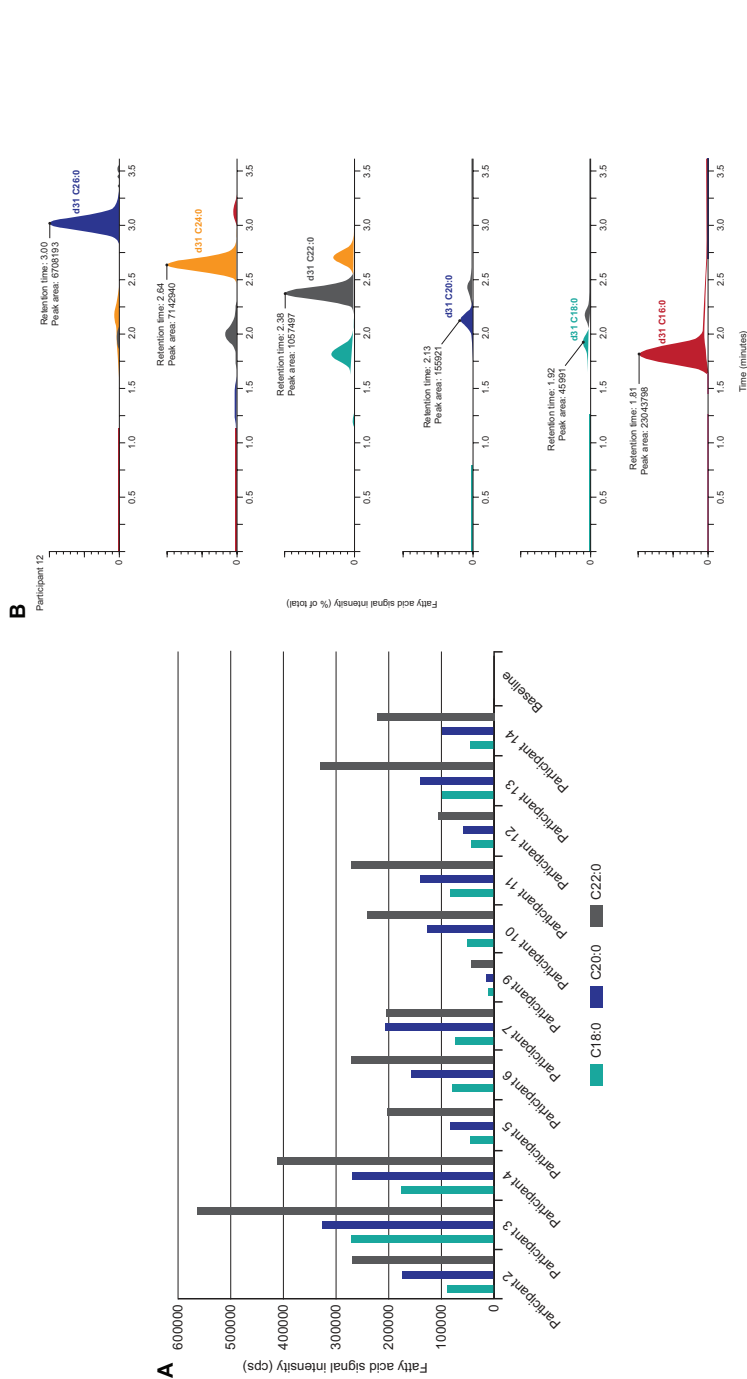
After four weeks application of the syndet bar formulation, deuterated elongated FFAs were detected in solvent extract samples from 9 out of 11 participants. Similar to the leave-on lotion, a trend was observed across participants. Higher levels of C20:0 were detected at Day 28 than C18:0 in all nine participants, and higher levels of C22:0 than C20:0 were detected in five of the nine participants (Figure 2a). Elongation up to C26:0 was not detected in any of the solvent extract samples. However, LC/MS/MS analysis of tape strips showed formation of C24:0 and C26:0 in a subset of three participants. MRM peak area assessment revealed that C24:0 was the most prevalent FFA in all tape strip extracts (Figure 2b).

### FFA ELONGATED FROM BODY WASH FORMULATIONS

After four weeks application of the body wash formulations to the arm and leg, detectable levels of elongated FFAs (C20:0 to C24:0) were found in tape strip samples for all body wash concentrations (Figure 3). Detectable levels of these elongated FFAs were found in all tape strip samples following application of the 2% d31-PA to the arm (Figure 3a) and leg (Figure 3b).

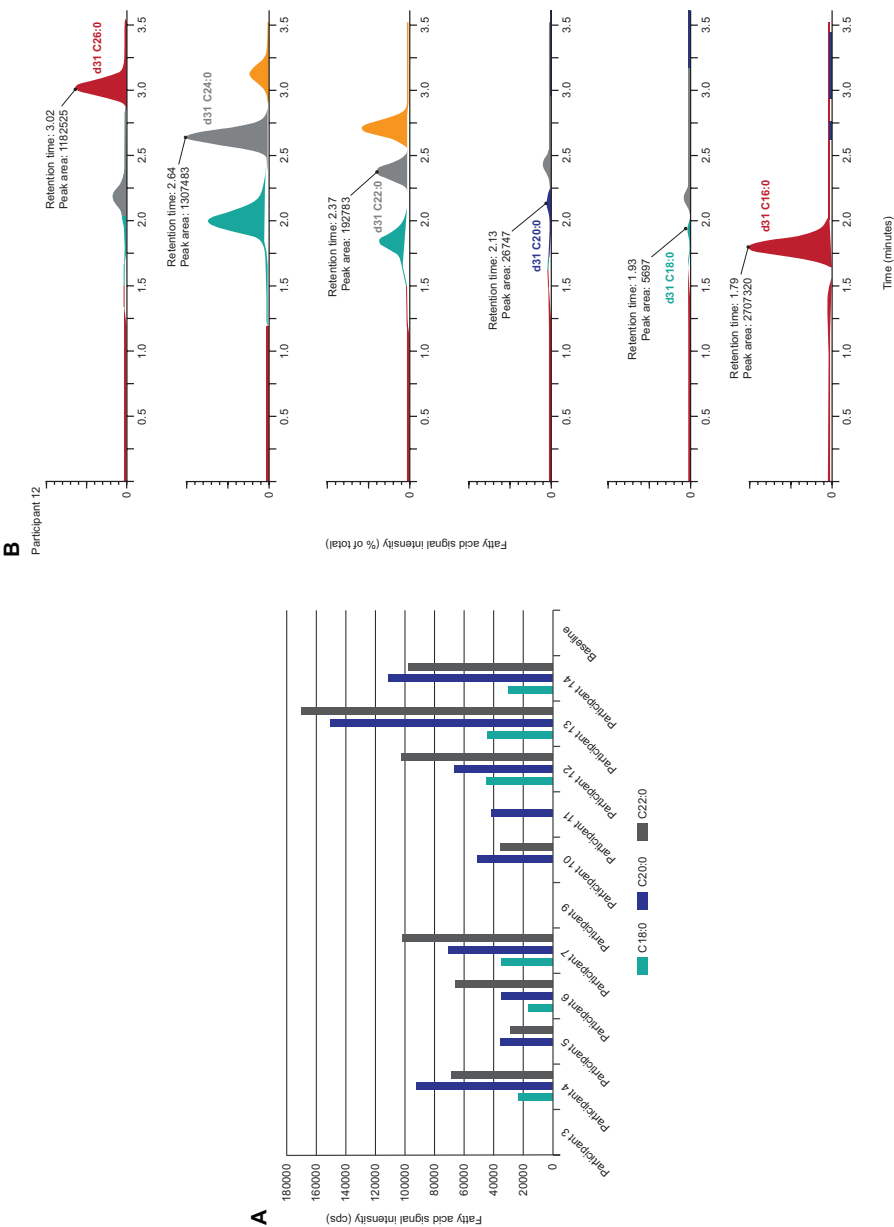
### DETECTION OF DEUTERATED SPHINGOSINE

Solvent extracts and skin tape strip extracts were analyzed for the presence of deuterated sphingosine following leave-on lotion, syndet bar and bodywash application. Detectable levels of d29 sphingosine were observed (Figure 4); these levels increased after acid hydrolysis for the leave-on lotion (Figure 4a), syndet bar (Figure 4b) and the different concentrations of body wash application (Figure 4c). This indicates that the d31-PA supplied by the topical

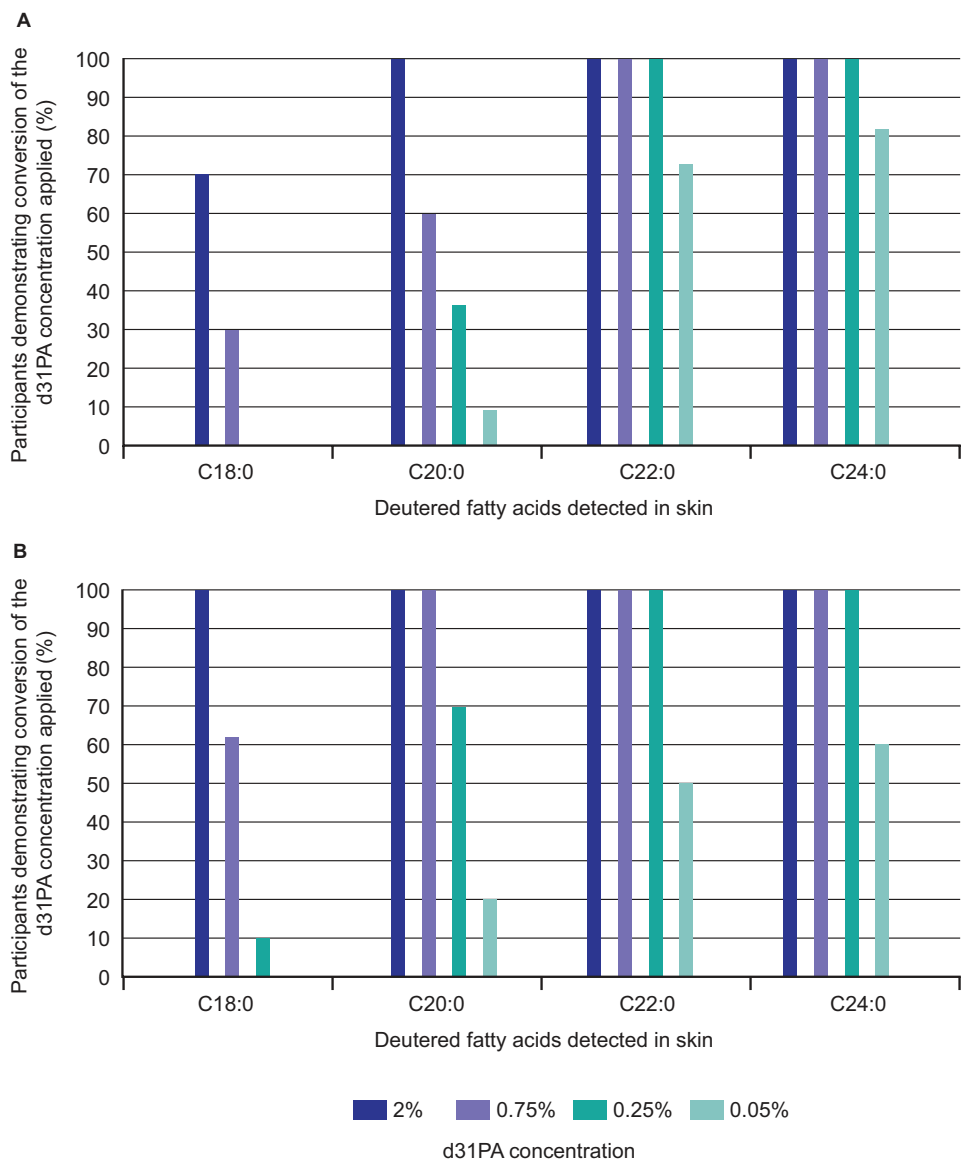


**Figure 1.** (A) Detection of d31-PA (C16:0) into elongated FFAs in solvent extract samples following leave-on lotion application at Day 28. Baseline levels were calculated as the average fatty acid signal intensity across participants at study initiation. Higher levels of C18:0, C20:0, and C22:0 were observed across all participants versus average baseline levels; (B) Representative MRM chromatogram of tape strip extracts obtained from a participant 12 of a subset of three participants at Day 28. Elongation up to C26:0 was detected in the subset following 4 weeks' application of the leave-on lotion. Elongated deuterated FFAs are labeled with retention time and peak area.

cps, counts per second; d31-PA; FFA, free fatty acid; MRM, multiple-reaction monitoring;



**Figure 2.** (A) Detection of d31-PA (C16:0) into elongated FFAs in solvent extract samples following syndet bar application at Day 28. Baseline levels were calculated as the average fatty acid signal intensity across participants at study initiation. Elongated FFAs were detected in 9 out of 11 participants versus average baseline levels; (B) Representative MRM chromatogram of tape strip extracts obtained from a participant 12 of a subset of three participants at Day 28. Elongation up to C26:0 was detected in the subset following 4 weeks' application of the syndet bar formulation. Elongated deuterated FFAs are labeled with retention time and peak area. cps, counts per second; d31-PA; FFA, free fatty acid; MRM, multiple-reaction monitoring.



**Figure 3.** Percentage of participants demonstrating conversion of different levels of d31-PA (C16:0) into elongated FFAs following application of a body wash formulation twice daily for four weeks on the arm (A) and leg (B). Detectable levels of elongated FFAs (C20:0 to C24:0) were found in tape strip samples for all body wash d31-PA concentrations.

d31-PA; FFA, free fatty acid.

formulations was incorporated into ceramides, as measured by the release of sphingosine via hydrolysis of ceramides. The concept of d31-PA integration into ceramides via the *de novo* pathway for ceramide and sphingosine synthesis was introduced by the authors at the AAD conference.<sup>25</sup>

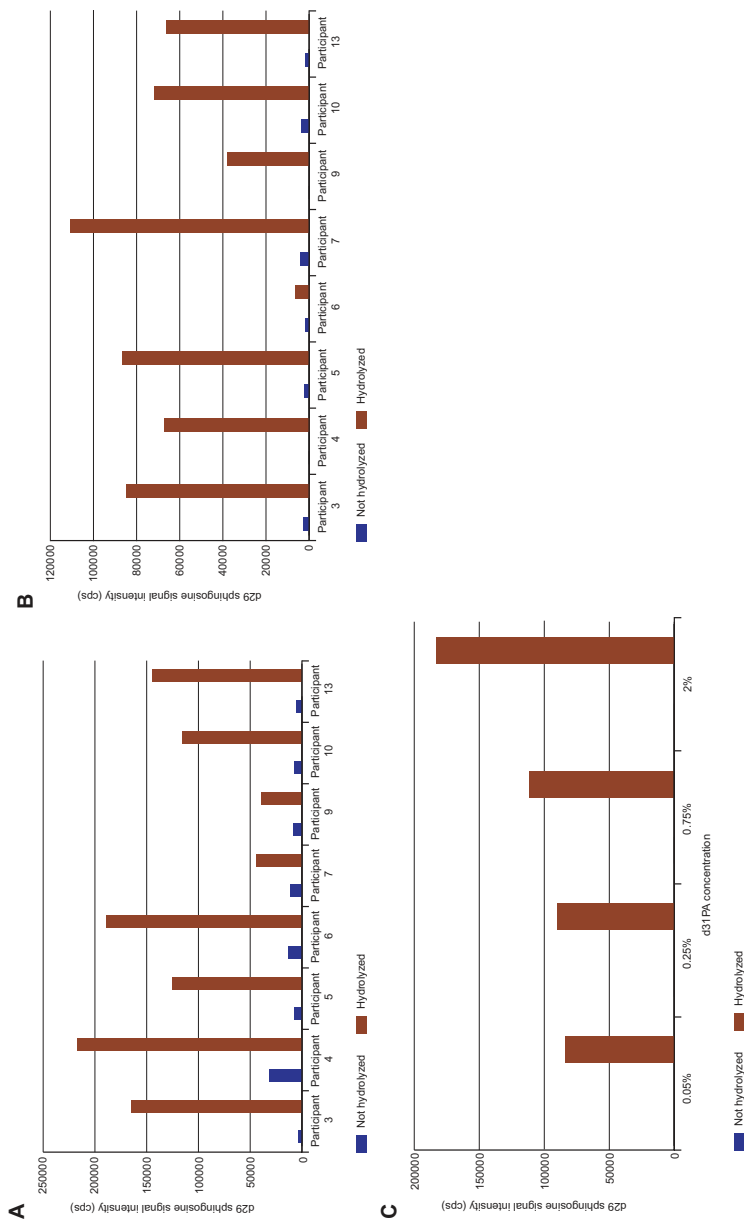


Figure 4. Formation of d29 sphingosine after application of leave-on lotion (A), syndet bar (B) and body wash formulations (C). Acid hydrolysis of the ceramides was performed using a modification of the Naoi, Lee and Roseman method. (A) Bar charts show d29 sphingosine signal intensity to be greater after acid hydrolysis following leave-on lotion application. (B) Bar charts show d29 sphingosine signal intensity to be greater after acid hydrolysis following syndet bar application. (C) Bar charts show the aggregate d29 sphingosine signal intensity to be greater after acid hydrolysis following application of all body wash formulations.

cps, counts per second.

## DISCUSSION

In the present report, we describe the first *in vivo* evidence that topically applied d31-PA from a range of skincare formulations (body wash, leave-on lotion, and syndet bar) promotes arm and leg skin barrier function via FFA elongation and successive incorporation into d29 sphingosine, a surrogate marker for ceramides.

Our data build upon previous *ex vivo* and *in vitro* findings that topically applied FFAs are elongated endogenously. Overall, there was a trend in our study for greater levels of C22:0 than C20:0 and greater levels of C20:0 than C18:0. These data could indicate that C18:0 and C20:0 are transient products in the FFA elongation process, in accordance with results from *ex vivo* and *in vitro* studies.<sup>4,26</sup> It may be reasonable to assume that providing shorter-chain FFAs delivers the substrates for sustained elongated FFA production.<sup>19</sup>

Between participants, we observed variability in the extent of FFA elongation. FFA elongation is determined by the activity of elongase enzymes. Elongase expression is regulated at the pre-translational level by tissue-specific and nutritional factors and during postnatal development; thus, individual differences in elongase expression may be accountable for the variation in FFA elongation.<sup>27</sup>

Elongation up to C24:0 was detected in the tape strip extracts of participants following body wash application, whilst elongation up to C26:0 was detected following leave-on lotion and syndet bar application. MRM LC/MS/MS is a gold-standard method used in this study for the detection and quantitation of FFAs and deuterated sphingosine as a surrogate marker of ceramides. However, C24:0 and C26:0 FFA levels were undetectable in the solvent extracts, despite *ex vivo* evidence of elongation of topically applied d31-PA to both C24:0 and C26:0.<sup>3,17</sup> There is limited evidence for the solubility of C24:0 in nonhalogenated solvents; therefore, its limited solubility in the methanol/ethyl acetate used for the solvent extracts may have resulted in inadequate recovery from the skin.<sup>28</sup> Enhanced detection of longer-chain FFAs from tape strips highlights an advantage of this sampling method for analyzing the full spectrum of SC FFAs.

The topical formulations tested in this study provided nourishment to the skin irrespective of the method of application. This demonstrates that wash-off products such as a syndet bar and body wash can mimic the performance of a leave-on formulation without compromising the benefits delivered to the skin when washed off up to 90 seconds later. Additionally, the body wash data show that even low levels of d31-PA incorporation in a wash-off product can result in detectable FFA elongation and the presence of d29 sphingosine. As our studies were conducted in healthy adults with minimal or moderate dry skin, these formulations could have particular utility for the nourishment of dry skin.

These data provide *in vivo* evidence that three different topical skincare formats, fully formulated to include d31-PA, are incorporated into the SC and used as building blocks to produce longer-chain FFAs. The formation of deuterated sphingosine from d31-PA offers direct evidence for the involvement of these topically applied FFAs in the ceramide biosynthesis pathway *in vivo*. Taken together, our findings demonstrate that leave-on lotion, body wash and syndet bar formulations can provide nourishment to the skin and may contribute to a healthy skin barrier.

## POSTERS PRESENTED AT TWO AMERICAN ACADEMY OF DERMATOLOGISTS MEETING

1. Hermanson K. et al. "Nutrient Benefits to the Skin are Delivered from both Bars and Liquid Cleansers" 2020 AAD e-poster.

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2. Yarova G. et al. "Delivering Nutrient Benefits from Cleansers to the Skin Through Enhanced Ceramide Production and Continues Nourishment" 2021 AAD e-poster.

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## CONFLICT OF INTEREST

The authors declare that no competing interest exist.

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## REFERENCES

- (1) Danso MO, Berkers T, Mieremet A, Hausil F, Bouwstra JA. An ex vivo human skin model for studying skin barrier repair. *Exp Dermatol*. 2015;24(1):48–54.
- (2) van Smeden J, Bouwstra JA. Stratum corneum lipids: their role for the skin barrier function in healthy subjects and atopic dermatitis patients. *Curr Probl Dermatol*. 2016;49:8–26.
- (3) Madison KC, Swartzendruber DC, Wertz PW, Downing DT. Presence of intact intercellular lipid lamellae in the upper layers of the stratum corneum. *J Invest Dermatol*. 1987;88(6):714–718.
- (4) Berkers T, van Dijk L, Absalah S, van Smeden J, Bouwstra JA. Topically applied fatty acids are elongated before incorporation in the stratum corneum lipid matrix in compromised skin. *Exp Dermatol*. 2017;26(1):36–43.
- (5) Knox S, O'Boyle NM. Skin lipids in health and disease: a review. *Chem Phys Lipids*. 2021;236:105055.
- (6) Cha HJ, He C, Zhao H, Dong Y, An IS, An S. Intercellular and intracellular functions of ceramides and their metabolites in skin (Review) [review]. *Int J Mol Med*. 2016;38(1):16–22.
- (7) Damien F, Boncheva M. The extent of orthorhombic lipid phases in the stratum corneum determines the barrier efficiency of human skin in vivo. *J Invest Dermatol*. 2010;130(2):611–614.
- (8) J.A. Bouwstra, P.L. Honeywell-Nguyen, G.S. Gooris, M. Ponc, Structure of the skin barrier and its modulation by vesicular formulations, *Prog Lipid Res*, 42, 1–36, (2003).
- (9) Bouwstra JA, Honeywell-Nguyen PL, Gooris GS, Ponc M. Structure of the skin barrier and its modulation by vesicular formulations. *Prog Lipid Res*. 2003;42(1):1–36.
- (10) van Smeden J, Janssens M, Kaye EC, et al. The importance of free fatty acid chain length for the skin barrier function in atopic eczema patients. *Exp Dermatol*. 2014;23(1):45–52.

- (11) Janssens M, van Smeden J, Gooris GS, et al. Increase in short-chain ceramides correlates with an altered lipid organization and decreased barrier function in atopic eczema patients. *J Lipid Res.* 2012;53(12):2755–2766.
- (12) Ishikawa J, Narita H, Kondo N, et al. Changes in the ceramide profile of atopic dermatitis patients. *J Invest Dermatol.* 2010;130(10):2511–2514.
- (13) Rogers J, Harding C, Mayo A, Banks J, Rawlings A. Stratum corneum lipids: the effect of ageing and the seasons. *Arch Dermatol Res.* 1996;288(12):765–770.
- (14) Wei KS, Stella C, Wehmeyer KR, et al. Effects of season on stratum corneum barrier function and skin biomarkers. *J Cosmet Sci.* 2016;67(3):185–203.
- (15) Ishikawa J, Yoshida H, Ito S, et al. Dry skin in the winter is related to the ceramide profile in the stratum corneum and can be improved by treatment with a Eucalyptus extract. *J Cosmet Dermatol.* 2013;12(1):3–11.
- (16) Ananthapadmanabhan KP, Yang L, Vincent C, et al. A novel technology in mild and moisturizing cleansing liquids. *Cosmet Dermatol.* 2009;22:307–316.
- (17) Ananthapadmanabhan KP, Mukherjee S, Chandar P. Stratum corneum fatty acids: their critical role in preserving barrier integrity during cleansing. *Int J Cosmet Sci.* 2013;35(4):337–345.
- (18) Zhang Q, Flach CR, Mendelsohn R, et al. Topically applied ceramide accumulates in skin glyphs. *Clin Cosmet Investig Dermatol.* 2015;8:329–337.
- (19) Murphy B, Grimshaw S, Hoptroff M, et al. Alteration of barrier properties, stratum corneum ceramides and microbiome composition in response to lotion application on cosmetic dry skin. *Sci Rep.* 2022;12(1):5223.
- (20) Mukherjee S, Edmunds M, Lei X, Ottaviani MF, Ananthapadmanabhan KP, Turro NJ. Stearic acid delivery to corneum from a mild and moisturizing cleanser. *J Cosmet Dermatol.* 2010;9(3):202–210.
- (21) Yarova G, Hermanson K, Lathrop W, et al. 27601 Delivering nutrient benefits from cleansers to the skin through enhanced ceramide production and continuous nourishment. *J Am Acad Dermatol.* 2021;85(3):AB154.
- (22) Ertel K., Keswick B., Bryant P. A forearm controlled application technique for estimating the relative mildness of personal cleansing products. *J Soc Cosmet Chem.* 1995;46:67–76.
- (23) Bligh EG, Dyer WJ. A rapid method of total lipid extraction and purification. *Can J Biochem Physiol.* 1959;37(8):911–917.
- (24) Naoi M, Lee YC, Roseman S. Rapid and sensitive determination of sphingosine bases and sphingolipids with fluorescamine. *Anal Biochem.* 1974;58(2):571–577.
- (25) Lathrop W, Yarova G, Dadd T, et al. 15959 Topically applied lotions containing fatty acids provide nourishment to the skin through maturation of the lipid matrix. *J Am Acad Dermatol.* 2020;83(6):AB165.
- (26) Thakoersing VS, van Smeden J, Boiten WA, et al. Modulation of stratum corneum lipid composition and organization of human skin equivalents by specific medium supplements. *Exp Dermatol.* 2015;24(9):669–674.
- (27) Wang Y, Botolin D, Xu J, et al. Regulation of hepatic fatty acid elongase and desaturase expression in diabetes and obesity. *J Lipid Res.* 2006;47(9):2028–2041.
- (28) Nechev JT, Edvinsen GK, Eilertsen KE. Fatty acid composition of the lipids from Atlantic salmon—comparison of two extraction methods without halogenated solvents. *Foods.* 2021;10(1):1–12.