Lipid Droplet Proteins in Acne Skin: A Sound Target for the Maintenance of Low Comedogenic Sebum and Acne-Prone Skin Health

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In adipocytes and sebocytes, lipid droplet proteins control the storage of lipids in organized droplets and their release on demand. The contribution of lipid droplet proteins to the pathogenesis of acne is plausible because they control the levels of comedogenic free fatty acids. The expression of two lipid droplet proteins, CIDEA and PLIN2, was analyzed in the skin of patients with acne by immunohistochemistry and western blotting. The design of clinical protocols allowed correlating the expression of CIDEA and PLIN2 with both comedogenesis and the release of free fatty acids. Both proteins were detected by immunohistochemistry in the sebaceous glands of patients with acne, with a disturbed expression pattern of PLIN2 compared with that in the controls. Higher levels of PLIN2 and CIDEA, as detected by western blotting in the infundibulum, significantly correlated with lower ongoing comedogenesis over 48 weeks of *Silybum marianum* fruit extract application. Accordingly, free fatty acid release from sebum triglycerides was significantly decreased, as shown with two distinct methods. The data are consistent with the expected role of PLIN2 and CIDEA in the prevention of comedogenic free fatty acid release. Modulation of PLIN2 and CIDEA expression appears as a sound target for the maintenance of low comedogenic sebum and acne-prone skin health.

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INTRODUCTION

Lipid droplets (LDs) are specialized organelles in the sebocyte cytoplasm in which sebum lipids are dispersed in an oil-in-water emulsion (Kory et al., 2016; Niemann and Horsley, 2012; Olzmann and Carvalho, 2019; Schneider and Paus, 2010). LDs are constituted by a lipid core containing triglycerides (TGs), wax esters, and cholesterol esters, surrounded by a monolayer of phospholipids in which various proteins are anchored (Figure 1) (Kory et al., 2016; Olzmann and Carvalho, 2019; Schneider, 2016). The perilipin (PLIN1–PLIN5) and CIDE proteins (CIDEA, CIDEB, and CIDEC) represent the main LD protein families. PLIN2, PLIN3, and CIDEA are expressed in significant amounts in sebocytes and are involved in the organization (number and the size) of LD within the cells (Dahlhoff et al., 2015; Schneider et al., 2016). An important function of CIDEA is to facilitate the fusion of small LD to larger droplets to avoid lipotoxicity, whereas PLIN2, a member of the perilipin proteins, is involved in the control of lipid accumulation in droplets and sebaceous gland size (Itabe et al., 2017; Olzmann and Carvalho, 2019; Zhang et al., 2014). These proteins are therefore required for lipids to be stored properly in sebocytes and may putatively contribute to controlling the levels of comedogenic free fatty acids (FFAs). Thus, their putative involvement in acne pathogenesis looks plausible, but knowledge on this field is currently limited by the lack of experimental clinical studies in humans (Schneider, 2016; Schneider et al., 2016).

We report a set of observations on PLIN2 and CIDEA, obtained through the profiling of a herbal extract of the well-known, natural, International Nomenclature Cosmetic Ingredients–registered *Silybum marianum*. This extract, called *S. marianum* fruit extract (SMFE), was identified through a program aimed at identifying the regulators of comedogenesis on the basis of human data derived from the extreme phenotype of Victor Yuschchenko dioxin poisoning (European Patent EP3478309). This extract induced a significant clinical effect on comedogenesis (Fontao et al., 2020) and was found by serendipity to regulate in vitro the transcription of two LD proteins PLIN2 and CIDEA. Therefore, we thought that further analyzing the expression of PLIN2 and CIDEA in human specimens obtained during the profiling of SMFE and correlating their expression with relevant clinical comedogenesis markers would provide some clues about the putative relevance of PLIN2 and CIDEA in comedogenesis.

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Abbreviations: FFA, free fatty acid; LD, lipid droplet; SMFE, Silybum marianum *fruit extract; TG, triglyceride*

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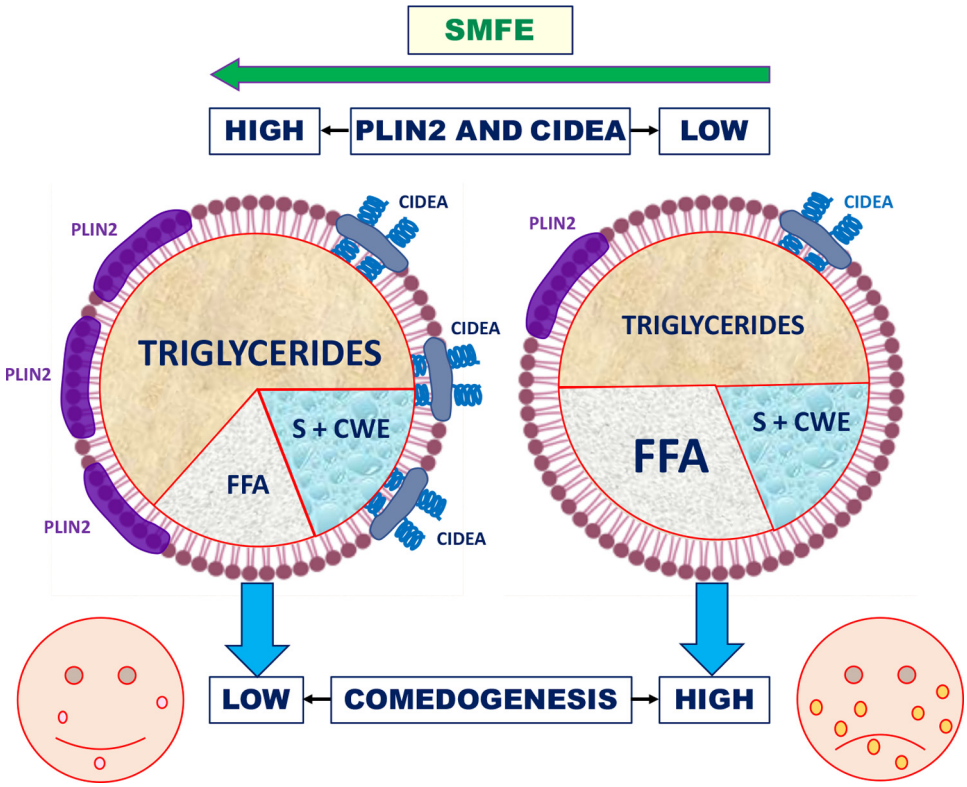

Figure 1. Summary diagram of data. PLIN2 and CIDEA are anchored on the phospholipid layer of lipid droplets. The lipid core contains mainly triglycerides, FFAs, S, as well as CWEs (Kory et al., 2016; Olzmann and Carvalho, 2019). During comedogenesis, the expression of PLIN2 and CIDEA is decreased, and a triglyceride lipase is activated, releasing FFAs from triglycerides. This process is partly reversed by topical application of SMFE. CWE, cholesterol and wax ester; FFA, free fatty acid; S, squalene; SMFE, *Silybum marianum* fruit extract.

When applied to the human face, SMFE is very well-tolerated, which is consistent with long term use. As an International Nomenclature of Cosmetic Ingredients natural compound, SMFE qualified for being a field cosmetic for the maintenance of low micro-comedogenesis in acne-prone skin. In a 48-week proof-of-concept study in 23 subjects (see cohort 1 in methods and our previous study [Fontao et al., 2020 #5407]), topical SMFE was associated with an initial decrease of microcomedone index (as shown in repeated skin surface biopsies) and the maintenance of a low microcomedone index over months. This lowering microcomedone index was correlated with a similar decrease in clinical lesions (Fontao et al., 2020 #5407). These observations, both on efficacy and tolerance, were confirmed and extended in a 12-month international study, which followed the same protocol as that of the proof-of-concept study (manuscript in preparation; EADV meeting abstract 2021 submitted).

RESULTS

SMFE modulates *PLIN2* and *CIDEA* gene expressions in vitro

In our previous study, cultured sebocytes were exposed to SMFE, then a transcriptomic analysis was performed by RNA sequencing (Fontao et al., 2020). Two LD proteins, PLIN2 and CIDEA, were distinguished by a strong induction of 14-fold and 9-fold, respectively (Table 1).

PLIN2 and CIDEA are expressed in human microcomedones

We have previously found that several proteins are identified by proteomic and western blot analysis of the cyanoacrylate skin surface biopsy extracts, including keratins 75 and 79, whose expressions in the study was linked to comedogenesis (Fontao et al., 2020). On the same samples series, we now measured and followed up the levels of PLIN2 and CIDEA. Both proteins were detected in all the 50 samples tested by western blot analysis. This indicates that the LD proteins are excreted within the flow of sebum—which is not surprising in a holocrine system—and persist, at least for part of them, in the infundibular microcomedone.

Lower expression of PLIN2 and CIDEA is associated with comedogenesis

We next explored the likelihood that the amounts of PLIN2 and CIDEA flowing out through the infundibulum and/or embedded into the microcomedones might be variable and related to the degree of comedogenesis, the in vivo

Table 1. Modulation of Lipid Droplet Proteins by SMFE in Cultured Sebocytes

Family	Gene	FC	c.p.m.	*P*-Value	FDR
Perilipins	*PLIN1*	—	—	—	—
	PLIN2	14.46	49.3	$6.37 \cdot 10^{-41}$	$2.03 \cdot 10^{-37}$
	PLIN3	0.84	153.6	0.250	0.578
	PLIN4	0.52	4.18	0.025	0.161
	PLIN5	0.35	1.10	$3.25 \cdot 10^{-3}$	0.040
CIDE	*CIDEA*	9.12	0.42	$3.91 \cdot 10^{-5}$	$1.23 \cdot 10^{-3}$
	CIDEB	0.86	3.23	0.628	0.852
	CIDECP	0.93	4.17	0.788	0.927

Abbreviations: c.p.m., counts per million; FC, fold change; FDR, false discovery rate; SMFE, *Silybum marianum* fruit extract.

Sebocytes were exposed to 30 μg/ml SMFE for 3 consecutive days; then a transcriptomic analysis was performed using RNA sequencing.

marker of which is the microcomedone index (Fontao et al., 2020). We had previously shown that the microcomedone index in nonlesional skin is highly correlated with ongoing clinical noninflammatory lesions and reflects the level of the actual comedogenic strengths (Fontao et al., 2020).

In this study, we characterized each of the 50 cyanoacrylate skin surface biopsy samples by its microcomedone index and PLIN2 or CIDEA content, as measured by western blotting. We found significant segregation of high PLIN2 or CIDEA content with a low microcomedone index (Figure 2a and b). This indicates that there are more PLIN2 and CIDEA in the upper part of the infundibulum when comedogenesis is weaker.

Lower FFA release in sebum is associated with lower comedogenesis

The different LD proteins act in concert to control the formation of large droplets and the number and the size of sebocytes and to prevent lipotoxicity (Kory et al., 2016; Zechner et al., 2017). FFAs, in particular polyunsaturated ones, are exposed to oxidative stress, and their products of oxidation play a key role in comedogenesis (Cunliffe et al., 2003; Hauck and Bernlohr, 2016; Melnik, 2015).

We wondered whether the increase in PLIN2 and CIDEA might, functionally, correlate with a decrease in FFA release. Sebum lipids were analyzed in a separate cohort of 40 patients with acne, 18 treated with SMFE and 18

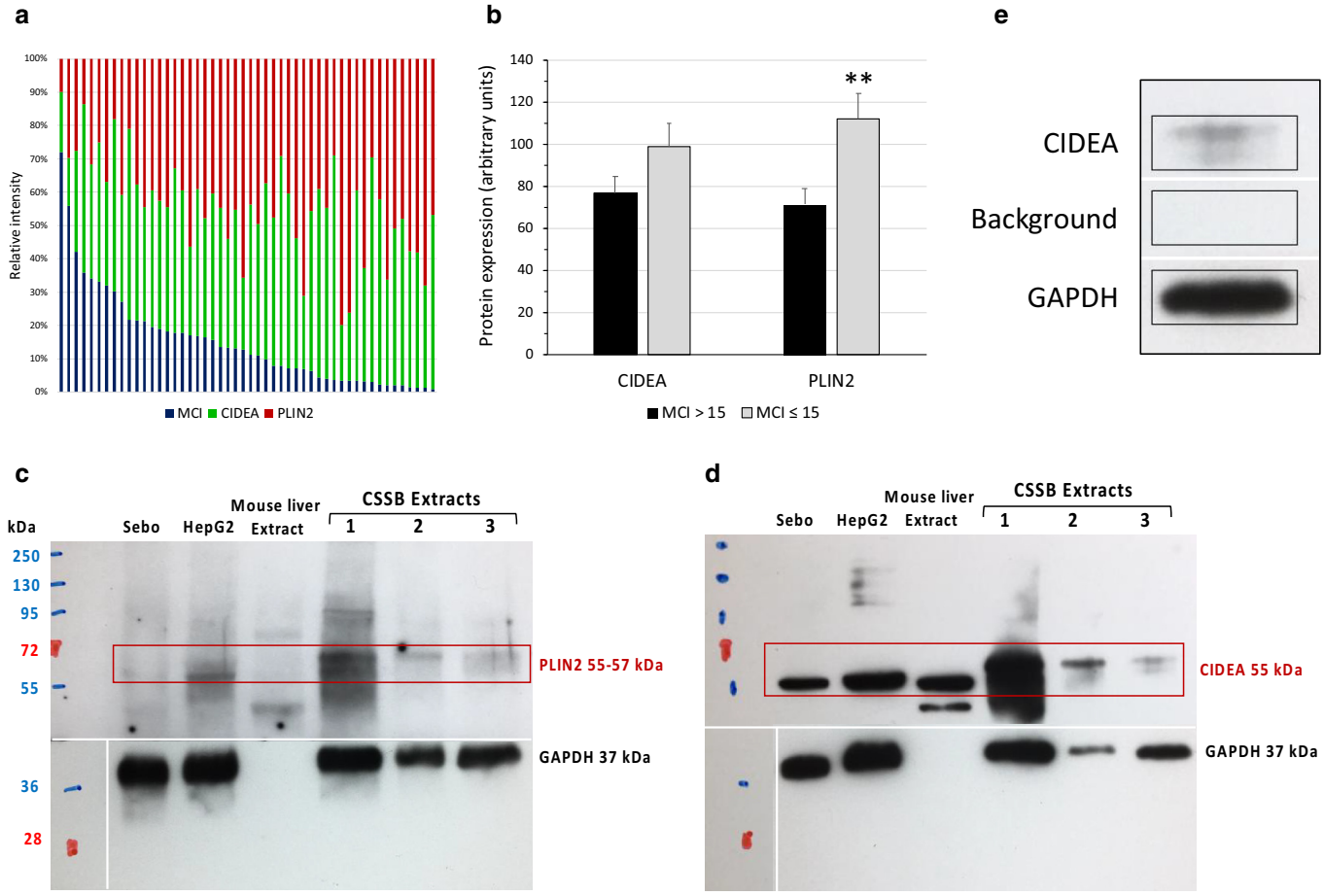

Figure 2. Correlation between microcomedone index and lipid droplet proteins in cohort 1 (CSSB biopsies). (**a**) PLIN2 and CIDEA are expressed in a nonmetric multidimensional scaling of 50 CSSB biopsies from 23 patients on the basis of the value of the MCI in decreasing order; for each sample, the sum of corresponding MCI value and the expression values for CIDEA and PLIN2 gives 100%, and the bars represent the respective percentages for MCI, CIDEA, and PLIN2 (Fontao et al., 2020). (**b**) The means ± SD of CIDEA and PLIN2 expressions are determined for 50 CSSB biopsies from 23 patients, with MCI values above and below 15. Positive control for (**c**) PLIN2 and (**d**) CIDEA expressions were analyzed in human Sebo cultures, human hepatocyte cell line HepG2, mouse liver extract (liver), and human CSSB extracts from three samples. For each of the gels in **c** and **d**, after the transfer to nitrocellulose membranes, the membranes were spliced to incubate the respective pieces with **(c)** PLIN2 and GAPDH antibodies or (**d**) CIDEA and GAPDH antibodies; white lines indicate where the membranes were spliced. (**e**) This image shows CIDEA and GAPDH bands from a gel to explain how the densitometric assessment was performed for all protein bands of interest; as for the gels in **c** and **d**, the nitrocellulose membrane was spliced to incubate each part with CIDEA or GAPDH antibodies; when comparing CIDEA and GAPDH (housekeeping protein), the background of the gel—corresponding to the rectangle in the middle of the image—is subtracted, and the density of the protein bands are calculated by subtracting their mean gray values from that of the background. The ratio between the LD protein and GAPDH gives the expression value of the LD protein. This ratio is then multiplied by 100 when creating the graphs shown in Figure 1a and b for clarity. Statistical significance in **b** is set as $^{**}P < 0.01$. CSSB, cyanoacrylate skin surface biopsy; LD, lipid droplet; MCI, microcomedone index; Sebo, sebocyte.

untreated. The release of FFAs from sebum TGs was significantly decreased in the treated group after two months, compared with that in the control group, as indicated by the FFA-to-TG ratio of lipids harvested on the forehead (Figure 3a).

Similarly, lipid contents of comedones isolated from the nose of patients were analyzed by gas chromatography–mass spectrometry at baseline and after 1 month of SMFE treatment. FFA-to-TG ratios were calculated. At baseline, FFA-to-TG ratio was not different between the groups. After 1 month of SMFE treatment, FFA-to-TG ratio decreased from 9.52 ± 4.85 in untreated patients to 7.54 ± 2.36 in treated ones.

The count of noninflammatory (comedonal) acne lesions was significantly decreased in the treated group, attesting objectively the decrease of comedogenesis associated with the decrease of FFA (Figure 3b–g).

These data indicate that a lower FFA release in the sebum is associated with lower comedogenesis and support the hypothesis.

Altered expression of PLIN2 in acne skin

The immunostaining of PLIN2 and CIDEA in nonacne controls is shown in Figure 4. As previously reported (Dahlhoff et al., 2015; Schneider et al., 2016), both proteins were detected in healthy human skin. The distribution over the sebaceous gland as well as the cellular pattern were similar but not identical for PLIN2 and CIDEA (Figure 4).

In the skin of patients with acne, the distribution of CIDEA over the sebaceous gland as well as the cellular pattern did not differ from that of controls without acne (not shown). In contrast, PLIN2 was more intensely expressed in all samples of acne skin (Figure 5). The hyperexpression was patchy with

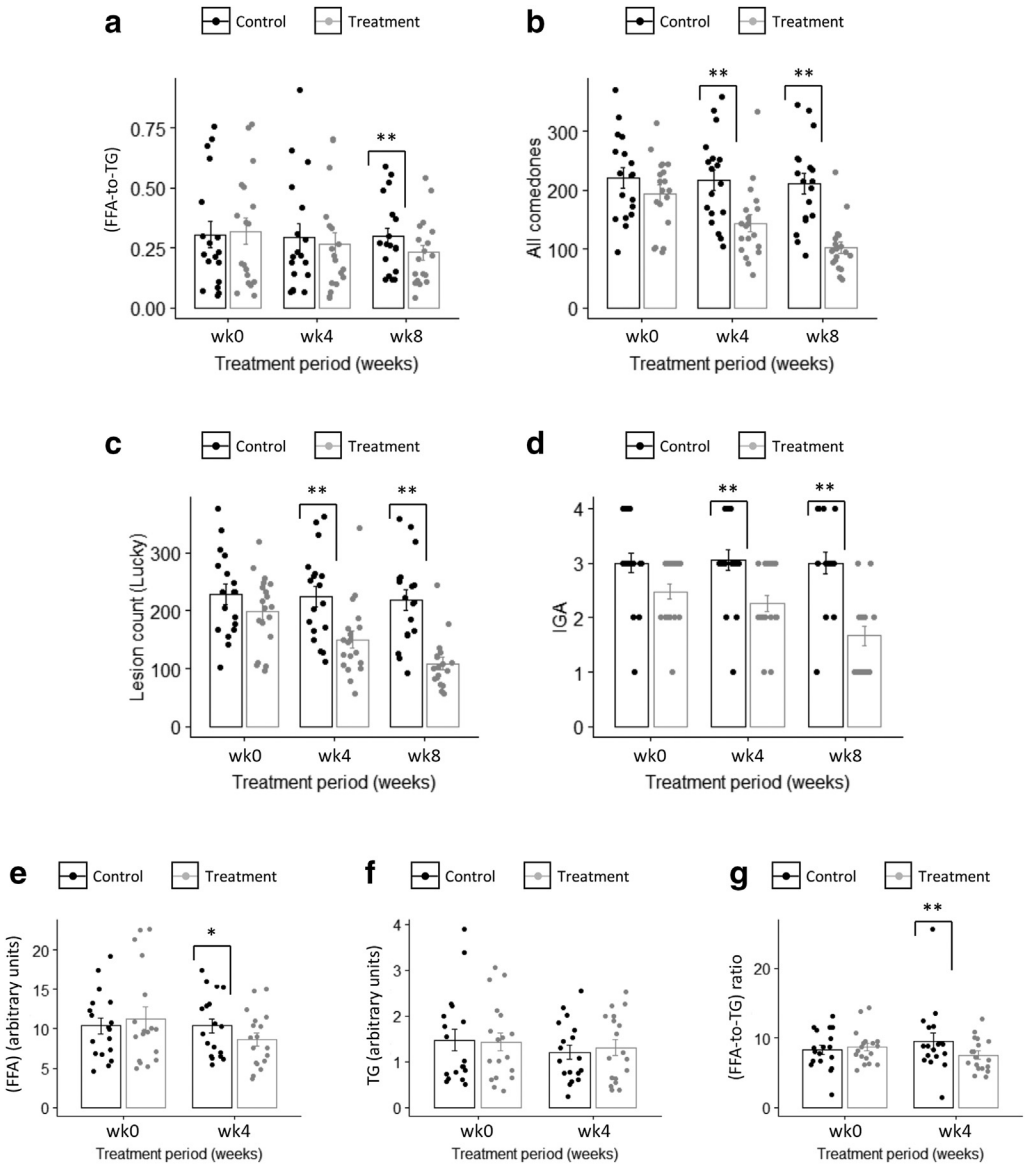

Figure 3. Assessment of acne in cohort 2 (skin surface lipids). (**a**) The lipid composition of sebum was analyzed by infrared spectroscopy to determine the FFAs-to-TGs ratio. (**b**) Noninflammatory acne lesions (open and closed comedones). **(c)** Acne lesion count as described by Lucky et al. (1996). (**d**) Acne assessment using the Investigator Global Assessment of Acne. (**e**–**g**) Data from FFAs and TGs for comedones collected from nose wings. FFA and TG were analyzed by gas chromatography–mass spectrometry. Results are expressed as means ± SD; statistical significance was set at $*P < 0.05$ and $**P < 0.01$. FFA, free fatty acid; TG, triglyceride; wk, week.

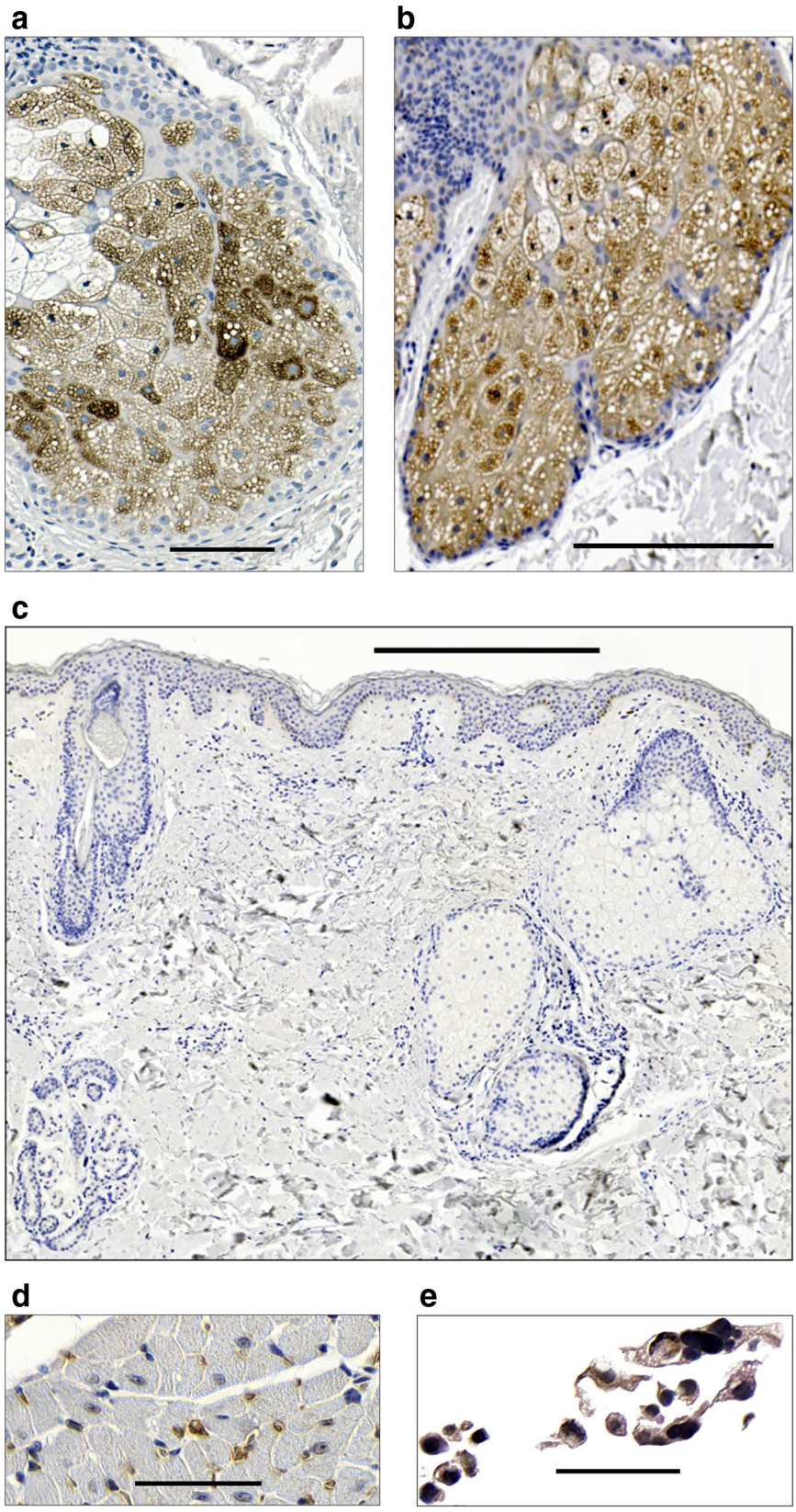

Figure 4. Immunohistochemical labelling of PLIN2 and CIDEA in human skin of nonacne controls (cohort 3). As previously reported, both proteins were detected in the skin of humans without acne (Dahlhoff et al., 2015; Schneider et al., 2016). The distribution over the sebaceous gland as well as the cellular pattern were similar but not identical for (**a**) PLIN2 and (**b**) CIDEA. Bar = 100 μm in **a** or 200 μm in **b**. (**c**) Human skin biopsy (used for negative control); absence of staining using only the second antibody (172-1019, 1:500; Bio-Rad Laboratories, Hercules, CA). Bar = 500 μm. (**d**) Mouse heart (used for positive control of CIDEA staining [PA5-29654, 1:200; Thermo Fisher Scientific, Waltham, MA]). Bar = 50 μm. (**e**) Sebocyte cultures (Zen-Bio, Research Triangle Park, NC) (used for positive control of PLIN2 staining [SAB4200452, 1:200; Sigma-Aldrich, St. Louis, MO]).

many zones without expression at all. Such a pattern might represent zones of either hyperproduction or retention of PLIN2 protein.

DISCUSSION

The information on the putative role of LD in the pathogenesis of acne from this work is presented in the following sections.

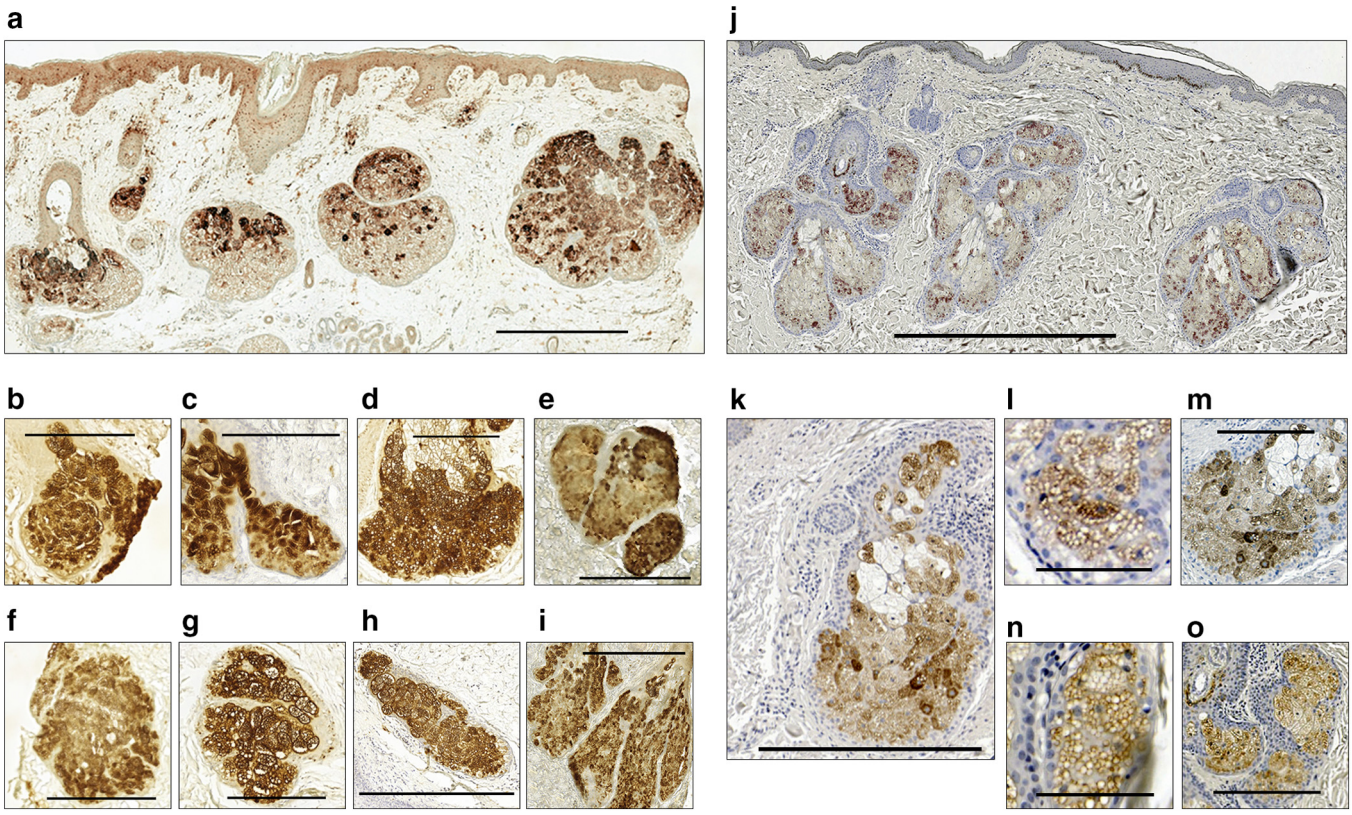

Figure 5. Immunohistochemical labeling of PLIN2 in human skin of patients with acne and nonacne controls (cohort 3). (**a**) A general view and (**b**) a close view of patient 1 highest expression field. (**c–i**) Close views of the highest expression field in the seven other patients with acne. (**j**) A general view of one control. (**k–o**) Close views of the highest expression field of the controls. Bar = 500 μm in **a, e, h, i, and k**. Bar = 200 μm in **b, d, f,** and **g**. Bar = 250 μm in **c, m,** and **o**. Bar = 1,000 μm in **l** and **n**. Bar = 1,000 μm in **j**.

Disorganized expression of PLIN2 in acne

The patchy hyperexpression pattern seen in all samples suggests a dysregulation in the maturation process within the sebaceous lobules. Zones with high expression might represent either hyperproduction or retention of PLIN2. Interestingly, this is not seen for CIDEA on the same samples, which argues against a technical problem or any factor unrelated specifically to PLIN2, such as inflammation. However, we consider these data as preliminary. Appropriate analysis of this disturbed in vivo distribution of a protein that prevents lipolysis in acne skin would necessitate many more samples, with related ethical problems. Of note, the experimental overexpression of PLIN2 reduces LD association with adipose TG lipase and thereby lipolysis (Wang et al., 2011).

Putative link of PLIN2 and CIDEA with comedogenesis

More PLIN2 and CIDEA proteins were found in the infundibulum when comedogenesis was weaker. Whether these two facts are causally related remains to be directly shown. It would be difficult to directly establish that PLIN2 and CIDEA, produced within the sebocytes, do retain some biological activity such as inhibition of lipases after their journey within the flow of sebum toward the microcomedone. The cyanoacrylate glue for microcomedones sampling prevents doing such direct biochemical tests. However, our study provides an indirect indication in favor of this hypothesis: indeed sebum collected at the skin surface in periods of low comedogenesis does have a low FFA-to-TG ratio (Figure 1) (Josse et al., 2020). This might indicate that during periods of low comedogenesis, small LDs of sebaceous glands reorganize to larger droplets. This would allow sebocytes to properly store more lipids and avoid lipotoxicity and cellular stress due to lipid constituents of microcomedones, for instance (Olzmann and Carvalho, 2019).

Several uncertainties should be further explored

In an in vitro experiment, pluripotent cells were incubated with a differentiation stimulus for 3 days; lipogenic activation of sebocytes was induced with arachidonic acid (20 μM) for 24 hours and 48 hours. In this system, SMFE decreased the levels of LD proteins and their mRNA, indicating that SMFE acts as a context-dependent regulator of lipid storage in sebocytes (Garidou et al., 2019).

The LD proteins PLIN2 and CIDEA are found at the interface between the cytosol and the surface of LDs. This means that their cellular concentration is related to the global surface of LDs rather than to their volume. In other words, any fusion or subdivision of intracellular LDs occurring without the modification of their global volume will affect the concentration of LD proteins to maintain their physiological concentration at the surface of LDs. This may explain apparent discrepancies of LD expression depending on the stage of differentiation of the sebocyte model used to assess the pharmacological effect of SMFE.

Overall

Our in vivo human data are consistent with the expected role of PLIN2 and CIDEA in the prevention of comedogenic FFAs release in human facial skin. Modulation of PLIN2 and CIDEA expression appears as a sound target for the maintenance of low comedogenic sebum and acne-prone skin health.

MATERIALS AND METHODS

Skin biopsies

Punch biopsies of 2 mm were obtained on the face under local anesthesia. Cyanoacrylate skin surface biopsies were harvested according to Piérard et al. (2014), as previously described (Fontao et al., 2020). The procedures were in accordance with Helsinki rules, with written consent from the patients and controls.

Assessing microcomedones (cohort 1)

The count of microcomedones, ranked by size on a 0–4 scale, and then the calculation of the microcomedone index were performed using the method described by Holmes et al. (1972), as previously reported (Fontao et al., 2020).

Protein extraction (cohort 1)

Cyanoacrylate skin surface biopsies were detached from the slides in distilled water (30 minutes at room temperature), and then proteins were extracted as previously described (Fontao et al., 2020).

Western blot analysis (cohort 1)

Protein samples were analyzed by western blot using a standard procedure using 10 μg total proteins on 12% polyacrylamide gels (456-1043; Bio-Rad Laboratories, Hercules, CA) (Fontao et al., 2020). The following primary antibodies were used: rabbit polyclonal anti-human IgG (NB110-40877, dilution 1:2,000; Novus Biologicals, Littleton, CO) for PLIN2, rabbit polyclonal anti-human IgG (PA5-29654, dilution 1:5,000; Thermo Fisher Scientific, Waltham, MA) for CIDEA, and mouse monoclonal anti-human GAPDH IgG1 (sc-47724, dilution 1:10,000l; Santa Cruz Biotechnology, Dallas, TX) for GAPDH. A goat anti-rabbit IgG (H + L)-horseradish peroxidase conjugate was used as a secondary antibody (172-1019, dilution 1:200; Bio-Rad Laboratories).

The densitometric analysis of protein bands was performed using ImageJ software (National Institutes of Health, Bethesda, MA), as shown in Figure 2e. The ratios between LD proteins and their respective GAPDH values were multiplied by 100 to get a better view of the nonmetric multidimensional scaling graph showing the evolution of LD proteins as a function of microcomedone index (Figure 2a).

Immunohistochemistry

Immunohistochemical analysis was performed with the use of an immunoperoxidase technique according to standard procedures (Lebeau et al., 2005). The following antibodies were used: a mouse monoclonal anti-human PLIN2 (SAB4200452, dilution 1:200; Sigma-Aldrich, St. Louis, MO), a rabbit polyclonal anti-human CIDEA (PA5-29654, dilution 1:200; Thermo Fisher Scientific), and a goat anti-rabbit IgG (H + L)-horseradish peroxidase conjugate was used as the secondary antibody (172-1019, dilution 1:500; Bio-Rad Laboratories).

Sebocyte cultures

Primary sebocytes from human origin were purchased from Zen-Bio (Research Triangle Park, NC) and cultured in the Sebocyte Growth Medium from Zen-Bio, according to the manufacturer's instructions (Fontao et al., 2017).

RNA extraction

Sebocyte cultures were washed with PBS; then total RNA was obtained using the TRIzol extraction method (Rio et al., 2010) using the TRIzol Reagent from Thermo Fisher Scientific (Reinach, Switzerland), according to the manufacturer's instructions.

Transcriptomic analysis

Sebocyte cultures were exposed to 300 μg/ml SMFE in triplicates for 3 consecutive days; then a transcriptomic analysis was performed by RNA sequencing technology at the Genomics Platform of the University of Geneva using the TruSeq RNA protocol from Illumina (Cambridge, United Kingdom) with reads of 100 bases (see Supplementary Materials of our previous study for more details [Fontao et al., 2020]).

SMFE

We had identified the comedolytic properties of a specific SMFE in the context of a patient-based, data-oriented program for fighting xenobiotic comedogens (European patent EP3478309) (Fontao et al., 2020). A topical skin care preparation based on SMFE was applied twice a day during the whole 48 weeks.

Sebum harvest and analysis: Quantification of FFA-to-TG ratio by infrared spectroscopy (cohort 2)

Forehead sebum was collected on absorbent paper, and attenuated total reflectance–Fourier transform infrared spectroscopy spectra were acquired in the 4,000–670 per cm range on a Perkin Frontier spectrometer (Spectrum 400 FT-IR, Perkin Elmer, Waltham, MA). The ratio of the peak areas of FFA (1,710 cm^{-1}) and TGs (1,740 per cm) was calculated.

Comedone lipid harvest and analysis: Quantification of FFA-to-TG ratio by gas chromatography–mass spectrometry (cohort 2)

Samples were collected with a patch on nose wings. Comedones were collected, and lipids were extracted according to the Bligh and Dyer extraction on the basis of a mixture of chloroform/methanol (Bligh and Dyer, 1959). The biochemical exploration was performed by the screening of neutral lipids on gas chromatography–mass spectrometry (Synelvia SAS, Labège, France).

Acne assessment (cohort 2)

Clinical acne severity was evaluated by Investigator Global Assessment of Acne score from 0 to 4 (absent, very mild, mild, moderate, severe, respectively) (Dréno et al., 2011). Efficacy was evaluated during noninflammatory and inflammatory clinical lesions count (i.e., open, closed comedonal, papules, and pustules) accordingly to Lucky method (Lucky et al., 1996) and by counting open and closed comedones (noninflammatory acne lesions).

Patients with acne

Cohort 1 (cyanoacrylate skin surface biopsies). A total of 23 patients (aged 14–45 years, mean age of 24.4 years, six males, 17 females) with moderate to severe acne were enrolled in a 48-week clinical trial (Fontao et al., 2020) aimed at complementing the prescription drugs with SMFE. The trial had been approved by the Geneva Ethics Committee for Clinical Trials (number 2016-01798), and written informed consent was obtained for all participating volunteers. SMFE was applied on the entire face in an oil-in-water emulsion at a concentration of 7%. The 23 patients reached a total

of 7,728 days of SMFE use. Prescription drugs aimed at controlling postcomedonal inflammatory/infectious lesions, as recommended by current acne therapeutic guidelines, that is, topical clindamycin or oral lymecycline, were used for 9.05% and 9.35% of the total days of the trial, respectively (Fontao et al., 2020). A weekly follow-up based on digital facilities was established. Cyanoacrylate skin surface biopsies were performed on the forehead at each visit. Clinical acne severity was evaluated by global evaluation of acne (Dréno et al., 2011) and lesions counts.

Cohort 2 (skin surface lipids). A total of 38 enrolled and 36 analyzed patients (aged 18–34 years, mean age of 24.2 years, four males, 32 females) with mild to moderate acne were enrolled in a clinical trial aimed at assessing the modulation of sebum lipids and acne score following topical treatment with a low concentration of SMFE on the face (1.75% in an oil-in-water emulsion). According to the French legislation, this trial using a topical cosmetic formulation (ClinicalTrials.gov NCT04873089) was not submitted to an Ethics Committee; written informed consent was obtained for all volunteers. Patients were randomly distributed into two groups (one treated twice a day with SMFE and the other one untreated) and followed for 8 weeks. Sebum was harvested on an absorbent paper; then the lipid composition was analyzed by infrared spectroscopy to determine the FFAs-to-TGs ratio.

Cohort 3. Skin biopsy samples for immunohistochemistry were obtained from a previous study aimed at detecting comedogenic markers in patients with severe acne. This trial had been approved by the Geneva University Hospital Ethics Committee (number 11-111), and written informed consent was obtained from all volunteers. There were eight patients (mean age of 33.4 years, four males, four females) and four matched controls. The biopsies were retroauricular on nonlesional skin. Sections were cut from formalin-fixed, paraffin-embedded skin biopsy specimens and stained with H&E. Immunohistochemical labeling was performed using rabbit polyclonal PLIN2 or CIDEA antibodies (Bio-Techne, Zug, Switzerland) at dilutions of 1:200 and an immunoperoxidase technique according to standard procedures (Lebeau et al., 2005).

Statistics

Cohort 1 (Figure 2b). To compare the amount of CIDEA and PLIN2 expressions between control and treated groups, a bilateral unpaired Student's *t*-test was performed; $^{**}P < 0.01$.

Cohort 2 (Figure 3a–c). To compare the changes from baseline between groups, a mixed model for repeated measures was made with groups (visit and interaction group), with visit group as fixed factors, subject as random factors, and baseline as covariate. Comparisons at each visit were made using the differences in estimated means. Significant differences between control and treated groups are indicated: $^{*}P < 0.05$, $^{**}P < 0.0001$.

Cohort 2 (Figure 3d). The comparison between groups on changes from baseline at each visit was made using Wilcoxon test.

Data availability statement

Datasets related to this article can be found at https://doi.org/10.26037/yareta:7jeav2e3lracfdwpabxvqnu2ke, hosted at Yareta.

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CONFLICT OF INTEREST

TN, NCR, LG, CL, JLD, and GJ are employees of Pierre Fabre Dermocosmetique. OS, FF, and JHS received financial support from Pierre Fabre Dermocosmetique. The remaining authors state no conflict of interest.

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AUTHOR CONTRIBUTIONS

Conceptualization: GJ, JHS, OS, TN; Data Curation: CL, GJ, JHS, JLD, LG, NCR, OS; Formal Analysis: CL, FF, JHS, JLD, OS; Funding Acquisition: GJ, JHS, NCR, TN; Investigation: FF, GJ, JHS, JLD, LG, NCR, OS, TN; Methodology: CL, FF, GJ, NCR, OS, TN; Project Administration: GJ, JHS, OS, TN; Resources: GJ, NCR, OS; Software: JLD; Supervision: GJ, JHS, NCR, OS, TN; Validation: FF, GJ, JHS, LG, NCR, OS, TN; Visualization: FF, GJ, JHS, JLD, LG, NCR, OS, TN; Writing - Original Draft Preparation: GJ, JHS, NCR, OS, TN; Writing - Review and Editing: GJ, JHS, NCR, OS, TN

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