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Effects of atopic dermatitis and gender on sebum lipid mediator and fatty acid profiles

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ABSTRACT

Skin disease alters cutaneous lipid mediator metabolism, and if skin secretions contain evidence of these changes, they may constitute useful clinical matrices with low associated subject burden. The influences of skin diseases on sebum lipid mediators are understudied. Here, sebum oxylipins, endocannabinoids, sphingolipids, and fatty acids were quantified from the non-lesional bilateral cheeks of subjects with and without quiescent atopic dermatitis (AD) using LC-MS/MS and GC–MS. AD decreased C36 [NS] and [NdS] ceramide concentrations. Compared to males, females demonstrated increased concentrations of oxylipin alcohols and ketones, and saturated and monounsaturated non-esterified fatty acids, as well as decreased concentrations of C42 [NS] and [NdS] ceramides. Additionally, contemporaneously collected sweat lipid mediator profiles were distinct, with sebum showing higher concentrations of most targets, but fewer highly polar lipids. Therefore, AD and gender appear to alter sebum lipid metabolism even in non-lesional skin of quiescent subjects.

1. Introduction

Sebum is an oily and waxy substance secreted by the sebaceous glands of the skin, and represents one of the two main cutaneous secretions (the other being sweat). Though sebum is secreted throughout the body (with the exception of the palms of the hand and soles of the feet), sebum production is most prominent at the forehead, nose and chin (the so-called "*t*-zone" of the face), where sebaceous gland density is the highest [1]. However, other sites such as the cheeks and upper torso also contain a high sebaceous gland density (and therefore produce appreciable amounts of sebum), and in general, sebaceous gland density decreases towards the extremities of the body [1]. The specific functions of sebum are yet to be fully elucidated, but the composition of sebum suggests that it (along with other epidermal surface lipids) plays a role in skin barrier formation, moderation of cutaneous inflammation, and anti-microbial defense [2].

Preliminary characterizations of sebum have indicated that it is a lipid-rich matrix, with its major components being glycerolipids (30–50%), free fatty acids (15–30%), cholesterol (1.5–2.5%), cholesterol esters (3–6%), squalene (12–20%), and wax esters (26–30%) [3]. Changes in the relative abundances of these sebum components have been associated with cutaneous diseases such as acne, papulopustular rosacea, atopic dermatitis and seborrheic dermatitis [4–7], though specific mechanisms explaining the role of sebum in these diseases have yet to be elucidated. More comprehensive characterizations of sebum content have recently been attempted, and the relative abundances and concentrations of individual species in each of the sebum lipid classes are now reported [4,6,8]. These recent reports have led to more nuanced understanding of the mechanism of diseases such as acne and papulopustular rosacea, as we now know that individuals with acne

Abbreviations: ad, atopic dermatitis; Aea, arachidonoylethanolamide (anandamide); Ag, arachidonoylglycerol; Cox, cyclooxygenase; %cv, coefficient of variance; Fame, fatty acid methyl ester; Lox, lipoxygenase; Lt, leukotriene; Ms, mass spectrometry; Ms/ms, tandem mass spectrometry; Mufa, mono-unsaturated fatty acid; NEFA, non-esterified fatty acid; PG, prostaglandin; PLS-DA, partial least squares discriminant analysis; PUFA, poly-unsaturated fatty acid; QC, quality control; TFA, total (aggregate-esterified) fatty acid; TLE, total lipid extract; UPLC, ultra-performance liquid chromatography

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demonstrate an increased proportion of sebum diacylglycerol species that is correlated to disease severity [7], and the sebum of individuals with papulopustular rosacea contains a greater proportion of saturated fatty acids compared to controls [6].

In addition to the lipids described above, there has been some indication that lipid-derivatives such as prostaglandins (PGs), endocannabinoids such as anandamide (AEA) and 2-arachidonylglycerol (2-AG), ceramides and other sphingolipids all play roles in cutaneous disease [9]. These and other related compounds are all members of the bioactive lipid mediator family, a super-class of fatty acid-derivatives that display biological activity and are capable of regulating a variety of processes, including inflammation, cell growth and differentiation, and vascular homeostasis [10]. With respect to sebum and sebum-associated diseases, 15-deoxy PGJ2, AEA and 2-AG are known to enhance lipid synthesis in sebocyte culture models in a manner resembling acne vulgaris [11,12], and several lipid mediator-generating enzyme systems such as cyclooxygenase (COX) − 2, 5-lipoxygenase (LOX) and leukotriene (LT) A4 hydrolase have increased expression in sebocytes cultured from acne lesions [13]. However, to the best of our knowledge bioactive lipid mediators in sebum have yet to be characterized.

In an effort to further the understanding of sebum composition and its relevance to cutaneous disease, the present study aims to quantitatively characterize the presence of 38 fatty acids in both their non-esterified ("NEFA") and aggregate esterified ("total fatty acid", TFA) forms, as well as > 150 bioactive lipid mediators including oxygenated lipids ("oxylipins"), nitrolipids, endocannabinoids and endocannabinoid-like compounds, and sphingolipids in subjects with and without atopic dermatitis (AD) from a single sebum sample extraction. Secondarily, we compare these profiles to those recently characterized in the sweat of many of the same subjects [14], to identify similarities and differences in the lipid mediator content of the two major cutaneous secretions. Characterization and quantitation of these compounds in sebum will enhance our understanding of cutaneous biochemistry, and may suggest novel biomarker and therapeutic targets for cutaneous diseases.

2. Materials and methods

2.1. Chemicals and reagents

Methanol, acetonitrile, isopropanol, toluene and hexane used during sample preparation, ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) analysis and/or gas chromatography-mass spectrometry (GC–MS) analysis were of UPLC-grade or better and were purchased from Fisher Scientific (Waltham, MA). Sodium methoxide (0.5 M), methanolic hydrochloric acid (3 N) and trimethylsilyl-diazomethane (2 M) used during sample preparation and glacial acetic acid, formic acid, ammonium hydroxide (10 M) used during UPLC-MS/MS analysis were purchased from either Fisher Scientific or Sigma Aldrich (St Louis, MO). Fatty acid and lipid mediator standards, analytical surrogates, and internal standards were synthesized or purchased from Cayman Chemicals (Ann Arbor, MI), Avanti Polar Lipids, Inc. (Alabaster, AL), Larodan (Malmo, Sweden), Nu-Chek Prep, Inc. (Elysian, MN), or CDN Isotopes (Pointe-Claire, Canada).

2.2. Subject recruitment and study design

This study uses the same study population as our previous work examining the sweat lipid mediator profile, and details on subject recruitment and inclusion and exclusion criteria can be found in the associated manuscript [14]. Briefly, 26 subjects (n = 13 each with and without AD) were recruited from the greater Sacramento, CA metropolitan area for this study between February 2015 and February 2016. Inclusion criteria included either a diagnosis of AD by a board-certified dermatologist or the absence of any inflammatory skin conditions, and exclusion criteria included current use of systemic immunosuppressive medications. All subjects with AD were sampled while they were in the quiescent state. Written informed consent was obtained from all subjects prior to participation in the study, and all study protocols were approved by the Institutional Review Board of the University of California–Davis (Protocol #605,131).

Subjects participated in a single study visit that lasted approximately one hour. Prior to their study visit, subjects were asked to refrain from use of any topical moisturizers or medications for at least 12 h. Sebum was collected from approximately 5.5 cm^2 areas located at non-lesional sites on the bilateral cheeks using Sebutape® Adhesive Patches (CuDerm Corporation, Dallas, TX). An image of the Sebutape® patch placed on the bilateral cheeks is available in **Supplemental Fig. S1**. Prior to sebum collection, subjects were acclimated to the ambient environment for 15 min, the collection area was wiped with a 70% isopropanol swab (Covidien, Minneapolis, MN) and one Sebutape® patch was placed on each cheek using a pair of methanol-rinsed forceps. Sebutape® patches were left in place for 1 h, after which they were removed using a pair of methanol-rinsed forceps and placed on Sebutape® Clear View PRO Storage Cards (CuDerm Corporation) that were stored at –80 °C in commercially available re-sealable zipper storage bags until analysis.

Skin sebum levels were measured at a non-lesional site immediately adjacent to each collection site using a Sebumeter® SM 815 (Courage and Khazaka Electronic GmbH, Cologne, Germany), and transepidermal water loss was measured in triplicate at the same sites using a Tewameter® TM 300 (Courage and Khazaka Electronic GmbH), both in accordance with manufacturer instructions. Sebum levels were reported in units of micrograms of sebum per square centimeter of skin (μg/cm^2) and transepidermal water loss was reported in units of grams of water lost per hour per square meter of skin (g/h/m^2). Sebum levels and transepidermal water loss measurements are available in Table 1.

Of the 26 subjects recruited, six were excluded. Two subjects with and three subjects without AD did not have measurable sebum levels as measured using the Sebumeter®, and one subject without AD had flared acne vulgaris at the time of sampling. Therefore, the study proceeded with 11 subjects with and 9 subjects without AD. Group characteristics are shown in Table 1.

2.3. Analysis of sebum lipid mediators and fatty acids

2.3.1. Preparation of the total lipid extract (TLE)

Oxylipins, nitrolipids, endocannabinoids and endocannabinoid-like

Table 1
Sampling and storage parameters of sebum collected from subjects with and without atopic dermatitis. All data reported as geometric mean [range].

Parameter	AD male (n = 6)	AD female (n = 5)	Control male (n = 5)	Control female (n = 4)
Age (yr)	30.3 [23.1 – 38.5]	42.0 [26.6 – 62.2][a]	32.0 [29.0 – 38.1]	28.8 [26.7 – 30.9]
Sebumeter® reading (μg/cm^2)[b]	33.1 [6 – 73]	8.8 [1 – 28]	36.1 [3 – 123]	39 [20 – 61]
Transepidermal water loss (g/h/m^2)[c]	18.6 [8.4 – 34.8]	9.5 [2.8 – 17.5]	13.6 [3.9 – 38.2]	9.5 [5.3 – 21.6]

[a] These data include two female subjects with AD who were outliers with respect to age (62.2 yr and 57.6 yr vs. $AD_{n=9}$ = 31.2 [23.1 – 46.3]), but not with respect to observed lipid mediators.

[b] $P > 0.05$ for all fixed effects when analyzed using a full factorial MANOVA approach, testing for the effects of disease, gender, and the interaction between them. Disease, gender and their interaction were included as fixed effects, and subject was included as a random effect.

[c] $P = 0.048$ for gender when analyzed using a full factorial MANOVA approach, testing for the effects of disease, gender, and the interaction between them. Disease, gender and their interaction were included as fixed effects, and subject was included as a random effect. $P > 0.05$ for all other fixed effects.