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Effects of stimulation technique, anatomical region, and time on human sweat lipid mediator profiles

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Abstract

Few studies compare sampling protocol effect on sweat composition. Here we evaluate the impact of sweat stimulation mode and site of collection on lipid mediator composition. Sweat from healthy males ($n = 7$) was collected weekly for three weeks from the volar forearm following either pilocarpine iontophoresis or exercise, and from the forearm, back and thigh following pilocarpine iontophoresis only. Sweat content of over 150 lipid mediators were measured by liquid chromatography-tandem mass spectrometry. Seventy lipid mediators were routinely detected, including prostanoids, alcohols, diols, epoxides, ketones, nitrolipids, N-acylethanolamides, monoacylglycerols, and ceramides. Detected lipid mediators appeared unaffected by sampling site, though the forearm was the most consistent source of sweat. Pilocarpine-induced sweat showed increased concentrations of most detected compounds. Moreover, lipid mediator concentrations and profiles were temporally stable over the study duration. Sweat therefore appears to be a consistent and anatomically-stable source of lipid mediators, but care must be taken in comparing results obtained from different stimulation techniques.

Keywords

oxygenated lipids; endocannabinoids; sphingolipids; metabolic profiling; exercise; pilocarpine

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Author Contributions

K.A., J.D.W., and J.W.N. designed the study; K.A. enrolled study participants; K.A. and J.D.W. conducted study visits; T.L.P. and J.W.N. developed the oxylipin/NAE/MAG fusion analytical method used in this study; K.A., T.L.P. and J.W.N. generated lipid mediator data and performed data analysis and interpretation; and K.A., J.D.W. and J.W.N. wrote the manuscript. All authors reviewed and approved the submitted manuscript

Conflicts of Interest

The authors declare no conflicts of interest.

Introduction

Sweat is a complex hypotonic biofluid produced by the eccrine, apocrine and apoeccrine glands of the skin, and along with sebum, is one of the two major cutaneous secretions. Reported components of sweat were traditionally limited to water, electrolytes, urea, lactate, amino acids, metals and xenobiotics [1], though recent studies have revealed that sweat also contains proteins, carbohydrates organic acids, and lipid-derived molecules, including PGE2 [2–6]. In fact, eccrine sweat contains numerous lipid mediators including a wide array of oxygenated lipids (i.e. oxylipins), nitrolipids, endocannabinoids and endocannabinoid-like N-acylethanolamides (NAEs) and monoacylglycerols (MAGs), ceramides and sphingoid bases [7]. Therefore, sweat is a rich source of small and large biomolecules, and omics analysis of sweat is a field with great interest and potential for future research [1]. It should be noted that most studies involving sweat bioanalysis have collected sweat from anatomical sites that contain only eccrine sweat glands, and a relatively low density of sebaceous glands [8], which has led to sweat being traditionally defined as predominantly eccrine sweat [9], a convention maintained in the current manuscript as well. However, one should always acknowledge that the liquid secretions of the skin which have physiologically important evaporative cooling properties may have mixed secretory compositions depending on the site and mode of collection.

To obtain sufficient quantities of sweat for analyses, sweating is usually stimulated either pharmacologically using pilocarpine iontophoresis, or physiologically with exercise or exposure to elevated temperatures in a sauna [9]. However, different methods of sweat stimulation can differentially affect sweat composition. For instance, both exercise and sauna increase interleukin (IL)-1 concentrations in sweat compared to pilocarpine iontophoresis, and sauna produces higher concentrations of sweat IL-1 compared to exercise [10]. Similarly, thermal exposure and running generate different sweat electrolyte, creatinine and urea profiles [11]. Moreover, sweat is generally collected from the forearm, though collection from other anatomical sites such as the leg, bicep, torso, forehead or axilla have been reported [9]. As with compositional variation associated with stimulation techniques, variations with collection site are also reported. For instance, the ratio of IL-1α to IL-1β appears to change depending on whether the palmar/plantar surface or torso is sampled [10], though sampling from the forearm, back, chest, forehead or thigh does not seem to affect sweat sodium or potassium concentrations [12]. Therefore, the effects of sampling site on sweat composition are less clear.

Given that IL-1 activity directly affects eicosanoid formation [13], it seems plausible that different sweat stimulation techniques and collection sites could impact sweat lipid mediator profiles, and such effects should be considered when interpreting these data. The primary goal of this study is to compare the sweat mediator lipidome obtained following either pharmacological (pilocarpine iontophoresis) or physiological (moderate exercise) stimulation, as well as the sweat mediator lipidome obtained from the forearm, back and thigh following pilocarpine iontophoresis. Secondary study goals compare the temporal stability and intra- and inter-individual variability of the pharmacologically stimulated sweat lipid mediator profile obtained from the volar forearm. Understanding the stability and

variability of sweat lipid mediators is critical given the preclinical diagnostic potential of these compounds in atopic dermatitis [7], and the possibility that sweat bioanalysis could enhance our understanding of cutaneous biochemistry, while ensuring minimal subject discomfort.

Materials and Methods

Subjects

Healthy male subjects aged 20–40 yr ($n = 10$) were recruited from the Sacramento, CA, USA metropolitan area between October and November 2016 to participate in this study. Eligibility for participation was assessed by an in-person screening questionnaire completed at recruitment. Exclusion criteria included: a diagnosed disease for which the subject was currently taking medication; recent hospitalization, surgery or antibiotic therapy; regular consumption of prescription or over-the-counter medications such as statins, steroids, weight loss aids, or non-steroidal anti-inflammatory drugs; BMI < 18.5 kg/m^2 or > 30 kg/m^2; orthopedic or cardiovascular limitations that precluded participation in moderate exercise; and regular performance of physical activity defined as “vigorous” by the Centers for Disease Control and Prevention [14]. Written informed consent was obtained from all subjects prior to participation, and all study protocols were approved by the Institutional Review Board of the University of California-Davis (Protocol #929370). This study is registered with ClinicalTrials.gov as NCT02935894.

Of the 10 subjects recruited, one did not comply with study requirements, and two did not produce sweat upon stimulation by pilocarpine iontophoresis. Therefore, the study proceeded with seven subjects. Baseline physiological characteristics of the subjects are listed in Supplemental Table S1.

Study Design and Sweat Collection

Subjects participated in three study visits, separated by approximately one week, at the United States Department of Agriculture’s Western Human Nutrition Research Center in Davis, CA, USA. All study visits started between 07:00 and 08:30 to avoid potential circadian effects on lipid mediators. Prior to each study visit, subjects were asked to fast for 12 h, not to apply any creams or topical medications for 24 h, and refrain from use of non-steroidal anti-inflammatory drugs such as aspirin, acetaminophen, or ibuprofen for 48 h. Subjects remained fasted during study visits, but *ad libitum* water intake was encouraged. During the first study visit, subjects completed a Physical Activity Readiness Questionnaire [15], and their height and weight were measured, to ensure compliance with study requirements.

Sweat was collected in environmentally regulated rooms (23 ± 1 °C; 35 ± 6% relative humidity) from an ~7 cm^2 area of skin by capillary action using Macroduct® sweat collectors (Wescor, Inc., Logan, UT, USA). At each study visit, sweat was stimulated by pilocarpine iontophoresis and collected from the right volar forearm (5–10 cm above the wrist) using the Macroduct® system’s Webster Sweat Inducer (Wescor, Inc.). During the first study visit, sweat was also stimulated with moderate exercise (see description below) and

collected from the left volar forearm (5–10 cm above the wrist). During the second study visit, sweat was also stimulated by pilocarpine iontophoresis and collected from the right anterior distal thigh (3–5 cm above the knee), and during the third study visit, sweat was also stimulated by pilocarpine iontophoresis and collected from the region of the back corresponding to the vertebrae T11 to L2 (5–10 cm to the right of the spine). All procedures involving the Macroduct® sweat collection system were in accordance with previously published protocols [7]. Images of the sampling sites and stimulation protocols are available in Supplemental Fig. S1.

Collected sweat was exuded into methanol-rinsed 2 mL amber vials with Teflon-lined closures (Waters Corporation, Milford, MA, USA) by passing air through the collection tubing three times using a 250 μL gastight syringe (Hamilton, Reno, NV, USA). Samples were stored at −80 °C for a maximum of one week, to control for and minimize storage duration.

Stimulation of Sweat by Moderate Exercise

Sweat stimulation by physiological methods was achieved by a 15-min exercise period on a Monark Ergomedic 828E cycle ergometer (Vansbro, Sweden). Prior to the start of the exercise period, subjects completed a 5-min warm-up on the cycle ergometer, during which time flywheel resistance was progressively increased so as to elicit a graded elevation in heart rate (HR). The increase in flywheel resistance was continued until an exercise HR equivalent to 60% heart rate reserve (HRR) was achieved. Exercise HR was determined using the Karvonen method, a model widely understood to correlate well with % maximum oxygen consumption (VO_2 max) and VO_2 reserve, both excellent predictors of exercise intensity [16, 17]. A range of 60–80% exercise HRR was selected to ensure sufficient sweat production and to standardize exercise intensity across participants. Achievement of 60% HRR commenced the exercise period, which consisted of 15 min of cadence-controlled (50 rpm) pedaling at a resistance sufficient to maintain exercise HR within the 60–80% HRR target zone. Following completion of the exercise period, a cooldown period was provided until subject was comfortable with exiting the cycle ergometer.

HR was monitored at rest and throughout exercise and cool down via a V800 Bluetooth HR monitor (Polar Electro, Inc., Lake Success, NY, USA) and TrueOne® 2400 HR sensor (Parvo Medics, Sandy, UT, USA). Respiratory exchange ratio and VO_2 were continuously monitored throughout exercise with a TrueOne® 2400 (Parvo Medics). Every three minutes, subjects provided a rating of perceived exertion (Borg scale), a subjective intensity measure shown to agree with exercise intensity and HR [18]. Measured HR, VO_2, and other exercise parameters are available in Supplemental Table S1, and demonstrate the uniformity of exercise intensity across subjects.

Prior to the start of the warm-up period, the left volar forearm was wiped with a 70% isopropanol swab (Fisher Scientific, Waltham, MA, USA) followed by a distilled water-saturated cotton gauze pad (Fisher Scientific) to keep sweat collection methodology consistent with those used after pilocarpine iontophoresis. Immediately prior to the start of the exercise period, the left volar forearm was again wiped with a fresh distilled water-saturated cotton gauze pad and then dried with another cotton gauze pad. After this, the

Macroduct® sweat collector was attached to the forearm 5–10 cm above the wrist, secured with an elastic band, and the subject was allowed to begin the exercise period. The Macroduct® sweat collector remained in place throughout the exercise period and was removed from the subject's forearm 15 min after the exercise period ended (total contact time of 30 min). Collected sweat was then exuded and stored as described in *Study Design and Sweat Collection* above.

Analysis of Sweat Lipid Mediators

Oxylipins, nitrolipids, free fatty acids, NAEs, MAGs, ceramides and sphingoid bases were isolated from sweat by direct evaporation of the matrix, resuspended in organic solvents and analyzed by ultra-performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS), using modifications of a previously published protocol [7]. Briefly, collected sweat volume was determined for each sample using either a 50 μL or 100 μL gastight syringe (Hamilton), and samples were enriched with 5 μL of an anti-oxidant solution (0.2 mg/mL butylated hydroxytoluene/EDTA in 1:1 methanol:water), 2 μL of a 500 nM deuterated-oxylipin/NAE/MAG surrogate solution in methanol, and 5 μL of a 1000 nM C17-analog ceramide surrogate solution in methanol. A complete list of analytical targets and their associated surrogates is shown in Supplemental Table S2A and Supplemental Table S3A. Each sample was then mixed with 100 μL of methanol and 10 μL of a 20% glycerol solution in methanol, and the solvent was evaporated under vacuum (GeneVac EZ-2 Personal Evaporator, SP Scientific, Warminster, PA, USA). Dried samples were reconstituted in 40 μL of an internal standard solution containing 50 nM each of 1-cyclohexyl-3-ureido dodecanoic acid (Sigma-Aldrich, St Louis, MO, USA) and 1-phenyl,3-ureido hexanoic acid (gift from B. D. Hammock, University of California-Davis) in 1:1 (v/v) methanol:acetonitrile prior to analysis. Reconstituted samples were injected (10 μL) onto a Shimadzu Nexera X2 series UPLC system (Columbia, MD) and oxylipins, nitrolipids, free fatty acids, NAEs and MAGs were separated on a 2.1 × 150 mm, 1.7 μm BEH C18 column (Waters) and detected with an API 6500 QTrap (Sciex, Framingham, MA, USA) with positive-negative mode switching electrospray ionization. A second 10 μL aliquot of the reconstituted sample was injected onto a Waters Acquity UPLC system and ceramides and sphingoid bases were separated on a 2.1 × 100 mm, 1.7 μm BEH C8 column (Waters) and detected with an API 4000 QTrap (Sciex) using positive mode electrospray ionization. Chromatographic solvent gradients are listed in Supplemental Table S2B and Supplemental Table S3B, and ionization voltages, MS/MS parameters, and retention times are listed in Supplemental Table S2A and Supplemental Table S3A.

Sample batches included method blanks and duplicate analysis of an in-house pooled sweat laboratory reference material (LRM) sample. Analytes were quantified using internal standard methodology with five- to seven-point calibration curves ($r \geq 0.997$). Data were processed using Sciex MultiQuant version 3.0.2 with lipid mediator concentrations reported in picomole per milliliter (i.e. nM) of collected sweat.

The nomenclature used to identify the lipid mediators examined in this study are described elsewhere [7], and all lipid mediator abbreviations are fully expanded in Supplemental Table S4.

Statistical Analysis

Statistical analysis generally followed a previously published protocol [19]. Prior to statistical analyses, data were curated such that analytes with >30% missing values across the data set were excluded. The remaining data were screened for outliers using Huber's maximum likelihood type estimates to determine the center and spread of each analyte [20]. Missing data were imputed by least squares multivariate normal imputation [21], after which data were transformed to normal using the generalized logarithm transform [22], and normality was verified using the Shapiro-Wilk test [23]. All data pretreatment was performed in JMP Pro 12 (SAS Institute, Inc., Cary, NC, USA).

To prevent differential storage times, samples were processed weekly along with a pooled sweat LRM. While within-batch precision was excellent, 16% of the LRM metabolites showed a significant correlation with batch ($r \geq 0.811$, $p \leq 0.05$, $n = 6$). Using the batch to concentration correlation for each lipid mediator detected in the LRM, sample results were batch adjusted calculating the batch-specific residuals and subtracting these from the measured data. Adjustment eliminated correlations between batch and lipid mediator concentration in the LRM and did not fundamentally alter the relationship in the experimental samples. Batch-adjusted data was then transformed to normal as described above. All batch adjustments were performed in Microsoft Excel 2010 (Redmond, WA, USA).

Comparisons of mean lipid mediator concentrations in sweat stimulated by pilocarpine iontophoresis and exercise were made by paired Student's *t*-tests. Comparisons of collection sites (anterior distal thigh, lower back and volar forearm) were made by one-way ANOVAs, while assessments of sweat lipid mediator temporal stability were made by repeated measures ANOVAs. Subject identification was included as a random effect in the one-way ANOVA models, as only two subjects produced sweat from all three collection sites. All univariate comparisons were performed in JMP Pro 12 (SAS Institute, Inc.) and adjusted for multiple comparisons using the Benjamini-Hochberg procedure at $q = 0.05$ [24].

Multivariate analyses were conducted by partial least squares discriminant analysis (PLS-DA), using two different algorithms in the R statistical environment (Vienna, Austria). Models were constructed using either the orthogonal scores algorithm with univariate scaling and leave-one-out cross-validation in imDEV version 1.42, a Microsoft Excel add-in [25], or using the *pls* and *caret* packages in R version 3.4.1 [26, 27]. In both cases sweat stimulation technique, collection site or study visit were used as classifiers, and variables were clustered by Spearman correlation coefficients using the Minkowski distance and Ward agglomeration. Models built using imDEV version 1.42 were evaluated using the Q2 score, with $Q2 \geq 0.4$ considered indicative of a predictive model [28]. Models built using the *pls* and *caret* packages in R version 3.4.1 were evaluated using model accuracy, and the significance of model accuracy was evaluated using a chi-squared test.

Results

Impact of Sweat Stimulation Method on the Lipid Mediator Profile

Pilocarpine- and exercise-induced sweat demonstrated different sweat lipid mediator profiles. Multivariate analysis demonstrated clear separation of groups in the PLS-DA scores plot (Fig. 1A), with a model Q2 = 0.8 and accuracy of 99% (χ^2 = 7.00, p = 0.009, n = 14), suggesting a highly predictive model. The PLS-DA loadings plot indicated increased concentrations of C18:1 sphingosine and C14-C24 [NS] ceramides, as well as alcohols, diols, epoxides, ketones and MAGs derived from arachidonate, linoleate, α-linolenate, eicosapentanoate and oleate in pilocarpine-induced sweat (Fig. 1B), and results were supported by univariate comparisons (Table 1). Exercise-induced sweat, on the other hand, contained increased concentrations of linoleate-derived triols (Table 1). The changes in individual sweat lipid mediator concentrations due to sweat stimulation method are fully documented in Supplemental Table S5A–C.

Examining the relative abundances of the detected lipid mediators within their respective classes, the relative proportions of sphingoid bases and MAGs were unaffected by sweat stimulation method. However, the class-specific proportions of oxylipins and NAEs were affected by sweat stimulation technique (Table 2). Changes in individual lipid mediator relative abundances are fully documented in Supplemental Table S5A–C.

Impact of Sampling Site on the Sweat Lipid Mediator Profile

Sweat collected from the volar forearm, the anterior distal thigh and the lower back all demonstrated similar lipid mediator profiles. Though the PLS-DA scores plot (Fig. 2) appears to show separation between the sweat lipid mediator profiles obtained from the lower back and anterior distal thigh, the model had a Q2 score of −0.1 and an accuracy of 55% (χ^2 = 0.21, p = 0.4, n = 21), suggesting lack of predictive power. Therefore, these apparent separations may be spurious. Univariate analyses of both the absolute concentrations and class-specific relative abundances of the lipid mediators also indicated no differences between sampling sites (Supplemental Table S6A–C).

However, as only four subjects were able to produce sweat from the thigh or lower back, and of these four subjects, only two subjects produced sweat from both sites, this analysis is likely under powered. By contrast, all seven subjects produced sweat from the forearm during the second study visit, and six of these subjects produced sweat from the forearm during the third study visit as well. Therefore, the volar forearm appears to be the most reliable source of sweat following stimulation by pilocarpine iontophoresis.

Temporal Stability of Sweat Lipid Mediators

Of the seven subjects sampled in this study, six produced sweat from the volar forearm following pilocarpine iontophoresis at all three study visits, and only their results are considered in this section. No temporal differences were detected between study visits by either multivariate analysis. Though the PLS-DA scores plot appears to show separation between the sweat lipid mediator profiles obtained at each study visit (Fig. 3), the model had a Q2 score of 0.2 and an accuracy of 57% (χ^2 = 0.11, p = 0.4, n = 18), suggesting a lack of

predictive power. Univariate analysis of absolute concentration or class-specific relative abundance of sweat lipid mediators at each study visit also indicated no difference between sweat lipid mediator profiles collected at each study visit (Supplemental Table S7A–C).

Inter- and Intra-Individual Variability of Sweat Lipid Mediators

As with temporal stability of sweat lipid mediators, only the results from the six subjects that produced sweat from the volar forearm at all three study visits are considered in this section. Considerable inter- and intra-individual variability in sweat lipid mediators (evaluated as the coefficient of variance) was observed when examining absolute concentrations, though intra-individual variance was always lower than inter-individual variance (Table 3). Coefficients of variance for individual sweat lipid mediators are available in Supplemental Table S8A–C, and a graphical representation of each subject's sweat lipid mediator concentrations at each study visit is available in Supplemental Fig. S2. These results suggest that individuals are more similar to themselves than each other with respect to their sweat lipid mediator concentrations.

Comparing the variability of the class-specific relative abundances of sweat lipid mediators, less variance was observed compared to absolute concentrations and inter- and intra-individual variance were almost equivalent for most analytes (Table 3 and Supplemental Table S8A–C). A graphical representation of each subject's sweat lipid mediator class-specific relative abundance profile is available in Supplemental Fig. S3. Collectively, these results suggest that while the concentration of sweat lipid mediators is highly variable, their pattern is generally more stable than their concentration across time and individuals.

Discussion

The emergence of sweat as a promising non-invasive clinical diagnostic matrix, and the knowledge that the method of sweat stimulation and site of collection can impact sweat composition, makes it important to understand how target compounds of interest are affected by the chosen sweat sampling protocol. With respect to sweat lipid mediators, it has been demonstrated that ceramides and sphingosines are elevated in the sweat of individuals with atopic dermatitis [7], and that IL-1 levels which can directly affect eicosanoid formation [13] are affected by sampling site and sweat stimulation method [10]. This makes it all the more important to understand the stability and variability of lipid mediators in sweat.

Comparison of sweat PGE2 levels collected from the forearm of healthy individuals following either pilocarpine iontophoresis (2.19 ± 3.02 nM, $n = 12$) [7] or thermal induction of sweating (1.77 ± 1.22 nM, $n = 14$) [6], suggests that sweat stimulation technique does not alter PGE2 concentration ($P = 0.7$, 2-tailed heteroscedastic Student's t-test). This study supports this finding, as sweat PGE2 levels were not affected by either pilocarpine iontophoresis or exercise stimulation in the same subjects ($P = 0.2$, Supplemental Table S5A). However, a suite of other lipid mediators including polyunsaturated fatty acid (PUFA)- derived alcohols, diols, triols, epoxides and ketones, as well as NAEs, MAGs and ceramides were affected by sweat stimulation technique, and with the exception of the linoleate-derived triols, concentrations of all of these lipid mediators increased in sweat collected following stimulation by pilocarpine iontophoresis. It is therefore advisable to

exercise caution when considering comparisons between studies involving sweat composition, particularly when sweat stimulation techniques differ between the studies.

The sources of sweat lipid mediators and the mechanism by which they are being incorporated into a predominantly aqueous matrix such as sweat are still unknown. Without this information, it is difficult to identify the mechanism by which pilocarpine increases sweat lipid mediator concentrations. However, pilocarpine has been shown to be a potent *in vitro* oxidant of linoleic acid [29] and an inducer of lipase activity in the liver [30]. A related muscarinic agonist, bethanechol, has also been demonstrated to increase permeability of the intestinal epithelium *in vitro* [31]. Therefore, it is possible to speculate that the increase in sweat lipid mediators following pilocarpine iontophoresis is due to either increased oxygenation of available fatty acid precursors, increased production of fatty acid precursors, increased permeability of the sweat gland, or some combination of these three possibilities. Oxidation of PUFAs by pilocarpine to form sweat lipid mediators appears less likely since neither of the major lipid mediator-associated auto-oxidative markers (i.e. F2-isoprostanes or linoleate-derived hydroperoxides) were detected in the samples. However, a minor auto-oxidative product, 9-HETE, was detected in sweat, so we cannot completely rule out pilocarpine-induced oxidation as a reason for increased concentrations of lipid mediators in pharmacologically-stimulated sweat. Further experimentation would be needed to establish the actual mechanism by which pilocarpine impacts sweat lipid mediator concentrations. Examples of such experiments could include sampling cultured dermal fibroblasts and sweat gland epithelial cells before and after the addition of pilocarpine, and permeability tests of cultured sweat gland epithelium in the presence and absence of pilocarpine.

Regarding sampling sites, the volar forearm has been the most popular site for sweat collection in omics studies using pilocarpine iontophoresis [9]. Advantages of this site include a low density of sebaceous glands, minimizing the contamination of collected sweat by sebum [8], its relatively hairless, sun-protected nature, and relative accessibility, which ensures minimal discomfort to subjects. For this study, we chose the lower back and anterior distal thigh as alternative sampling sites because these sites have resting and exercise-induced sweating rates that are greater than or equal to the forearm [32], and like the volar forearm are also relatively hairless, contain a low density of sebaceous glands, and are generally sun-protected. Additionally, few cultural barriers exist for accessing these anatomical locations, compared to other sites such as the chest or buttocks that have been examined in other sweat studies [9]. Collectively, the choice of these alternative sites allowed for the evaluation of the anatomical variability of sweat lipid mediators, without the presence of confounders such as sebaceous contamination or differential sweating rates, and also allows direct comparison of the sweat lipid mediator profile at these sites to the previously characterized sweat lipid mediator profile at the volar forearm [7].

Our findings that sampling site appeared not to impact the sweat lipid mediator profile is in contrast to the strong impact sampling site did have on the ratio of IL-1α to IL-1β [10], which is perhaps surprising given the strong associations between IL-1 and eicosanoids [13]. However, our sample size was small, particularly for the lower back and anterior distal thigh, and sweat production was very inconsistent from these alternative sites, which could confound our findings. Furthermore, our findings were in pilocarpine-induced sweat,

whereas the IL-1 ratios were measured in sweat collected following either exercise or sauna [10], and the use of different sweat stimulation techniques may help explain the differences in the two studies, as has already been pointed out in this manuscript. It is therefore difficult to make conclusive statements regarding the impact of the chosen collection sites on pilocarpine-induced eccrine sweat. Therefore, while weak, our data do not support an anatomical difference in sweat lipid mediator profiles.

Some of the inconsistencies in our sweat collection from sites other than the volar forearm may be explained by the fact that the Macroduct® sweat collection system was optimized for sweat collection from the forearm [33], and not sites such as the thigh or back. Therefore, application of this device to alternative anatomical sites may not result in optimal pilocarpine delivery to the cutaneous muscarinic receptors, possibly due to differential skin thickness or resistance, which could impact sweat yield. Additionally, to assure a flat surface for sweat collector placement as specified by the manufacturer [33], subjects were asked to bend the knee at a 90° angle or lean forward for the 30 min collection period, which caused discomfort to all subjects, although not so much discomfort as to be unbearable. It is possible that minor adjustments made by the subjects during this collection period to increase comfort could have displaced the sweat collector, which again would impact sweat yield. Finally, our choice of a 5–10 cm distance from the spine to ensure a flat collection surface for sweat collection from the back could have resulted in a lower sweat yield, as sweating rates on the back are inversely proportional to the distance from the spine [34]. The use of exercise and/or sauna as sweat stimulation techniques would avoid some of these difficulties associated with using the Macroduct® sweat collection system at an anatomical location other than the volar forearm, and would also allow for a more direct comparison to the results of Didierjean et al [10]. Therefore, to definitively address the anatomical stability of sweat lipid mediator profiles, future studies should consider addressing these experimental limitations.

It should also be noted that no evaluation of the impact of sampling site on sweat composition is complete without characterizing the lipid mediator profile of sweat collected from sites such as the forehead or axilla, which contain a high density of sebaceous glands or apocrine sweat glands, respectively. Sebum and apocrine sweat are other major cutaneous secretions, but they have distinct stimulatory and secretory mechanisms [9, 35, 36], and at this time their composition is poorly characterized. Evidence exists for the presence of lipid mediators in sebum [37], and to the best of our knowledge, no evidence exists documenting the presence of lipid mediators in apocrine sweat. However, for this study we have chosen to ignore sites that are rich in other cutaneous secretions in addition to eccrine sweat, as without a comprehensive understanding of the lipid mediator profile in each matrix (i.e. eccrine sweat, apocrine sweat and sebum), it is difficult to appreciate the individual impacts these matrices may have on a mixed sweat lipid mediator profile, which could affect data analysis and interpretation. Our previous work has focused on eccrine sweat lipid mediators [7], and therefore we have chosen to continue with this matrix for the present study. However, we have evidence that the sebum lipid mediator profile is distinct from the sweat lipid mediator profile (unpublished results), and therefore hope to incorporate sebum-rich anatomical sites such as the face and upper torso in future studies skin lipid mediator secretions. In the interim, it is hoped that other researchers will continue to evaluate the

impact of anatomical site on the sweat lipid mediator profile, with a focus on areas where it is possible to collect a more mixed sweat sample.

The high inter-individual variability and comparatively lower intra-individual variability of sweat lipid mediators is consistent with other studies examining individual variability of lipid mediators in other matrices such as skin, plasma and serum [38–41]. Factors which could promote metabolic variance in lipid mediator profiles may include genetic, metabolic, microbiotic and/or lifestyle variables (e.g. diet, medication and cosmetic use), subtle differences in the dose of pilocarpine delivery, or subject hydration. However, the comparatively low variability of class-specific relative abundances of sweat lipid mediators, a finding that is consistent with published literature examining skin lipid mediators [41], suggests that despite metabolic and lifestyle differences and/or potential sampling confounders, the relative proportions of lipid mediators in predominantly eccrine sweat are more consistent within and between individuals. Additionally, given that no differences were observed in concentrations of sweat lipid mediators sampled from the volar forearm at each of the three study visits following pilocarpine iontophoresis, it can be inferred that eccrine sweat is a reliable and consistent source of lipid mediators.

Interestingly, despite the high inter-individual variability of sweat lipid mediators, these metabolites have shown diagnostic potential, as concentrations of sweat ceramides and sphingoid bases are increased in individuals with atopic dermatitis compared to controls [7]. In the cited study, individual diets, medications and cosmetic use were again not controlled, which more closely simulates real-world conditions. However, if the data from our previous work [7] are re-examined looking at lipid mediator class-specific relative abundances, there appear to be no differences between individuals with and without atopic dermatitis (Supplemental Table S9A–C). This discrepancy may be due to the fact that individuals with atopic dermatitis demonstrate increased ceramide synthase 4 activity, which uniformly increases synthesis of C30-C40 [NS] and [NdS] ceramides [42], and therefore a study such as that by Agrawal et al. [7] which measures only these ceramides would not be able to detect any changes in their relative proportions. Therefore, despite their high variability, it is still worth considering absolute concentrations of sweat lipid mediators, though in some cases, consideration of class-specific relative abundances of lipid mediators may be more appropriate, particularly where a complete biological system is affected rather than a discrete pathway.

Overall, this study finds that eccrine sweat is a consistent source of lipid mediators, but care must be taken in comparing results obtained from different sweat stimulation techniques. Results involving eccrine sweat collected from different anatomical sites appear less variable, though further study are needed to fully evaluate the impact of sampling site on sweat lipid mediator composition. Future studies should include understanding the sources from and mechanisms by which lipid mediators are incorporated into eccrine sweat, further characterization of the impact of sampling site on sweat lipid mediators, and evaluation of the diagnostic potential of sweat in inflammatory skin diseases such as acne and psoriasis. It is hoped that these studies, along with previously published work, will culminate in a more comprehensive understanding of the utility of sweat and its lipid mediator profile, and the

presentation of a non-invasive tool to better understand cutaneous immunomodulation and biochemistry.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

HR	heart rate
HRR	heart rate reserve
IL	interleukin
LRM	laboratory reference material
MAGs	monoacylglycerols
MS/MS	tandem mass spectrometry
NAEs	N-acylethanolamides
PLS-DA	partial least squares discriminant analysis
PUFA	polyunsaturated fatty acid
UPLC	ultra-performance liquid chromatography
VO_2	oxygen capacity

References

1. Mena-Bravo A, Luque de Castro MD. Sweat: a sample with limited present applications and promising future in metabolomics. J Pharm Biomed Anal. 2014; 90:139–47. DOI: 10.1016/j.jpba.2013.10.048 [PubMed: 24378610]
2. Calderon-Santiago M, Priego-Capote F, Jurado-Gamez B, Luque de Castro MD. Optimization study for metabolomics analysis of human sweat by liquid chromatography-tandem mass spectrometry in high resolution mode. J Chromatogr A. 2014; 1333:70–8. DOI: 10.1016/j.chroma.2014.01.071 [PubMed: 24529403]

3. Calderon-Santiago M, Priego-Capote F, Turck N, Robin X, Jurado-Gamez B, Sanchez JC, Luque de Castro MD. Human sweat metabolomics for lung cancer screening. Anal Bioanal Chem. 2015; 407(18):5381–92. DOI: 10.1007/s00216-015-8700-8 [PubMed: 25935675]
4. Delgado-Povedano MM, Calderón-Santiago M, Priego-Capote F, Luque de Castro MD. Development of a method for enhancing metabolomics coverage of human sweat by gas chromatography-mass spectrometry in high resolution mode. Anal Chim Acta. 2016; 905:115–125. DOI: 10.1016/j.aca.2015.11.048 [PubMed: 26755145]
5. Harker M, Coulson H, Fairweather I, Taylor D, Daykin CA. Study of metabolite composition of eccrine sweat from healthy male and female human subjects by 1H NMR spectroscopy. Metabolomics. 2006; 2(3):105–112. DOI: 10.1007/s11306-006-0024-4
6. Forstrom L, Goldyne ME, Winkelmann RK. Prostaglandin activity in human eccrine sweat. Prostaglandins. 1974; 7(6):459–64. [PubMed: 4415343]
7. Agrawal K, Hassoun LA, Foolad N, Pedersen TL, Sivamani RK, Newman JW. Sweat lipid mediator profiling: a noninvasive approach for cutaneous research. J Lipid Res. 2017; 58(1):188–195. DOI: 10.1194/jlr.M071738 [PubMed: 27875258]
8. Strauss, JS., Downing, DT., Ebling, JF., Stewart, ME. Sebaceous glands. In: La, G., editor. Physiology Biochemistry and Molecular Biology of the Skin. Oxford University Press; New York: 1991. p. 712-740.
9. Hussain JN, Mantri N, Cohen MM. Working up a good sweat - the challenges of standardising sweat collection for metabolomics analysis. Clin Biochem Rev. 2017; 38(1):13–34. [PubMed: 28798503]
10. Didierjean L, Gruaz D, Frobert Y, Grassi J, Dayer JM, Saurat JH. Biologically active interleukin 1 in human eccrine sweat: Site-dependent variations in α/β ratios and stress-induced increased excretion. Cytokine. 1990; 2(6):438–446. DOI: 10.1016/1043-4666(90)90053-V [PubMed: 2104237]
11. Fukumoto T, Tanaka T, Fujioka H, Yoshihara S, Ochi T, Kuroiwa A. Differences in composition of sweat induced by thermal exposure and by running exercise. Clin Cardiol. 1988; 11(10):707–709. DOI: 10.1002/dc.49601U010 [PubMed: 3224454]
12. Baker LB, Stofan JR, Hamilton AA, Horswill CA. Comparison of regional patch collection vs. whole body washdown for measuring sweat sodium and potassium loss during exercise. J Appl Physiol. 2009; 107(3):887–895. DOI: 10.1152/japplphysiol.00197.2009 [PubMed: 19541738]
13. Godessart N, Vila L, Puig L, de Moragas JM. Interleukin-1 increases 15-hydroxyeicosatetraenoic acid production in human dermal fibroblasts. J Invest Dermatol. 1994; 102(1):98–104. DOI: 10.1111/1523-1747.ep12371740 [PubMed: 8288917]
14. Centers for Disease Control and Prevention. Physical activity. 2015. https://www.cdc.gov/physicalactivity/basics/adults/. Accessed September 30, 2016
15. National Academy of Sports Medicine. Physical activity readiness questionnaire. https://www.nasm.org/docs/default-source/PDF/nasm_par-q-(pdf-21k).pdf (Accessed July 26 2016)
16. Swain DP, Leutholtz BC. Heart rate reserve is equivalent to %VO2 reserve, not to %VO2max. Medicine and science in sports and exercise. 1997; 29(3):410–4. [PubMed: 9139182]
17. King CN, Senn MD. Exercise testing and prescription. Sports Medicine. 1996; 21(5):326–336. doi: 610.2165/00007256-199621050-00002. [PubMed: 8724201]
18. Noble BJ. Clinical applications of perceived exertion. Medicine and Science in Sports and Exercise. 1982; 14(5):406–11. DOI: 10.1249/00005768-198205000-00016 [PubMed: 7154897]
19. Dunn T, Keenan A, Thomas A, Newman J, Adams S. A diet containing a nonfat dry milk matrix significantly alters systemic oxylipins and the endocannabinoid 2-arachidonoylglycerol (2-AG) in diet-induced obese mice. Nutrition & Metabolism. 2014; 11(1):24.doi: 10.1186/1743-7075-11-24 [PubMed: 24963334]
20. Huber, PJ., Ronchetti, EM. Robust Statistics. Second. John Wiley & Sons, Inc.; Hoboken, NJ: 2009. The Basic Types of Estimates; p. 53-84.
21. SAS Institute Inc.. JMP(R) 12 Basic Analysis. SAS Institute Inc.; Cary, NC: 2015. Modeling Utilities: Exploring Data for Outliers, Missingness, and Strong Predictors; p. 313-342.
22. Durbin BP, Hardin JS, Hawkins DM, Rocke DM. A variance-stabilizing transformation for gene-expression microarray data. Bioinformatics (Oxford, England). 2002; 18(Suppl 1):S105–10. DOI: 10.1093/bioinformatics/18.suppl_1.S105

23. Shapiro SS, Wilk MB. An analysis of variance test for normality (complete samples). Biometrika. 1965; 52(3–4):591–611. DOI: 10.1093/biomet/52.3-4.591
24. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J R Stat Soc Series B Stat Methodol. 1995; 57(1):289–300.
25. Grapov D, Newman JW. imDEV: a graphical user interface to R multivariate analysis tools in Microsoft Excel. Bioinformatics (Oxford). 2012; 28(17):2288–90. DOI: 10.1093/bioinformatics/bts439
26. Kuhn, M., Wing, J., Williams, A., Keefer, C., Engelhardt, A., Cooper, T., Mayer, Z., Kenkel, B., the R Core Team. Benesty, M., Lescarbeau, R., Ziem, A., Scrucca, L., Tang, Y., Candan, C., Hunt, T. caret: Classification and regression training R package version 6.0-76. 2017. https://cran.r-project.org/package=caret
27. Mevik, BH., Wehrens, R., Liland, KH. pls: Partial least squares and principal component regression R package version 2.6-0. 2016. https://cran.r-project.org/package=pls
28. Westerhuis JA, Hoefsloot HCJ, Smit S, Vis DJ, Smilde AK, van Velzen EJJ, van Duijnhoven JPM, van Dorsten FA. Assessment of PLSDA cross validation. Metabolomics. 2008; 4(1):81–89. DOI: 10.1007/s11306-007-0099-6
29. Matsushita S, Ibuki F. Antioxidative and prooxidative abilities of some biological substances and physiologically active substances on the oxidation of unsaturated fatty acids. Agric Biol Chem. 1965; 29(9):792–795. DOI: 10.1080/00021369.1965.10858470
30. Singh H, Appert HA. Effect of pilocarpine and pancreatectomy on amylase and lipase levels of serum and hepatic lymph. Proc Soc Exp Biol Med. 1969; 130(4):1122–1125. DOI: 10.3181/00379727-130-33733 [PubMed: 5779988]
31. Cameron HL, Perdue MH. Muscarinic acetylcholine receptor activation increases transcellular transport of macromolecules across mouse and human intestinal epithelium in vitro. Neurogastroenterology & Motility. 2007; 19(1):47–56. DOI: 10.1111/j.1365-2982.2006.00845.x
32. Taylor NAS, Machado-Moreira CA. Regional variations in transepidermal water loss, eccrine sweat gland density, sweat secretion rates and electrolyte composition in resting and exercising humans. Extreme Physiology & Medicine. 2013; 2:4.doi: 10.1186/2046-7648-2-4 [PubMed: 23849497]
33. Wescor, Inc. Macroduct Sweat Collection System Model 3700 SYS Instruction/Service Manual. 2004. http://www.wescor.com/translations/Translations/M2551-7A-EN.pdf. Accessed July 11, 2016
34. Smith CJ, Havenith G. Body mapping of sweating patterns in male athletes in mild exercise induced hyperthermia. Eur J Appl Physiol. 2011; 111(7):1391–1404. DOI: 10.1007/s00421-010-1744-8 [PubMed: 21153660]
35. Ebling FJ. Hormonal control and methods of measuring sebaceous gland activity. J Invest Dermatol. 1974; 62(3):161–71. [PubMed: 4361983]
36. Beier K, Ginez I, Schaller H. Localization of steroid hormone receptors in the apocrine sweat glands of the human axilla. Histochem Cell Biol. 2005; 123(1):61–65. DOI: 10.1007/s00418-004-0736-3 [PubMed: 15609040]
37. Iwata C, Akimoto N, Sato T, Morokuma Y, Ito A. Augmentation of lipogenesis by 15-deoxy-δ12,14-Prostaglandin J2 in hamster sebaceous glands: Identification of cytochrome P-450-mediated 15-deoxy-δ12,14-Prostaglandin J2 production. J Invest Dermatol. 2005; 125(5):865–872. DOI: 10.1111/j.0022-202X.2005.23866.x [PubMed: 16297182]
38. Jergovic M, Bendelja K, Savic Mlakar A, Vojvoda V, Aberle N, Jovanovic T, Rabatić S, Sabioncello A, Vidović A. Circulating levels of hormones, lipids, and immune mediators in post-traumatic stress disorder - a 3-month follow-up study. Frontiers in Psychiatry. 2015; 6(49)doi: 10.3389/fpsyt.2015.00049
39. Nording ML, Yang J, Georgi K, Hegedus Karbowski C, German JB, Weiss RH, Hogg RJ, Trygg J, Hammock BD, Zivkovic AM. Individual variation in lipidomic profiles of healthy subjects in response to omega-3 fatty acids. PLoS One. 2013; 8(10)doi: 10.1371/journal.pone.0076575
40. Zivkovic AM, Wiest MM, Nguyen U, Nording ML, Watkins SM, German JB. Assessing individual metabolic responsiveness to a lipid challenge using a targeted metabolomic approach. Metabolomics. 2008; 5(2):209.doi: 10.1007/s11306-008-0136-0

41. Kendall AC, Pilkington SM, Massey KA, Sassano G, Rhodes LE, Nicolaou A. Distribution of bioactive lipid mediators in human skin. J Invest Dermatol. 2015; 135(6):1510–1520. DOI: 10.1038/jid.2015.41 [PubMed: 25668241]

42. Ito S, Ishikawa J, Naoe A, Yoshida H, Hachiya A, Fujimura T, Kitahara T, Takema Y. Ceramide synthase 4 is highly expressed in involved skin of patients with atopic dermatitis. J Eur Acad Dermatol Venereol. 2016; 31(1):135–141. DOI: 10.1111/jdv.13777 [PubMed: 27358008]

HIGHLIGHTS

- Exercise- and pilocarpine-induced sweat show different lipid mediator profiles.
- Collection site differences in sweat lipid mediator content were not detected.
- Pilocarpine-induced sweat collection from volar forearm was most consistent.
- Lipid mediators are temporally stable across three-week study duration.
- Analyte composition varied less than concentration within and between subjects.

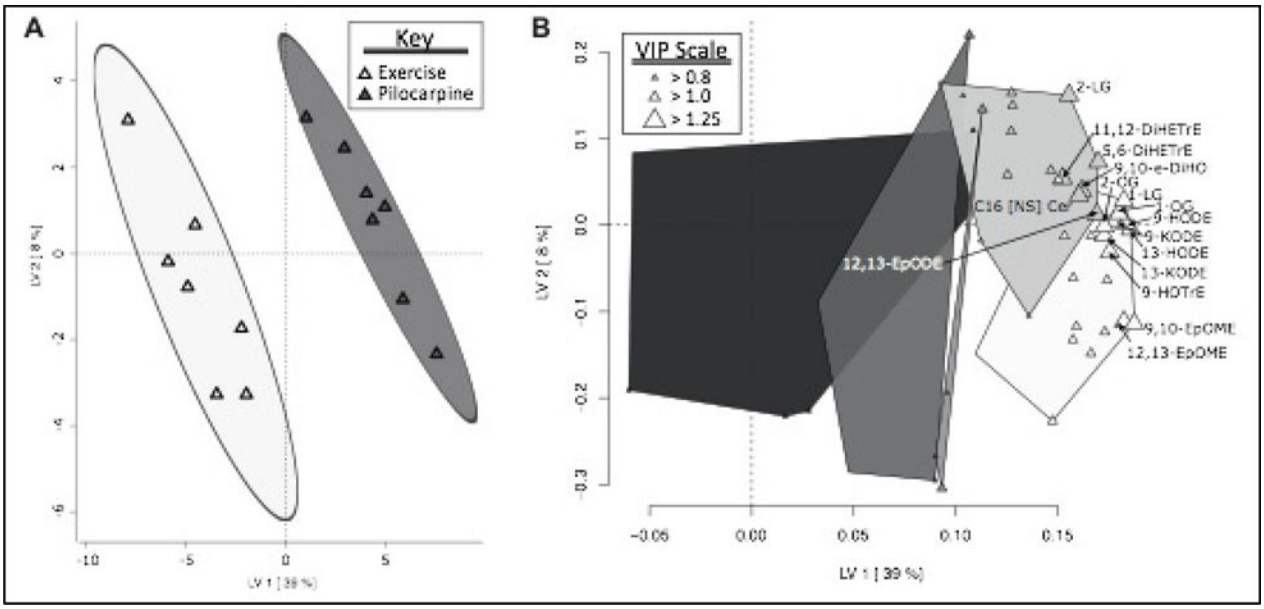

Fig. 1.

(2 columns). Pilocarpine-, as compared to exercise-induced sweat contained elevated concentrations of [NS]-type ceramides and PUFA-derived alcohols, diols and epoxides. *(A)* The PLS-DA scores plot generated in imDEV version 1.42 showing discrimination of groups by sweat stimulation technique (Q2 = 0.8), and *(B)* The PLS-DA loadings plot generated in imDEV v1.42 showing variable weight in discrimination, with variables having VIP score > 1.25 labelled. Variables are grouped by hierarchical cluster analysis of Spearman's ρ with clusters identified by unique tones of gray

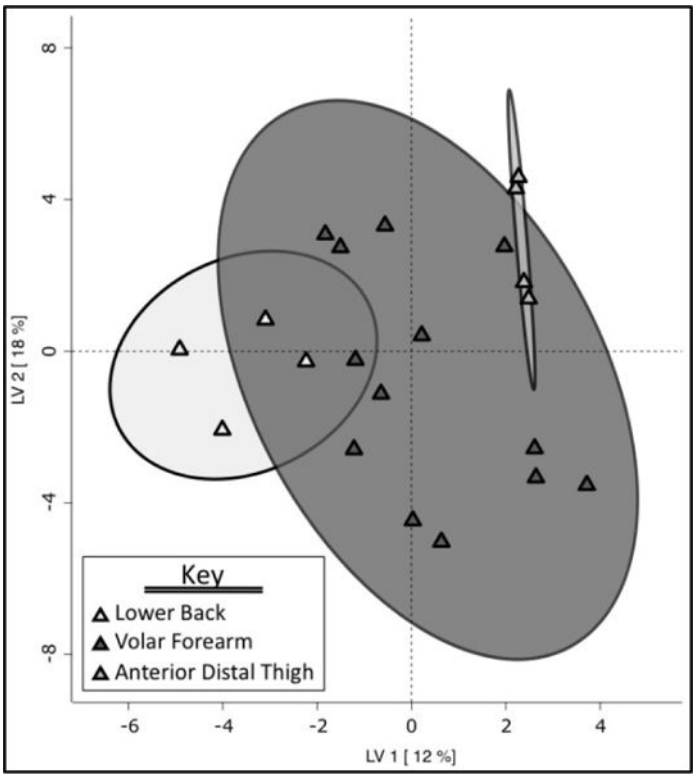

Fig. 2.

(1 column). The volar forearm, lower back and anterior distal thigh all demonstrate similar sweat lipid mediator profiles. The PLS-DA scores plot generated in imDEV version 1.42 shows apparent discrimination of groups by sampling site, but the Q2 score of −0.1 suggests a model with no predictive power.

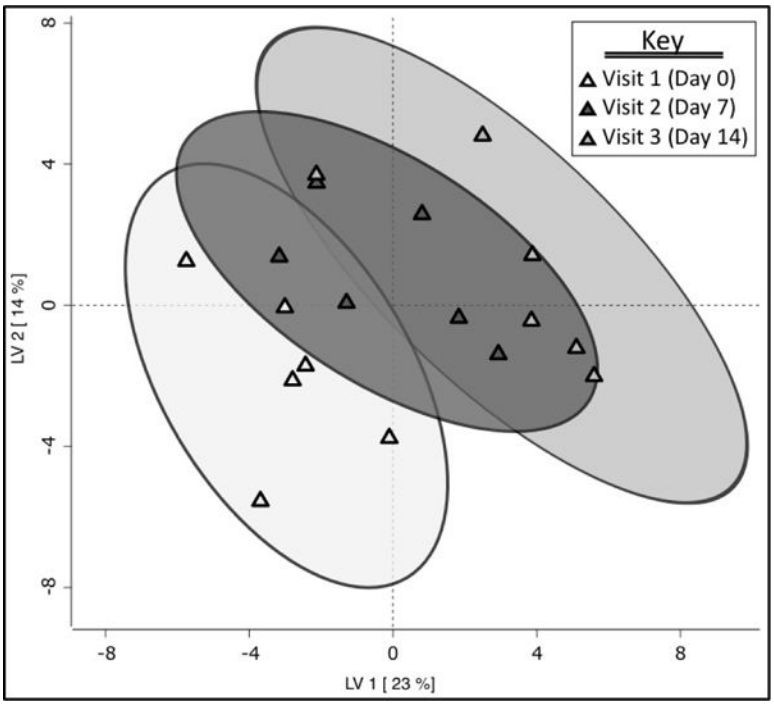

Fig. 3.

(1 column). Sweat collected from the volar forearm following pilocarpine iontophoresis demonstrates similar lipid mediator profiles at each of the three study visits. The PLS-DA scores plot generated in imDEV version 1.42 shows apparent discrimination of groups by sampling visit, but the Q2 score of 0.2 suggests a model with low predictive power.

Table 1

Lipid mediator changes in eccrine sweat collected from healthy males following sweat stimulation by either pilocarpine iontophoresis or moderate exercise on a cycle ergometer. Concentrations represent the sum of concentrations of lipid mediators belonging to the same sub-class or derived from the same fatty acid precursor.

Lipid Mediator	Concentration (nM)[a]		Fold Change[b]	*P*[c]
	Exercise (*n* = 7)	Pilocarpine (*n* = 7)		
Alcohols				
C18:2n6	7.67 ± 5.8	65.9 ± 37.9	0.106 [0.0404–0.214]	0.002
C18:3n3	2.09 ± 1.86	5.72 ± 3.03	0.333 [0.136–0.899]	0.015
C20:4n6	0.254 ± 0.136	1.7 ± 1.17	0.165 [0.125–0.261]	0.00003
C20:5n3	0.0363 ± 0.0197	0.212 ± 0.118	0.21 [0.074–1.91]	0.02
Diols				
C18:0	21.2 ± 12	85.2 ± 37.5	0.238 [0.0586–0.84]	0.009
C18:2n6	5.91 ± 3.36	7.79 ± 5.06	0.766 [0.253–2.08]	0.5
C18:3n3	2.75 ± 3.12	4.06 ± 5.28	0.773 [0.353–2.05]	0.5
C20:4n6	0.282 ± 0.259	7.2 ± 3.41	0.023 [0.000612–0.121]	0.0052
Epoxides				
C18:2n6	0.82 ± 0.525	2.67 ± 1.06	0.27 [0.156–0.641]	0.002
C18:3n3	0.994 ± 1.04	4.18 ± 3.89	0.195 [0.0737–0.415]	0.002
Ketones				
C18:2n6	2.38 ± 1.98	21 ± 9.68	0.107 [0.0527–0.34]	0.0046
C18:3n3	3.36 ± 1.81	3.65 ± 2.82	1.17 [0.314–6.12]	0.9
C20:4n6	0.02 ± 0.0172	0.0245 ± 0.0192	0.754 [0.0711–3.11]	0.7
Leukotrienes				
C20:5n3	0.0991 ± 0.082	0.1 ± 0.0941	0.96 [0.479–1.91]	0.97
Nitrolipids				
C18:0	0.0743 ± 0.0425	0.0957 ± 0.0542	0.708 [0.284–1.42]	0.3
C18:2n6	0.0874 ± 0.0782	0.108 ± 0.069	0.7 [0.229–1.55]	0.7
Prostanoids				
C20:3n6	0.849 ± 1.05	0.237 ± 0.15	1.29 [0.0907–24.5]	0.8
C20:4n6	1.29 ± 1.73	1.93 ± 1.96	0.3 [0.0439–18.8]	0.4
C20:5n3	0.178 ± 0.206	0.128 ± 0.169	1.35 [0.837–4.2]	0.3
Triols				
C18:2n6	133 ± 129	57.1 ± 55.8	2.5 [1.42–4.25]	0.0046
NAEs and MAGs				
Monoacylglycerols	21.3 ± 26	1390 ± 2150	0.0248 [0.00145–0.186]	0.003
Acylethanolamides	29.5 ± 50	16.3 ± 13.6	1.02 [0.0992–4.38]	0.97
Acylglycines	0.163 ± 0.129	0.494 ± 0.216	0.272 [0.0774–0.696]	0.02
Sphingolipids				
Sphingosines	4.92 ± 4.62	15.1 ± 8.92	0.185 [0.00964–0.834]	0.04

Lipid Mediator	Concentration (nM)[a]		Fold Change[b]	P[c]
	Exercise (n = 7)	Pilocarpine (n = 7)		
Ceramides	2.4 ± 2.5	16.8 ± 10.8	0.0734 [0.000402–0.773]	0.052

[a] Values are reported as Mean ± Standard Deviation

[b] Ratio of each individual's lipid mediator concentrations following exercise to each individual's lipid mediator concentrations following pilocarpine iontophoresis. Data are presented as Geometric Mean [Range]

[c] Reported p-values are adjusted for multiple comparisons using the Benjamini-Hochberg procedure at q = 0.05

Table 2

Changes in class-specific relative abundances of lipid mediators in eccrine sweat collected from healthy males following sweat stimulation by either pilocarpine iontophoresis or moderate exercise on a cycle ergometer. Concentrations of lipid mediators belonging to the same sub-class or derived from the same fatty acid precursor were summed, and the relative abundance of each fatty acid precursor or sub-class within the lipid mediator class is reported.

Lipid Mediator	Relative Abundance (%)[a]		Fold Change[b]	P[c]
	Exercise (*n* = 7)	Pilocarpine (*n* = 7)		
Alcohols				
C18:2n6	74.2 ± 8.66 %	88.6 ± 3.14 %	0.833 [0.695–0.961]	0.011
C18:3n3	22.5 ± 8.45 %	8.73 ± 4.05 %	2.62 [1.53–4.08]	0.006
C20:4n6	2.94 ± 1.56 %	2.34 ± 1.51 %	1.29 [0.48–3.12]	0.5
C20:5n3	0.42 ± 0.287 %	0.29 ± 0.154 %	1.45 [0.46–10.2]	0.5
Diols				
C18:0	67.9 ± 19.1 %	82.1 ± 5.61 %	0.793 [0.442–1.03]	0.14
C18:2n6	19.9 ± 9.27 %	7.54 ± 2.6 %	2.55 [0.866–7.32]	0.051
C18:3n3	11.3 ± 16.1 %	3.44 ± 3.36 %	2.58 [1.28–5.84]	0.011
C20:4n6	0.864 ± 0.814 %	6.96 ± 2.58 %	0.0768 [0.00462–0.259]	0.006
Epoxides				
C18:2n6	53.3 ± 16.2 %	46.4 ± 14.4 %	1.16 [0.93–1.52]	0.2
C18:3n3	46.7 ± 16.2 %	53.6 ± 14.4 %	0.837 [0.401–1.05]	0.3
Ketones				
C18:2n6	38.7 ± 15.1 %	80.7 ± 22.2 %	0.476 [0.272–1.05]	0.011
C18:3n3	60.9 ± 15 %	19.2 ± 22.2 %	5.2 [0.97–13.8]	0.011
C20:4n6	0.414 ± 0.372 %	0.0969 ± 0.0578 %	3.36 [0.264–25.6]	0.12
Leukotrienes				
C20:5n3	100 ± 0 %	100 ± 0 %	1 [1–1]	1.0
Nitrolipids				
C18:0	49.1 ± 19.5 %	48.5 ± 8.17 %	0.947 [0.462–1.55]	0.8
C18:2n6	50.9 ± 19.5 %	51.5 ± 8.17 %	0.937 [0.505–1.63]	0.7
Prostanoids				
C20:3n6	31.5 ± 8.74	17.5 ± 11	2.09 [0.737–8.55]	0.12
C20:4n6	49.8 ± 18.3	78.7 ± 11	0.557 [0.2–1.07]	0.14
C20:5n3	18.8 ± 17.5	8.14 ± 6.18	2.19 [0.238–13]	0.3
Triols				
C18:2n6	100 ± 0	100 ± 0	1 [1–1]	1.0
NAEs and MAGs				
Monoacylglycerols	52.9 ± 27.9	89.5 ± 17.3	0.471 [0.0951–0.907]	0.10
Acylethanolamides	46.6 ± 28.1	10.3 ± 16.9	19.3 [2.1–154]	0.011
Acylglycines	0.572 ± 0.38	0.251 ± 0.436	5.17 [0.0396–143]	0.3
Sphingolipids				

Lipid Mediator	Relative Abundance (%)[a]		Fold Change[b]	P[c]
	Exercise (*n* = 7)	Pilocarpine (*n* = 7)		
Sphingosines	67.5 ± 15.7	48.3 ± 10.8	1.4 [1.03–3.18]	0.12
Ceramides	32.5 ± 15.7	51.7 ± 10.8	0.556 [0.133–0.959]	0.13

[a] Values calculated as the relative abundance of each compound within its class (e.g. relative abundance of Sphingosines within all Sphingolipids). Values are reported as Mean ± Standard Deviation

[b] Ratio of each individual's lipid mediator relative abundances following pilocarpine iontophoresis to each individual's lipid mediator concentrations following exercise. Data are presented as Geometric Mean [Range]

[c] Reported p-values are adjusted for multiple comparisons using the Benjamini-Hochberg procedure at q = 0.05

Table 3

Inter- and intra-individual variability of lipid mediators detected at each of the three study visits (separated by one week) in the eccrine sweat of healthy males ($n = 6$) collected from the volar forearm following sweat stimulation by pilocarpine iontophoresis. Concentrations of lipid mediators belonging to the same sub-class or derived from the same fatty acid precursor were summed, and the relative abundance of each fatty acid precursor or sub-class within the lipid mediator class is reported.

Lipid Mediator	Absolute Concentration		Relative Abundance	
	Intra-Individual Variability[a] (% CV)	Inter-Individual Variability[b] (% CV)	Intra-Individual Variability[a] (% CV)	Inter-Individual Variability[b] (% CV)
Alcohols				
C18:2n6	34%	62%	4%	4%
C18:3n3	27%	51%	35%	37%
C20:4n6	44%	57%	51%	61%
C20:5n3	51%	100%	66%	108%
Diols				
C18:0	27%	47%	3%	6%
C18:2n6	39%	59%	25%	36%
C18:3n3	53%	107%	35%	73%
C20:4n6	36%	47%	30%	30%
Epoxides				
C18:2n6	38%	40%	59%	55%
C18:3n3	43%	101%	79%	89%
Ketones				
C18:2n6	40%	52%	12%	17%
C18:3n3	39%	72%	53%	103%
C20:4n6	77%	143%	85%	132%
Leukotrienes				
C20:5n3	44%	69%	0%	0%
Nitrolipids				
C18:0	93%	111%	32%	34%
C18:2n6	84%	83%	41%	41%
Prostanoids				
C20:3n6	80%	125%	64%	72%
C20:4n6	90%	148%	16%	16%
C20:5n3	46%	110%	96%	87%
Triols				
C18:2n6	51%	80%	0%	0%
NAEs and MAGs				
Monoacylglycerols	64%	192%	19%	26%
Acylethanolamides	85%	232%	77%	143%
Acylglycines	69%	86%	75%	205%
Sphingolipids				

Lipid Mediator	Absolute Concentration		Relative Abundance	
	Intra-Individual Variability[a] (% CV)	Inter-Individual Variability[b] (% CV)	Intra-Individual Variability[a] (% CV)	Inter-Individual Variability[b] (% CV)
Sphingosines	36%	60%	19%	23%
Ceramides	74%	93%	27%	33%

[a]Calculated as ratio of the square root of the within-sample variance (based on one-way ANOVA) to the mean of all samples

[b]Calculated as ratio of the standard deviation of all samples to the mean of all samples