

## **SUPPLEMENTAL INFORMATION:**

### **Sweat lipid mediator profiling: a non-invasive approach for cutaneous research**

Karan Agrawal<sup>1,2</sup>, Lauren A. Hassoun<sup>3</sup>, Negar Foolad<sup>3</sup>,

Theresa L. Pedersen<sup>2,4</sup>, Raja K. Sivamani<sup>3</sup>, John W. Newman<sup>1,2,4</sup>

<sup>1</sup> Department of Nutrition, University of California-Davis, Davis, CA 95616, USA

<sup>2</sup> NIH West Coast Metabolomics Center, Davis, CA 95616, USA

<sup>3</sup> Department of Dermatology, University of California-Davis Medical Center, Sacramento, CA 95816, USA

<sup>4</sup> United States Department of Agriculture, Agricultural Research Service,  
Western Human Nutrition Research Center, Davis, CA 95616, USA

## Lipid Mediator Nomenclature

The abbreviated nomenclature for oxylipins described in this study is adopted from the International Union of Pure and Applied Chemistry (IUPAC) based on the recommendations of Smith et al (1, 2). These abbreviations are used in order to improve manuscript readability. Briefly, the numbers prior to the hyphen indicate the positions of the functional group substituent on the fatty acid chain, the first one to three letters of the after the hyphen indicate functional group substituent (e.g. Ep: epoxide, DiH: dihydroxide, Hp: hydroperoxide, H: hydroxide, and K: ketone) and the remaining letters of the abbreviation indicating the fatty acid chain length and degree of unsaturation (e.g. DPE: docosapentaenoate, ETE: eicosatetraenoate, ETrE: eicosatrienoate, ODE: octadecadienoate, and OME: octadecenoate). Examples of this form of nomenclature include the epoxy fatty acid 16,17-epoxydocosapentaenoate which is abbreviated to 16,17-EpDPE, the dihydroxy fatty acid 8,15-dihydroxyeicosatetraenoate which can be reduced to 8,15-DiHETE, the hydroxy fatty acid 15-hydroxyeicosatrienoate which becomes 15-HETrE and the keto fatty acid 9-ketooctadecadienoate which can be abbreviated as 9-KODE. Other common abbreviations of oxylipins used in this study are TX for thromboxanes, LT for leukotrienes and PG for prostaglandins.

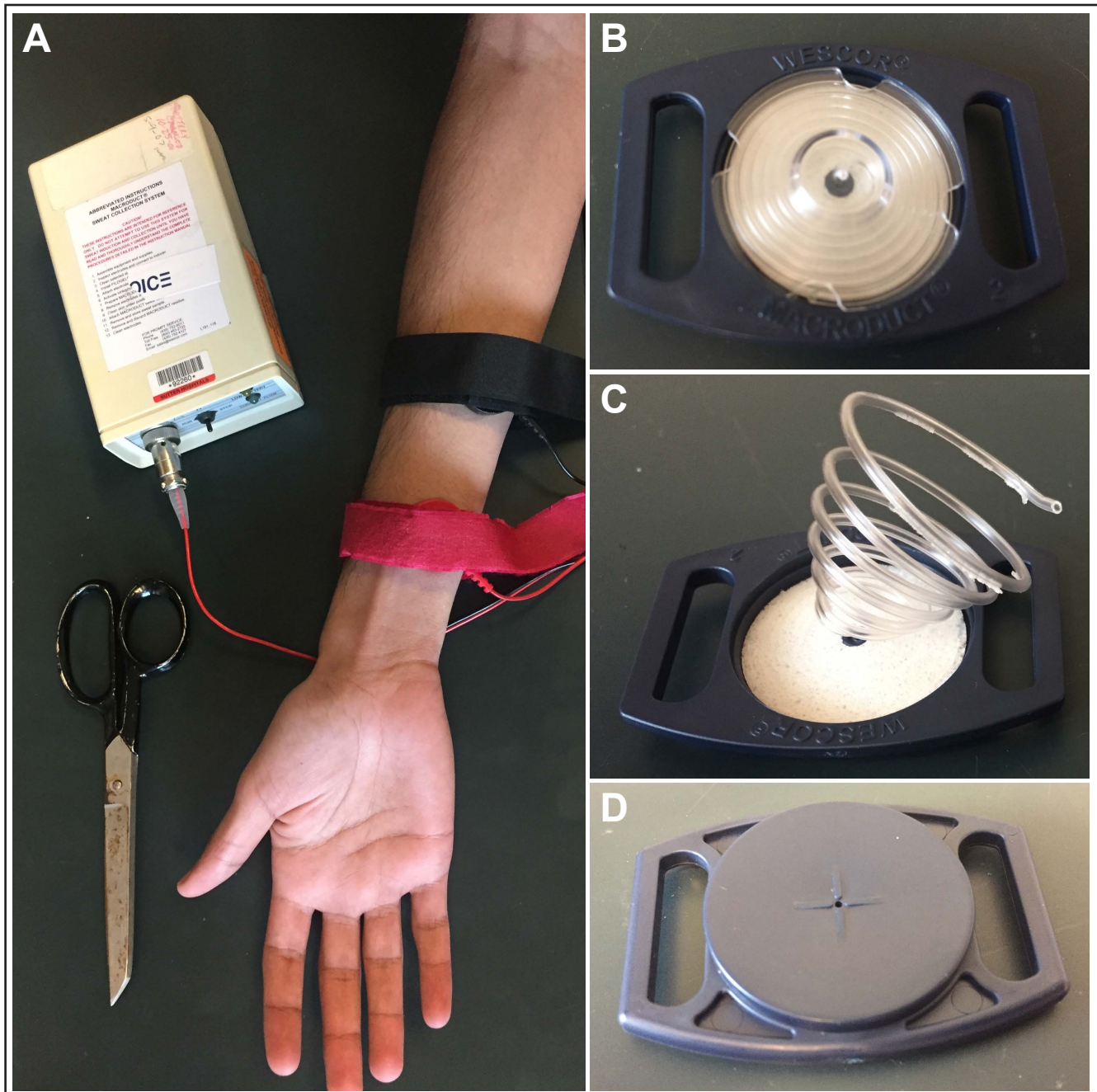
Endocannabinoid abbreviated nomenclature has not been standardized by IUPAC, but generally the first letters of the abbreviation refer to the fatty acid precursor (e.g. A: arachidonate, L: linoleate, O: oleate, P: palmitate, S: stearate, aL:  $\alpha$ -linolenate, D: docosatetraenoate, and DH: docosahexaenoate) and the last one to three letters refer to the substituent (e.g. G: glycerol, EA: ethanolamide, and Gly: glycine). Any numbers prior to the abbreviation refer to the position on the substituent to which the fatty acid chain is attached. Therefore 2-arachidonylglycerol can be abbreviated to 2-AG and linoleoylethanolamide can be abbreviated to LEA.

Ceramide nomenclature used in this manuscript generally follows the nomenclature used by Janssens et al (3). Chain lengths (which are preceded by “C”) refer to the length of the fatty acid chain of the ceramides (unless specifically referred to as “total carbons”), and letters in brackets refer to the substituents on the fatty acid and sphingosine chains, respectively. Briefly, fatty acid chains associated with ceramides can either be non-hydroxylated (N),  $\alpha$ -hydroxylated (A) or  $\omega$ -esterified (EO) whereas sphingosine chains may either be unsubstituted (S), dihydro-substituted (dS), 6-hydroxy-substituted (H) or dihydro-hydroxy-substituted (P). Example structures of the various ceramide classes, which better elucidate the nomenclature, may be found in supplemental Fig. S8.

## **References**

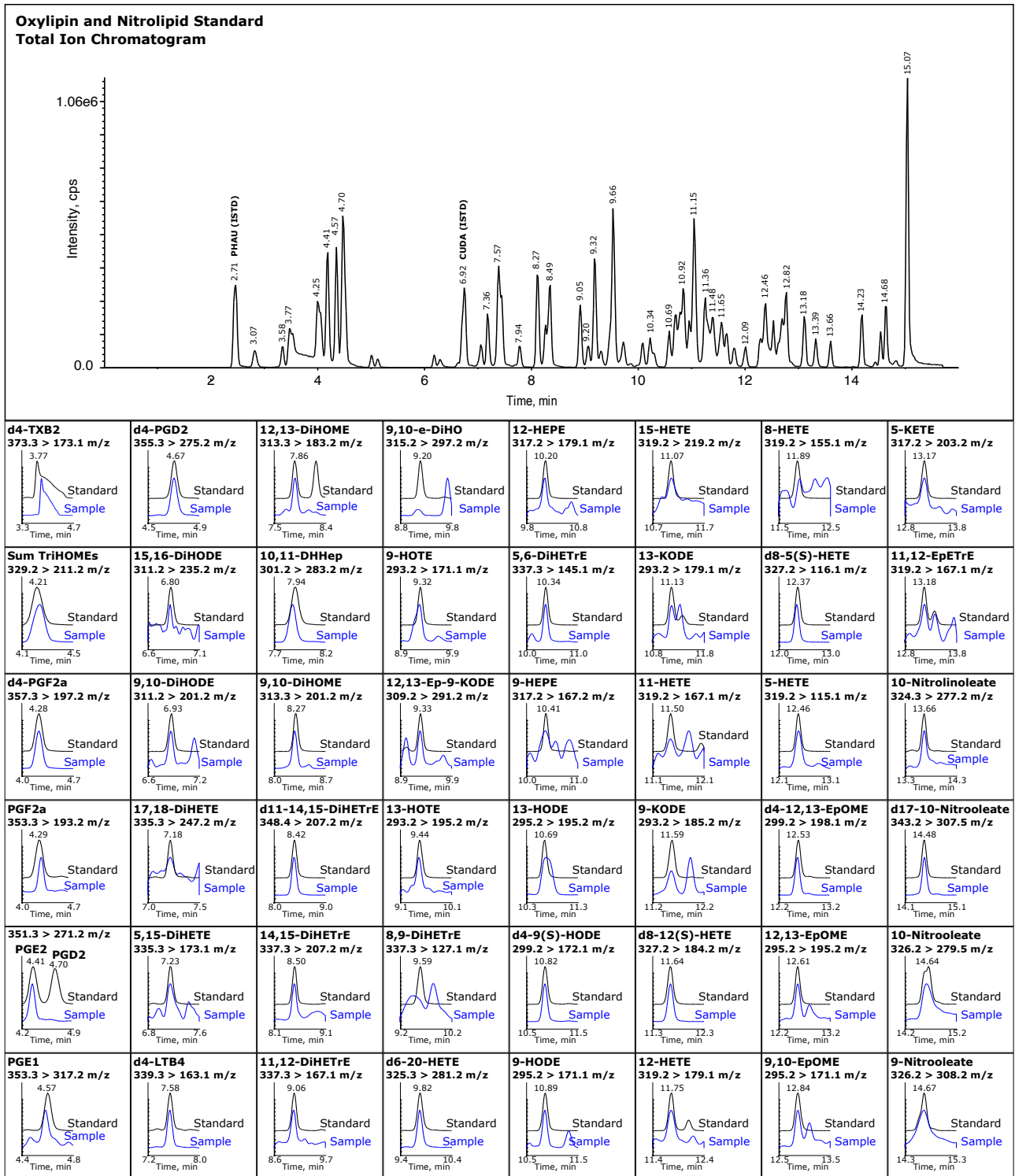
1. Smith, D. L., and A. L. Willis. 1987. A suggested shorthand nomenclature for the eicosanoids. *Lipids* **22**: 983-986.
2. Smith, W. L., P. Gorgeat, M. Hamberg, L. J. Roberts II, A. L. Willis, S. Yamamoto, P. W. Ramwell, J. Rokach, B. Samuelsson, E. J. Corey, and C. R. Pace-Asciak. 1990. Nomenclature. *In* Methods in Enzymology. F. A. Fitzpatrick, editor. Academic Press, Inc., San Diego, CA. 1-9.
3. Janssens, M., J. van Smeden, G. S. Gooris, W. Bras, G. Portale, P. J. Caspers, R. J. Vreeken, T. Hankemeier, S. Kezic, R. Wolterbeek, A. P. Lavrijsen, and J. A. Bouwstra. 2012. Increase in short-chain ceramides correlates with an altered lipid organization and decreased barrier function in atopic eczema patients. *J. Lipid Res.* **53**: 2755-2766.

## Supplemental Figure S1



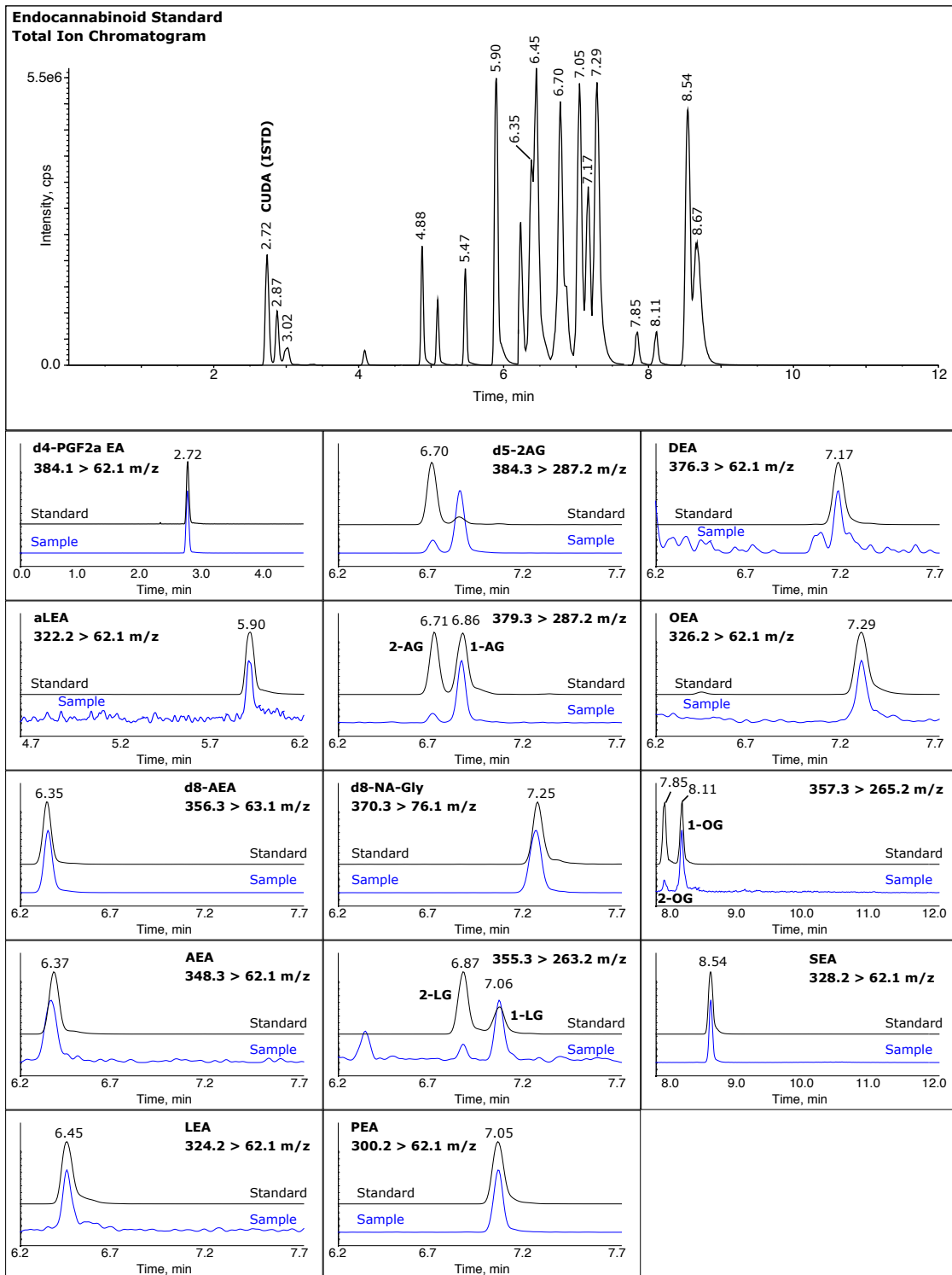
**Supplemental Fig. S1. The Macroduct® sweat collection system.** Sweat production is stimulated by pilocarpine iontophoresis using the Webster Sweat Inducer (A), which consists of two electrodes into which propriety gel disks containing pilocarpine nitrate are placed. Transdermal diffusion of pilocarpine is induced by a 1.5 mA current produced two 9 V batteries. The red (positive) electrode is placed at the desired site of sweat collection, while the black (negative) electrode must be placed within close proximity of the red electrode. Following pilocarpine iontophoresis, a propriety collector (B) is placed at the site of the red electrode for sweat collection by capillary action. Sweat is collected into capillary tubing (C) and is introduced into the tubing by a small hole present at the back of the collection device (D). For the purposes of this study, all collections took place on the volar forearm and the red electrode and collection device were placed on a 7 cm<sup>2</sup> area located within 8 cm of the wrist, as shown in the figure above (A).

# Supplemental Figure S2



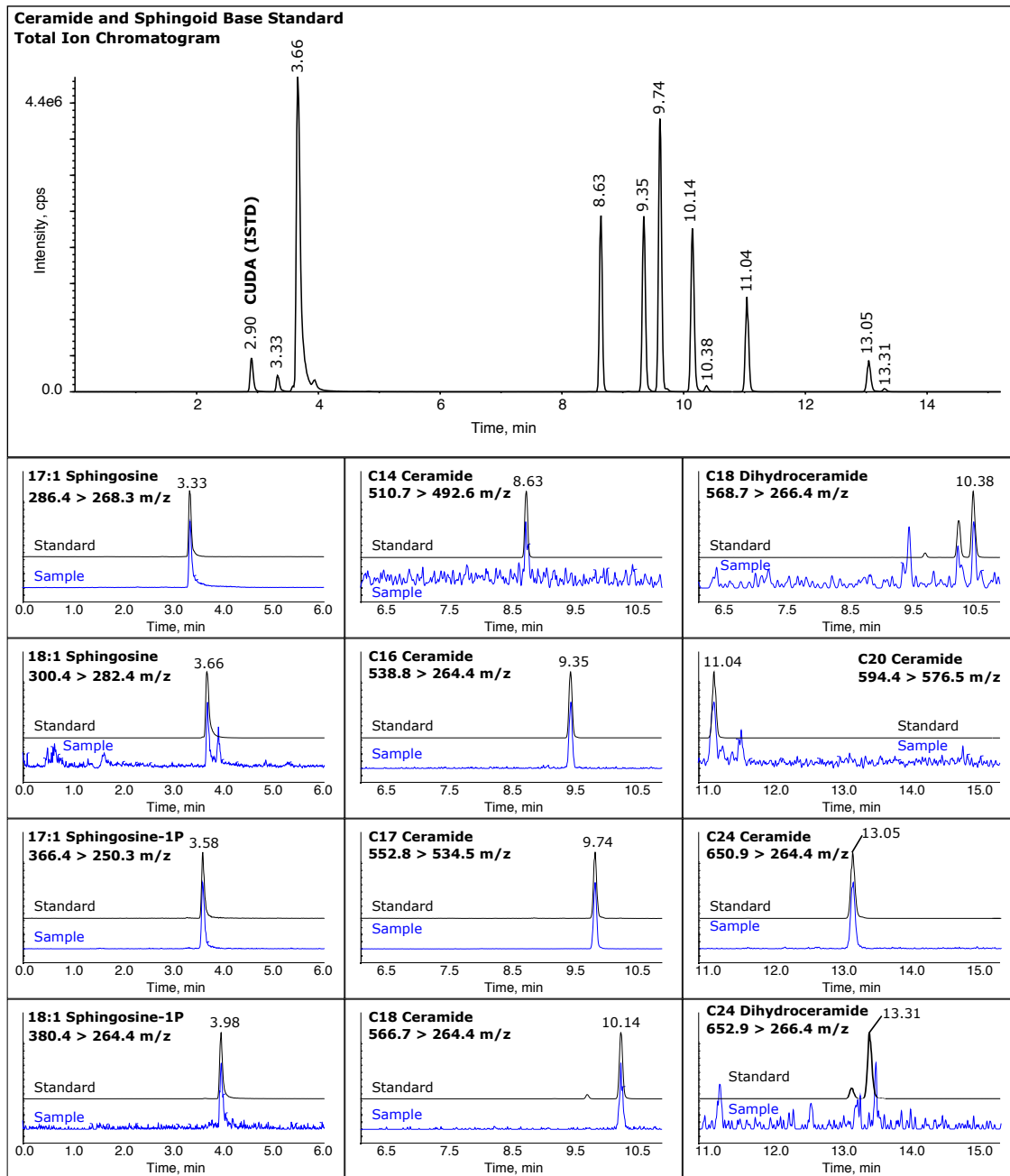
**Supplemental Fig. S2. Representative sweat oxylipin and nitrolipid chromatograms.** The total ion chromatogram is that of a representative oxylipin and nitrolipid standard to show separation of individual analytes by the UPLC-MS/MS method used in the study. The extracted ion chromatograms indicate detected oxylipins and nitrolipids in a representative sweat sample obtained from a subject with atopic dermatitis and are overlaid with extracted ion chromatograms from the representative standard to demonstrate appropriateness of peak selection in the samples.

## Supplemental Figure S3



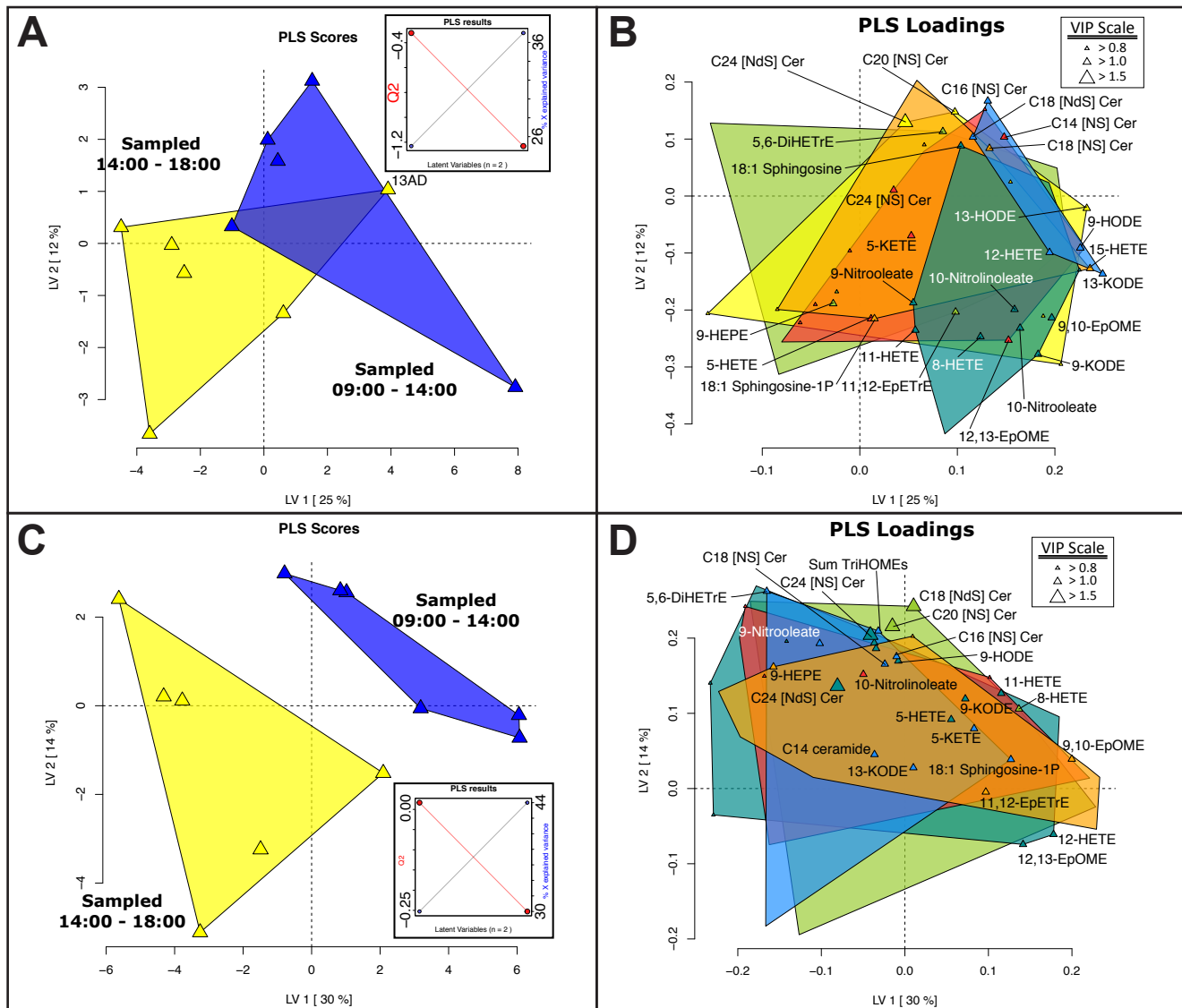
**Supplemental Fig. S3. Representative sweat endocannabinoid chromatograms.** The total ion chromatogram is that of a representative endocannabinoid standard to show separation of individual analytes by the UPLC-MS/MS method used in the study. The extracted ion chromatograms indicate detected endocannabinoids in a representative sweat sample obtained from a subject with atopic dermatitis and are overlaid with extracted ion chromatograms from the representative standard to demonstrate appropriateness of peak selection in the samples.

## Supplemental Figure S4



**Supplemental Fig. S4. Representative sweat ceramide and sphingoid base chromatograms.** The total ion chromatogram is that of a representative ceramide and sphingoid base standard to show separation of individual analytes by the UPLC-MS/MS method used in the study. The extracted ion chromatograms indicate detected ceramides and sphingoid bases in a representative sweat sample obtained from a subject with atopic dermatitis and are overlaid with extracted ion chromatograms from the representative standard to demonstrate appropriateness of peak selection in the samples.

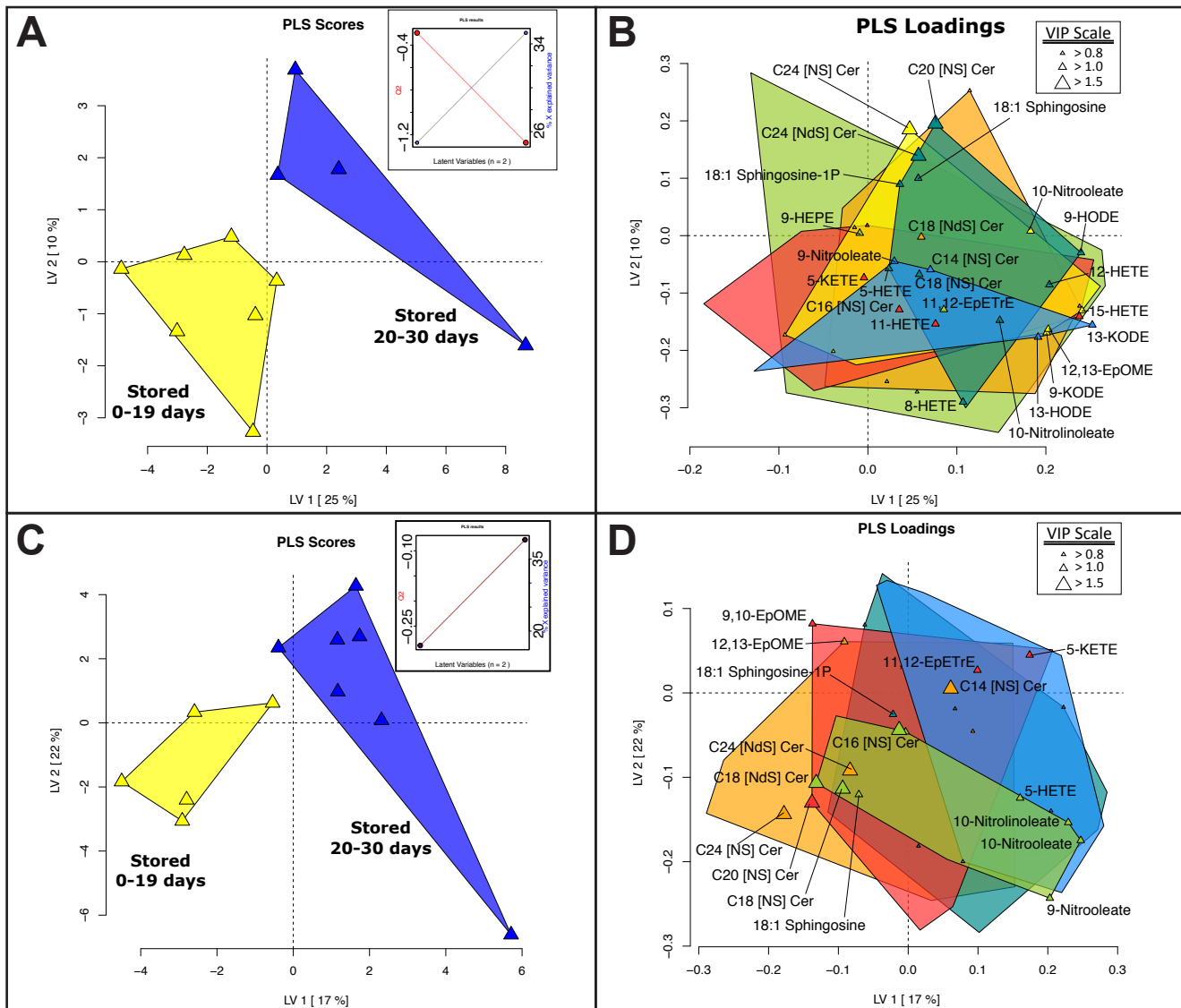
## Supplemental Figure S5



**Supplemental Fig. S5. Effect of time of sampling on eccrine sweat lipid mediators in subjects with and without atopic dermatitis.** The Partial Least Squares Discriminant Analysis (PLS-DA) Scores Plot shows apparent discrimination in subjects with (A) and without (C) AD, however negative Q2 scores (inset) indicate lack of a predictive model. (B & D) The PLS-DA Loadings Plot showing variable weight in discrimination. Point sizes are defined by the variable importance in projection (VIP) scores, with VIPs >1 considered significant factors in the discriminant model. Variables were grouped by their correlation (Spearman's  $\rho$ ) using a hierarchical cluster analysis with cluster identified by a unique color. No analytes appear different between subject groups.

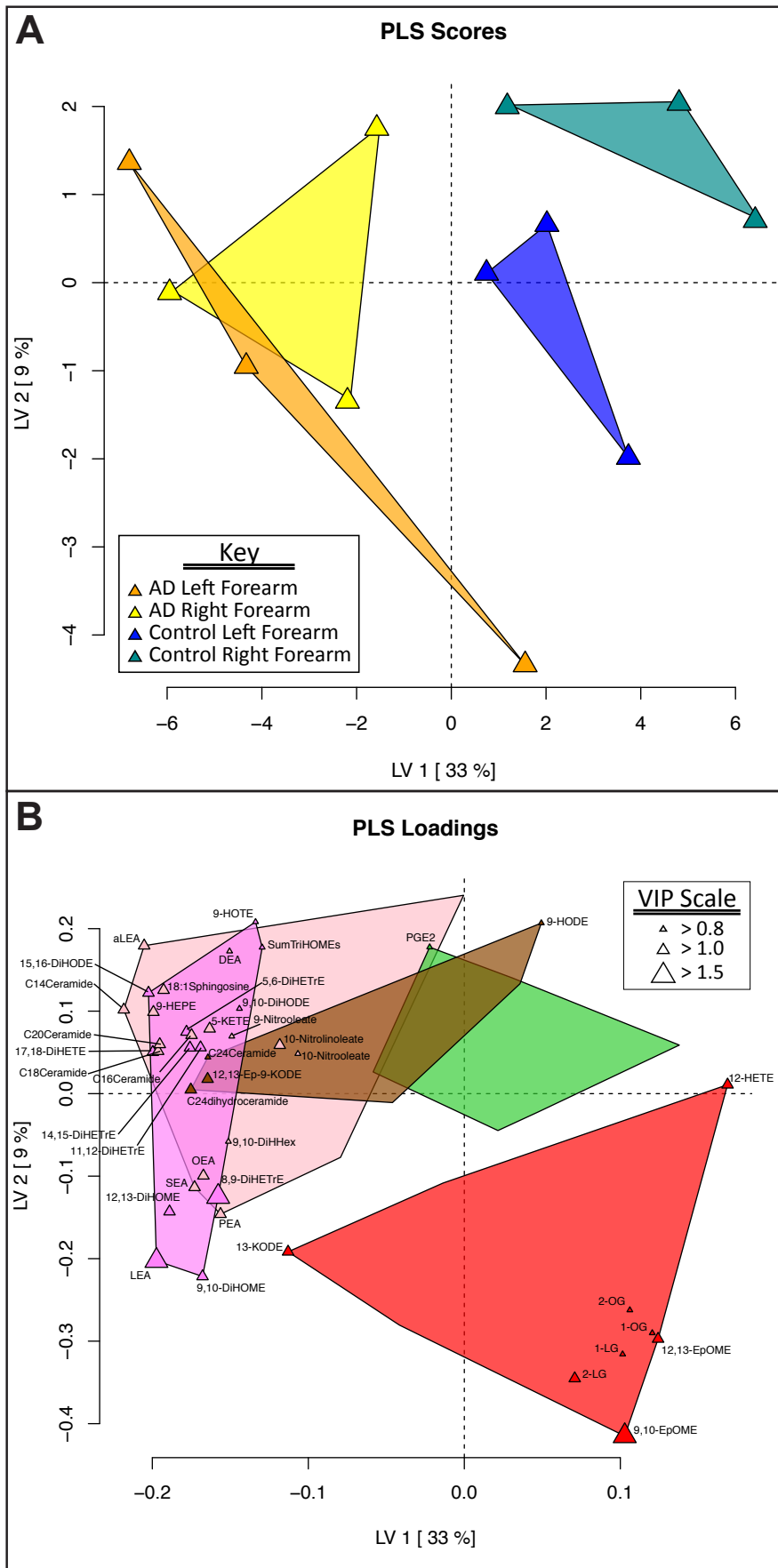


## Supplemental Figure S6



**Supplemental Fig. S6. Effect of storage time at  $-80^{\circ}\text{C}$  on eccrine sweat lipid mediators in subjects with and without atopic dermatitis.** The Partial Least Squares Discriminant Analysis (PLS-DA) Scores Plot shows apparent discrimination in subjects with (A) and without (C) AD, however negative Q2 scores (inset) indicate lack of a predictive model. (B & D) The PLS-DA Loadings Plot showing variable weight in discrimination. Point sizes are defined by the variable importance in projection (VIP) scores, with VIPs >1 considered significant factors in the discriminant model. Variables were grouped by their correlation (Spearman's  $\rho$ ) using a hierarchical cluster analysis with cluster identified by a unique color. No analytes appear different between subject groups.

## Supplemental Figure S7



**Supplemental Fig. S7. Effect of volar forearm sampled on eccrine sweat lipid mediators in subjects with and without atopic dermatitis.** (A) The Partial Least Squares Discriminant Analysis (PLS-DA) Scores Plot shows discrimination in subjects with and without AD and apparent differences in forearm sampled for subjects without AD. Q2 scores for forearm sampled for subjects with and without AD were -0.1 and -0.2, respectively indicating lack of a predictive model. (B) The PLS-DA Loadings Plot showing variable weight in discrimination. Point sizes are defined by the variable importance in projection (VIP) scores, with VIPs >1 considered significant factors in the discriminant model. Variables were grouped by their correlation (Spearman's  $\rho$ ) using a hierarchical cluster analysis with cluster identified by a unique color. No analytes appear different between arms sampled, but subjects with atopic dermatitis show elevated ceramides and sphingoid bases.

## Supplemental Figure S8

		Fatty Acid Chain		
		Non-hydroxy fatty acid [N]	$\alpha$ -hydroxy fatty acid [A]	Esterified $\omega$ -hydroxy fatty acid [EO]
Sphingoid Base	Dihydrosphingosine [dS]	 [NdS] Ceramide / Cer 10 (8.6%)	 [AdS] Ceramide / Cer 11 (1.9%)	 [EOdS] Ceramide (1.3%)
	Sphingosine [S]	 [NS] Ceramide / Cer 2 (6.7%)	 [AS] Ceramide / Cer 5 (3.8%)	 [EOS] Ceramide / Cer 1 (5.4%)
	Phytosphingosine [P]	 [NP] Ceramide / Cer 3 (25.8%)	 [AP] Ceramide / Cer 6 (13.4%)	 [EOP] Ceramide / Cer 9 (2.7%)
	6-hydroxy sphingosine [H]	 [NH] Ceramide / Cer 8 (12.4%)	 [AH] Ceramide / Cer 7 (12.4%)	 [EOH] Ceramide / Cer 4 (5.4%)

**Supplemental Fig. S8. Ceramide classes and their relative abundance in healthy human epidermis.** Twelve classes of ceramides have thus far been described consisting of four possible sphingoid base moieties and three possible fatty acid chain moieties. Numbers in parentheses represent known proportions of each ceramide class in healthy human epidermis as estimated by van Smeden et al (1). Short-chain (30-40 total carbon) ceramides highlighted in orange increase in the skin of subjects with AD whereas all known chain lengths of ceramides highlighted in blue decrease in the skin of subjects with AD, as determined by Ishikawa et al and Janssens et al (2, 3). Ceramides highlighted in grey are not demonstrated to either increase or decrease in the skin of subjects with AD. This figure is adapted from Janssens et al (3).

### References

- van Smeden, J., W. A. Boiten, T. Hankemeier, R. Rissmann, J. A. Bouwstra, and R. J. Vreeken. 2014. Combined LC/MS-platform for analysis of all major stratum corneum lipids, and the profiling of skin substitutes. *Biochim. Biophys. Acta* **1841**: 70-79.
- Ishikawa, J., H. Narita, N. Kondo, M. Hotta, Y. Takagi, Y. Masukawa, T. Kitahara, Y. Takema, S. Koyano, S. Yamazaki, and A. Hatamochi. 2010. Changes in the ceramide profile of atopic dermatitis patients. *J. Invest. Dermatol.* **130**: 2511-2514.
- Janssens, M., J. van Smeden, G. S. Gooris, W. Bras, G. Portale, P. J. Caspers, R. J. Vreeken, T. Hankemeier, S. Kezic, R. Wolterbeek, A. P. Lavrijsen, and J. A. Bouwstra. 2012. Increase in short-chain ceramides correlates with an altered lipid organization and decreased barrier function in atopic eczema patients. *J. Lipid Res.* **53**: 2755-2766.

## Supplemental Table S1

**Supplemental Table S1A.** Mobile phase gradient conditions for the analysis of oxylipins and nitrolipids by UPLC-MS/MS. A Waters Acquity UPLC® BEH C18 2.1 x 150 mm, 1.7 µm column was used to separate analytes and column temperature was maintained at 60°C.

Time (min)	Mobile Phase A <sup>a</sup> (%)	Mobile Phase B <sup>b</sup> (%)	Flow Rate (mL/min)
0.00	75	25	0.25
1.00	60	40	0.25
2.50	58	42	0.25
4.50	50	50	0.25
10.50	35	65	0.25
12.50	25	75	0.25
14.00	15	85	0.25
14.50	5	95	0.25
15.00	75	25	0.25
16.00	75	25	0.25

<sup>a</sup> Mobile Phase A: 0.1% Acetic Acid in Water

<sup>b</sup> Mobile Phase B: 90:10 Acetonitrile:Isopropanol

Supplemental Table S1

Supplemental Table S1B. UPLC/electrospray ionization QTRAP analyte and instrument specific parameters for assayed oxylipins and nitrolipids.

Lipid Mediator	Analyte Class	Fatty Acid Precursor	Internal Standard	Retention Time (min)	Q1 > Q3 Transition (m/z)	Collision Energy (V)	Declustering Potential (V)
PHAU	ISTD <sup>a</sup>			2.67	249.2 > 130.1	-20	-45
6-keto PGF1a	Prostanoid	C20:4n6	d4-PGD2	3.04	369.3 > 163.1	-40	-70
PGF3a	Prostanoid	C20:5n3	d4-PGF2a	3.56	351.3 > 307.4	-26	-80
PGE3	Prostanoid	C20:5n3	d4-PGF2a	3.69	349.3 > 269.2	-22	-45
d4-TXB2	SSTD <sup>b</sup>		PHAU	3.74	373.3 > 173.1	-25	-50
TXB2	Prostanoid	C20:4n6	d4-TXB2	3.75	369.3 > 169.1	-25	-50
Sum TriHOMEs	Triol	C18:2n6	d4-PGF2a	4.22	329.2 > 211.2	-32	-70
d4-PGF2a	SSTD <sup>b</sup>		PHAU	4.27	357.3 > 197.2	-35	-65
PGF2a	Prostanoid	C20:4n6	d4-PGF2a	4.28	353.3 > 193.2	-35	-65
PGE2	Prostanoid	C20:4n6	d4-PGD2	4.40	351.3 > 271.2	-25	-35
PGE1	Prostanoid	C20:3n6	d4-PGD2	4.58	353.3 > 317.2	-22	-50
d4-PGD2	SSTD <sup>b</sup>		PHAU	4.69	355.3 > 275.2	-26	-45
PGD2	Prostanoid	C20:4n6	d4-PGD2	4.71	351.3 > 271.2	-25	-35
15-keto PGE2	Prostanoid	C20:4n6	d4-PGD2	4.75	349.2 > 331.3	-16	-45
Resolvin D1	Triol	C20:4n6	d4-PGF2a	5.23	375.3 > 121.1	-40	-50
Lipoxin A4	Triol	C20:4n6	d4-PGF2a	5.35	351.3 > 217.2	-26	-50
LTB5	Leukotriene	C20:5n3	d4-LTB4	6.40	333.3 > 195.2	-20	-70
15,16-DiHODE	Diol	C18:3n3	d11-14,15-DiHETrE	6.85	311.2 > 235.2	-22	-65
12,13-DiHODE	Diol	C18:3n3	d11-14,15-DiHETrE	6.93	311.2 > 183.2	-30	-70
8,15-DiHETE	Diol	C20:5n3	d11-14,15-DiHETrE	6.95	335.3 > 235.2	-22	-65
9,10-DiHODE	Diol	C18:3n3	d11-14,15-DiHETrE	6.98	311.2 > 201.2	-30	-65
17,18-DiHETE	Diol	C20:5n3	d11-14,15-DiHETrE	7.23	335.3 > 247.2	-25	-60
5,15-DiHETE	Diol	C20:5n3	d11-14,15-DiHETrE	7.28	335.3 > 173.1	-21	-45
6-trans-LTB4	Leukotriene	C20:4n6	d4-LTB4	7.40	335.3 > 195.2	-21	-70
14,15-DiHETE	Diol	C20:5n3	d11-14,15-DiHETrE	7.57	335.3 > 207.2	-25	-55
CUDA	ISTD <sup>a</sup>			7.62	339.4 > 214.2	-35	-65
d4-LTB4	SSTD <sup>b</sup>		CUDA	7.63	339.3 > 163.1	-38	-70
LTB4	Leukotriene	C20:4n6	d4-LTB4	7.67	335.3 > 195.2	-21	-70
12,13-DiHOME	Diol	C18:2n6	d11-14,15-DiHETrE	7.94	313.3 > 183.2	-30	-70
10,11-DHHep	SSTD <sup>b</sup>		CUDA	8.02	301.2 > 283.2	-30	-70
9,10-DiHOME	Diol	C18:2n6	d11-14,15-DiHETrE	8.35	313.3 > 201.2	-30	-70
d11-14,15-DiHETrE	SSTD <sup>b</sup>		CUDA	8.51	348.4 > 207.2	-28	-64
19,20-DiHDoPA	Diol	C22:6n3	d11-14,15-DiHETrE	8.57	361.3 > 273.2	-24	-74
14,15-DiHETrE	Diol	C20:4n6	d11-14,15-DiHETrE	8.60	337.3 > 207.2	-25	-65
11,12-DiHETrE	Diol	C20:4n6	d11-14,15-DiHETrE	9.16	337.3 > 167.1	-27	-60
9,10-e-DiHO	Diol	C18:0	10,11-DHHep	9.31	315.2 > 297.2	-33	-30
9-HOTE	Alcohol	C18:3n3	d4-9(S)-HODE	9.42	293.2 > 171.1	-22	-65
12,13-Ep-9-KODE	Ketone	C18:3n3	d4-9(S)-HODE	9.43	309.2 > 291.2	-20	-65
13-HOTE	Alcohol	C18:3n3	d4-9(S)-HODE	9.55	293.2 > 195.2	-25	-65
8,9-DiHETrE	Diol	C20:4n6	d11-14,15-DiHETrE	9.70	337.3 > 127.1	-30	-55
15-deoxy PGJ2	Prostanoid	C20:4n6	d11-14,15-DiHETrE	9.77	315.2 > 271.2	-20	-60
d6-20-HETE	SSTD <sup>b</sup>		CUDA	9.93	325.3 > 281.2	-25	-70
20-HETE	Alcohol	C20:4n6	d6-20-HETE	9.97	319.2 > 275.2	-24	-65
15-HEPE	Alcohol	C20:5n3	d8-12(S)-HETE	9.97	317.2 > 219.2	-20	-60
12-HEPE	Alcohol	C20:5n3	d8-12(S)-HETE	10.33	317.2 > 179.1	-20	-60

Supplemental Table S1

Lipid Mediator	Analyte Class	Fatty Acid Precursor	Internal Standard	Retention Time (min)	Q1 > Q3 Transition (m/z)	Collision Energy (V)	Decustering Potential (V)
5,6-DiHETrE	Diol	C20:4n6	d11-14,15-DiHETrE	10.46	337.3 > 145.1	-25	-70
9-HEPE	Alcohol	C20:5n3	d4-9(S)-HODE	10.53	317.2 > 167.2	-18	-85
13-HODE	Alcohol	C18:2n6	d4-9(S)-HODE	10.83	295.2 > 195.2	-25	-65
5-HEPE	Alcohol	C20:5n3	d4-9(S)-HODE	10.91	317.2 > 115.1	-20	-60
d4-9(S)-HODE	SSTD <sup>a</sup>		CUDA	10.95	299.2 > 172.1	-26	-70
9-HODE	Alcohol	C18:2n6	d4-9(S)-HODE	11.01	295.2 > 171.1	-25	-60
15,16-EpODE	Epoxide	C18:3n3	d4-12,13-EpOME	11.09	293.2 > 275.2	-20	-60
17,18-EpETE	Epoxide	C20:5n3	d4-12,13-EpOME	11.19	317.2 > 259.2	-15	-55
15-HETE	Alcohol	C20:4n6	d8-12(S)-HETE	11.21	319.2 > 219.2	-16	-70
13-KODE	Ketone	C18:2n6	d4-9(S)-HODE	11.27	293.2 > 179.1	-26	-70
9,10-EpODE	Epoxide	C18:3n3	d4-12,13-EpOME	11.28	293.2 > 275.2	-20	-60
17-HDoHE	Alcohol	C22:6n3	d8-12(S)-HETE	11.32	343.3 > 281.2	-20	-45
12,13-EpODE	Epoxide	C18:3n3	d4-12,13-EpOME	11.48	293.2 > 183.2	-25	-60
13-HpODE	Hydroperoxide	C18:2n6	d4-9(S)-HODE	11.48	311.2 > 179.1	-20	-40
15-HpETE	Hydroperoxide	C20:4n6	d8-12(S)-HETE	11.48	335.2 > 113.1	-20	-58
15-KETE	Ketone	C20:4n6	d8-12(S)-HETE	11.55	317.2 > 273.2	-20	-65
14-HDoHE	Alcohol	C22:6n3	d8-12(S)-HETE	11.62	343.3 > 281.2	-20	-45
11-HETE	Alcohol	C20:4n6	d8-12(S)-HETE	11.64	319.2 > 167.1	-15	-55
14,15-EpETE	Epoxide	C20:5n3	d4-12,13-EpOME	11.64	317.2 > 247.2	-15	-45
9-KODE	Ketone	C18:2n6	d4-9(S)-HODE	11.71	293.2 > 185.2	-30	-70
d8-12(S)-HETE	SSTD <sup>a</sup>		CUDA	11.78	327.2 > 184.2	-21	-60
11,12-EpETE	Epoxide	C20:5n3	d4-12,13-EpOME	11.80	317.2 > 167.3	-16	-70
12-HETE	Alcohol	C20:4n6	d8-12(S)-HETE	11.88	319.2 > 179.1	-21	-60
9-HpODE	Hydroperoxide	C18:2n6	d4-9(S)-HODE	11.88	311.2 > 185.2	-20	-40
12-HpETE	Hydroperoxide	C20:4n6	d8-12(S)-HETE	12.00	335.2 > 153.1	-20	-58
8-HETE	Alcohol	C20:4n6	d8-12(S)-HETE	12.02	319.2 > 155.1	-15	-55
9-HETE	Alcohol	C20:4n6	d8-12(S)-HETE	12.23	319.2 > 167.1	-15	-55
d8-5(S)-HETE	SSTD <sup>a</sup>		CUDA	12.49	327.2 > 116.1	-22	-55
19,20-EpDPE	Epoxide	C22:6n3	d4-12,13-EpOME	12.57	343.3 > 281.2	-20	-45
5-HETE	Alcohol	C20:4n6	d8-5(S)-HETE	12.58	319.2 > 115.1	-20	-50
d4-12(13)-EpOME	SSTD <sup>a</sup>		CUDA	12.65	299.2 > 198.1	-25	-65
12,13-EpOME	Epoxide	C18:2n6	d4-12,13-EpOME	12.74	295.2 > 195.2	-25	-65
14,15-EpETrE	Epoxide	C20:4n6	d4-12,13-EpOME	12.83	319.2 > 219.2	-16	-70
4-HDoHE	Alcohol	C22:6n3	d8-5(S)-HETE	12.88	343.3 > 281.2	-20	-45
16,17-EpDPE	Epoxide	C22:6n3	d4-12,13-EpOME	12.96	343.5 > 273.5	-15	-55
9,10-EpOME	Epoxide	C18:2n6	d4-12,13-EpOME	12.97	295.2 > 171.1	-25	-60
5-HpETE	Hydroperoxide	C20:4n6	d8-5(S)-HETE	13.18	335.2 > 155.1	-20	-58
5-KETE	Ketone	C20:4n6	d8-5(S)-HETE	13.28	317.2 > 203.2	-25	-70
11,12-EpETrE	Epoxide	C20:4n6	d4-12,13-EpOME	13.31	319.2 > 167.1	-15	-55
8,9-EpETrE	Epoxide	C20:4n6	d4-12,13-EpOME	13.51	319.2 > 155.1	-15	-55
10-Nitrooleate	Nitrolipid	C18:2n6	d17-10-Nitrooleate	13.79	324.3 > 277.2	-16	-60
d17-10-Nitrooleate	SSTD <sup>a</sup>		CUDA	14.60	343.2 > 307.5	-20	-70
10-Nitrooleate	Nitrolipid	C18:1n9	d17-10-Nitrooleate	14.70	326.2 > 279.5	-24	-60
9-Nitrooleate	Nitrolipid	C18:1n9	d17-10-Nitrooleate	14.80	326.2 > 308.2	-18	-70

<sup>a</sup> ISTD = Internal Standard. This compound is added to the samples at the reconstitution step

<sup>b</sup> SSTD = Surrogate. This compound is added to the samples prior to extraction

## Supplemental Table S2

**Supplemental Table S2B.** Mobile phase gradient conditions for the analysis of endocannabinoids by UPLC-MS/MS. A Waters Acquity UPLC® BEH C18 2.1 x 150 mm, 1.7 µm column was used to separate analytes and column temperature was maintained at 60°C.

Time (min)	Mobile Phase A <sup>a</sup> (%)	Mobile Phase B <sup>b</sup> (%)	Flow Rate (mL/min)
0.00	75	25	0.25
0.25	75	25	0.25
0.50	60	40	0.25
1.50	50	50	0.25
3.00	45	55	0.25
3.50	20	80	0.25
8.00	15	85	0.25
9.00	5	95	0.25
9.25	5	95	0.25
9.35	0	100	0.25
10.35	0	100	0.25
10.50	75	25	0.25
12.00	75	25	0.25

<sup>a</sup> Mobile Phase A: 0.1% Acetic Acid in Water

<sup>b</sup> Mobile Phase B: 90:10 Acetonitrile:Isopropanol

## Supplemental Table S2

**Supplemental Table S2B.** UPLC/electrospray ionization QTRAP analyte and instrument specific parameters for assayed endocannabinoids.

Lipid Mediator	Analyte Class	Fatty Acid Precursor	Internal Standard	Retention Time (min)	Q1 > Q3 Transition (m/z)	Collision Energy (V)	Declustering Potential (V)
PGF2a EA	Acylethanolamide	C20:4n6	d4-PGF2a EA	2.77	380.3 > 62.1	41	55
d4-PGF2a EA	SSTD <sup>b</sup>		CUDA	2.77	384.3 > 62.1	40	55
PGD2 EA	Acylethanolamide	C20:4n6	d4-PGF2a EA	2.79	378.3 > 62.1	38	58
PGE2 EA	Acylethanolamide	C20:4n6	d4-PGF2a EA	2.91	378.3 > 62.1	38	58
PGF2a 1G	Monoacylglycerol	C20:4n6	d5-2-AG	3.03	411.3 > 301.2	19	55
PGE2 1G	Monoacylglycerol	C20:4n6	d5-2-AG	3.07	409.3 > 317.2	19	55
CUDA	ISTD <sup>a</sup>			4.92	341.3 > 216.2	25	58
15-HETE EA	Acylethanolamide	C20:4n6	d4-PGF2a EA	5.16	346.3 > 62.1	39	58
11,12-EpETre EA	Acylethanolamide	C20:4n6	d4-PGF2a EA	5.56	364.3 > 62.1	40	58
aLEA	Acylethanolamide	C18:3n3	d8-AEA	6.00	322.2 > 62.1	32	72
DHEA	Acylethanolamide	C22:6n3	d8-AEA	6.35	372.3 > 62.1	36	61
d8-AEA	SSTD <sup>b</sup>		CUDA	6.47	356.3 > 63.1	30	60
AEA	Acylethanolamide	C20:4n6	d8-AEA	6.50	348.3 > 62.1	33	65
LEA	Acylethanolamide	C18:2n6	d8-AEA	6.57	324.2 > 62.1	31	72
d5-2-AG	SSTD <sup>b</sup>		CUDA	6.84	384.3 > 287.2	19	63
2-AG	Monoacylglycerol	C20:4n6	d5-2-AG	6.85	379.3 > 287.2	19	53
Dihomo GLA EA	Acylethanolamide	C18:3n6	d8-AEA	6.92	350.3 > 62.1	36	65
2-LG	Monoacylglycerol	C18:2n6	d5-2-AG	7.00	355.3 > 263.2	18	52
1-AG	Monoacylglycerol	C20:4n6	d5-2-AG	7.00	379.3 > 287.2	19	53
d8-NA-Gly	SSTD <sup>b</sup>		CUDA	7.00	370.3 > 76.1	35	79
NA-Gly	Acylamide	C20:4n6	d8-NA-Gly	7.05	362.3 > 76.1	35	79
PEA	Acylethanolamide	C16:0	d8-AEA	7.19	300.2 > 62.1	31	80
1-LG	Monoacylglycerol	C18:2n6	d5-2-AG	7.20	355.3 > 263.2	18	52
DEA	Acylethanolamide	C22:4n6	d8-AEA	7.32	376.3 > 62.1	36	66
OEA	Acylethanolamide	C18:1n9	d8-AEA	7.44	326.2 > 62.1	32	80
2-OG	Monoacylglycerol	C18:1n9	d5-2-AG	8.02	357.3 > 265.2	18	52
NO-Gly	Acylamide	C18:1n9	d8-NA-Gly	8.28	340.2 > 76.2	26	80
1-OG	Monoacylglycerol	C18:1n9	d5-2-AG	8.30	357.3 > 265.2	18	52
SEA	Acylethanolamide	C18:0	d8-AEA	8.74	328.2 > 62.1	35	80

<sup>a</sup> ISTD = Internal Standard. This compound is added to the samples at the reconstitution step

<sup>b</sup> SSTD = Surrogate. This compound is added to the samples prior to extraction



## Supplemental Table S3

**Supplemental Table S3C.** Mobile phase gradient conditions for the analysis of ceramides and sphingoid bases by UPLC-MS/MS. A Waters Acquity UPLC<sup>®</sup> BEH C8 2.1 x 100 mm, 1.7  $\mu$ m column was used to separate analytes and column temperature was maintained at 60°C.

Time (min)	Mobile Phase A <sup>a</sup> (%)	Mobile Phase B <sup>b</sup> (%)	Flow Rate (mL/min)
0.00	30	70	0.25
2.00	20	80	0.25
5.00	15	85	0.25
5.50	10	90	0.25
13.50	5	95	0.25
13.75	1	99	0.25
14.50	1	99	0.25
14.70	30	70	0.25
15.20	30	70	0.25

<sup>a</sup> Mobile Phase A: 5 mM Ammonium Formate and 0.2% Formic Acid in Water

<sup>b</sup> Mobile Phase B: 5 mM Ammonium Formate and 0.2% Formic Acid in Methanol

## Supplemental Table S3

**Supplemental Table S3B.** UPLC/electrospray ionization QTRAP analyte and instrument specific parameters for assayed ceramides and sphingoid bases.

Lipid Mediator	Analyte Class	Fatty Acid Precursors <sup>a</sup>	Internal Standard	Retention Time (min)	Q1 > Q3 Transition (m/z)	Collision Energy (V)	Decustering Potential (V)
CUDA	ISTD <sup>b</sup>			3.02	341.3 > 216.2	24.00	60
17:1 Sphingosine	SSTD <sup>c</sup>		CUDA	3.43	286.4 > 268.3	15.00	40
17:1 Sphingosine-1P	SSTD <sup>c</sup>		CUDA	3.69	366.4 > 250.3	23.00	50
18:1 Sphingosine	Sphingoid Base		17:1 Sphingosine	3.76	300.4 > 282.4	21.00	40
18:0 Sphinganine-1P	Sphingoid Base		17:1 Sphingosine-1P	4.03	382.4 > 266.4	25.00	50
18:1 Sphingosine-1P	Sphingoid Base		17:1 Sphingosine-1P	4.03	380.4 > 264.4	25.00	50
C14 Ceramide	[NS] Ceramide	d C18:1/C14:0	C17 Ceramide	8.82	510.7 > 492.6	21.00	50
C16 Ceramide	[NS] Ceramide	d C18:1/C16:0	C17 Ceramide	9.54	538.8 > 264.4	37.00	55
C18:1 Ceramide	[NS] Ceramide	d C18:1/C18:1	C17 Ceramide	9.81	564.5 > 546.4	24.00	60
C17 Ceramide	SSTD <sup>c</sup>		CUDA	9.94	552.8 > 534.5	24.00	55
C18 Ceramide	[NS] Ceramide	d C18:1/C18:0	C17 Ceramide	10.3	566.7 > 264.4	37.00	55
C18 dihydroceramide	[NdS] Ceramide	d C18:0/C18:0	C17 Ceramide	10.6	568.7 > 266.4	33.00	85
C20 Ceramide	[NS] Ceramide	d C18:1/C20:0	C17 Ceramide	11.3	594.4 > 576.5	21.00	55
C24 Ceramide	[NS] Ceramide	d C18:1/C24:0	C17 Ceramide	13.3	650.9 > 264.4	42.00	55
C24 dihydroceramide	[NdS] Ceramide	d C18:0/C24:0	C17 Ceramide	13.6	652.9 > 266.4	42.00	55

<sup>a</sup> Ceramides are synthesized by the addition of a fatty acid chain (represented by the carbon chain to the right of the slash) to a sphingoid base (represented by the carbon chain to the left of the slash)

<sup>b</sup> ISTD = Internal Standard. This compound is added to the samples at the reconstitution step

<sup>c</sup> SSTD = Surrogate. This compound is added to the samples prior to extraction

Supplemental Table S4

Supplemental Table S4A. Oxylipins and nitrolipids screened for and detected in the eccrine sweat of men and women with and without atopic dermatitis.

Lipid Mediator	Fatty Acid Precursor	Cluster <sup>a</sup>	Concentration (nM) <sup>b</sup>				p <sup>c</sup>
			Atopic Dermatitis		Control		
			Males (n=7)	Females (n=4)	Males (n=7)	Females (n=5)	
<i>Alcohols</i>							
9-HODE	C18:2n6	4	17.4 ± 14.2	9.87 ± 3.11	9.5 ± 6.66	13.1 ± 7.02	0.7
13-HODE	C18:2n6	4	20.6 ± 16.2	12.8 ± 6.7	9.44 ± 6.65	11.1 ± 4.85	0.5
9-HOTE	C18:3n3	2	1.03 ± 1.06	0.651 ± 0.0808	0.536 ± 0.519	0.753 ± 0.186	0.5
13-HOTE	C18:3n3	2	2.98 ± 4.57	1.73 ± 0.39	1.22 ± 1.11	1.34 ± 0.224	0.7
5-HETE	C20:4n6	1	0.147 ± 0.119	0.0604 ± 0.0173	0.0869 ± 0.0337	0.0604 ± 0.0252	0.4
8-HETE	C20:4n6	4	0.283 ± 0.184 <sup>A</sup>	0.0946 ± 0.0459 <sup>A</sup>	0.108 ± 0.0314 <sup>B</sup>	0.0942 ± 0.0471 <sup>B</sup>	<b>0.05</b>
9-HETE	C20:4n6		ND <sup>d</sup>	ND	ND	ND	
11-HETE	C20:4n6	4	0.223 ± 0.202	0.114 ± 0.0632	0.104 ± 0.0566	0.0831 ± 0.0429	0.4
12-HETE	C20:4n6	3	0.377 ± 0.294	0.392 ± 0.372	0.393 ± 0.509	0.403 ± 0.287	0.9
15-HETE	C20:4n6	4	0.648 ± 0.478	0.35 ± 0.29	0.884 ± 1.35	0.162 ± 0.059	0.5
20-HETE	C20:4n6		ND	ND	ND	ND	
5-HEPE	C20:5n3		ND	ND	ND	ND	
9-HEPE	C20:5n3	2	0.12 ± 0.165	0.0277 ± 0.0212	0.0353 ± 0.0322	0.037 ± 0.0157	0.4
12-HEPE	C20:5n3	4	0.125 ± 0.127	0.128 ± 0.146	0.0614 ± 0.0285	0.0753 ± 0.0128	0.7
15-HEPE	C20:5n3		ND	ND	ND	ND	
4-HDoHE	C22:6n3		ND	ND	ND	ND	
14-HDoHE	C22:6n3		ND	ND	ND	ND	
17-HDoHE	C22:6n3		ND	ND	ND	ND	
<i>Diols</i>							
9,10-e-DiHO	C18:0	3	872 ± 2020	44.2 ± 21.1	180 ± 114	69.4 ± 28.4	0.4
9,10-DiHOME	C18:2n6	2	5.73 ± 4.54	3.3 ± 1.22	2.13 ± 1.31	2 ± 0.781	0.12
12,13-DiHOME <sup>e</sup>	C18:2n6	2	0.1 ± 0.086	0.0536 ± 0.0157	0.0495 ± 0.0379	0.046 ± 0.0188	0.4
9,10-DiHODE	C18:3n3	2	0.325 ± 0.37	0.144 ± 0.092	0.0968 ± 0.107	0.116 ± 0.0961	0.4
12,13-DiHODE	C18:3n3		ND	ND	ND	ND	
15,16-DiHODE	C18:3n3	2	1.55 ± 2.83	0.233 ± 0.111	0.419 ± 0.396	0.46 ± 0.278	0.5
5,6-DiHETrE	C20:4n6	2	4.14 ± 1.9	3.18 ± 1.96	3.06 ± 2.37	3.76 ± 1	0.8
8,9-DiHETrE	C20:4n6	2	0.928 ± 0.312	0.657 ± 0.276	0.617 ± 0.35	0.773 ± 0.184	0.5
11,12-DiHETrE	C20:4n6	2	0.391 ± 0.341	0.184 ± 0.151	0.209 ± 0.196	0.258 ± 0.135	0.5
14,15-DiHETrE	C20:4n6	2	0.474 ± 0.313	0.233 ± 0.227	0.258 ± 0.264	0.356 ± 0.216	0.6
5,15-DiHETE	C20:5n3	2	0.135 ± 0.184	0.0141 ± 0.0115	0.0275 ± 0.0379	0.0187 ± 0.0161	0.4
8,15-DiHETE	C20:5n3		ND	ND	ND	ND	
14,15-DiHETE	C20:5n3		ND	ND	ND	ND	
17,18-DiHETE	C20:5n3	2	1.68 ± 1.29	0.656 ± 0.548	0.785 ± 0.848	1.04 ± 0.888	0.5
19,20-DiHDoPA	C22:6n3		ND	ND	ND	ND	

Supplemental Table S4

Lipid Mediator	Fatty Acid Precursor	Cluster <sup>a</sup>	Concentration (nM) <sup>b</sup>				p <sup>c</sup>	
			Atopic Dermatitis		Control			
			Males (n=7)	Females (n=4)	Males (n=7)	Females (n=5)		
<i>Epoxides</i>								
9,10-EpOME	C18:2n6	3	1.97 ± 2.91	1.09 ± 0.895	0.805 ± 0.311	0.949 ± 0.258	0.9	
12,13-EpOME	C18:2n6	3	0.828 ± 0.856	0.998 ± 0.805	0.621 ± 0.311	0.858 ± 0.308	0.8	
9,10-EpODE	C18:3n3		ND	ND	ND	ND		
12,13-EpODE	C18:3n3		ND	ND	ND	ND		
15,16-EpODE	C18:3n3		ND	ND	ND	ND		
8,9-EpETrE	C20:4n6		ND	ND	ND	ND		
11,12-EpETrE	C20:4n6	1	0.0601 ± 0.0709	0.0121 ± 0.0142	0.0176 ± 0.00741	0.0104 ± 0.00513	0.5	
14,15-EpETrE	C20:4n6		ND	ND	ND	ND		
11,12-EpETE	C20:5n3		ND	ND	ND	ND		
14,15-EpETE	C20:5n3		ND	ND	ND	ND		
17,18-EpETE	C20:5n3		ND	ND	ND	ND		
16,17-EpDPE	C22:6n3		ND	ND	ND	ND		
19,20-EpDPE	C22:6n3		ND	ND	ND	ND		
<i>Hydroperoxides</i>								
9-HpODE <sup>e</sup>	C18:2n6		ND	ND	ND	ND		
13-HpODE <sup>e</sup>	C18:2n6		ND	ND	ND	ND		
5-HpETE <sup>e</sup>	C20:4n6		ND	ND	ND	ND		
12-HpETE <sup>e</sup>	C20:4n6		ND	ND	ND	ND		
15-HpETE <sup>e</sup>	C20:4n6		ND	ND	ND	ND		
<i>Ketones</i>								
9-KODE	C18:2n6	4	13 ± 13.2	6.92 ± 5.11	5.8 ± 7	4.89 ± 3.87	0.7	
13-KODE	C18:2n6	4	5.52 ± 6.78	2.77 ± 2.81	1.45 ± 0.882	1.1 ± 0.752	0.4	
12,13-Ep-9-KODE	C18:3n3	2	5.68 ± 9.13	1.29 ± 0.779	1.15 ± 1.01	1.12 ± 0.464	0.4	
5-KETE	C20:4n6	1	0.0838 ± 0.0481	0.0454 ± 0.0274	0.0336 ± 0.0205	0.0207 ± 0.0202	0.10	
15-KETE	C20:4n6		ND	ND	ND	ND		
<i>Leukotrienes</i>								
6-trans-LTB4	C20:4n6		ND	ND	ND	ND		
LTB4	C20:4n6		ND	ND	ND	ND		
LTB5	C20:5n3		ND	ND	ND	ND		
<i>Nitrolipids</i>								
9-Nitrooleate	C18:1n9	1	0.531 ± 0.366	0.154 ± 0.2	0.643 ± 1.36	0.0644 ± 0.0639	0.2	
10-Nitrooleate	C18:1n9	1	0.578 ± 0.424 <sup>A</sup>	0.251 ± 0.215 <sup>A,B</sup>	1.05 ± 1.94 <sup>A</sup>	0.0413 ± 0.0371 <sup>B</sup>	0.02	
10-Nitrolinoleate	C18:2n6	1	0.274 ± 0.218	0.133 ± 0.135	0.156 ± 0.131	0.0515 ± 0.0378	0.3	

Supplemental Table S4

Lipid Mediator	Fatty Acid Precursor	Cluster <sup>a</sup>	Concentration (nM) <sup>b</sup>				p <sup>c</sup>
			Atopic Dermatitis		Control		
			Males (n=7)	Females (n=4)	Males (n=7)	Females (n=5)	
<b>Prostanoids</b>							
PGE1	C20:3n6	2	0.351 ± 0.214	0.299 ± 0.167	0.164 ± 0.0896	0.55 ± 0.246	0.08
PGD2	C20:4n6		ND	ND	ND	ND	
PGE2	C20:4n6	2	1.43 ± 1.32	2.04 ± 1.83	0.733 ± 0.754	4.23 ± 3.91	0.2
15-keto PGE2	C20:4n6		ND	ND	ND	ND	
6-keto PGF1a	C20:4n6		ND	ND	ND	ND	
PGF2a	C20:4n6	2	0.408 ± 0.313	0.273 ± 0.183	0.124 ± 0.117	0.225 ± 0.154	0.4
15-deoxy PGJ2	C20:4n6		ND	ND	ND	ND	
TXB2	C20:4n6		ND	ND	ND	ND	
PGE3	C20:5n3		ND	ND	ND	ND	
PGF3a	C20:5n3		ND	ND	ND	ND	
<b>Triols</b>							
Sum TriHOMEs <sup>f</sup>	C18:2n6	2	89.4 ± 118	25.3 ± 12.6	15.1 ± 8.07	25.3 ± 7.47	0.08
Lipoxin A4	C20:4n6		ND	ND	ND	ND	
Resolvin D1	C20:4n6		ND	ND	ND	ND	

<sup>a</sup> Analytes were clustered by Spearman's  $\rho$  correlation coefficients using the Minkowski distance and Ward agglomeration

<sup>b</sup> Values are reported as Mean ± Standard Deviation. Upper-case superscript letters indicate differences between group means as determined by one-way ANOVA with Tukey's post-hoc HSD.

<sup>c</sup> The reported p values are false detection rate-corrected at  $q = 0.05$

<sup>d</sup> ND= Not Detected

<sup>e</sup> Analytes are evaluated on a semi-quantitative basis due to lack of authentic standards

<sup>f</sup> This analyte represents the sum of the peaks of all observed isomers of the linoleate-derived triols. Concentrations were calculated relative to concentrations of authentic 9,12,13-TriHOME standards

Supplemental Table S4

Supplemental Table S4B. Endocannabinoids screened for and detected in the eccrine sweat of men and women with and without atopic dermatitis.

Lipid Mediator	Fatty Acid Precursor	Cluster <sup>a</sup>	Concentration (nM) <sup>b</sup>				p <sup>c</sup>
			Atopic Dermatitis		Control		
			Males (n=7)	Females (n=4)	Males (n=7)	Females (n=5)	
<b>Acylethanolamides</b>							
PEA	C16:0	1	3.62 ± 2.22	2.47 ± 2.18	3.26 ± 4.21	12.6 ± 27.7	0.8
SEA	C18:0	1	4.45 ± 4.49	2.78 ± 2.56	2.95 ± 3.55	13.5 ± 29.5	0.8
OEA	C18:1n9	1	0.46 ± 0.466	0.32 ± 0.176	0.73 ± 1.56	0.109 ± 0.174	0.3
LEA	C18:2n6	1	0.0825 ± 0.036	0.066 ± 0.0175	0.122 ± 0.201	0.0305 ± 0.0242	0.5
aLEA	C18:3n3	1	0.0313 ± 0.0203	0.0128 ± 0.013	0.0245 ± 0.0362	0.00632 ± 0.0044	0.11
Dihomo GLA EA	C18:3n6		ND <sup>d</sup>	ND	ND	ND	
11,12-EpETre EA	C20:4n6		ND	ND	ND	ND	
15-HETE EA	C20:4n6		ND	ND	ND	ND	
AEA	C20:4n6	2	0.0465 ± 0.0536	0.02 ± 0.0113	0.0384 ± 0.0406	0.0173 ± 0.00956	0.6
PGD2 EA	C20:4n6		ND	ND	ND	ND	
PGE2 EA	C20:4n6		ND	ND	ND	ND	
PGF2a EA	C20:4n6		ND	ND	ND	ND	
DEA	C22:4n6	1	0.0339 ± 0.0236	0.022 ± 0.0156	0.0317 ± 0.0518	0.00485 ± 0.00434	0.3
DHEA	C22:6n3		ND	ND	ND	ND	
<b>Monoacylglycerols</b>							
1-OG	C18:1n9	3	15300 ± 39100	405 ± 614	816 ± 988	118 ± 99	0.7
2-OG	C18:1n9	3	2000 ± 5110	43.4 ± 59.3	108 ± 132	14.1 ± 11.2	0.8
1-LG	C18:2n6	3	2590 ± 6200	120 ± 165	146 ± 177	34.5 ± 25.3	0.7
2-LG	C18:2n6	3	234 ± 551	19.8 ± 27.2	20.6 ± 26.2	5.37 ± 3.67	0.8
1-AG	C20:4n6	3	476 ± 1210	57.7 ± 25.5	36.6 ± 41.7	20.1 ± 15.2	0.8
2-AG	C20:4n6	3	65 ± 164	9.16 ± 5.24	6.48 ± 6.41	2.55 ± 1.81	0.8
PGE2 1G	C20:4n6		ND	ND	ND	ND	
PGF2a 1G	C20:4n6		ND	ND	ND	ND	
<b>Acylamides</b>							
NO-Gly	C18:1n9		ND	ND	ND	ND	
NA-Gly	C20:4n6		ND	ND	ND	ND	

<sup>a</sup> Analytes were clustered by Spearman's ρ correlation coefficients using the Minkowski distance and Ward agglomeration

<sup>b</sup> Values are reported as Mean ± Standard Deviation. Superscript letters indicate differences between group means as determined by one-way ANOVA with Tukey's post-hoc HSD.

<sup>c</sup> The reported p values are false detection rate-corrected at q = 0.05

<sup>d</sup> ND= Not Detected

Supplemental Table S4

Supplemental Table S4C. Ceramides and sphingoid bases screened for and detected in the eccrine sweat of men and women with and without atopic dermatitis.

Lipid Mediator	Fatty Acid Precursor <sup>a</sup>	Cluster <sup>b</sup>	Concentration (nM) <sup>c</sup>				p <sup>d</sup>
			Atopic Dermatitis		Control		
			Males (n=5)	Females (n=4)	Males (n=5)	Females (n=5)	
<b>[NdS] Ceramides</b>							
C18 dihydroceramide	d C18:0/C18:0	5	0.822 ± 0.304 <sup>A</sup>	0.335 ± 0.123 <sup>B</sup>	0.265 ± 0.126 <sup>B</sup>	0.289 ± 0.131 <sup>B</sup>	<b>0.003</b>
C24 dihydroceramide	d C18:0/C24:0	5	7.2 ± 5.99 <sup>A</sup>	2.79 ± 2.13 <sup>AB</sup>	0.889 ± 0.334 <sup>B</sup>	1.93 ± 1.16 <sup>B</sup>	<b>0.006</b>
<b>[NS] Ceramides</b>							
C14 Ceramide	d C18:1/C14:0	5	1.99 ± 1.81 <sup>A</sup>	0.463 ± 0.185 <sup>AB</sup>	0.273 ± 0.236 <sup>B</sup>	0.373 ± 0.25 <sup>AB</sup>	<b>0.02</b>
C16 Ceramide	d C18:1/C16:0	5	10.6 ± 4.08 <sup>A</sup>	3.45 ± 1.44 <sup>B</sup>	2.44 ± 1.54 <sup>B</sup>	3.33 ± 1.71 <sup>B</sup>	<b>0.0008</b>
C18 Ceramide	d C18:1/C18:0	5	2.51 ± 1.15 <sup>A</sup>	0.898 ± 0.33 <sup>AB</sup>	0.339 ± 0.167 <sup>B</sup>	0.649 ± 0.189 <sup>B</sup>	<b>0.0008</b>
C18:1 Ceramide	d C18:1/C18:1		ND <sup>e</sup>	ND	ND	ND	
C20 Ceramide	d C18:1/C20:0	5	3.5 ± 1.91 <sup>A</sup>	1.41 ± 0.72 <sup>AC</sup>	0.489 ± 0.256 <sup>B</sup>	1.08 ± 0.399 <sup>BC</sup>	<b>0.0008</b>
C24 Ceramide	d C18:1/C24:0	5	9.82 ± 5.67 <sup>A</sup>	3.28 ± 2.18 <sup>B</sup>	1.25 ± 0.519 <sup>B</sup>	2.6 ± 0.977 <sup>B</sup>	<b>0.0008</b>
<b>Sphingoid Bases</b>							
18:0 Sphinganine-1P			ND	ND	ND	ND	
18:1 Sphingosine		5	31.1 ± 16 <sup>A</sup>	9.05 ± 3.84 <sup>B</sup>	3.16 ± 2.75 <sup>B</sup>	6.27 ± 2.63 <sup>B</sup>	<b>0.0008</b>
18:1 Sphingosine-1P		1	28.6 ± 24.7	7.96 ± 4.26	9.58 ± 9.21	5.7 ± 3.86	0.12

<sup>a</sup> Ceramides are synthesized by the addition of a fatty acid chain (represented by the carbon chain to the right of the slash) to a sphingoid base (represented by the carbon chain to the left of the slash)

<sup>b</sup> Analytes were clustered by Spearman's  $\rho$  correlation coefficients using the Minkowski distance and Ward agglomeration

<sup>c</sup> Values are reported as Mean ± Standard Deviation. Superscript letters indicate differences between group means as determined by one-way ANOVA with Tukey's post-hoc HSD.

<sup>d</sup> The reported p values are false detection rate-corrected at  $q = 0.05$

<sup>e</sup> ND= Not Detected

**Supplemental Table S5**

**Supplemental Table S5.** Impact of storage and sampling conditions used on eccrine sweat lipid mediators in subjects without atopic dermatitis. Samples were stored at -80°C for 0-19 days or 20-30 days, and subjects were sampled between 09:00-14:00 or 14:00-18:00, respectively. Eccrine sweat lipid mediators in subjects with atopic dermatitis were unaffected by sampling and storage conditions.

Lipid Mediator	Fatty Acid Precursor		Storage Time (n = 5/7)		Sampling Time (n = 6/6)	
			Unadjusted p <sup>a</sup>	FDR-Corrected p <sup>b</sup>	Unadjusted p <sup>a</sup>	FDR-Corrected p <sup>b</sup>
<b>Endocannabinoids</b>						
<i>Acylethanolamides</i>						
	PEA	C16:0	<b>0.04</b>	0.62	0.06	0.32
	SEA	C18:0	<b>0.05</b>	0.62	0.06	0.32
<i>Monoacylglycerols</i>						
	1-OG	C18:1n9	0.93	0.96	<b>0.02</b>	0.32
	2-OG	C18:1n9	0.84	0.92	<b>0.04</b>	0.32
	1-LG	C18:2n6	0.83	0.92	<b>0.03</b>	0.32
	2-LG	C18:2n6	0.84	0.92	<b>0.04</b>	0.32
	1-AG	C20:4n6	0.72	0.92	<b>0.03</b>	0.32
	2-AG	C20:4n6	0.77	0.92	<b>0.04</b>	0.32
<b>Oxylipins and Nitrolipids</b>						
<i>Diols</i>						
	9,10-DiHHex	C16:0	0.08	0.73	<b>0.03</b>	0.32
<i>Epoxides</i>						
	9,10-EpO	C18:0	0.30	0.89	<b>0.05</b>	0.32
<i>Ketones</i>						
	13-KODE	C18:2n6	<b>0.02</b>	0.47	0.98	0.98
	12,13-Ep-9-KODE	C18:3n3	0.28	0.88	<b>0.01</b>	0.32
<i>Prostanoids</i>						
	PGE1	C20:3n6	<b>0.01</b>	0.47	0.91	0.94
	PGE2	C20:4n6	<b>0.02</b>	0.47	0.74	0.94

<sup>a</sup>The reported p values were determined by Student's t-test, and are unadjusted for multiple comparisons

<sup>b</sup>The reported p values adjust the p values from the preceding column for multiple comparisons by a false discovery rate correction (q = 0.05)