

SUPPLEMENTARY MATERIAL

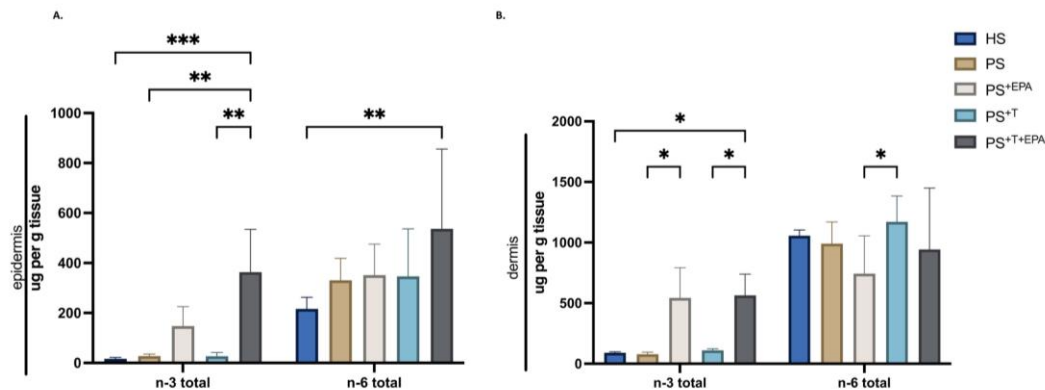


Figure S1. Epidermal and dermal phospholipid quantification of the skin substitutes. (A) Characterization of epidermal total fatty acids using gas chromatography. Impact of EPA supplementation on total n-3 and n-6 PUFAs quantities; (B) Characterization of dermal total fatty acids using gas chromatography. Impact of EPA supplementation on total n-3 and n-6 PUFAs quantities. The values are presented as $\mu\text{g/g}$ of tissue; (N=3 donors, n=2 skin substitutes per donor). Statistical significance was determined using two-way ANOVA followed by Tukey's *post-hoc* test. Significant differences are indicated by asterisks (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$). Abbreviations: HS: healthy substitutes; EPA: eicosapentaenoic acid; PS: psoriatic substitutes; PS^{+EPA}: psoriatic substitutes supplemented with EPA; PS^{+T}: psoriatic substitutes produced with T cells; PS^{+T+EPA}: psoriatic substitutes produced with T cells and supplemented with EPA; T: T cells.

The comparison of the levels of lipid mediators derived from n-3 and n-6 PUFAs between the PS and PS^{+T} conditions was carried out. As for the n-3 PUFA lipid mediators, no difference between n-3 prostaglandins levels was noted between PS and PS^{+T}. As for n-3 HFAs, the amounts of 12-HEPE were decreased in PS^{+T} as compared with PS, suggesting that T cells might affect the efficiency of the 12-LOX enzyme (Figure S2.A). As for the n-6 PUFA lipid mediators, prostaglandin levels were relatively similar between PS and PS^{+T}, except for PGE₂ where a significant increase was quantified in PS^{+T}. Moreover, 12-HETE was diminished in PS^{+T} compared with PS, reinforcing the hypothesis that the addition of T cells affects the function of 12-LOX preventing it from producing both 12-HEPE and 12-HETE (Figure S2.A and S2.B).

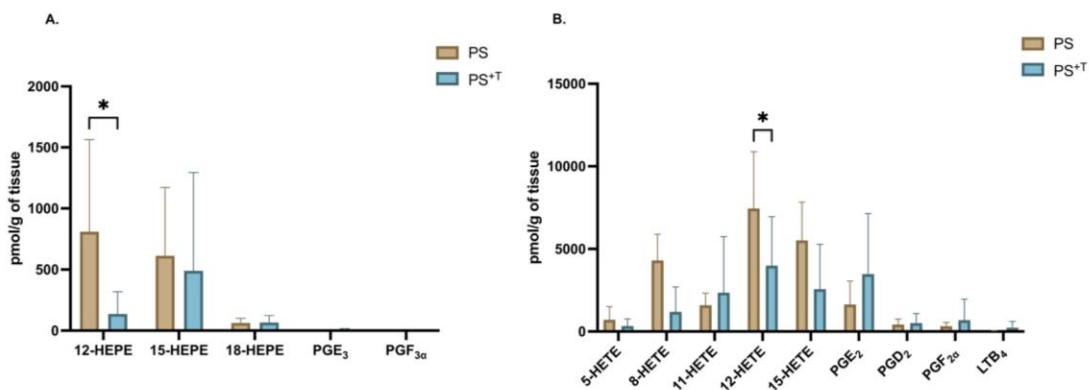


Figure S2. n-3 and n-6-PUFA lipid mediator profile of the psoriatic skin conditions. Influence of the addition of T cells to psoriatic substitutes the levels of n-3 and n-6-PUFA lipid derivatives in the epidermis of PS and PS⁺; (A) Characterization of epidermal n-3 lipid mediators derived from EPA; (B) Characterization of epidermal n-6 lipid mediators derived from AA. (N=3 donors; n=2 skin substitutes per donor). Statistical significance was determined using two-way

ANOVA followed by Bonferroni's *post-hoc* test. Significant differences are indicated by asterisks (* $p < 0.05$). Abbreviations: AA: arachidonic acid; EPA: eicosapentaenoic acid; HEPE: hydroxyeicosapentaenoic acid; HETE: hydroxyeicosatetraenoic acid; T: T cells; n-3: omega-3; n-6: omega-6; PG: prostaglandin; PUFAs: polyunsaturated fatty acids; PS: psoriatic substitutes; PS^T: psoriatic substitutes produced with T cells.

Supplementary tables

Table S1. Levels of fatty acids in phospholipids of the epidermis of the skin substitutes after EPA supplementation.

Epidermal phospholipids						
Mean \pm SD (%)						
Fatty acids	HS	PS	PS ^{EPA}	PS ^T	PS ^{T+EPA}	P-value
Saturated FAs						
14:0 (Myristic acid)	1.786 \pm 0.374	2.185 \pm 0.216	2.235 \pm 0.154	2.048 \pm 0.542	1.922 \pm 0.401	NS
16:0 (Palmitic acid)	14.070 \pm 1.043	14.458 \pm 0.483	15.625 \pm 0.741	16.29 \pm 1.614	16.51 \pm 1.77	NS
18:0 (Stearic acid)	11.365 \pm 0.783	10.852 \pm 0.501	11.228 \pm 0.632	11.68 \pm 0.978	13.618 \pm 2.74	NS
20:0	0.325 \pm 0.187	0.307 \pm 0.052	0.347 \pm 0.06	0.405 \pm 0.142	0.433 \pm 0.107	NS
22:0	0.667 \pm 0.301	0.510 \pm 0.082	0.345 \pm 0.272	0.637 \pm 0.221	0.773 \pm 0.299	NS
24:0	1.755 \pm 0.591	1.378 \pm 0.122	1.328 \pm 0.101	1.678 \pm 0.491	2.012 \pm 0.646	NS
n-3 PUFAs						
t-18:3n-3	ND	ND	ND	ND	ND	NS
18:3n-3 (ALA)	ND	ND	ND	ND	ND	NS
20:3n-3 (ETE)	ND	ND	ND	ND	ND	NS
20:4n-3 (ETA)	ND	ND	ND	ND	ND	NS
20:5n-3 (EPA)	ND	0.185 \pm 0.102	2.838 \pm 1.201	0.082 \pm 0.09	2.645 \pm 0.601	<0.0001
22:3n-3	ND	ND	ND	ND	ND	NS
22:5n-3 (DPA)	0.438 \pm 0.073	0.445 \pm 0.069	2.942 \pm 2.042	0.457 \pm 0.165	4.045 \pm 1.895	NS
22:6n-3 (DHA)	0.388 \pm 0.054	0.437 \pm 1.178	1.818 \pm 62.5	0.402 \pm 0.097	2.505 \pm 1.294	<0.0001
n-5 PUFAs						
t-14:1n-5	ND	ND	ND	ND	ND	NS
14:1n-5	ND	ND	ND	ND	ND	NS
18:1n-5	0.472 \pm 0.138	0.320 \pm 0.0054	0.328 \pm 0.067	0.437 \pm 0.08	0.240 \pm 0.196	NS
n-6 PUFAs						
18:1	ND	ND	ND	ND	ND	NS
t-18:2n-6	0.12 \pm 0.134	0.357 \pm 0.203	0.255 \pm 0.141	0.160 \pm 0.137	0.07 \pm 0.109	NS
9c12t-18:2n-6	0.170 \pm 0.137	0.365 \pm 0.067	0.255 \pm 0.051	0.257 \pm 0.175	0.112 \pm 0.131	NS
8t12c-18:2n-6	0.103 \pm 0.161	ND	0.183 \pm 0.299	0.302 \pm 0.387	0.040 \pm 0.098	NS
18:2n-6 (LA)	3.750 \pm 0.296	5.408 \pm 0.870	4.547 \pm 0.866	4.332 \pm 1.115	4.980 \pm 0.188	0.0023
18:3n-6 (γ -linolenic acid)	0.048 \pm 0.118	0.462 \pm 0.276	0.252 \pm 0.221	0.097 \pm 0.150	0.065 \pm 0.107	NS
20:2n-6	ND	0.045 \pm 0.071	0.037 \pm 0.058	0.042 \pm 0.066	0.043 \pm 0.07	NS
20:3n-6 (dihomo- γ -linolenic acid)	0.915 \pm 0.256	1.255 \pm 0.175	1.170 \pm 0.132	1.360 \pm 0.493	1.548 \pm 0.434	NS
20:4n-6 (AA)	1.783 \pm 1.412	3.962 \pm 0.359	3.312 \pm 0.461	4.030 \pm 1.187	3.498 \pm 0.460	<0.0001
22:2n-6	ND	ND	ND	ND	ND	NS
22:4n-6	0.623 \pm 0.564	1.020 \pm 0.231	0.743 \pm 0.107	1.118 \pm 0.329	0.903 \pm 0.399	NS
22:5n-6	0.917 \pm 0.123	0.195 \pm 0.096	0.040 \pm 0.062	0.178 \pm 0.139	0.088 \pm 0.110	NS
n-7 MUFAs						
t-16:1n-7	ND	ND	ND	ND	ND	NS
16:1n-7 (Palmitoleic acid)	8.557 \pm 1.733	10.452 \pm 2.072	10.355 \pm 1.884	8.813 \pm 2.643	6.975 \pm 3.303	NS
t-18:1n-7	0.025 \pm 0.061	0.05 \pm 0.078	0.037 \pm 0.057	0.028 \pm 0.069	0.037 \pm 0.057	NS
18:1n-7 (Vaccenic acid)	15.847 \pm 1.809	13.723 \pm 0.954	12.83 \pm 0.789	13.847 \pm 1.00	10.2 \pm 3.807	0.0101
n-9 MUFA						

t-18:1n-9	0.09 ± 0.143	0.067 ± 0.103	0.052 ± 0.08	0.075 ± 0.117	0.053 ± 0.084	NS
18:1n-9 (Oleic acid)	29.92 ± 1.901	27.723 ± 2.776	27.02 ± 2.105	28.482 ± 0.76	28.88 ± 2.946	NS
20:1n-9	0.337 ± 0.263	0.408 ± 0.064	0.353 ± 0.072	0.445 ± 0.169	0.258 ± 0.203	NS
22:1n-9	ND	ND	ND	ND	ND	NS
24:1n-9	1.053 ± 0.209	0.732 ± 0.107	0.745 ± 0.176	0.992 ± 0.293	0.983 ± 0.278	NS
n-12 MUFA						
t-18:1n-12	ND	ND	ND	ND	ND	NS
18:1n-12	ND	ND	ND	ND	ND	NS
20:1n-12	ND	ND	ND	ND	ND	NS
Totals						
Total SFAs	31.323 ± 2.153	31.22 ± 0.638	32.295 ± 1.00	33.629 ± 1.129	35.937 ± 3.169	0.0003
Total PUFAs	11.852 ± 1.165	14.172 ± 1.751	15.112 ± 2.142	12.812 ± 2.65	18.535 ± 7.272	<0.0001
Total MUFAs	58.565 ± 3.241	55.338 ± 2.147	53.178 ± 2.797	54.738 ± 2.096	46.697 ± 10.117	<0.0001

Abbreviations: AA, arachidonic acid; ALA, α-linolenic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; FA, fatty acid; LA, linoleic acid; MUFA, monounsaturated fatty acid; PUFAs, polyunsaturated fatty acids; SFA, saturated fatty acid; SD, standard deviation.

Table S2. Levels of fatty acids in phospholipids of the dermis of the skin substitutes after EPA supplementation.

Dermal phospholipids						
Mean ±SD (%)						
Fatty acids	HS	PS	PS+EPA	PS ^T	PS ^T +EPA	P-value
Saturated FAs						
14:0 (Myristic acid)	2.096 ± 0.161	2.145 ± 0.153	2.035 ± 0.179	2.078 ± 0.42	1.722 ± 0.398	NS
16:0 (Palmitic acid)	14.522 ± 0.607	14.557 ± 0.457	14.425 ± 0.842	15.585 ± 1.744	14.098 ± 0.817	NS
18:0 (Stearic acid)	16.632 ± 0.27	16.652 ± 0.298	17.785 ± 0.323	18.343 ± 1.253	20.047 ± 2.046	0.0012
20:0	0.182 ± 0.013	0.227 ± 0.025	0.22 ± 0.033	0.170 ± 0.096	0.237 ± 0.05	NS
22:0	0.572 ± 0.047	0.69 ± 0.072	0.7 ± 0.091	0.63 ± 0.179	0.745 ± 0.152	NS
24:0	1.15 ± 0.069	1.325 ± 0.103	1.625 ± 0.266	1.192 ± 0.186	1.582 ± 0.387	NS
n-3 PUFAs						
t-18:3n-3	ND	ND	ND	ND	ND	NS
18:3n-3 (ALA)	ND	ND	ND	ND	ND	NS
20:3n-3 (ETE)	ND	ND	ND	ND	ND	NS
20:4n-3 (ETA)	ND	ND	ND	ND	ND	NS
20:5n-3 (EPA)	0.050 ± 0.046	0.018 ± 0.045	3.588 ± 2.138	0.092 ± 0.156	4.077 ± 2.568	<0.0001
22:3n-3	ND	ND	ND	ND	ND	NS
22:5n-3 (DPA)	1.54 ± 0.06	1.437 ± 0.236	7.02 ± 1.599	1.688 ± 0.189	6.798 ± 1.552	<0.0001
22:6n-3 (DHA)	1.094 ± 0.109	1.158 ± 0.115	2.587 ± 1.012	1.284 ± 0.28	3.28 ± 1.323	0.0001
n-5 PUFAs						
t-14:1n-5	ND	ND	ND	ND	ND	NS
14:1n-5	ND	ND	ND	ND	ND	NS
18:1n-5	0.48 ± 0.051	0.357 ± 0.065	0.328 ± 0.079	0.347 ± 0.269	0.257 ± 0.158	NS
n-6 PUFAs						
18:1	ND	ND	ND	ND	ND	NS
t-18:2n-6	0.054 ± 0.05	ND	ND	0.012 ± 0.029	ND	NS
9c12t-18:2n-6	0.12 ± 0.019	0.092 ± 0.073	0.023 ± 0.057	0.045 ± 0.07	0.068 ± 0.075	NS
8t12c-18:2n-6	0.088 ± 0.082	0.065 ± 0.102	ND	0.022 ± 0.053	ND	NS
18:2n-6 (LA)	2.554 ± 0.222	4.642 ± 0.604	4.583 ± 0.692	3.362 ± 0.186	4.642 ± 0.692	0.0096
18:3n-6 (γ-linolenic acid)	0.136 ± 0.026	0.125 ± 0.101	0.058 ± 0.092	0.062 ± 0.068	0.078 ± 0.092	NS
20:2n-6	0.164 ± 0.032	0.238 ± 0.044	0.182 ± 0.1	0.183 ± 0.144	0.148 ± 0.132	NS

20:3n-6 (dihomo- γ -linolenic acid)	0.414 \pm 0.053	0.54 \pm 0.061	0.592 \pm 0.09	0.455 \pm 0.036	0.663 \pm 0.122	NS
20:4n-6 (AA)	14.152 \pm 1.688	20.395 \pm 0.523	14.813 \pm 2.657	19.715 \pm 1.163	14.162 \pm 3.66	<0.0001
22:2n-6	ND	ND	ND	ND	ND	NS
22:4n-6	2.992 \pm 1.256	5.53 \pm 0.627	2.223 \pm 0.994	5.55 \pm 0.748	1.957 \pm 1.212	<0.0001
22:5n-6	0.952 \pm 0.447	1.575 \pm 0.283	0.363 \pm 0.246	1.815 \pm 0.361	0.272 \pm 0.3	NS
n-7 MUFAs						
t-16:1n-7	ND	ND	ND	ND	ND	NS
16:1n-7 (Palmitoleic acid)	2.438 \pm 0.326	2.707 \pm 0.661	2.655 \pm 0.923	1.968 \pm 0.282	1.758 \pm 0.485	NS
t-18:1n-7	0.112 \pm 0.033	0.133 \pm 0.066	0.087 \pm 0.096	0.04 \pm 0.063	0.097 \pm 0.077	NS
18:1n-7 (Vaccenic acid)	7.516 \pm 0.917	6.635 \pm 0.627	5.485 \pm 0.576	6.357 \pm 0.856	4.54 \pm 1.435	0.0006
n-9 MUFA						
t-18:1n-9	0.196 \pm 0.038	0.185 \pm 0.023	0.062 \pm 0.098	0.123 \pm 0.096	0.087 \pm 0.1	NS
18:1n-9 (Oleic acid)	17.702 \pm 0.793	16.363 \pm 0.557	16.08 \pm 0.439	16.802 \pm 0.793	16.825 \pm 1.064	0.0462
20:1n-9	0.474 \pm 0.072	0.363 \pm 0.074	0.285 \pm 0.084	0.317 \pm 0.170	0.178 \pm 0.169	NS
22:1n-9	ND	ND	ND	ND	ND	NS
24:1n-9	2.01 \pm 0.071	1.858 \pm 0.183	1.74 \pm 0.351	1.748 \pm 0.228	1.518 \pm 0.37	NS
n-12 MUFA						
t-18:1n-12	ND	ND	ND	ND	ND	NS
18:1n-12	ND	ND	ND	ND	ND	NS
20:1n-12	ND	ND	ND	ND	ND	NS
Totals						
Total SFAs	36.38 \pm 0.432	36.817 \pm 0.542	37.862 \pm 1.124	38.805 \pm 2.295	39.345 \pm 1.756	0.0003
Total PUFAs	33.822 \pm 0.826	35.808 \pm 0.883	36.273 \pm 1.62	34.303 \pm 2.065	36.153 \pm 2.245	0.0004
Total MUFAs	32.424 \pm 1.284	30.068 \pm 1.129	27.725 \pm 1.216	28.778 \pm 1.498	26.018 \pm 2.688	<0.0001

Abbreviations: AA, arachidonic acid; ALA, α -linolenic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; FA, fatty acid; LA, linoleic acid; MUFA, monounsaturated fatty acid; PUFAs, polyunsaturated fatty acids; SFA, saturated fatty acid; SD, standard deviation.

Table S3. Levels of bioactive lipids in the skin substitutes after EPA supplementation.

Bioactive lipids	Mean \pm SD (pmol/g of tissue)			P-value	
	HS	PS	PS ^{+EPA}	PS vs PS ^{+EPA}	PS vs HS
EPA derivatives					
PGE ₃	0.853 \pm 2.09	ND	360.83 \pm 442.99	0.0191	NS
PGF _{3α}	1.227 \pm 3.005	ND	ND	NS	NS
12-HEPE	210.65 \pm 278.51	809.65 \pm 754.32	3721.27 \pm 3011.2	0.0038	NS
15-HEPE	363.66 \pm 763.37	612.63 \pm 560.14	2169.45 \pm 2097.3	NS	NS
18-HEPE	22.17 \pm 10.35	62.38 \pm 38.06	668.49 \pm 326.43	NS	NS
LTB ₅	ND	ND	ND	NS	NS
DHA derivatives					
14-HDHA	887.45 \pm 555.26	835.71 \pm 736.33	1219.96 \pm 969.95	NS	NS
17-HDHA	1142.95 \pm 1132.65	2475.9 \pm 1809.7	10561.7 \pm 9171.6	0.0008	NS
RvD ₅	ND	27.9 \pm 36.4	1.22 \pm 2.45	NS	NS
Maresin 2	2.43 \pm 3.77	ND	ND	NS	NS
LA derivatives					
9-HODE	7347.5 \pm 3457.5	25210.8 \pm 11934.1	12532.7 \pm 14265.4	NS	NS
13-HODE	28383.5 \pm 21301.3	148139.3 \pm 141509.9	55890.9 \pm 34010.8	NS	0.0175
DGLA derivatives					
PGE ₁	8.32 \pm 20.37	25.6 \pm 51.3	80.1 \pm 46.5	NS	NS
6-keto PGF _{1α}	12.5 \pm 15.9	4.8 \pm 5.6	5.4 \pm 6.2	NS	NS
15-HETrE	72.3 \pm 57.04	334.9 \pm 179.2	113.8 \pm 24.6	<0.0001	<0.0001
LTB ₃	ND	ND	ND	NS	NS

AA derivatives

PGE ₂	134.37 ± 52.9	1638.1 ± 1408.4	488.3 ± 330.6	NS	NS
PGD ₂	39.9 ± 66.1	422.5 ± 325.3	250.8 ± 427.1	NS	NS
1a1b-dihomo PGF _{2α}	4.1 ± 1.79	6.83 ± 2.7	2.0 ± 2.35	NS	NS
PGF _{2α}	11.9 ± 13.5	319.7 ± 219.6	599.3 ± 814	NS	NS
5-HETE	630.9 ± 249.1	706.2 ± 805.4	302.9 ± 261.6	NS	NS
8-HETE	1004.1 ± 559.8	4300.6 ± 1587.2	801.8 ± 528.1	0.0284	0.0286
11-HETE	626.1 ± 209.9	1586.3 ± 733.9	702.7 ± 199.2	NS	NS
12-HETE	899.4 ± 510.8	7446.8 ± 3428.6	1885.2 ± 945.4	<0.0001	<0.0001
15-HETE	644.9 ± 485.2	5516.7 ± 2306.3	2173.8 ± 2167.3	0.0251	0.0002
LTB ₄	17.57 ± 16.7	211.5 ± 381.9	194.4 ± 393.3	NS	NS

Abbreviations: AA, arachidonic acid; ALA, α-linolenic acid; DGLA, dihomo-gamma-linolenic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; HEPE, hydroxyeicosapentaenoic acid; HETE, hydroperoxyeicosatetraenoic acid; HETRe, hydroxyeicosatrienoic acid; HDHA, hydroxydocosahexaenoic acid; HODE, hydroxyoctadecadienoic acid; LA, linoleic acid; LT, Leukotriene; N.D., not detected; PG, prostaglandin; Rv, Resolvin; SD, standard deviation.

Table S4. Levels of bioactive lipids in the skin substitutes produced with T cells after EPA supplementation.

Bioactive lipids	Mean ±SD (pmol/g of tissue)		P-value
	PS ⁺ T	PS ⁺ T+EPA	PS ⁺ T vs PS ⁺ TEPA
EPA derivatives			
PGE ₃	6.67 ± 8.967	293.32 ± 405.06	NS
PGF _{3α}	ND	22.82 ± 55.90	NS
12-HEPE	137.27 ± 181.53	1665.95 ± 2344.6	NS
15-HEPE	489.5 ± 803.55	1532.95 ± 2350.13	NS
18-HEPE	65.65 ± 58.58	538.77 ± 203.93	NS
LTB ₅	ND	ND	NS
DHA derivatives			
14-HDHA	151.72 ± 211.34	410.99 ± 549.47	NS
17-HDHA	2030.9 ± 2271.9	4284.2 ± 3994.6	NS
RvD ₅	ND	ND	NS
Maresin 2	ND	ND	NS
LA derivatives			
9-HODE	13521.7 ± 16007.7	8134.3 ± 10863.7	NS
13-HODE	87296 ± 132641.6	44923.6 ± 45115.6	NS
DGLA derivatives			
PGE ₁	40.9 ± 58.4	80.3 ± 81.7	NS
6-keto PGF _{1α}	32.05 ± 48.9	2.4 ± 4.9	NS
15-HETRe	138.8 ± 160.1	77.3 ± 45.8	NS
LTB ₃	6.9 ± 17.1	ND	NS
AA derivatives			
PGE ₂	2813.3 ± 3500.4	911.2 ± 990.4	0.0085
PGD ₂	507.9 ± 577.7	219.3 ± 490.4	NS
1a1b-dihomo PGF _{2α}	6.67 ± 2.6	0.79 ± 1.59	NS
PGF _{2α}	677.2 ± 1274.9	396.7 ± 766.3	NS
5-HETE	327.1 ± 427.3	130.9 ± 153.4	NS
8-HETE	1188.5 ± 1510.2	1525.3 ± 3026.7	NS
11-HETE	2355.1 ± 3387.2	448.9 ± 364.1	NS
12-HETE	3986.5 ± 2955.5	411.9 ± 301.1	0.0232
15-HETE	2567.8 ± 2701.5	1619.6 ± 2035.1	NS
LTB ₄	225.1 ± 373.5	113.3 ± 235.6	NS

Abbreviations: AA, arachidonic acid; ALA, α-linolenic acid; DGLA, dihomo-gamma-linolenic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; HEPE, hydroxyeicosapentaenoic acid; HETE, hydroperoxyeicosatetraenoic acid; HETRe, hydroxyeicosatrienoic acid; HDHA, hydroxydocosahexaenoic acid; HODE, hydroxyoctadecadienoic acid; LA, linoleic acid; LT, Leukotriene; N.D., not detected; PG, prostaglandin; Rv, Resolvin; SD, standard deviation.