

## SUPPLEMENTARY MATERIAL

**Table S1. Levels of epidermal phospholipid fatty acids following DHA supplementation.**

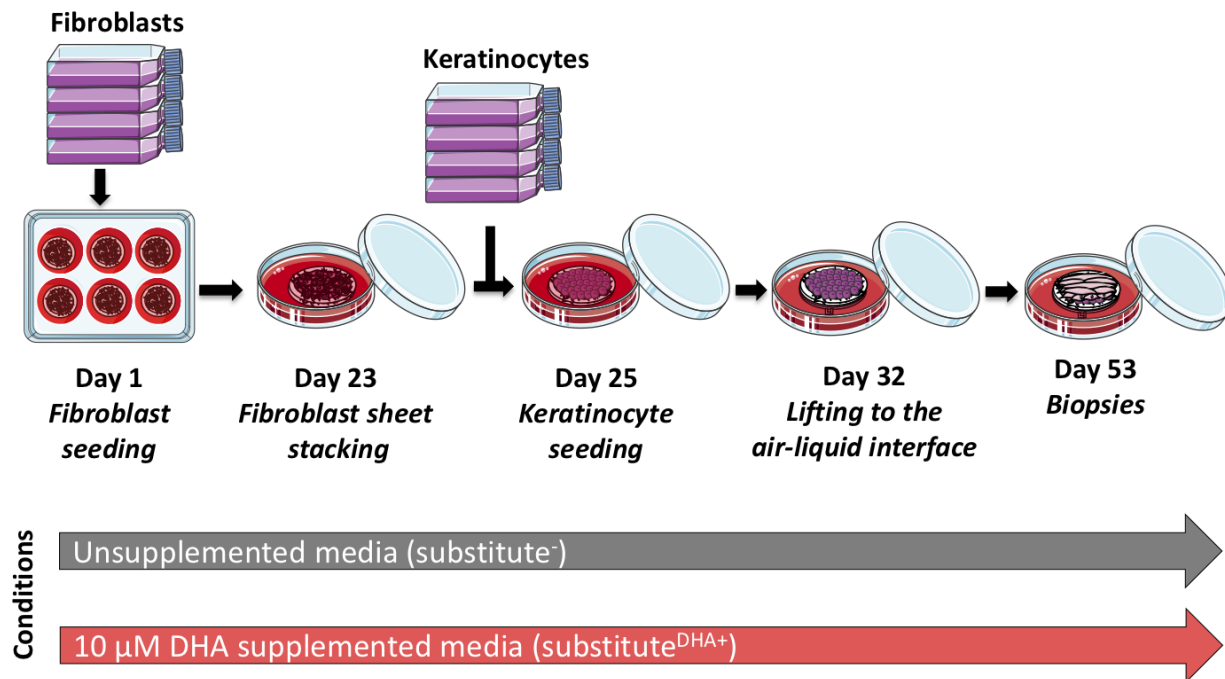
	Epidermis		
	Mean ± SD (µg per g of tissue)		
Fatty acids	Substitute <sup>-</sup>	Substitute <sup>DHA+</sup>	p-value
<b>Saturated FAs</b>			
14:0 (Myristic acid)	38,8 ± 11,0	33,7 ± 8,7	NS
16:0 (Palmitic acid)	292,5 ± 69,9	233,0 ± 62,2	NS
18:0 (Stearic acid)	236,2 ± 61,6	199,2 ± 45,9	NS
<b>n-3 PUFAs</b>			
18:3n-3(ALA)	0 ± 0	2,3 ± 2,6	NS
20:3n-3 (ETE)	0 ± 0	0 ± 0	NS
20:4n-3 (ETA)	0 ± 0	0,9 ± 1,4	NS
20:5n-3 (EPA)	1,3 ± 2,1	29,2 ± 5,0	<0,0001
22:5n-3 (DPA)	11,8 ± 1,9	14,9 ± 4,1	NS
22:6n-3 (DHA)	11,8 ± 4,0	64,7 ± 11,2	<0,0001
<b>n-6 PUFAs</b>			
18:2n-6 (LA)	107,8 ± 23,1	115,4 ± 28,5	NS
18:3n-6 (GLA)	0,0 ± 0,0	2,8 ± 3,0	NS
20:3n-6 (DGLA)	20,4 ± 4,6	13,6 ± 4,0	NS
20:4n-6 (AA)	78,6 ± 19,7	44,2 ± 6,7	0,0001
<b>n-7 MUFAs</b>			
16:1n-7 (Palmitoleic acid)	179,4 ± 56,3	152,4 ± 35,8	NS
18:1n-7 (Vaccenic acid)	317,6 ± 104,9	198,3 ± 72,7	0,0469
<b>n-9 MUFAs</b>			
18:1n-9 (Oleic acid)	603,5 ± 158,3	518,0 ± 85,9	NS
<b>Totals</b>			
Total PUFAs	291,7 ± 60,4	342,3 ± 54,3	NS
Total MUFAs	902,0 ± 571,4	716,2 ± 351,4	NS
Total FAs	2073,9 ± 511,2	1757,6 ± 347,9	<0,0001

Abbreviations : AA, arachidonic acid; ALA,  $\alpha$ -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.

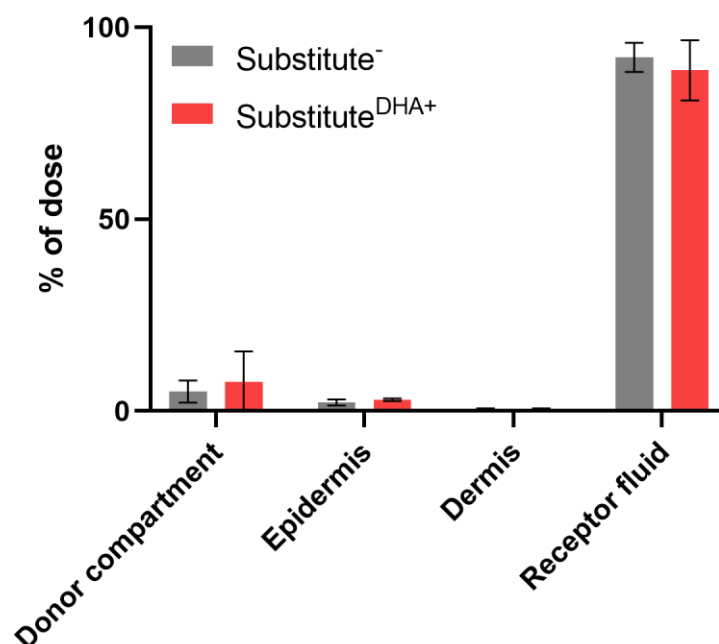
**Table S2. Flux of testosterone in skin substitutes following DHA supplementation.**

Time (h)	Mean $\pm$ SEM ( $\mu\text{g}/\text{cm}^2/\text{h}$ )		p-value
	Substitute <sup>-</sup>	Substitute <sup>DHA+</sup>	
1	28,3 $\pm$ 2,6	25,8 $\pm$ 2,1	NS
2	63,0 $\pm$ 4,4	54,9 $\pm$ 3,8	NS
3	101,0 $\pm$ 4,8	83,8 $\pm$ 4,0	0,0101
4	128,5 $\pm$ 4,8	111,2 $\pm$ 6,2	0,0387
6	111,6 $\pm$ 2,2	109,2 $\pm$ 3,3	NS
8	72,2 $\pm$ 2,9	78,6 $\pm$ 1,9	NS
24	7,6 $\pm$ 0,6	10,3 $\pm$ 1,0	NS

All data are mean  $\pm$  SEM. Statistical analyses were performed with two-way ANOVA followed by Sidak's post-hoc test. Abbreviation: SEM, standard error of the mean.



**Figure S1. Schematic overview of the preparation of tissue-engineered skin substitutes according to the self-assembly method.** A) Fibroblasts were cultured with ascorbic acid to form manipulatable sheets. B) Two fibroblast sheets were stacked to form the dermal layer. C) Keratinocytes were seeded on the dermal layer and skin substitutes were cultured for 1 week in submerged conditions and then D) for 21 days at the air-liquid interface. E) Biopsies were taken from the skin substitutes and were analyzed.



**Figure S2. Percutaneous absorption of testosterone through skin substitutes.** Testosterone was recovered in the different compartments after 24 h and is expressed as percentage of dose. Values are mean  $\pm$  standard error of the mean (SEM).  $n=15$  (3 donors, 5 skin substitutes per donor), Two-way ANOVA followed by Tukey's *post-hoc* test.