

Supplementary Information for

Prolonged experimental sleep disturbance affects the inflammatory resolution pathways in healthy humans

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Table S1. Formal names and CAS registry numbers of lipid mediators.

Analyte	Formal name	CAS RN
17-HDHA	17 <i>R/S</i> -hydroxy-4 <i>Z</i> ,7 <i>Z</i> ,10 <i>Z</i> ,13 <i>Z</i> ,15 <i>E</i> ,19 <i>Z</i> -docosahexaenoic acid	90780-52-2
RvD1	7 <i>S</i> ,8 <i>R</i> ,17 <i>S</i> -trihydroxy-4 <i>Z</i> ,9 <i>E</i> ,11 <i>E</i> ,13 <i>Z</i> ,15 <i>E</i> ,19 <i>Z</i> -docosahexaenoic acid	872993-05-0
RvD2	7 <i>S</i> ,16 <i>R</i> ,17 <i>S</i> -trihydroxy-4 <i>Z</i> ,8 <i>E</i> ,10 <i>Z</i> ,12 <i>E</i> ,14 <i>E</i> ,19 <i>Z</i> -docosahexaenoic acid	810668-37-2
RvD3	4 <i>S</i> ,11 <i>R</i> ,17 <i>S</i> -trihydroxy-5 <i>Z</i> ,7 <i>E</i> ,9 <i>E</i> ,13 <i>Z</i> ,15 <i>E</i> ,19 <i>Z</i> -docosahexaenoic acid	916888-47-6
RvD4	4 <i>S</i> ,5 <i>R</i> ,17 <i>S</i> -trihydroxy-6 <i>E</i> ,8 <i>E</i> ,10 <i>Z</i> ,13 <i>Z</i> ,15 <i>E</i> ,19 <i>Z</i> -docosahexaenoic acid	1025684-60-9
RvD5	7 <i>S</i> ,17 <i>S</i> -dihydroxy-4 <i>Z</i> ,8 <i>E</i> ,10 <i>Z</i> ,13 <i>Z</i> ,15 <i>E</i> ,19 <i>Z</i> -docosahexaenoic acid	578008-43-2
18-HEPE	18 <i>R/S</i> -hydroxy-5 <i>Z</i> ,8 <i>Z</i> ,11 <i>Z</i> ,14 <i>Z</i> ,16 <i>E</i> -eicosapentaenoic acid	141110-17-0
RvE1	5 <i>S</i> ,12 <i>R</i> ,18 <i>R</i> -trihydroxy-6 <i>Z</i> ,8 <i>E</i> ,10 <i>E</i> ,14 <i>Z</i> ,16 <i>E</i> -eicosapentaenoic acid	552830-51-0
RvE2	5 <i>S</i> ,18 <i>R</i> -dihydroxy-6 <i>E</i> ,8 <i>Z</i> ,11 <i>Z</i> ,14 <i>Z</i> ,16 <i>E</i> -eicosapentaenoic acid	865532-70-3

Table S2. Lower limit of quantification (LOQ) and limit of detection (LOD) information.

Analyte	Per plasma sample		Samples <LOQ, ≥LOD ^a		Samples <LOD ^b	
	LOQ	LOD	out of 180		out of 180	
	pg/ml	pg/ml	Count	Percentage	Count	Percentage
17-HDHA	1.042	0.521	—	—	—	—
RvD1	0.130	0.065	2	1.1%	1	0.6%
RvD2	0.260	0.130	19	11%	11	6%
RvD3	0.130	0.065	24	13%	7	4%
RvD4	0.130	0.065	26	14%	26	14%
RvD5	0.130	0.130	—	—	42	23%
18-HEPE	0.521	0.260	—	—	—	—
RvE1	0.130	0.065	16	9%	47	26%
RvE2	0.130	0.065	—	—	1	0.6%

^a Samples <LOQ, ≥LOD were left in the dataset as is.

^b Samples <LOD were set to half the LOD for GLMM analysis.

Table S3. Intra-assay CV, inter-assay CV, and recovery rate.

Analyte	Intra-assay CV %	Inter-assay CV %	Recovery rate %
17-HDHA	13.0	12.3	85.1
RvD1	6.5	7.9	103.7
RvD2	4.3	5.1	104.3
RvD3	0.8	4.1	107.6
RvD4	4.6	5.2	107.7
RvD5	6.0	6.6	98.4
18-HEPE	9.0	12.9	102.0
RvE1	5.6	6.3	102.8
RvE2	N/A	N/A	N/A

Table S4. Daily diet composition.

Variable, mean (SD)	Control	ESD	p-value^a
Energy [kcal]	2258.7 (402.1)	2258.8 (356.3)	.997
Protein [g]	89.7 (19.2)	91.4 (16.3)	.191
Total lipids [g]	76.5 (14.3)	76.8 (14.0)	.807
Carbohydrate, by diff. [g]	318.1 (56.1)	315.6 (49.6)	.534
Calcium, Ca [mg]	752.9 (268.6)	734.9 (285.0)	.386
Phosphorus, P [mg]	1196.0 (270.7)	1214.8 (269.6)	.354
Potassium, K [mg]	3203.0 (731.7)	3158.2 (664.6)	.394
Sodium, Na [mg]	2985.2 (745.7)	2974.0 (588.7)	.824
Omega-3 fatty acids [g]	1.44 (0.62)	1.39 (0.55)	.218
Omega-6 fatty acids [g]	13.18 (4.58)	12.72 (4.46)	.176
Omega-6/Omega-3 ratio	9.88 (3.23)	9.69 (3.01)	.406

^a *Independent samples t-test, two-sided.*

Table S5. Baseline comparisons by condition and by sex.

By Condition <i>Variable, mean (SD) in pg/ml</i>	Control	ESD	p-value^a
17-HDHA	159.3 (111.0)	129.3 (141.4)	.123
RvD1	0.59 (0.47)	0.73 (0.41)	.137
RvD2	0.94 (1.00)	0.68 (0.77)	.286
RvD3	0.50 (0.39)	0.30 (0.24)	.047
RvD4	0.37 (0.29)	0.33 (0.24)	.847
RvD5	0.76 (0.71)	1.10 (3.74)	.027
18-HEPE	135.0 (86.7)	301.1 (684.2)	.910
RvE1	1.80 (1.47)	0.90 (1.08)	.029
RvE2	1.60 (0.99)	2.92 (3.83)	.163
By Sex <i>Variable, mean (SD) in pg/ml</i>	Female	Male	p-value^a
17-HDHA	127.6 (66.6)	161.0 (164.4)	.683
RvD1	0.48 (0.23)	0.83 (0.53)	.011
RvD2	0.66 (0.88)	0.96 (0.90)	.109
RvD3	0.32 (0.27)	0.47 (0.39)	.128
RvD4	0.25 (0.20)	0.45 (0.28)	.008
RvD5	0.59 (0.70)	1.25 (3.64)	.829
18-HEPE	271.3 (667.0)	163.5 (194.6)	.134
RvE1	1.43 (1.24)	1.29 (1.49)	.555
RvE2	2.75 (3.86)	1.76 (1.08)	.650

^a Mann-Whitney U test, two-sided.

Table S6. Results of generalized linear mixed model analyses.

Variable	Model term	F	df1	df2	P	
17-HDHA (pg/ml) ^a	Corrected Model	5.758	12	42	<0.001	
	Condition	35.648	1	52	<0.001	***
	Day	0.810	2	36	0.453	
	Sex	1.317	1	30	0.260	
	Condition × Day	0.896	2	45	0.415	
	Condition × Sex	2.572	1	53	0.115	
	Day × Sex	2.212	2	37	0.124	
	Condition × Day × Sex	2.785	2	45	0.072	
Baseline (pg/ml)	0.938	1	86	0.336		
RvD1 (pg/ml) ^b	Corrected Model	2.016	12	54	0.041	
	Condition	7.842	1	107	0.006	**
	Day	0.393	2	61	0.677	
	Sex	12.692	1	24	0.002	**
	Condition × Day	0.842	2	62	0.436	
	Condition × Sex	0.488	1	107	0.486	
	Day × Sex	1.114	2	61	0.335	
	Condition × Day × Sex	0.214	2	61	0.808	
Baseline (pg/ml)	2.310	1	82	0.132		
RvD2 (pg/ml) ^c	Corrected Model	9.271	12	35	<0.001	
	Condition	0.689	1	71	0.409	
	Day	8.621	2	30	0.001	**
	Sex	9.170	1	24	0.006	**
	Condition × Day	17.050	2	38	<0.001	***
	Condition × Sex	3.680	1	61	0.060	
	Day × Sex	1.024	2	29	0.372	
	Condition × Day × Sex	3.894	2	38	0.029	*
Baseline (pg/ml)	21.369	1	59	<0.001	***	
RvD3 (pg/ml) ^d	Corrected Model	3.447	12	61	<0.001	
	Condition	18.541	1	105	<0.001	***
	Day	2.764	2	65	0.070	
	Sex	0.289	1	15	0.599	
	Condition × Day	0.908	2	65	0.408	
	Condition × Sex	1.117	1	92	0.293	
	Day × Sex	5.869	2	65	0.005	**
	Condition × Day × Sex	1.021	2	65	0.366	
Baseline (pg/ml)	0.981	1	65	0.326		
RvD4 (pg/ml) ^d	Corrected Model	3.142	12	57	0.002	
	Condition	19.261	1	97	<0.001	***
	Day	0.427	2	56	0.655	
	Sex	3.608	1	23	0.070	
	Condition × Day	0.100	2	59	0.905	
	Condition × Sex	5.629	1	97	0.020	*
	Day × Sex	0.032	2	57	0.968	
	Condition × Day × Sex	0.632	2	59	0.535	
Baseline (pg/ml)	0.825	1	79	0.367		
RvD5 (pg/ml) ^e	Corrected Model	3.673	12	57	<0.001	
	Condition	29.243	1	80	<0.001	***
	Day	0.245	2	50	0.784	
	Sex	7.599	1	54	0.008	**
	Condition × Day	0.554	2	51	0.578	
	Condition × Sex	2.853	1	81	0.095	
	Day × Sex	0.686	2	50	0.508	
	Condition × Day × Sex	0.549	2	51	0.581	
Baseline (pg/ml)	0.117	1	47	0.734		

Table S6 (continued)

Variable	Model term	F	df1	df2	P	
18-HEPE (pg/ml) ^c	Corrected Model	4.260	12	28	<0.001	
	Condition	7.775	1	28	0.009	**
	Day	2.570	2	22	0.099	
	Sex	0.761	1	29	0.390	
	Condition × Day	0.096	2	29	0.909	
	Condition × Sex	1.863	1	28	0.183	
	Day × Sex	1.274	2	22	0.299	
	Condition × Day × Sex	0.254	2	29	0.778	
Baseline (pg/ml)	4.809	1	40	0.034	*	
RvE1 (pg/ml) ^b	Corrected Model	1.897	12	54	0.056	
	Condition	0.479	1	99	0.490	
	Day	0.291	2	66	0.749	
	Sex	0.728	1	19	0.404	
	Condition × Day	0.599	2	67	0.553	
	Condition × Sex	1.109	1	102	0.295	
	Day × Sex	0.101	2	66	0.904	
	Condition × Day × Sex	2.754	2	67	0.071	
Baseline (pg/ml)	1.942	1	91	0.167		
RvE2 (pg/ml) ^e	Corrected Model	2.574	12	42	0.012	
	Condition	11.350	1	42	0.002	**
	Day	0.609	2	29	0.551	
	Sex	0.228	1	21	0.638	
	Condition × Day	3.191	2	32	0.055	
	Condition × Sex	0.500	1	39	0.484	
	Day × Sex	0.647	2	30	0.531	
	Condition × Day × Sex	2.345	2	32	0.112	
Baseline (pg/ml)	1.338	1	47	0.253		

^a Normal distribution with identity link function

^b Normal distribution with log link function

^c Inverse Gaussian distribution with log link function

^d Gamma distribution with log link function

^e Gamma distribution with power(-1) link function

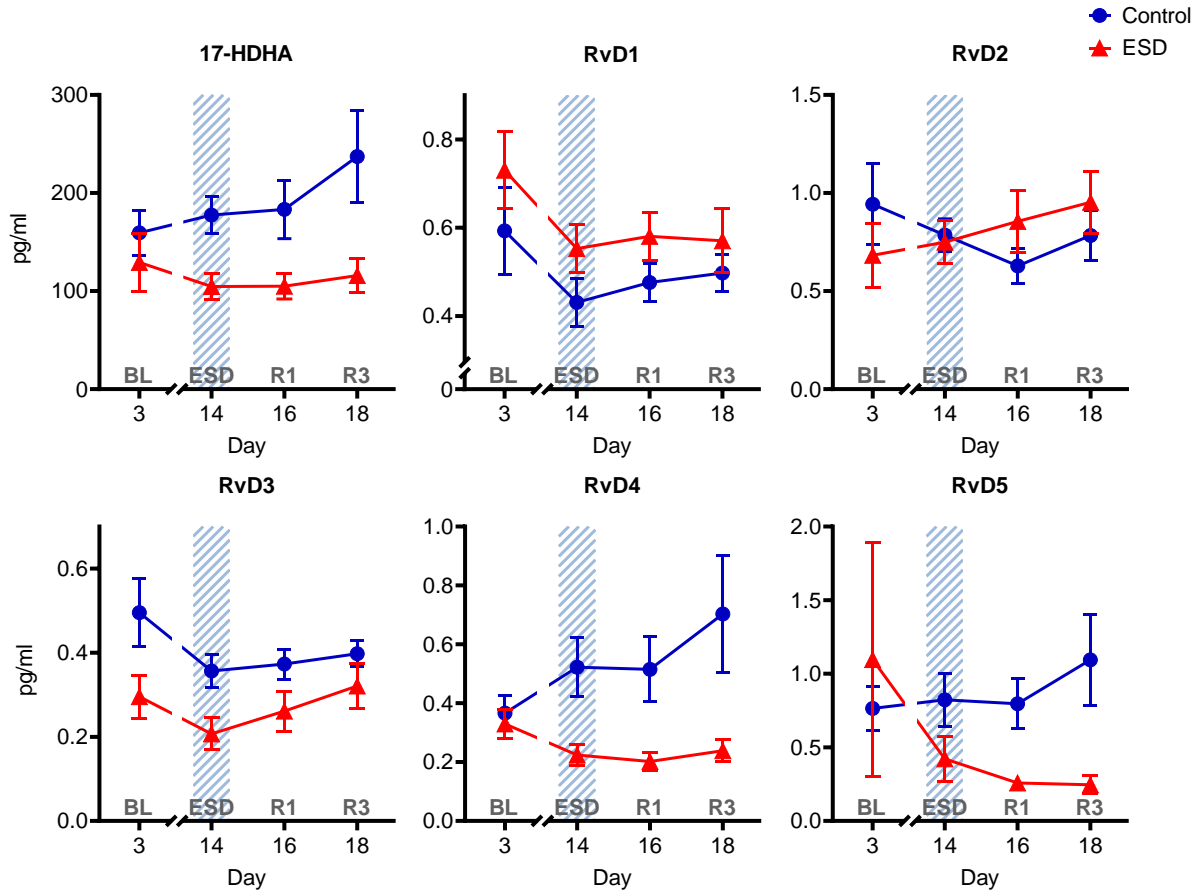


Figure S1. D-series resolvins and their precursor by condition in healthy humans. The graphs show original data (mean \pm SEM) of plasma concentrations in pg/ml at each time point of measurement of the precursor 17-hydroxydocosahexaenoic acid (17-HDHA) and the D-series resolvins RvD1, RvD2, RvD3, RvD4, and RvD5. Shaded area indicates ESD. Red triangles indicate ESD condition (n = 22), blue dots indicate control condition (n = 23). Blood samples were collected at baseline (day 3 = BL), during the 3rd ESD cycle (day 14 = ESD), after 1 night of recovery sleep (day 16 = R1), and after 3 nights of recovery sleep (day 18 = R3). N = 24 (12F/12M) participants in total.

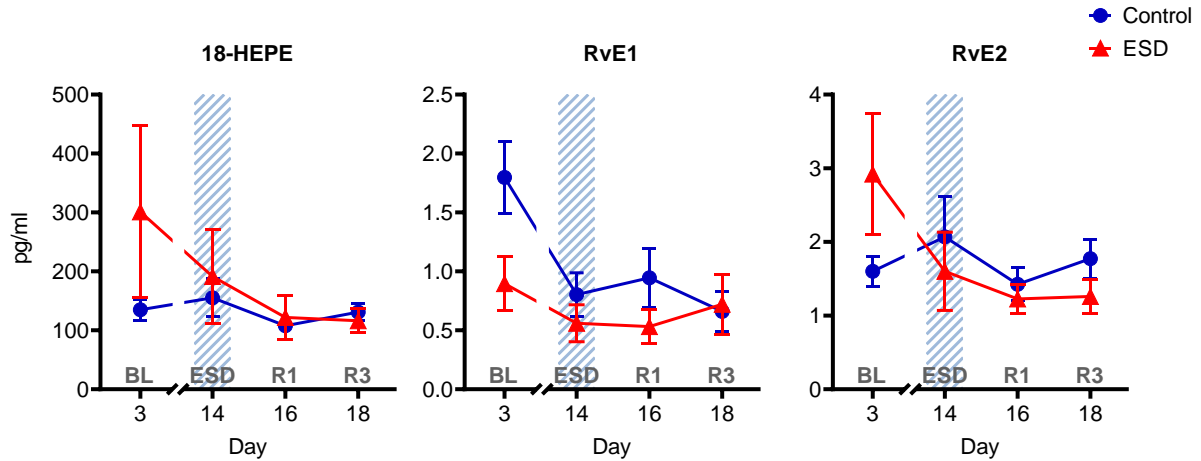


Figure S2. E-series resolvins and their precursor by condition in healthy humans. The graphs show original data (mean \pm SEM) of plasma concentrations in pg/ml at each time point of measurement of the precursor 18-hydroxyeicosapentaenoic acid (18-HEPE) and the E-series resolvins RvE1 and RvE2. Shaded area indicates ESD. Red triangles indicate ESD condition ($n = 22$), blue dots indicate control condition ($n = 23$). Blood samples were collected at baseline (day 3 = BL), during the 3rd ESD cycle (day 14 = ESD), after 1 night of recovery sleep (day 16 = R1), and after 3 nights of recovery sleep (day 18 = R3). $N = 24$ (12F/12M) participants in total.