SUPPLEMENTAL FIGURES

FIGURE 1S: **Cytochrome P450-dependent metabolism of arachidonic acid (AA, 20:4 n-6), eicosapentaenoic acid (EPA, 20:5 n-3) and docosahexaenoic acid (DHA, 22:6 n-3).** Among the hydroxy-metabolites that are primarily produced by CYP4A/4F isoforms, only those resulting from the hydroxylation of the terminal methyl group are shown. In general, the same enzymes also catalyze the hydroxylation of the first subterminal CH_2-group leading to isoform-specific mixtures of 20-/19-HETE, 20-/19-HEPE and 22-/21-HDoHE. Among the epoxy-metabolites that are primarily produced by CYP2C/2J isoforms, only one out of the various possible regioisomers is shown as an example. The whole set of regioisomeric epoxides involves 5,6-, 8,9-, 11,12- and 14,15-EET from AA, 5,6-, 8,9-, 11,12-, 14,15 and 17,18-EEQ from EPA, and 4,5-, 7,8-, 10,11-, 13,14-, 16,17- and 19,20-EDP from DHA. Each individual CYP-isoform displays a unique regioselectivity that is, moreover, dependent on the polyunsaturated fatty acid used as substrate.

FIGURE 2S: **Analysis of CYP-dependent AA, EPA, DPA and DHA metabolites by reversed-phase (RP)- and normal-phase (NP)-HPLC.** Shown are representative HPLC chromatograms for the resolution of the metabolites produced by human recombinant CYP2C8 (panel A) and mouse recombinant Cyp4a12a (B). The assay, extraction and HPLC conditions were as described in Experimental Procedures. RP-HPLC allowed to separate and quantify the major epoxy- and hydroxy-metabolite classes. Subsequent NP-HPLC of product peaks collected from RP-HPLC served to resolve and identify the regioisomers resulting from epoxidation of the individual double bonds or from ω-/(ω-1)-hydroxylation. All fatty acids used as substrates were [1-^{14}C]-labeled and the metabolites eluting from HPLC were monitored using an on-line radiodetector.

FIGURE 3S: **Competition between AA, EPA and DHA for the metabolism by human recombinant CYP4F2, CYP2E1 and CYP2J2.** The CYP isoforms were incubated with a substrate mixture consisting of equal amounts of non-labeled AA, EPA and DHA (10 μM each) using the standard conditions described in Experimental Procedures. The metabolites were extracted with ethylacetate and quantified by LC-MS/MS. The bars show the relative contribution of AA-, EPA- and DHA-derived metabolites to total product formation (the sum of all epoxy- and hydroxy-metabolites derived from the three different substrates was set to 100 %). No product formation was detectable in control assays performed in the absence of NADPH. Data are mean ± SD values from three assays.

FIGURE 4S: **Effect of dietary EPA/DHA-supplementation on the total levels of AA-, EPA- and DHA-derived monoepoxides in different organs and tissues of rat.** Male Sprague-Dawley rats (n=6 per group) received for three weeks a diet either supplemented with 5 % sunflower-oil alone (omega-6 fatty acid-rich diet; OM-6 group) or additionally with 2.5 % OMACOR®-oil consisting of purified EPA- and DHA-ethylesters in a molar ratio of 1.4: 1 (OM-3 group). AA-derived EETs, EPA-derived EEQs and DHA-derived EDPs were determined by LC-MS/MS in the harvested organs and tissues of six animals per group and the data represent the corresponding mean ± SEM values for the sum of the individual metabolites in each class.

FIGURE 5S: **Effect of dietary EPA/DHA-supplementation on the individual (A) and total levels (B) of AA-, EPA- and DHA-derived monoepoxides in different regions of the rat brain and heart (C, D).** The animals received either an omega-6 fatty acid-rich (OM-6 group) or an EPA/DHA-supplemented diet (OM-3 group) as described in Fig. 1S. AA-derived EETs, EPA-derived EEQs and DHA-derived EDPs were determined by LC-MS/MS in the harvested tissues of six animals per group and the data represent the corresponding mean ± SEM values for the individual metabolites.

FIGURE 1S

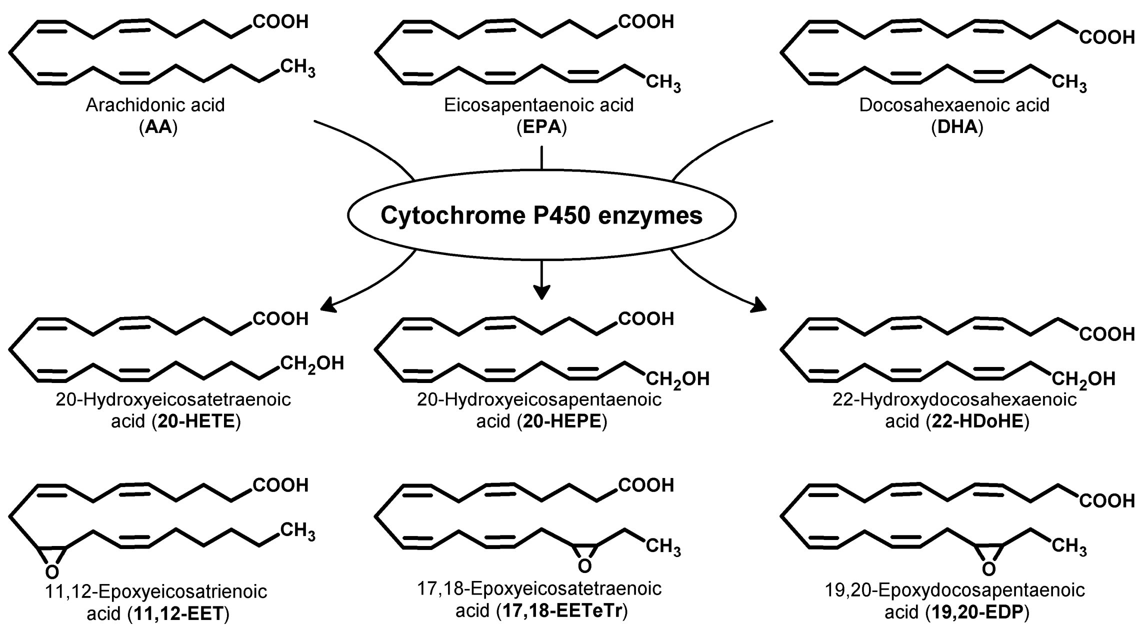
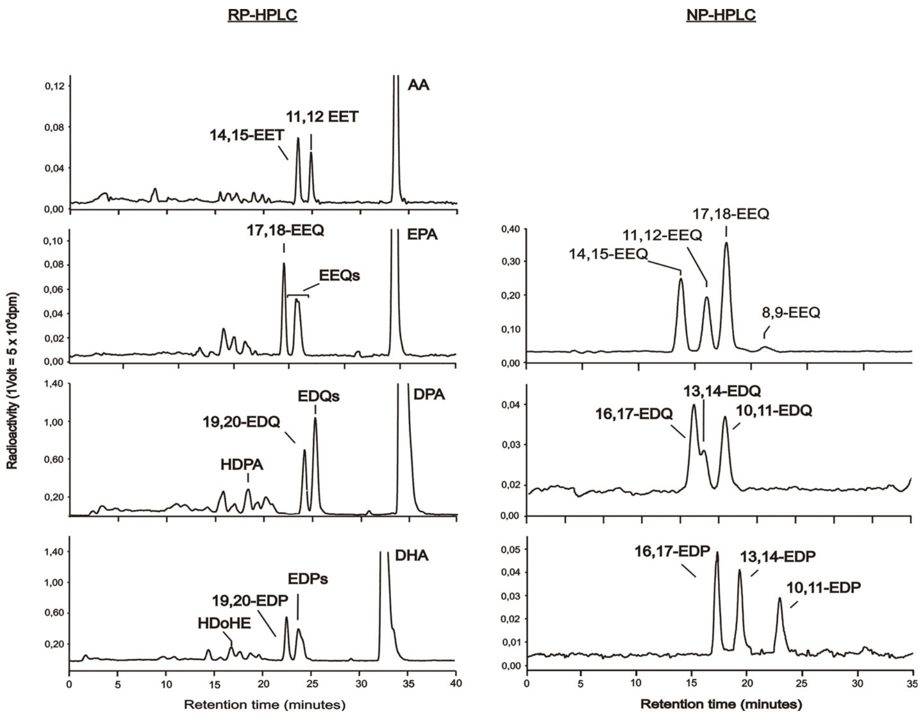

COOH
CH3
Arachidonic acid
(AA)
Eicosapentaenoic acid
(EPA)
Docosahexaenoic acid
(DHA)
Cytochrome P450 enzymes
CH2OH
20-Hydroxyeicosatetraenoic acid (20-HETE)
20-Hydroxyeicosapentaenoic acid (20-HEPE)
22-Hydroxydocosahexaenoic acid (22-HDoHE)
O
11,12-Epoxyeicosatrienoic acid (11,12-EET)
17,18-Epoxyeicosatetraenoic acid (17,18-EETeTr)
19,20-Epoxydocosapentaenoic acid (19,20-EDP)

FIGURE 2S

A


RP-HPLC
NP-HPLC
AA
14,15-EET
11,12 EET
EPA
17,18-EEQ
EEQs
DPA
19,20-EDQ
EDQs
HDPA
DHA
19,20-EDP
EDPs
HDoHE
14,15-EEQ
11,12-EEQ
17,18-EEQ
8,9-EEQ
16,17-EDQ
13,14-EDQ
10,11-EDQ
16,17-EDP
13,14-EDP
10,11-EDP
Radioactivity (1Volt = 5 x 10⁶dpm)
Retention time (minutes)

B

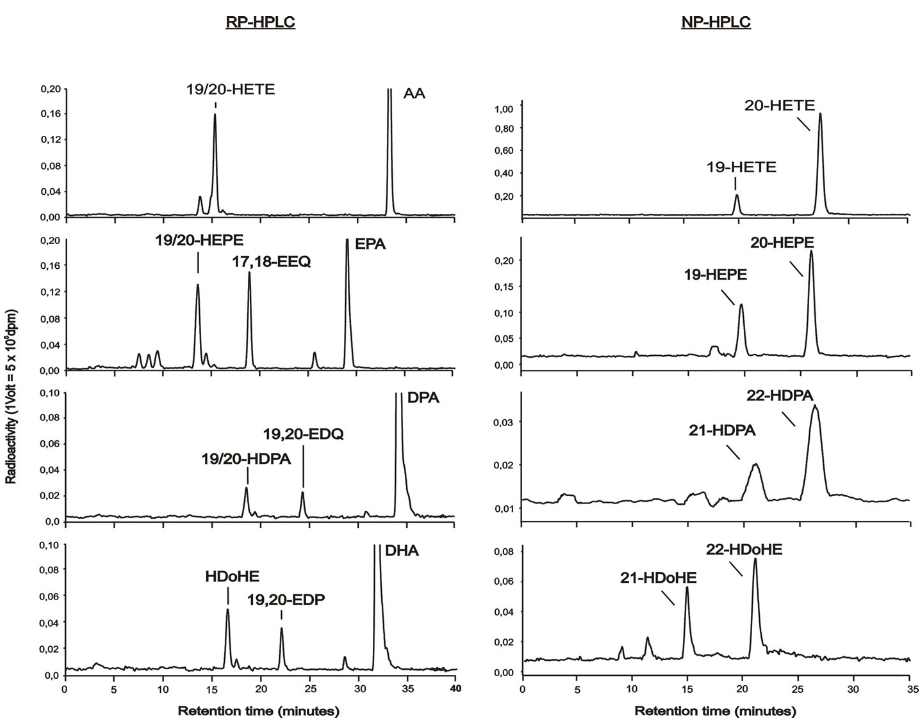

RP-HPLC
NP-HPLC
19/20-HETE
AA
20-HETE
19-HETE
19/20-HEPE
17,18-EEQ
EPA
20-HEPE
19-HEPE
DPA
19,20-EDQ
19/20-HDPA
22-HDPA
21-HDPA
DHA
HDoHE
19,20-EDP
22-HDoHE
21-HDoHE
Radioactivity (1Volt = 5 x 10⁶dpm)
Retention time (minutes)

FIGURE 3S

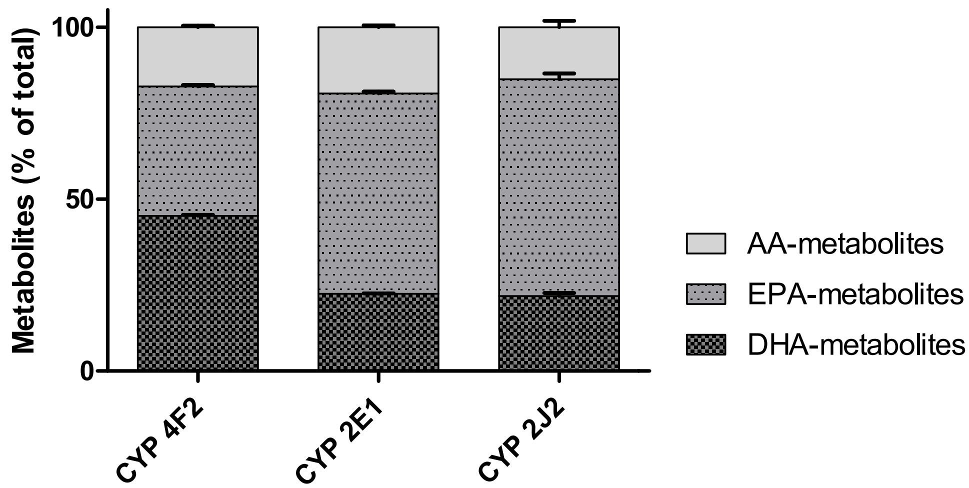

Metabolites (% of total)
100
50
0
CYP 4F2
CYP 2E1
CYP 2J2
AA-metabolites
EPA-metabolites
DHA-metabolites

FIGURE 4S

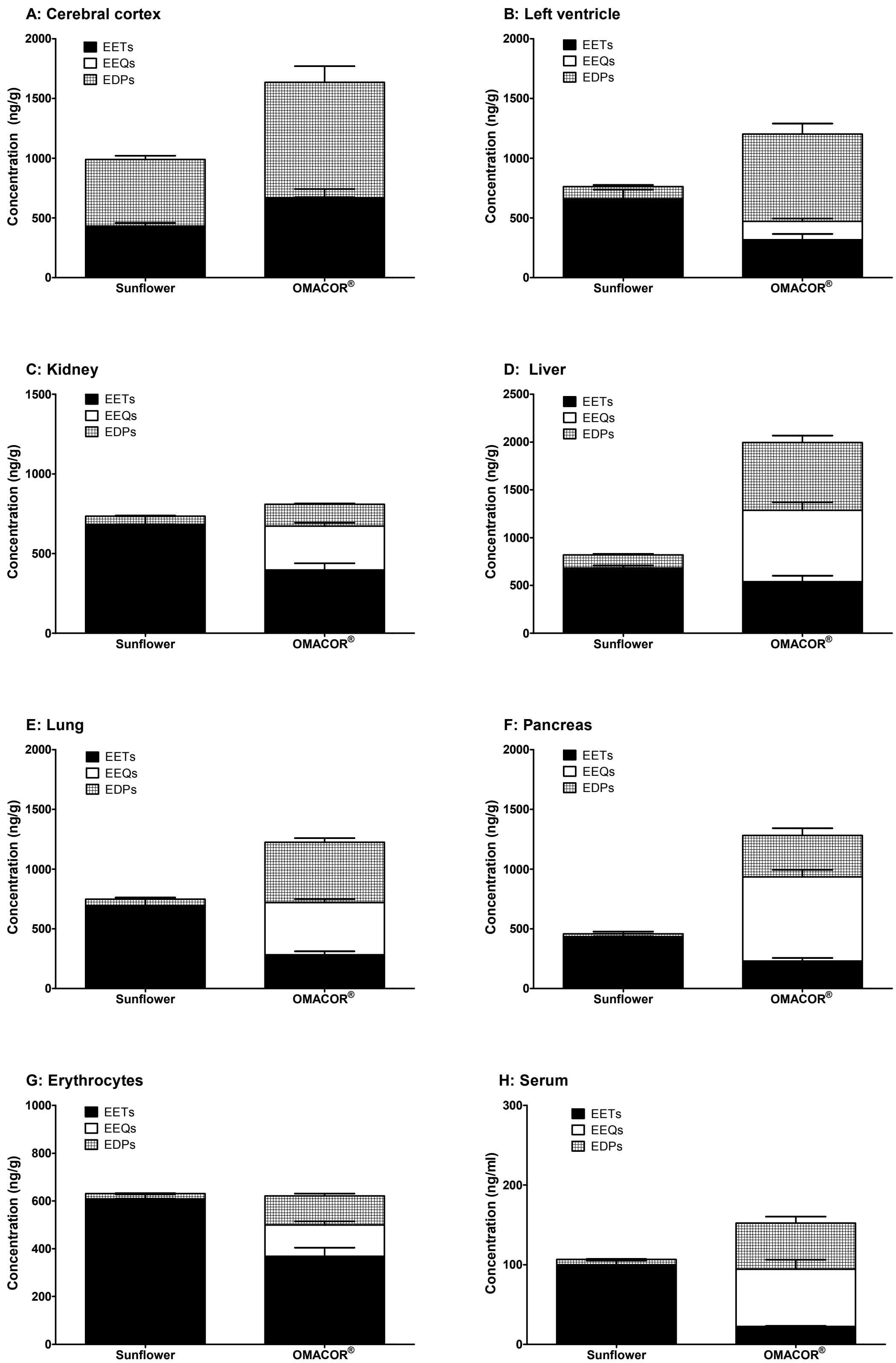
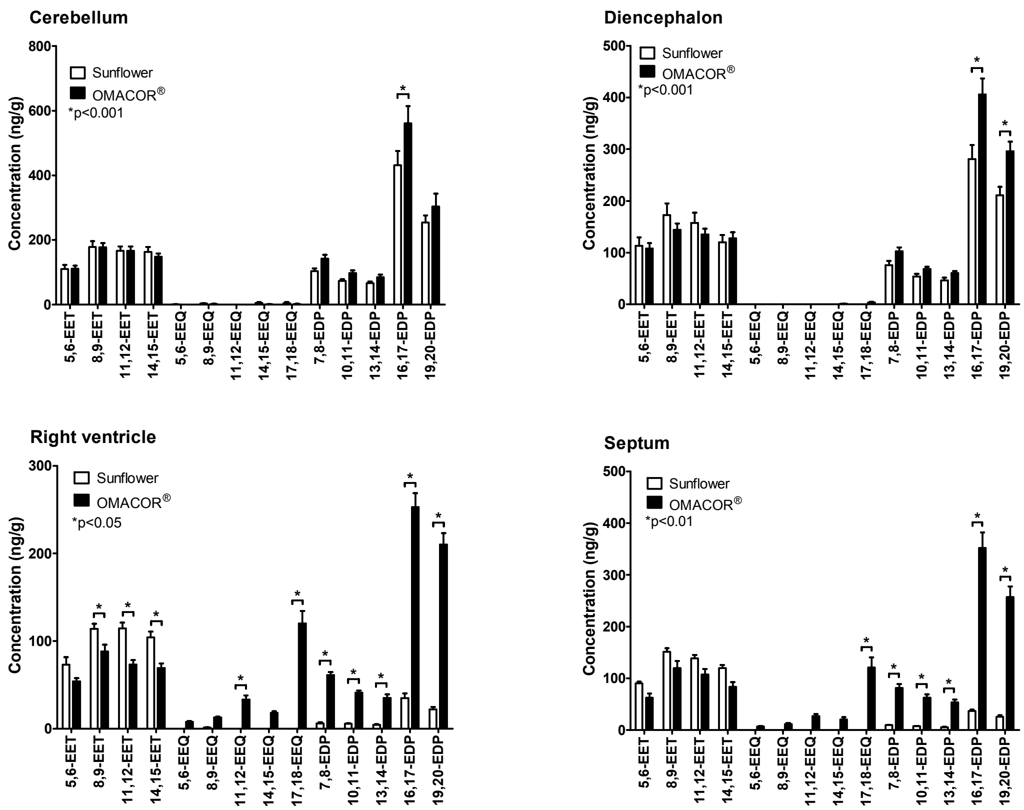

A: Cerebral cortex
B: Left ventricle
C: Kidney
D: Liver
E: Lung
F: Pancreas
G: Erythrocytes
H: Serum
EETs
EEQs
EDPs
Concentration (ng/g)
Concentration (ng/ml)
Sunflower
OMACOR®

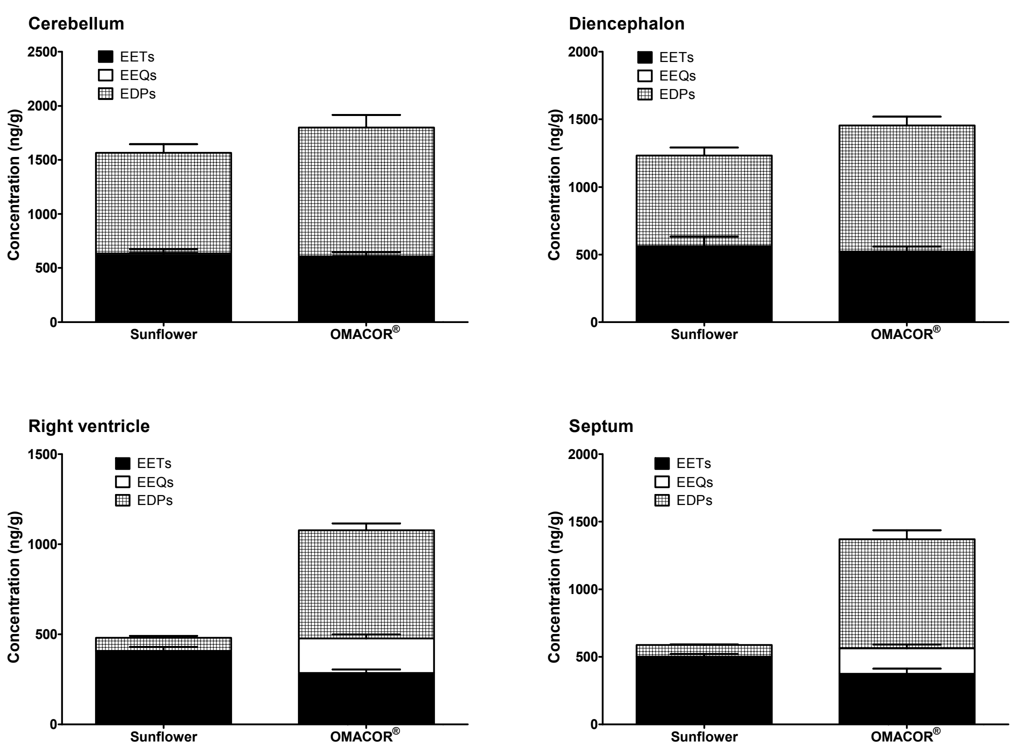
FIGURE 5S

A



Cerebellum
Sunflower
OMACOR®
*p<0.001
Concentration (ng/g)
0
200
400
600
800
5,6-EET
8,9-EET
11,12-EET
14,15-EET
5,6-EEQ
8,9-EEQ
11,12-EEQ
14,15-EEQ
17,18-EEQ
7,8-EDP
10,11-EDP
13,14-EDP
16,17-EDP
19,20-EDP
Diencephalon
Sunflower
OMACOR®
*p<0.001
Concentration (ng/g)
0
100
200
300
400
500
Right ventricle
Sunflower
OMACOR®
*p<0.05
Concentration (ng/g)
0
100
200
300
Septum
Sunflower
OMACOR®
*p<0.01
Concentration (ng/g)
0
100
200
300
400
500

B



Cerebellum
EETs
EEQs
EDPs
Concentration (ng/g)
0
500
1000
1500
2000
2500
Sunflower
OMACOR®
Diencephalon
EETs
EEQs
EDPs
Concentration (ng/g)
0
500
1000
1500
2000
Sunflower
OMACOR®
Right ventricle
EETs
EEQs
EDPs
Concentration (ng/g)
0
500
1000
1500
Sunflower
OMACOR®
Septum
EETs
EEQs
EDPs
Concentration (ng/g)
0
500
1000
1500
2000
Sunflower
OMACOR®

<u>Supplemental Table 1:</u> Composition of the experimental diets as prepared for the present study by the ssniff Spezialdiäten GmbH, Soest, Germany.

		S8586-E020 EF rat (= "OM-6")	**S8586-E021 EF rat (= "OM-3")**
Casein. %		20.000	20.000
Corn starch mod.. %		51.600	49.100
Sucrose. %		10.000	10.000
pur. Celullose powder. %		6.000	6.000
L-Cystine. %		0.200	0.200
Minerals & trace elements. %		6.000	6.000
Vitamin premix. %		1.000	1.000
Choline Chloride. %		0.200	0.200
Sunflower oil. %		**5.000**	**5.000**
OMACOR® oil. % [1]		——	**2.500**
Crude protein. %		17.8	17.8
Crude fat. %		5.1	7.4
Crude fibre. %		6.0	6.0
Crude ash. %		5.3	5.3
Starch. %		50.4	48.0
Sugar. %		11.0	11.0
N free Extracts. %		58.7	56.5
FATTY ACIDS (%)	C14:0	0.01	0.01
	C16:0	0.33	0.33
	C18:0	0.24	0.24
	C20:0	0.02	0.02
	C16:1	0.03	0.03
	C18:1	1.02	1.02
	C18:2	3.15	3.15
	C18:3 n-3	0.03	0.03 [2]
	C20:4 n-6	——	——
	C20:5 n-3	——	**1.04**
	C22:6 n-3	——	**0.86**
Energy (Atwater). MJ/kg [3]		15.4	15.9
Kcal % Protein		19	19
Kcal % Fat		13	17
Kcal % Carbohydrates		68	64

1) contains 46 % EPA, 38 % DHA, 6 % other n-3-fatty acids
2) contains also 0.13 % not specified n-3-fatty acids from Omacor®-oil
3) physiological fuel value

<u>Supplemental Table 2:</u> LC-MS/MS conditions used to determine the ω-hydroxy- and monoepoxy-metabolites of AA. EPA and DHA.

Metabolite	Transition	CE [V]	Fragmentor [V]	LOD [ng]	Slope of calibration curve	r^2	Retention Time[1)] [min]
20-HEPE	317.3→287.2	12	140	0.10	1.05	0.9936	7.52
20-HETE	319.3→289.3	14	140	0.10	1.09	0.9917	7.98
22-HDoHE	343.3→269.2	11	130	0.50	0.48	0.9904	8.37
17.18-EEQ	317.3→259.2	6	140	0.05	1.19	0.9991	9.04
5.6-EEQ	317.3→189.2	4	140	0.10	22.19	0.9900	9.04
14.15-EEQ	317.3→219.2	7	140	0.10	3.48	0.9941	9.42
8.9-EEQ	317.3→157.2	4	120	0.05	7.21	0.9899	9.46
11.12-EEQ	317.3→167.2	6	140	0.10	2.95	0.9921	9.47
19.20-EDP	343.3→299.3	6	140	0.05	1.49	0.9973	9.92
7.8-EDP	343.3→189.2	7	160	0.05	3.67	0.9941	10.34
14.15-EET	319.3→219.2	5	140	0.10	1.87	0.9992	10.34
13.14-EDP	343.3→193.2	7	160	0.10	3.85	0.9968	10.36
16.17-EDP	343.3→233.2	8	140	0.10	2.83	0.9959	10.36
5.6-EET	319.3→191.1	8	140	0.05	1.57	0.9961	10.36
10.11-EDP	343.3→153.1	8	140	0.10	8.33	0.9972	10.38
11.12-EET	319.3→167.2	6	140	0.10	1.96	0.9987	10.72
8.9-EET	319.3→257.0	7	140	0.50	0.99	0.9944	10.73
20-HETE-D6 [2)]	325.3→289.3	14	140	-	-	-	8.04
14.15-EET-D8 [3)]	327.3→226.2	9	140	-	-	-	10.24

[1]Retention times refer to the Zorbax Eclipse Plus-C18, 4.6 x 150 mm, 1.8 µm column and the acetonitrile/0.01 M ammonia acetate solvent system used. Gradient elution was started with 30 % acetonitrile. Acetonitrile was increased within 10 min to 90% and held for further 10 min with a flow rate of 0.8 ml/min.
[2]Used as internal standard for all ω-hydroxy-metabolites
[3]Used as internal standard for all monoepoxy-metabolites
CE- collision energy; LOD- limit of detection

<u>Supplemental table 3:</u> Fatty acid composition of various organs and tissues of rats. Individual fatty acids are given as percentage of total fatty acids and data represent the mean values from three animals per feeding group. t- trans. c- cis.

	Diet		C14:0	C16:0	C16:1. n-7. t	C16:1. n-7	C18:0	C18:1. t	C18:1. n-9	C18:2. n-6. t. t	C18:2. n-6. c t	C18:2. n-6. t c	C18:2. n-6	C18:3. n-6	C20:1. n-9	C18:3. n-3	C20:2. n-6	C20:3. n-6	C20:4. n-6	C24:0	C20:5. n-3	C24:1. n-9	C22:4. n-6	C22:5. n-6	C22:5. n-3	C22:6. n-3
Cerebral Cortex	OM-6	mean	0.00	19.93	0.02	0.34	19.40	0.30	17.81	0.11	0.00	0.25	3.73	0.03	0.77	0.46	0.85	0.32	12.62	0.11	0.11	0.50	3.94	2.69	0.12	15.58
		SEM	0.00	0.35	0.00	0.00	0.22	0.06	0.20	0.01	0.00	0.02	0.87	0.00	0.02	0.03	0.17	0.01	0.23	0.01	0.00	0.09	0.07	0.03	0.00	0.37
	OM-3	mean	0.00	20.02	0.02	0.38	19.31	0.29	17.65	0.11	0.01	0.36	3.42	0.05	0.73	0.51	0.90	0.43	11.41	0.12	0.11	0.41	2.89	0.79	0.48	19.61
		SEM	0.00	0.09	0.00	0.02	0.11	0.05	0.31	0.01	0.00	0.07	0.59	0.00	0.03	0.04	0.13	0.01	0.06	0.01	0.01	0.12	0.03	0.02	0.01	0.55
Left ventricle	OM-6	mean	0.00	11.83	0.03	0.30	21.53	0.20	8.94	0.09	0.02	0.07	28.07	0.03	0.06	0.05	0.28	0.22	19.40	0.08	0.03	0.01	1.82	3.56	0.49	2.88
		SEM	0.00	0.09	0.00	0.02	0.38	0.03	0.24	0.01	0.01	0.00	0.72	0.01	0.02	0.02	0.01	0.01	0.34	0.00	0.00	0.01	0.04	0.17	0.08	0.31
	OM-3	mean	0.00	12.89	0.04	0.08	21.94	0.17	5.44	0.07	0.01	0.04	21.54	0.02	0.07	0.07	0.30	0.30	9.62	0.05	1.62	0.04	0.12	0.33	2.13	23.11
		SEM	0.00	0.22	0.00	0.01	0.41	0.02	0.18	0.01	0.00	0.02	1.51	0.00	0.00	0.01	0.01	0.01	0.13	0.00	0.05	0.01	0.01	0.02	0.07	0.90
Kidney	OM-6	mean	0.00	19.19	0.01	0.88	17.17	0.23	9.95	0.04	0.01	0.07	13.56	0.08	0.10	0.04	0.45	0.69	33.09	0.47	0.08	0.14	0.98	0.94	0.16	1.68
		SEM	0.00	0.14	0.00	0.16	0.44	0.02	0.64	0.01	0.00	0.01	0.25	0.01	0.00	0.00	0.03	0.05	0.51	0.04	0.01	0.01	0.02	0.12	0.01	0.07
	OM-3	mean	0.00	18.01	0.02	0.30	18.22	0.32	7.21	0.05	0.03	0.19	20.42	0.10	0.07	0.33	0.44	0.90	17.75	0.73	7.89	0.12	0.17	0.05	1.23	5.45
		SEM	0.00	0.15	0.01	0.06	0.46	0.06	0.46	0.02	0.01	0.11	0.10	0.04	0.01	0.11	0.03	0.06	0.37	0.06	0.37	0.01	0.04	0.01	0.13	0.28
Liver	OM-6	mean	0.00	23.12	0.05	3.15	13.19	0.12	18.00	0.01	0.03	0.12	15.90	0.20	0.16	0.03	0.44	0.40	18.64	0.10	0.03	0.03	0.81	2.79	0.19	2.48
		SEM	0.00	0.21	0.00	0.48	0.49	0.01	0.80	0.00	0.00	0.00	1.45	0.02	0.01	0.01	0.02	0.02	0.07	0.01	0.00	0.00	0.03	0.48	0.02	0.23
	OM-3	mean	0.00	18.21	0.07	0.39	15.52	0.10	7.18	0.01	0.01	0.05	21.54	0.10	0.14	0.70	0.68	1.36	10.65	0.17	5.65	0.09	0.19	0.18	3.26	13.75
		SEM	0.00	0.14	0.01	0.08	0.89	0.03	0.52	0.00	0.01	0.01	0.40	0.01	0.02	0.03	0.03	0.06	0.39	0.01	0.45	0.02	0.04	0.03	0.13	0.26
Lung	OM-6	mean	0.00	30.38	0.06	1.66	11.05	0.18	20.89	0.05	0.01	0.07	15.71	0.17	0.23	0.08	0.51	0.56	12.18	0.17	0.07	0.14	3.87	1.14	0.31	0.53
		SEM	0.00	0.49	0.02	0.43	1.41	0.02	4.63	0.01	0.00	0.01	2.45	0.02	0.02	0.01	0.09	0.12	3.07	0.04	0.02	0.04	1.13	0.35	0.07	0.11
	OM-3®	mean	0.00	29.07	0.08	1.61	10.59	0.21	13.98	0.02	0.01	0.03	19.36	0.11	0.16	1.11	0.66	0.73	5.71	0.28	4.07	0.15	0.54	0.16	4.32	7.03
		SEM	0.00	0.87	0.01	0.14	0.13	0.04	0.11	0.00	0.01	0.02	1.02	0.01	0.01	0.11	0.02	0.04	0.30	0.02	0.08	0.02	0.04	0.01	0.17	0.54
Pancreas	OM-6	mean	0.00	24.87	0.03	4.66	7.18	0.18	21.50	0.02	0.01	0.13	25.35	0.23	0.15	0.24	0.31	0.26	12.59	0.07	0.08	0.05	0.86	0.61	0.14	0.47
		SEM	0.00	0.25	0.00	0.85	0.87	0.02	1.48	0.00	0.00	0.01	1.05	0.03	0.00	0.04	0.03	0.05	2.10	0.01	0.03	0.01	0.24	0.14	0.03	0.08
	OM-3	mean	0.00	23.98	0.05	1.29	10.08	0.30	11.23	0.02	0.01	0.07	26.08	0.12	0.15	1.37	0.49	0.36	6.38	0.15	9.22	0.07	0.10	0.12	1.36	7.01
		SEM	0.00	0.58	0.01	0.36	0.76	0.01	0.99	0.00	0.00	0.01	0.18	0.01	0.01	0.14	0.02	0.04	0.51	0.02	0.38	0.02	0.01	0.01	0.05	0.22
Erythrocytes	OM-6	mean	0.00	25.64	0.14	0.79	12.97	0.46	10.29	0.23	0.09	0.51	11.31	0.21	0.25	0.10	1.28	0.45	26.69	0.52	0.21	0.58	2.88	2.37	0.62	1.42
		SEM	0.00	0.59	0.04	0.12	1.52	0.08	0.89	0.03	0.06	0.33	0.35	0.11	0.10	0.03	0.76	0.11	0.64	0.05	0.12	0.27	0.25	0.41	0.05	0.05
	OM-3	mean	0.00	24.93	0.14	0.15	15.01	0.51	7.55	0.23	0.03	0.17	14.95	0.11	0.13	0.07	0.60	0.67	17.15	0.70	5.97	0.29	0.66	0.30	3.38	6.31
		SEM	0.00	0.07	0.02	0.01	0.76	0.06	0.28	0.03	0.01	0.02	0.68	0.01	0.01	0.01	0.04	0.02	0.04	0.08	0.23	0.04	0.02	0.01	0.14	0.16
Plasma	OM-6	mean	0.00	27.48	0.14	0.69	21.79	1.25	11.02	0.05	0.10	0.24	20.31	0.04	0.10	0.16	0.28	0.41	10.51	0.10	0.08	0.14	0.71	2.54	0.15	1.72
		SEM	0.00	0.13	0.07	0.03	1.14	0.25	0.89	0.01	0.08	0.04	1.57	0.00	0.00	0.01	0.01	0.01	0.36	0.01	0.00	0.04	0.03	0.41	0.02	0.14
	OM-3	mean	0.00	25.48	0.06	0.28	24.45	1.76	10.05	0.06	0.08	0.26	22.65	0.19	0.19	0.48	0.47	0.84	3.17	0.19	2.46	0.07	0.06	0.34	1.22	5.19
		SEM	0.00	1.73	0.01	0.08	0.59	0.62	1.38	0.00	0.05	0.01	1.64	0.00	0.02	0.04	0.06	0.11	0.55	0.04	0.28	0.03	0.01	0.28	0.23	0.98