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Brain and Liver Headspace Aldehyde Concentration Following Dietary Supplementation with n-3 Polyunsaturated Fatty Acids

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Abstract Reactive oxygen species react with unsaturated fatty acids to form a variety of metabolites including aldehydes. Many aldehydes are volatile enough to be detected in headspace gases of blood or cultured cells and in exhaled breath, in particular propanal and hexanal which are derived from omega-3 and omega-6 polyunsaturated fatty acids, respectively. Aldehydes are therefore potential non-invasive biomarkers of oxidative stress and of various diseases in which oxidative stress is thought to play a role including cancer, cardiovascular disease and diabetes. It is unclear, however, how changes in the abundance of the fatty acid precursors, for example by altered dietary intake, affect aldehyde concentrations. We therefore fed male Wistar rats diets supplemented with either palm oil or a combination of palm oil plus an n-3 fatty acid (alpha-linolenic, eicosapentaenoic, or docosahexaenoic acids) for 4 weeks. Fatty acid analysis revealed large changes in the abundance of both n-3 and n-6 fatty acids in the liver with smaller changes observed in the brain. Despite the altered fatty acid abundance, headspace concentrations of C1–C8 aldehydes, and tissue concentrations of thiobarbituric acid reactive substances, did not differ between the 4 dietary groups. Our data suggest that tissue aldehyde concentrations are independent of fatty acid abundance, and further support their use as volatile biomarkers of oxidative stress.

Abbreviations
ALA	Alpha-linolenic acid
ANOVA	Analysis of variance
DHA	Docasahexaenoic acid
EPA	Eicosapentaenoic acid
ID	Internal diameter
MDA	Malondialdehyde
OD	Outer diameter
PUFA	Polyunsaturated fatty acid
PPBV	Parts per billion by volume
ROS	Reactive oxygen species
TBARS	Thiobarbituric acid reactive substances
VOC	Volatile organic compounds

Introduction

The analysis of volatile organic chemicals (VOC) offer a means to non-invasively investigate cellular processes either *in vitro* or *in vivo* [1]. One such process is the reaction between cellular constituents and reactive oxygen species (ROS) otherwise known as oxidative stress [2]. Oxidative stress has been implicated in a variety of common diseases including cancer, heart disease and diabetes, with the quantification of oxidative stress being proposed to have diagnostic and prognostic value [3–5]. Monitoring of lipid peroxidation also has biotechnological applications including in the food industry to monitor spoilage [6, 7]. ROS react with unsaturated fatty acids to produce lipid peroxides which subsequently give rise to a variety of secondary metabolites some of which are volatile including alkanes and aldehydes [1, 8]. The specific compounds

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produced are dependent on where carbon–carbon double bonds are located within the fatty acid molecule, with ethane and propanal being the predominant products of n-3 fatty acid oxidation, and pentane and hexanal deriving from n-6 fatty acids, due to the position of the terminal unsaturated bond [2, 8]. Other aldehydes can also be produced via reaction of ROS with other C=C double bonds at various positions producing volatile aldehydes such as malondialdehyde (MDA) and saturated aldehydes ranging from C2 (ethanal) to C8 (octanal) [2, 8]. In particular the autoxidation of the docosahexaenoic acid (22:5n-3) results in the production of butanal, pentanal and heptanal as well as propanal, while arachidonic acid (20:4n-6) autoxidation produces pentanal [8]. It is presently unclear whether aldehydes such as pentanal, butanal and heptanal are produced in significant quantities in biological systems however.

Methanal and ethanal, have been suggested to be tumour biomarkers. Beside oxidative stress, however, methanal and ethanal can derive from microbial action in the gut and from dietary sources making interpretation of altered concentrations of these aldehydes difficult [9]. Moreover even for aldehydes, which are thought to derive exclusively from the reaction of fatty acids with ROS, such as propanal and hexanal, it is unclear whether their concentrations are influenced by the degree of oxidative stress, the abundance of their fatty acid precursors, or a combination of the two. That is, if the concentration of the fatty acids from which the aldehydes derive changes, will the concentration of the aldehydes also change? We have therefore investigated the effect of altered n-3 and n-6 fatty acid abundance *in vivo* on aldehyde concentrations by feeding animals with diets containing either no n-3 fatty acids, or high purity alpha-linolenic acid (ALA; 18:3n-3), eicosapentaenoic acid (EPA; 20:4n-3), or docasahexaenoic acid (DHA; 22:6n-3), diets which we expected to increase total relative n-3 fatty abundance and/or decrease total relative n-6 fatty acid abundance compared to that resulting from feeding animals an n-3 fatty acid free diet.

Methods

Dietary Intervention

Animal experimentation was carried according to an animal utilization protocol approved by the Lakehead University animal care committee. Male Wistar rats were randomly assigned into pairs and placed into cages with free access to food and water. Rats were fed for 8 weeks on supplementation diets containing 90 % (by weight) rat chow containing all required nutrients except for fats (TD99159 basal mix, Harlan Laboratories) plus 10 % added fat. The fat component for the control group was exclusively palm

Table 1 Composition of test diet

Component	Weight per kg
Added fat[a]	100.00 g
Casein	181.90 g
DL-Methionine	2.84 g
Sucrose	636.11 g
Cellulose	47.37 g
Calcium monohydrogen phosphate	3.32 g
Choline bitartrate	2.38 g
Calcium carbonate	11.84 g
Potassium dihydrogen phosphate	6.50 g
Potassium citrate monohydrate	2.35 g
Sodium chloride	2.45 g
Potassium sulphate	1.55 g
Magnesium oxide	0.81 g
Ferric citrate	0.20 g
Zinc carbonate	54.72 mg
Manganese carbonate	20.89 mg
Copper carbonate	10.28 mg
Potassium iodate	0.33 mg
Sodium selenate	0.34 mg
Ammonium paramolybdate, tetrahydrate	0.27 mg
Sodium meta-silicate, nonahydrate	48.09 mg
Chromium potassium sulphate, dodecahydrate	9.12 mg
Lithium chloride	0.58 mg
Boric acid	2.70 mg
Sodium fluoride	2.11 mg
Nickel carbonate hydroxide, tetrahydrate	1.05 mg
Ammonium meta-vanadate	0.22 mg
Niacin	28.43 mg
Calcium pantothenate	15.16 mg
Pyroxidine hydrochloride	6.63 mg
Thiamin hydrochloride	5.69 mg
Riboflavin	5.69 mg
Folic acid	1.90 mg
Biotin	0.19 mg
Vitamin B12 (0.1 % in mannitol)	23.69 mg
Vitamin E, DL-alpha tocopheryl acetate (500 IU/g)	142.16 mg
Vitamin A palmitate (500,000 IU/g)	7.58 mg
Vitamin D3, cholecalciferol (500,000 IU/g)	1.90 mg
Vitamin K1, phylloquinone	0.71 mg

[a] The added fat contained either 100 g palm oil or 90 g palm oil plus 10 g n-3 fatty acid

oil (New Directions Aromatics, Canada), containing (as per our own analysis) 1.2 % (mol%) 14:0, 42.2 % 16:0, 3.5 % 18:0, 41.9 % 18:1, 11.2 % 18:2, n-6, while other groups received palm oil plus n-3 fatty acids ("95 % purity" preparations from Equatech, UK which contained as per our own analysis over 97 % of the described fatty acid) in the

ratio 9 parts palm oil to 1 part n-3 fatty acid as described in Table 1. Food was prepared ahead of time and stored in canisters at −20 °C until use. After completion of the intervention the rats were euthanized by first anaesthetizing with isofluorane gas followed by decapitation using a small animal guillotine. The brain and liver were rapidly removed and tissue stored in a −80 °C freezer until required. Body weight was monitored throughout the experiment and did not differ significantly (ANOVA; $P > 0.05$) between the dietary groups.

the methylation tubes in a boiling water bath. The resulting fatty acid methyl esters were analyzed on a Varian 3400 gas–liquid chromatograph, with a 60-m DB-23 capillary column (0.32 mm internal diameter), and quantified using a flame ionisation detector. Results are expressed as relative concentration of each fatty acid species. The unsaturation index gives a measure of the prevalence of carbon–carbon double bonds in the tissue lipids and is calculated as

$$\frac{\sum \text{Relative concentration of fatty acid} \cdot \text{number of unsaturated bonds in fatty acid}}{100}$$

Tissue Preparation

Brain or liver was ground in liquid nitrogen using a mortar and pestle to produce a fine powder with approximately 300 mg being used for fatty acid analysis, 200 mg for headspace aldehyde concentration measurement and 100 mg for TBARS analysis.

Fatty Acid Analysis

Fatty acids were analysed essentially as described previously [10]. Briefly, tissue powder was were homogenized in ice cold water using a Polytron homogenizer. Lipids were then extracted from the samples according to the method of Bligh and Dyer [11] in the presence of the internal standard, tritridecanoin. Fatty acid methyl esters were prepared using boron trichloride in methanol, and heating

TBARS

Thiobarbituric acid reactive substances (TBARS) were measured using an assay kit (Cayman Chemical Company, USA) according to the manufacturer's instructions [12]. Briefly, approximately 100 mg tissue powder was homogenised in 350 µl degassed water using a Polytron homogenizer, transferred to glass tubes and 100 µl sodium dodecyl sulphate and 4 ml colour reagent added as supplied by the manufacturer. The tubes were capped and boiled for an hour in a 100 °C water bath, cooled on ice for 10 min, transferred to plastic centrifuge tubes and centrifuged for 10 min at 1600×g. Supernatant (150 µl) from each tube was transferred to a 96-well assay plate and absorbance readings were taken at 530 nm using the plate spectrophotometer. Blank (no tissue) subtracted absorbance was compared with an MDA standard curve

Table 2 Precursor ions, product ions and reaction rate coefficients used to quantify aldehyde concentrations

Aldehyde	Precursor ion (m/z)	Product ions [multiplier] (m/z)	Reaction rate coefficient ($cm^3 \ s^{-1}$)
Methanal	19, 32[a]	31 [1.0], 33 [−100.4], 83 [−100.9]	3.4×10^{-9}
Ethanal	19, 37, 55, 73[b]	45 [1.576], 81 [1.831][c]	3.7×10^{-9}
Propanal	30, 48[d]	55 [−0.006], 57 [1.0]	3.0×10^{-9}
Butanal	30, 48	71 [1.0]	3.5×10^{-9}
Pentanal	30, 48	85 [1.0]	3.2×10^{-9}
Hexanal	30, 48	99 [1.0]	2.5×10^{-9}
Heptanal	30, 48	113 [1.0]	3.1×10^{-9}
Octanal	30, 48	127 [1.0]	3.0×10^{-9}

The data in the table are derived from Refs. [37, 38]. Please note that alternative reaction rate coefficients have been published which would alter the absolute value of the concentrations reported [39]

[a] The primary precursor ion is H_3O^+ (m/z 19) however $O_2^{+\cdot}$ (32) is also counted to allow accurate quantification of methanal concentrations in the presence of methanol and ethanol as described in Spaněl and Smith [9]

[b] m/z 37, 55 and 73 are the hydrates of H_3O^+ (m/z 19)

[c] See Spaněl and Smith [9] for further details

[d] m/z 48 is the hydrate of NO^+ (m/z 30)

processed simultaneously to calculate tissue TBARS concentration in MDA equivalents.

Headspace Aldehyde Quantification

Headspace aldehydes were quantified essentially as described previously using selected ion flow tube mass spectrometry (SIFT-MS), a soft-ionisation mass spectrometry technique, which can quantify aldehyde concentrations in real time with a limit of detection of approximately 1 PPBV [8]. Briefly, 300 mg tissue powder was transferred to a 250 ml flask and the flask covered with aluminum foil and sealed with Scotch tape. The flask was then incubated at 60 °C for 10 min prior

to analysis of the headspace. SIFT-MS analysis was performed using a Profile 3 SIFT-MS (Instrument Science, Crewe, UK) using a flow tube pressure of 1 Torr and temperature of 300 K, while headspace gases were sampled at a rate of 0.2 ml/s with an inlet temperature of 100 °C. Purity of the precursor ions was routinely evaluated and was observed to be higher than 99 %, with precursor ion count rates being approximately 600,000 counts per second for H_3O^+ (including hydrates) and 500,000 counts per second for NO^+. To sample headspace gases from foil sealed glass flasks, the foil was covered with a small piece of scotch tape to prevent ripping, and punctured with a 22 gauge needle connected to the SIFT-MS sampling head via a 1 inch length of PTFE tubing

Table 3 Composition of esterified fatty acids in the liver following dietary intervention

Fatty acid	Dietary group			
	Ctrl	ALA	EPA	DHA
14:0	0.9 ± 0.2	0.7 ± 0.1	0.6 ± 0.1*	0.5 ± 0.1*
16:0	25.7 ± 2.4	26.1 ± 1	27.1 ± 2.1	27.0 ± 2.4
16:1	4.6 ± 1.8	3.6 ± 1.0	3.4 ± 1.3	2.8 ± 1.1
18:0	10.7 ± 1.5	11.6 ± 2.1	10.8 ± 1.6	13.0 ± 1.2
18:1	28.5 ± 3.3	24.9 ± 5.6	22.8 ± 2.4*	20.0 ± 2.8*
18:2n-6	7.0 ± 2.2	9.0 ± 1.2	8.6 ± 1.8	8.5 ± 1.1
18:3n-6	0.2 ± 0.1	0.1 ± 0.0*	0.1 ± 0.0*	0.2 ± 0.1
18:3n-3	0.1 ± 0.0	1.3 ± 0.4*	0.2 ± 0.1	0.2 ± 0.3
20:0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
20:1	0.3 ± 0.1	0.3 ± 0.0	0.3 ± 0.1	0.2 ± 0.0
20:2n-6	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
20:3n-6	0.4 ± 0.1	0.8 ± 0.2*	0.6 ± 0.1	0.9 ± 0.2*
20:4n-6	15.2 ± 2.7	10.1 ± 2.3*	6.1 ± 0.9**	5.7 ± 0.7**
20:5n-3	0.1 ± 0.1	2.1 ± 0.4**	7.8 ± 2.7***	4.2 ± 1.1**
22:1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
22:4n-6	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
22:5n-6	0.9 ± 0.3	0.0 ± 0.0***	0.0 ± 0.0	0.0 ± 0.0
22:5n-3	0.3 ± 0.2	1.3 ± 0.5	3.0 ± 1.5*	1.0 ± 0.3
22:6n-3	4.3 ± 0.9	7.1 ± 2.2*	10.1 ± 0.8**	15.3 ± 2.6***
24:0	0.2 ± 0.0	0.2 ± 0.1	0.9 ± 2.0	0.3 ± 0.1
24:1	0.2 ± 0.0	0.1 ± 0.0	0.2 ± 0.1	0.2 ± 0.0
Total n-3	4.8 ± 0.8	11.8 ± 1.8***	18.1 ± 2.4***	20.7 ± 2.6***
Total n-6	23.9 ± 3.1	20.2 ± 3.4	18.6 ± 2.8***	15.5 ± 2.3***
n-3/n-6	0.20 ± 0.04	0.59 ± 0.10***	0.97 ± 0.18***	1.34 ± 0.21***
Total saturated	37.6 ± 3.4	38.7 ± 3.9	38.8 ± 3.1	40.9 ± 3.2**
Total monosaturated	33.6 ± 4.1	28.9 ± 3.2*	26.7 ± 3.2***	23.2 ± 3.0***
Total polyunsaturated	28.7 ± 2.1	32.0 ± 3.4	34.6 ± 4.0*	36.2 ± 3.9***
Unsaturation index	1.44 ± 0.17	1.55 ± 0.14	1.85 ± 0.18***	1.86 ± 0.20***

Values shows are mean ($n = 8$) liver fatty acid relative concentrations (mol%) ± SD. Only species with a relative abundance of >0.2 % in at least one dietary group in brain and/or liver are shown

Ctrl palm oil control, *EPA* eicosapentaenoic acid, *DHA* docosahexaenoic acid, *ALA* alpha-linolenic acid. The unsaturation index is the sum of the abundance of each fatty acid multiplied by the number of double bonds it contains

* $P < 0.05$, ** $P < 0.01$, and *** $P < 0.005$, as compared to palm oil control group using a post-ANOVA Tukey test

(OD 1/16″, ID 0.031″). Aldehyde concentrations were measured using the multi-ion monitoring mode of the instrument which quantified the count rates of the appropriate precursor and product ions as described in Table 2. Trace gas concentrations were calculated as described with the effects of ion diffusion taken into account using a calculated rather than empirical correction [13].

Statistical Analysis

The mean measurement in each dietary group was compared using one-way ANOVA with follow-up Tukey tests to compare one group with another.

Results

Fatty Acid Concentrations

The fatty acid content of the brain and liver of rats which had received a diet supplemented with either palm oil or palm oil plus either ALA, EPA or DHA for a period of 8 weeks was determined (Tables 3, 4). In the liver (Table 3), compared to the n-3 PUFA free group, all 3 supplementation groups showed a significantly ($P < 0.05$) increased abundance of total n-3 PUFA (to 432, 377 and 248 % of that in the control diet for DHA, EPA and ALA respectively). DHA supplementation also significantly

Table 4 Composition of esterified fatty acids in the brain following dietary intervention

Fatty acid	Dietary group			
	Ctrl	ALA	EPA	DHA
14:0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.1
16:0	22.2 ± 1.2	22.6 ± 1.1	22.4 ± 1.0	22.5 ± 1.0
16:1	0.5 ± 0.0	0.5 ± 0.1	0.5 ± 0.1	0.6 ± 0.1
18:0	19.1 ± 0.5	19.5 ± 0.2	19.4 ± 0.6	19.3 ± 0.4
18:1	24.1 ± 1.2	23.9 ± 0.8	24.3 ± 1.4	24.2 ± 0.4
18:2n-6	0.7 ± 0.1	0.8 ± 0.1	0.8 ± 0.2	0.8 ± 0.1
18:3n-6	0.1 ± 0.1	0.0 ± 0	0.0 ± 0.0	0.0 ± 0.0
18:3n-3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
20:0	0.6 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	0.5 ± 0.1
20:1	3.7 ± 1.0	3.3 ± 0.6	3.4 ± 0.4	3.4 ± 0.4
20:2n-6	0.5 ± 0.1	0.5 ± 0.1	0.7 ± 0.5	0.5 ± 0.2
20:3n-6	0.4 ± 0.1	0.5 ± 0.0*	0.5 ± 0.1*	0.6 ± 0.1*
20:4n-6	9.6 ± 0.6	9.3 ± 0.5	8.2 ± 0.9*	8.2 ± 0.5*
20:5n-3	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.1*	0.1 ± 0.1*
22:1	0.4 ± 0.1	0.3 ± 0.2	0.3 ± 0.1	0.3 ± 0.1
22:4n-6	2.6 ± 0.1	2.3 ± 0.1*	2.0 ± 0.2**	1.9 ± 0.2**
22:5n-6	0.5 ± 0.1	0.1 ± 0.1*	0.1 ± 0.1*	0.0 ± 0.1**
22:5n-3	0.1 ± 0.0	0.3 ± 0.0*	0.7 ± 0.1**	0.4 ± 0.0*
22:6n-3	11.9 ± 0.9	12.8 ± 0.5	12.6 ± 1.0	13.7 ± 0.6*
24:0	1.0 ± 0.2	0.9 ± 0.1	0.9 ± 0.2	1.0 ± 0.3
24:1	1.7 ± 0.4	1.4 ± 0.4	1.9 ± 0.9	1.6 ± 0.6
Total n-3	12.0 ± 1.2	13.1 ± 0.9	13.5 ± 1.0*	14.2 ± 0.8***
Total n-	14.4 ± 0.9	13.5 ± 0.6	12.9 ± 1.2*	12.0 ± 1.0***
n-3/n-6	0.83 ± 0.09	0.97 ± 0.10	1.05 ± 0.12**	1.18 ± 0.14***
Total saturated	43.1 ± 6.0	43.7 ± 3.2	44.4 ± 4.6	43.0 ± 5.2
Total monounsaturated	30.4 ± 2.8	29.4 ± 3.7	30.4 ± 2.2	30.1 ± 3.3
Total polyunsaturated	26.4 ± 1.9	26.6 ± 2.2	26.6 ± 2.4	26.2 ± 1.9
Unsaturation index	1.58 ± 0.14	1.59 ± 0.15	1.60 ± 0.18	1.60 ± 0.14

Values shows are mean ($n = 8$) liver fatty acid relative concentrations (mol%) ± SD. Only species with a relative abundance of >0.2 % in at least one dietary group in brain and/or liver

Ctrl palm oil control, *EPA* eicosapentaenoic acid, *DHA* docosahexaenoic acid, *ALA* α-linolenic acid. The unsaturation index is the sum of the abundance of each fatty acid multiplied by the number of double bonds it contains

* $P < 0.05$, ** $P < 0.01$, and *** $P < 0.005$, as compared to palm oil control group using a post-ANOVA Tukey test

increased the total amount of saturated fatty acids relative to the control group, while all three n-3 PUFA significantly decreased the abundance of total mono-unsaturated fatty acids, and EPA and DHA significantly increased the abundance of total polyunsaturated fatty acids. The so-called unsaturation index (see the "Methods" section) was significantly increased in the EPA relative to control group. For individual fatty acid species the largest change in liver n-3 PUFA in each group compared to the palm oil control was ALA for the ALA supplemented group, EPA for the EPA group and DHA for the DHA group. As expected animals supplemented with ALA and EPA showed significantly altered abundance of the metabolic products of these fatty acids (22:5n-3 and 22:6n-3 (DHA) for EPA supplementation, and 20:5n-3 (EPA) for ALA). Rats supplemented with DHA also showed elevated EPA concentration from a value of close to zero in the control group. The abundance of n-6 PUFA inversely correlated that of n-3 PUFA with the n-6 to n-3 ratio, and the concentration of arachidonic acid (20:4n-6), the major n-6 fatty acid, being decreased (although the minor species 20:3n-6 was increased in the DHA and ALA supplemented groups) in all three n-3 PUFA groups. Total n-6 concentrations decreased to 78 and 65 % of that in the control group in the EPA and DHA supplemented animals respectively.

Brain fatty acid analysis (Table 4) revealed much less pronounced differences compared to the liver. Total saturated, monounsaturated and polyunsaturated fatty acid concentrations, and the unsaturation index, were not significantly altered ($P < 0.05$). Total n-3 fatty acid concentration was increased to only 118 and 114 % of the control diet for DHA and EPA respectively, while ALA supplementation resulted in no significant change ($P < 0.05$). Indeed ALA did not significantly alter concentrations of brain ALA, EPA or DHA, while DHA supplementation increased DHA concentrations by only a small amount. In contrast with liver, EPA concentrations, although slightly increased by EPA supplementation, were very low in all 4 groups. Similarly, n-6 fatty acid abundance was also much less affected in the brain, with DHA and EPA, but not ALA, significantly reducing the abundance of arachidonic acid and total n-6 fatty acid concentration (to 87 and 84 % of that in the control group for DHA and EPA respectively). In addition, the concentrations of the minor n-6 fatty acids 22:4n-6 and 22:5n-6 were reduced in all three groups.

Aldehyde Concentrations

The liver and brain tissue was pulverised in liquid nitrogen to produce a homogenous preparation. The concentration of TBARS did not differ significantly between each dietary group in neither tissue (Table 5). Using SIFT-MS headspace saturated aldehyde concentrations were also found

Table 5 Brain and liver headspace aldehyde concentrations following dietary intervention

Tissue	Aldehyde	Dietary group			
		Ctrl	ALA	EPA	DHA
Liver	TBARS	56 ± 6	63 ± 8	62 ± 8	59 ± 9
	Methanal	1 ± 3	2 ± 3	1 ± 1	0 ± 3
	Ethanal	268 ± 71	233 ± 75	281 ± 80	242 ± 96
	Propanal	54 ± 32	49 ± 30	42 ± 27	43 ± 39
	Butanal	7 ± 3	10 ± 4	8 ± 5	6 ± 5
	Pentanal	22 ± 16	22 ± 14	26 ± 15	24 ± 14
	Hexanal	133 ± 47	164 ± 80	138 ± 54	170 ± 76
	Heptanal	14 ± 7	17 ± 10	18 ± 8	19 ± 13
	Octanal	6 ± 7	5 ± 4	4 ± 5	4 ± 3
Brain	TBARS	61 ± 12	50 ± 22	51 ± 10	49 ± 8
	Methanal	1 ± 3	2 ± 2	1 ± 1	3 ± 2
	Ethanal	214 ± 72	269 ± 49	224 ± 42	244 ± 24
	Propanal	7 ± 3[2]	8 ± 3[2]	9 ± 6[2]	10 ± 5[1]
	Butanal	3 ± 6	2 ± 3	4 ± 5	4 ± 6
	Pentanal*	34 ± 7	38 ± 14	33 ± 13	48 ± 14
	Hexanal	162 ± 44	190 ± 58	189 ± 59	188 ± 95
	Heptanal	14 ± 7	21 ± 12	16 ± 14	19 ± 12
	Octanal	4 ± 3	6 ± 7	7 ± 5	8 ± 5

Values shown are the means ± SD of eight samples analysed in duplicate. TBARS concentration is the tissue concentration expressed in MDA equivalents (nM). The concentration of the other aldehydes is the headspace concentration in units of PPB

Ctrl palm oil control, *EPA* eicosapentaenoic acid, *DHA* docosahexaenoic acid, *ALA* α-linolenic acid

* $P < 0.05$ by one-way ANOVA. No significant ($P > 0.05$) intergroup differences were observed using a *post hoc* Tukey test

[1] $P < 0.05$ and [2] $P < 0.01$ by paired t test comparing brain and liver aldehyde concentrations in the same dietary group

to not differ significantly between dietary group ($P > 0.05$) (Table 5) with the exception of brain headspace pentanal which differed between groups as assessed using a one-way ANOVA ($P < 0.05$) although no *post hoc* Tukey tests were statistically significant. Comparison of aldehyde concentrations between brain and liver within each dietary group revealed significantly ($P < 0.05$) lower brain headspace propanal concentration compared to liver.

Discussion

Our major finding was that while esterified concentrations could be markedly altered (in the liver at least) by supplementation with purified fatty acids, volatile aldehyde concentrations did not differ. The dietary intervention resulted in pronounced changes in the fatty acid profile of the liver. As expected (for example see Refs. [14–17]) all three n-3 fatty acids increased total liver n-3 concentrations with the

greatest effect being produced by DHA. This increase was mostly balanced by decreased monounsaturated fatty acid concentrations although the concentration of the n-6 polyunsaturated fatty acid, arachidonic acid, was also decreased by all three n-3 fatty acids compared to the control group with EPA and DHA having a greater effect than ALA similar as has been reported by others [18]. Such a more pronounced effect upon arachidonic acid concentrations by the longer chain length n-3 fatty acids agrees with the finding that oils containing EPA and/or DHA they have a greater inhibitory effect on the production of arachidonic acid derived inflammatory mediators compared to ALA containing oils [19]. This may occur due to the differing substrate preferences of the lysophospholipid acyltransferase enzymes which results in greater competition between the 20 carbon arachidonic acid and 20 carbon EPA and 22 carbon DHA than occurs with the 18 carbon ALA [20]. In addition, ALA increased the concentration its metabolites EPA and DHA although not to the same extent as with supplementation with DHA or EPA themselves, likely due to the low rates of conversion between n-3 species [21, 22]. These results mimic experiments comparing crude flax seed (high-ALA) and fish oils (high-EPA/DHA) although are somewhat in contrast to a report that ALA supplementation increased plasma EPA but not DHA concentration [14]. EPA similarly elevated DHA concentrations but unexpectedly DHA was observed to significantly increase EPA concentration compared to the control group. Evidence for the retro-conversion of DHA into EPA has been reported previously and was suggested to occur through partial β-oxidation and saturation of the resulting trans-double bond [23]. Such a pathway is also suggested by observations that docosapentaenoic acid supplementation increases concentrations of the metabolically downstream DHA but also the upstream EPA [24]. To date, however, researchers have not identified the enzymes needed to perform this retro-conversion and it is doubtful that such a mechanism exists. Alternatively, since the DHA used did contain impurities it is possible, particularly since only one dose of DHA was used, that small quantities of EPA in the supplement could have resulted in our observation, a possibility which will be tested in future work.

As has been observed by others [25], n-3 fatty acid supplementation had a much lesser effect on brain fatty acid concentrations than that in the liver. Although DHA supplementation significantly increased DHA concentration compared to the control groups, EPA and ALA-supplementation did not. Indeed, ALA supplementation had no effect at all on any brain n-3 fatty acid concentration. Furthermore, even in the DHA-free palm oil control group DHA concentrations were only slightly less than that in the DHA supplementation group. It is likely that the brain possesses a homeostatic mechanism for maintaining high DHA concentrations even in the absence of DHA in the diet. Such a hypothesis is consistent with the fact that DHA makes up approximately one-third of the total fatty acid content of phospholipids in cerebral gray matter, and is thought to be very important for proper brain functioning [26, 27].

Although brain EPA concentrations were significantly increased by dietary supplementation with EPA and DHA, the actual EPA abundance was much lower than in liver. The consistently low concentrations of EPA in brain (for example see Engström *et al*. [28]), are thought to occur due to an active preferential and rapid de-esterification and oxidation of EPA in the brain for reasons which are currently unclear [29]. It is notable, however, that EPA supplementation did reduce arachidonic acid concentration in brain to the same extent as DHA suggesting that EPA has a biochemical effect on the brain despite its low abundance.

Fatty acid supplementation therefore successfully changed the concentration of tissue, in particular liver, fatty acids which allowed us to conclude that aldehyde concentrations were independent of fatty acid abundance over the range of fatty acid concentrations observed. We cannot rule out that other aldehydes, such as alpha- and beta-unsaturated aldehydes, are affected by diet, a possibility that could be investigated in future studies. In particular, liver propanal concentrations did not differ between groups even though n-3 fatty acid concentrations varied markedly. Similarly, hexanal concentrations were not correlated with n-6 fatty acid abundance. Indeed, although brain total n-6 fatty acid concentration differed from that in liver, headspace hexanal concentrations did not differ significantly between the two tissues in any of the dietary groups. Interestingly, headspace propanal concentrations did differ by approximately one order of magnitude between liver and brain. This is similar what we observed in mouse tissue headspace which also showed low propanal concentrations in lung and kidney headspace [8]. Although this could be due to a higher degree of oxidative stress in the liver the similar concentrations of TBARS (which includes MDA) and hexanal in brain and liver are not supportive of such a conclusion. Instead other factors may underlie this observation including a higher rate of propanal metabolism in brain, again an unlikely scenario given the role of the liver as a detoxifying organ, or that propanal is being produced in another metabolic pathway other than n-3 fatty acid peroxidation. Future work will in our laboratory will investigate this matter further. This anomaly aside, our observations are in agreement with our work using human subjects in which the breath concentrations of another volatile n-3 fatty acid oxidation product, ethane, correlated with plasma hydroperoxides concentrations but not with erythrocyte n-3 fatty acid abundance [30]. Taken together with the data presented in this paper and our previous work showing

increased propanal and hexanal concentration in the headspace of peroxidised cultured cells [8], strongly suggesting that the concentration of volatile fatty acid peroxidation products is related predominantly to oxidative stress rather than that of fatty acid abundance.

Tissue TBARS concentrations also did not differ between dietary groups. This, and the lack of change of headspace aldehyde concentrations suggests that oxidative stress was not increased by the extent of polyunsaturated fatty acid supplementation in this particular dietary paradigm. No change was observed even though the unsaturation of the liver phospholipids increased, a change which would indeed be expected to increase oxidative stress [2]. Additional and more specific measures of oxidative stress, such as the concentration of hydroxynonenal, would strengthen this conclusion. Moreover, our observations differ from the observations of others suggesting that PUFA supplementation induces oxidative stress [31, 32], but are in agreement with those who report that n-3 fatty acid supplementation results in unaltered or even lowered oxidative stress [33, 34]. Importantly, it is not known how oxidative stress varied throughout the supplementation period, whether different doses of n-3 fatty acids would have had a different effect, and whether all body tissues are affected in the same way as the liver and brain. For example, it cannot be ruled out that oxidative stress was higher earlier in the supplementation regime but that homeostatic mechanisms increased the anti-oxidant defense in response to the pro-oxidant fatty acids, a mechanism which has been suggested elsewhere [34–36].

In summary, although our use of dietary supplementation resulted in a marked difference between dietary groups of n-3 and n-6 fatty acids, headspace aldehyde concentrations did not vary. This suggests that measurement of volatile aldehydes may be used as a measure of oxidative stress in situations in which dietary fatty acid intake cannot be easily controlled, such as human clinical studies. However more investigation is required of the metabolic sources and fates of the aldehydes, as to how these contribute to determining tissue concentrations in both health and disease.

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