Biochemical Pharmacology, Vol. 42, No. 7, pp. 1353–1360, 1991.
Printed in Great Britain.

0006-2952/91 $3.00 + 0.00

THE EFFECT OF A FISH OIL ENRICHED DIET ON OXYGEN TOXICITY AND LIPID PEROXIDATION IN MICE

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(*Received* 1 *March* 1991; *accepted* 10 *May* 1991)

Abstract—Mice were fed a chow diet or diets enriched in fish oil, sunflower oil or beef tallow for 3 weeks. Fatty acid analysis was carried out in samples of plasma, brain and lungs from these animals and large changes were found in plasma and lungs with relatively small dietary-induced changes in brain tissue. Bleeding times were increased very significantly in the fish oil group, and slightly increased in the sunflower oil group. Endogenous lipid peroxidation (measured as thiobarbituric acid reactive substances) was unchanged in lung and brain, but lung tissue from fish oil fed mice produced more lipid peroxides *in vitro* during incubation at 37° than those of other dietary groups. Mice fed the four different diets were exposed to hyperbaric oxygen at 618, 585 and 515 kPa and convulsive activity and lung damage was recorded. No dietary-induced alterations in susceptibility to oxygen toxicity were found.

The ingestion of dietary fish oils provides many physiological effects that may protect the cardiovascular system. However, the incorporation of fish oil polyunsaturated fatty acids into lipid membranes has also been reported to increase the susceptibility of these membranes to lipid peroxidation [1–4], a process which can be initiated by reactive oxygen metabolites [5–8]. The fish oil fatty acids apparently exert their physiological influence by displacement and substitution of arachidonic acid (AA) in lipid membranes with eicosapentanoic acid (EPA) which, together with docosahexanoic acid (DHA), is found in high concentrations in fish oil [9]. The change in the EPA/AA ratio following consumption of diets rich in fish oils causes the competitive inhibition of AA-derived eicosanoids [10] and the formation of EPA-derived eicosanoids. The latter have generally weaker pharmacological properties, with the exception that the EPA derived PGI_3 has similar potency to PGI_2 produced from AA [11].

The new balance between the thromboxane-like and prostacyclin-like effects is thought to lead to a state less conducive to atherogenesis. There is a decreased systolic blood pressure [12, 13], decreased tendency for platelet aggregation to occur with a consequent increase in bleeding time [13, 14] and decreased cholesterol with a rise in HDL [15–17]. In addition decreased migration and activation of neutrophils are claimed to occur as the EPA-based leukotrienes possess weaker chemotactic activity than the corresponding AA-derived compounds and anti-inflammatory properties have been reported in animals and man consuming diets rich in fish oil [18, 19].

However, some negative factors exist that may counterbalance the beneficial effects of dietary fish oil. Ingestion of a diet containing high levels of fish oil containing large amounts of highly unsaturated fatty acids may increase the tendency for a tissue to undergo lipid peroxidation [20]. Several investigators have reported increased peroxidation in tissues of animals fed high levels of fish oil [1–4] and suggested that the potentially advantageous effects of dietary fish oils may therefore be offset by the deleterious consequence of lipid peroxidation. Lipid peroxidation has been implicated as the link between the production of excess reactive oxygen species and resulting tissue damage in various situations [21–25]. For example, overproduction of oxygen radicals is believed to be involved in ischaemic-reperfusion injury, ageing, liver toxicity following ethanol or CCl_4 administration, Parkinson's disease and oxygen toxicity [5, 6, 26]. However, it has not yet been proved that lipid peroxidation is a major mechanism mediating cell damage [5, 8, 27, 28].

When mammals are exposed to oxygen at pressures exceeding about 300 kPa convulsions occur, and if the hyperoxia is maintained gross pulmonary oedema and finally haemorrhage eventuate [29]. In a previous investigation we examined the role of lipid peroxidation in central nervous system disruption (measured as convulsive activity) and pulmonary pathology in hyperbaric oxygen and our failure to detect increased peroxidation products caused us to doubt that lipid peroxidation is a significant factor in hyperoxic toxicity [30]. While the role of lipid peroxidation in hyperoxia is thus not clear, there is no doubt that increased mitochondrial and microsomal generation of oxygen radicals can occur as the surrounding oxygen tension is raised [31–35]. In addition some investigators believe that activated polymorphonuclear leukocytes (PMNL) are an additional source of radicals which may at least exacerbate, if not initiate, hyperoxic lung damage [36–40]. Thus, both the contribution of the neutrophil and the role of lipid peroxidation in the cellular injury caused by hyperoxia are controversial, and

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both these factors could be affected by changes in the membrane composition following consumption of a fish oil enriched diet.

In the present experiments we have measured the latency to convulsions as an indicator of central nervous system damage, lung wet weight and dry weight increases as an indicator of pulmonary damage, and *ex vivo* thiobarbituric acid reactive substances (TBARS) as an indicator of lipid peroxidation in brains and lungs from mice exposed to hyperbaric oxygen. Using these parameters mice fed diets containing 10% fish oil were compared to mice fed diets containing 10% sunflower oil, 10% beef tallow or 10% cellulose added to chowfeed.

MATERIALS AND METHODS

Animals and compositions of diets

Male Balb/c specific pathogen free mice (UNSW Animal Breeding and Holding Unit), initially weighing 18–23 g, were fed on one of four diets for a period of 3 weeks. The diets consisted of 90% ground chow (Milling Industries Ltd) with addition of either 10% cellulose, 10% saturated fat or 10% unsaturated fat (by weight). The saturated fat diet consisted of purified beef tallow rich in 16:0 and 18:0 fatty acids, while the unsaturated fat diets contained either sunflower oil rich in 18:2n-6 (linoleic acid) or fish oil (sardine oil containing 21% 20:5n-3 and 10% 22:6n-3). The Milling Industry Ltd basic chow diet contained 4.8% fat, consisting of a mixture of tallow, fish meal and vegetable oil. The diets were made fresh twice weekly, wrapped in aluminium foil and stored in the dark at 4°. The mixture was formed into a dough with water and given fresh to the animals each day, with any leftover food being discarded. The added fats were free of antioxidants, but the basic chow diet with which they were mixed contained 35 I.U./kg tocopherol and 50 mg/kg butylated hydroxytoluene (BHT). Food and water were provided *ad lib.* and mice were weighed twice weekly to determine growth rates.

Exposure to hyperbaric oxygen

The procedure and apparatus have been previously described in detail [41, 42]. Briefly, four mice, i.e. one mouse from each dietary group, were pressurized simultaneously to a final pressure of 515, 585 or 618 kPa. Compression time was 3 min in each case. As described previously our Balb/c mice show a biphasic convulsive pattern on exposure to hyperbaric oxygen [43]. Latency to the initial convulsion (Convulsion 1) was measured from the time of reaching the nominated pressure to the loss of the righting reflex. Latency to the second, sustained generalized convulsion (Convulsion 2) was also noted. Two investigators monitored all experiments. After exactly 30 min and a decompression lasting 3 min, mice were removed from the hyperbaric chamber, immediately killed by cervical fracture and lungs and brains were excised. Lungs were weighed, and the results are presented on an adjusted body weight basis. Lungs and brains were immediately placed on ice, and lung wet weight recorded prior to biochemical analysis or overnight heating to 300° for dry weight estimations.

The number of samples generated by using mice on four different diets made it impossible to perform biochemical determinations of tissues from non-pressurized and pressurized animals simultaneously, as had been done in the previous investigation [30]. However, we carried out experiments at one of the pressures, namely 585 kPa, interspersed on a daily basis with experiments using non-pressurized animals. The data from the mice fed four different diets and pressurized to 585 kPa is thus presented in the figures together with relevant data from the corresponding non-pressurized mice, while data from a preliminary experimental set at 618 kPa, and another set at 515 kPa are presented in the tables.

Assay of lipid peroxidation products

Lungs. For lung tissue the method of Boehme *et al.* [44] was used allowing for the increased lung weight during hyperoxic damage, as described in a previous communication [30]. A series of detailed control experiments have previously shown that this method is suitable for TBARS estimations in the presence of considerable quantities of blood, such as is found in hyperoxic damaged lungs [30]. Two assays of tissue homogenate were carried out: one immediately (unincubated samples), and the other after 2.5 hr incubation at 37° to provide some indication of the auto-oxidative potential of the tissue.

Brain. For brain homogenates the method of Ohkawa *et al.* [45], which solubilizes the samples with sodium dodecyl sulphate during homogenization, was used. Again, unincubated aliquots to determine endogenous levels and incubated aliquots for oxidizing potential were assayed.

Bleeding times in mice

Bleeding times were measured by removing 2 mm from the tip of the tail of mice using a pair of surgical scissors. Fifteen mice from each dietary group were used for this part of the experiment. Timing began immediately on abscission and finished when the touch of filter paper failed to show absorption of blood. The tail was kept at a constant level throughout the measurement. Mice were unrestrained during the procedure, and exhibited no indication of discomfort. Tissues from some of these mice were subsequently used for fatty acid analyses, or for determination of haematocrit values.

Determination of haematocrit values

Mice were killed by cervical fracture and the thoracic cavity was immediately opened and blood withdrawn into a heparinized syringe by cardiac puncture. The blood was transferred to a micro-centrifuge tube and the haematocrit determined.

Analyses of fatty acids

Pooled lungs, brains and plasma from two mice were combined for each analysis. Tissues were homogenized in a chloroform–methanol solution (8:4:3 of CH_3Cl:CH_3OH:tissue by mass). Fatty acid analysis was performed by gas–liquid chromatography by the method of Neumann *et al.* [46].

Table 1. Summary of fatty acid analysis in plasma, lungs and brains. All results are expressed as percentages of the total fatty acid content

Fatty acid	Chow	Fish oil	Sunflower oil	Beef tallow
Plasma				
Total saturates	31.06	29.70	27.82	30.99
Total mono	23.7	18.65	16.85	27.29
PUFAS	45.2	51.6	55.33	42.70
Total n-6	34.77	22.18	49.41	31.97
Linoleic acid (18:2n-6)	25.08 ± 0.25	12.06 ± 0.63	37.11 ± 0.69	23.10 ± 0.12
AA (20:4n-6)	8.09 ± 0.43	9.22 ± 0.53	10.69 ± 1.0	6.94 ± 0.21
Total n-3	10.32	29.61	6.17	9.47
Linolenic acid (18:3n-3)	0.54 ± 0.06	0.31 ± 0.02	0.28 ± 0.03	0.35 ± 0.03
EPA (20:5n-3)	1.54 ± 0.04	10.96 ± 1.35	0.44 ± 0.01	1.38 ± 0.02
DHA (22:6n-3)	7.63 ± 0.77	16.12 ± 1.90	5.04 ± 0.15	7.20 ± 0.32
EPA/AA	0.19	1.19	0.04	0.20
Lung				
Total saturates	52.85	55.89	53.19	52.15
Total mono	16.38	14.27	12.40	18.85
PUFAS	30.77	31.44	34.41	29.00
Total n-6	15.07	8.35	22.59	13.81
Linoleic acid (18:2n-6)	4.00 ± 0.25	2.62 ± 0.02	7.80 ± 0.05	3.43 ± 0.3
AA (20:4n-6)	8.14 ± 0.30	4.66 ± 0.20	10.41 ± 0.02	7.61 ± 0.53
Total n-3	15.59	22.04	11.82	15.01
Linolenic acid (18:3n-3)	0.16 ± 0.02	0.16 ± 0.00	0.14 ± 0.00	0.14 ± 0.01
EPA (20:5n-3)	0.66 ± 0.01	3.05 ± 0.03	0.27 ± 0.01	0.69 ± 0.07
DHA (22:6n-3)	11.66 ± 0.48	11.75 ± 0.21	9.40 ± 0.18	11.31 ± 0.47
EPA/AA	0.08	0.67	0.03	0.09
Brain				
Total saturates	50.50	48.99	47.84	48.06
Total mono	23.85	22.21	21.60	22.48
PUFAS	25.65	29.80	31.56	29.46
Total n-6	10.17	10.50	13.38	11.99
Linoleic acid (18:2n-6)	0.57 ± 0.07	0.34 ± 0.01	0.94 ± 0.00	0.54 ± 0.04
AA (20:4n-6)	7.08 ± 0.21	7.18 ± 0.10	8.49 ± 0.26	7.88 ± 0.11
Total n-3	15.72	19.21	17.19	17.47
Linolenic acid (18:3n-3)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
EPA (20:5n-3)	0.00 ± 0.00	0.31 ± 0.01	0.00 ± 0.00	0.00 ± 0.00
DHA (22:6n-3)	15.10 ± 0.69	18.14 ± 0.70	16.76 ± 0.25	16.98 ± 0.28
EPA/AA	0.00	0.04	0.00	0.00

Statistical analysis

Results were analysed initially using SPSS and ANOVA one-way analysis. If any significant differences occurred results were re-analysed using the Student's *t*-test with the Bonferroni correction for multiple comparisons applied. Thus the level of significance corresponding to $P = 0.05$ for a simple *t*-test becomes $P = 0.05/N$, and with three repeated comparisons as used for comparison of diets in these experiments, the significant level of P is taken as $P < 0.0167$. All values are expressed as mean ± SEM.

RESULTS

Growth rates

Mice fed with the fat supplemented diets gained weight slightly but significantly faster than those fed a chow plus cellulose diet. The final mean weight of the latter group was 27.8 ± 0.5 g while those of the other three groups were between 29.2 and 29.5 g. All data have been expressed on a normalized weight basis where relevant.

Fatty acid analysis

A summarized version of the results of the fatty acid determinations is shown in Table 1. The total saturated fatty acid content of tissues remains quite constant in spite of the very different levels of saturated or unsaturated fats consumed, but the monounsaturated, n-6 and n-3 polyunsaturated fatty acids substituted for each other depending on the composition of the diet. There were substantial increases in the EPA levels of the plasma and lung following fish oil administration. There was a detectable, albeit small amount of EPA in brain tissue following fish oil administration whereas none was detected in animals on other diets.

There were also alterations in AA levels in the lungs of mice fed different diets, where the content of AA from mice fed fish oil was less than half of that in sunflower oil fed mice, with chow and beef tallow fed mice showing values between these two extremes. Indeed, AA altered much more in lung tissue than in the plasma (Table 1). The changes in the brain were slight, although, as in the lung and

Table 2. Convulsive activity, lung weight* and TBARS data from groups of mice fed different diets for 3 weeks then exposed to hyperbaric oxygen at 618 or 515 kPa

	Chow	Fish oil	Sunflower oil	Beef tallow
618 kPa†				
Latency to Convulsion 1 (min)	2.1 ± 0.2	2.3 ± 0.2	2.1 ± 0.2	2.3 ± 0.2
Latency to Convulsion 2 (min)	10.0 ± 0.9	8.7 ± 0.5	8.1 ± 0.5	9.6 ± 1.0
Lung wet weight (mg)	297 ± 25	309 ± 15	282 ± 12	304 ± 18
Lung dry weight (mg)	43 ± 2	48 ± 3	43 ± 1	45 ± 2
515 kPa‡				
Latency to Convulsion 1 (min)	2.4 ± 0.2	2.8 ± 0.2	2.7 ± 0.2	2.7 ± 0.1
Latency to Convulsion 2 (min)	18.4 ± 1.7	17.0 ± 1.6	16.8 ± 2.0	14.0 ± 2.6
Lung wet weight (mg)	191 ± 14	225 ± 47	222 ± 37	262 ± 56
Lung TBARS (unincubated)	45 ± 5	37 ± 4	34 ± 5	36 ± 2
Lung TBARS (incubated)	430 ± 24	515 ± 29	438 ± 31	402 ± 33
Brain TBARS (unincubated)	466 ± 54	427 ± 39	465 ± 59	452 ± 53
Brain TBARS (incubated)	1590 ± 67	1557 ± 62	1605 ± 63	1519 ± 67

* Lung weights normalized to 25 g body weight.
† There were 15 mice in each dietary group exposed to 618 kPa.
‡ There were seven mice in each dietary group exposed to 515 kPa.

plasma, AA tended to be higher in the brain of the sunflower oil fed group. There were considerable alterations also in the content of linoleic acid (18:2 n-6), the precursor of AA, which were primarily related to the fatty acid composition of the diets. Lung was found to contain approximately 7–8-fold higher amounts of linoleic acid than brain.

To summarize, large alterations in EPA and AA contents, with very large changes in the EPA/AA ratio were achieved in plasma and lung tissue as a result of the dietary manipulations, while the corresponding changes in brain tissue were slight. Linoleic acid was altered in all tissues, although this latter substance is present in only low concentrations in brain phospholipids.

Bleeding times and haematocrit determinations

Bleeding times (average 7.3 ± 0.4 min) were significantly prolonged ($P < 0.001$) in mice fed a fish oil enriched diet compared to mice of any other dietary group. Mice fed diets high in sunflower oil also showed a significant ($P < 0.0167$) though less marked increase in bleeding times (5.3 ± 0.7 min) compared to chow (3.6 ± 0.3 min) or beef tallow (4.2 ± 0.3 min) fed animals. There was no change in the haematocrit value (which ranged from 42 to 44%) between any dietary group.

Effects of diet on oxygen toxicity at 618 kPa

In the first experiment 15 mice from each of the four diets were exposed to 618 kPa as described in Materials and Methods and their convulsive activity and lung damage, measured by increases in wet and dry weight, assessed. No biochemical analyses were carried out on tissues from these animals. The different diets did not alter any of the parameters of brain or lung damage assessed in these experiments (Table 2).

Effect of diet on oxygen toxicity at 585 kPa

All data described in this section are from the same groups of mice with eight non-pressurized mice in each dietary group, and a corresponding seven mice exposed to 585 kPa of oxygen.

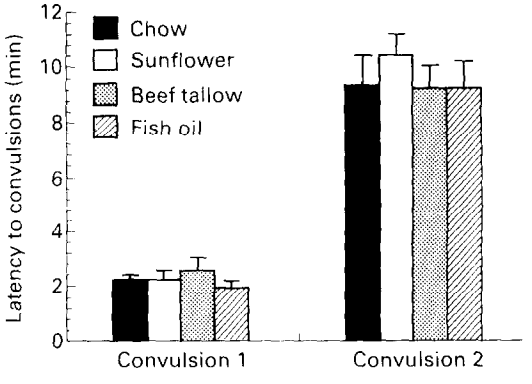

Fig. 1. Time to loss of righting reflex (Convulsion 1) and to start of severe clonic generalized convulsive activity (Convulsion 2) in groups of mice fed different diets and exposed to 585 kPa oxygen. Values are mean ± SE, N = 7 for each group of mice, and no significant differences were observed.

Convulsions. The latency to either the initial, brief convulsion, or to the second, sustained and more violent convulsive period was no different in any dietary group (Fig. 1).

Lung weight. The wet weights of the lungs of non-pressurized mice fed four different diets and those of corresponding mice after exposure to oxygen at 585 kPa are shown in Fig. 2. None of the the diets inhibited nor exacerbated lung damage caused by hyperbaric oxygen.

TBARS estimations in brain tissue. The results of assays of TBARS in unincubated brain homogenates and those of the same homogenates after 2.5 hr incubation at 37° are depicted in Fig. 3. For both unincubated and incubated samples the TBARS

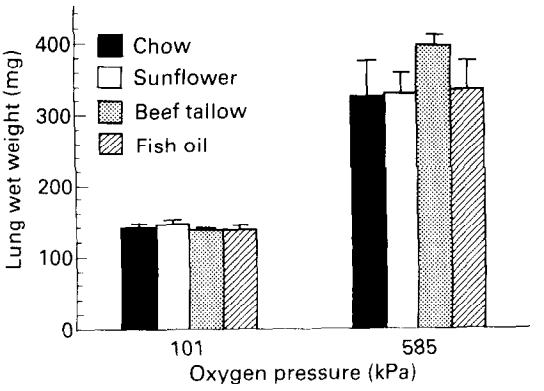

Fig. 2. Wet lung weight of non-pressurized mice fed different diets and of corresponding mice exposed to 585 kPa oxygen for 30 min. Values are mean ± SE with N = 8 in all non-pressurized dietary groups and N = 7 for each group of pressurized mice. All lung weights are normalized to 25 g body weight mouse. There were no significant dietary alterations.

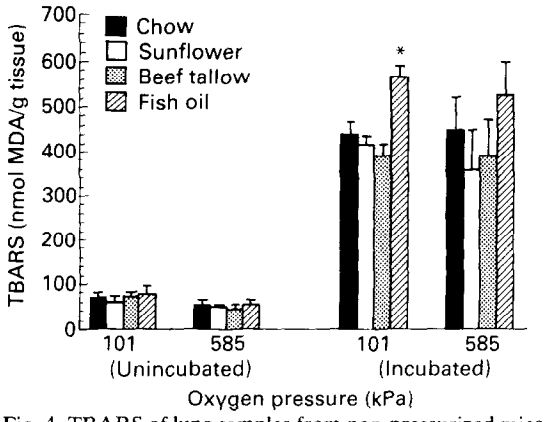

Fig. 4. TBARS of lung samples from non-pressurized mice and mice exposed to 585 kPa oxygen from each of the different diets (unincubated) and TBARS in the same samples after 2.5 hr incubation in air at 37° (incubated). TBARS measured according to Ref. 44. Values shown are means ± SE, N = 8 for non-pressurized groups and N = 7 for each group exposed to 585 kPa. * $P < 0.0167$ between chow fed and fish oil fed animals.

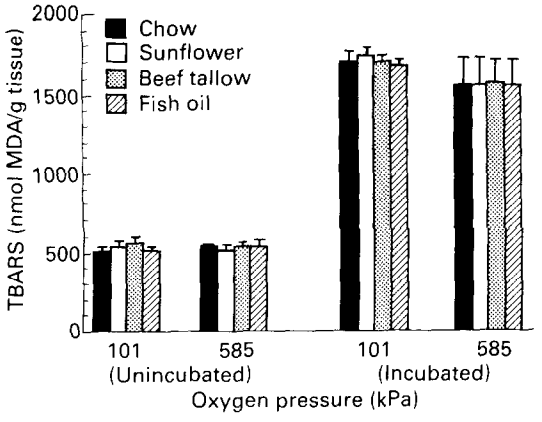

Fig. 3. TBARS (unincubated or following 2.5 hr incubation at 37°) of brain samples from non-pressurized mice fed different diets and of corresponding mice exposed to 585 kPa oxygen for 30 min). TBARS measured according to Ref. 45. Values are mean ± SE, N = 8 for each non-pressurized group and N = 7 for each group exposed to 585 kPa. No significant differences between diets was found.

levels did not vary between any of the dietary groups in either non-pressurized mice or those mice exposed to 585 kPa. As found in a previous series of experiments [30] there was no increase in TBARS levels in either unincubated nor incubated sample of brains of mice following exposure to hyperbaric oxygen compared to control, non-pressurized mice.

TBARS estimations in lung tissue. There were no significant differences in TBARS of unincubated lung homogenates between mice fed different dietary fats, whether samples came from non-pressurized or pressurized mice (Fig. 4).

The incubated lung homogenates did, however, demonstrate a significant effect of diet on the amount of TBARS formed. Lung homogenates from non-pressurized sunflower oil and beef tallow fed mice did not differ from chow fed animals, while those from the fish oil group were significantly higher ($P < 0.0167$). As found for brain tissue there was no increase in unincubated nor in incubated TBARS levels in lungs of mice following exposure to hyperbaric oxygen, compared to control, non-pressurized animals (Fig. 4).

Effect of diet on oxygen toxicity at 515 kPa

This last series of experiments was carried out due to some concern that the lung damage resulting from the exposure of mice to 585 kPa was somewhat greater than anticipated. Thus a separate group of seven mice from each diet was pressurized to 515 kPa and the results are presented in Table 2. It is clear that the results at 515 kPa were essentially in agreement with those at 585 kPa, in that changes in fatty acids in the diet and tissues did not alter convulsive activity or lung damage during hyperbaric oxygenation. TBARS values were also unaltered between dietary groups. Although the values for TBARS in the incubated lung samples were again highest in the fish oil group, none of the results achieved statistical significance at this pressure.

DISCUSSION

Incorporation of different fatty acids into lungs, brain and plasma of mice was clearly related to ingestion of diets of differing fatty acid composition. Total saturated or unsaturated content was not greatly influenced by diet, however, the unsaturated fatty acids substitute for each other depending on the type of unsaturated fat ingested, such that the ratio of n-3 to n-6 fatty acids is highest in the fish oil fed group and lowest in the tissues of sunflower oil fed mice. Such overall findings are in agreement

with those reported by Gibson *et al.* [47] for microsomal and mitochondrial membranes from various tissues of rats fed different diets and with other reports where a variety of tissues and subcellular membranes of tissues were analysed [1–4].

Brain tissue was found to be more resistant to change than other tissues and our dietary induced alterations were generally similar to other reported values [48, 49]. The changes induced in brain tissue were slight compared to the alterations in fatty acid composition in lung. Large decreases in AA (down to 50% of sunflower oil group levels), markedly increased EPA levels (10-fold greater than levels in sunflower oil fed rats), and a decreased linoleic acid content (one-third of that of the sunflower oil group) occurred in the lungs of animals on the fish oil supplemented diet. The values for the chow fed mice in our experiments were in all instances intermediate between these extremes. The differences in EPA and AA found in lung tissue are similar in magnitude to those reported for kidney and liver tissues in rats fed fish oil rich diets compared to other diets [4].

Bleeding times in our mice increased when fish oil diets were ingested. Correlation between the high levels of membrane n-3 fatty acids and longer bleeding times is well established [9, 50] presumably due to decreased thromboxane production resulting from substitution of EPA for AA in platelet membranes. Such changes have been confirmed for rats fed diets high in fish oil [2] but there is also evidence that other less transient factors than tissue eicosanoids may be involved [51].

These and other alterations to the cardiovascular system mentioned in the introduction may contribute to the reduced risk of cardiovascular disease in populations which ingest a large proportion of their fats from marine sources, but the increased polyunsaturation and different composition of these fats may lead to other less desirable effects. Polyunsaturated fatty acids are peroxidized in preference to saturated and monounsaturated fatty acids [20] with docosahexanoic acid (22:6n-3) being particularly sensitive [52]. In 1978, Hammer and Wills [1] demonstrated that microsomes prepared from fish oil fed rats had considerably greater rates of *in vitro* (incubated) malonaldehyde formation than those from corn oil, coconut oil or lard supplemented animals. The varying propensities to peroxidize reflected the large changes in fatty acid composition of the liver microsomal lipids which were obtained in that study. Similar increases in TBARS found in, or formed by, homogenates of several tissues from rats fed fish oil diets confirmed these findings [2, 4] and led Herbert and Wills [2] to conclude that there was reason for caution in extolling the virtues of fish oil ingestion, as the beneficial effects, such as the decreased platelet activity which they also demonstrated in their experiments, may be offset by the possibly harmful effects of the highly polyunsaturated fat diet. Augmented lipid peroxidation was seen also in heart tissue from pigs fed fish oil compared to those fed lard, and in the heart sarcolemma large dietary induced changes in fatty acid composition occurred [3]. In the latter experiments susceptibility to ischaemic-reperfusion injury was tested along with malonaldehyde production, but in spite of the increased peroxidation in the fish oil group, there was no correspondingly greater injury resulting from the oxidative stress imposed.

In the experiments reported here we also found no dietary influence on susceptibility to another form of oxidative stress, i.e. that resulting from hyperoxia. There was no dietary influence on the time taken to produce any form of convulsions. Also, there was no difference in TBARS values for unincubated *ex vivo* brain samples in non-pressurized mice nor in samples of brain from mice following exposure to two different pressures of hyperbaric oxygen. In addition, incubated homogenates of brain showed no influence of diet on the peroxidizability of this tissue. These results probably reflect the homeostasis of the brain phospholipids which demonstrate relatively minor changes compared to those which take place in other tissues following dietary modifications of fatty acid intakes, as discussed above.

Although the unincubated levels of TBARS were similar in lungs from non-pressurized or pressurized mice fed four different diets, the lungs from mice on the fish oil enriched diet did produce more TBARS during *in vitro* incubation, suggesting that they could more readily be induced to peroxidize. This, however, did not produce any measurable effect on leakage of fluid or blood into lungs, judged by the weight increase, during hyperbaric oxygen exposure. In many earlier studies it has been shown that wet and dry lung weight correlates closely with histological and biochemical indicators of hyperbaric oxygen damage [53–55]. In the present experiments no effects of diet were seen in weights of lungs after exposure of mice to 618 or 585 kPa. However, at both these pressures lungs were quite severely oedematous and haemorrhagic with an increase in wet weight greater than 100%. It seemed possible that if conditions were chosen so that less damage was produced, i.e. if results were to fall on the steep portion of the pressure/damage dose–response curve rather than near its plateau, there would be a better chance of detecting an inhibitory (or potentiating) effect on oxygen toxicity. Such an interaction has been seen previously in hyperbaric oxygen studies [42]. Thus, a further group of animals was exposed to a pressure of 515 kPa, which produced only moderate damage within 30 min. However, even with this lower pressure neither fish oil, nor any other dietary fat, influenced the degree of damage produced.

Thus, any possible deleterious effect of increased susceptibility to lipid peroxidation due to fish oil ingestion was not apparent in this hyperoxic model of radical induced damage. This is perhaps not surprising as we had previously failed to find a correlation between hyperoxic brain or lung damage *in vivo*, and *ex vivo* measurements of either incubated or unincubated brain and lung TBARS. In this study we confirmed the lack of raised TBARS levels in lungs or brains following hyperbaric oxygenation. In the present experiments the commonly used method of Ohkawa *et al.* [45] was employed for TBARS estimations in brain tissue to

further verify our earlier findings. Ohkawa's method was, however, not suitable for measurement of TBARS in the blood laden lungs of mice which had been exposed to the higher pressures of oxygen, whereas Boehme's method can be used satisfactorily in these cirmcumstances [30].

While there was no potentiation of tissue injury due to hyperbaric oxygenation, neither was any protection afforded by the high fish oil, nor any other diet. Such protection may perhaps be expected if indeed neutrophils are an important contributing factor to pulmonary pathology in hyperoxia, as has been claimed by several investigators [36–38]. Reduced chemo-attraction should result from the production of the weaker chemoattractant LTB_5 derived from EPA, in place of LTB_4 resulting from AA metabolism, in the lungs of the fish oil fed mice [56, 57]. However, it is possible that LTB_4 may not be the critical compound for neutrophil attraction in this situation.

In conclusion, the results of our study suggest that dietary fish oils neither reduced nor increased the susceptibility of mice to the radical-mediated injury of hyperbaric oxygen. The beneficial effects of fish oil consumption on the cardiovascular system do not appear to be compromised by the potentially deleterious consequence of increased membrane polyunsaturation.

Acknowledgements—The authors are grateful to Mr Mark Neumann for the capillary GLC analysis of tissue fatty acids. This work was supported by a grant from the ARC.

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