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Menhaden Fish Oil, n-3 Polyunsaturated Fatty Acids, and Protection of Newborn Rats from Oxygen Toxicity

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ABSTRACT. We have previously reported that newborn rats born to mothers fed a high n-6 polyunsaturated fatty acid (PUFA) (safflower oil) diet demonstrated increased n-6 PUFA in lung lipids and superior tolerance to high oxygen exposure. In the present study, we explored whether high n-3 PUFA might also protect against hyperoxic damage and by what mechanism. Adult female rats were fed either regular rat chow, a high n-3 (menhaden fish oil-based) diet, or a high n-6 (safflower oil-based) diet for 6 wk before and then throughout pregnancy and lactation. Newborn offspring of the high n-3 (fish oil) dams demonstrated increased n-3 PUFA (*i.e.* eicosapentaenoic and docosahexaenoic acid) and decreased n-6 PUFA (*i.e.* linoleic and arachidonic acid) in their lung lipids compared to the other two diet groups. The high n-6 (safflower oil) offspring had the opposite PUFA lung lipid pattern (with increases in total n-6 fatty acids and decreases in total n-3 fatty acids). The high n-3 offspring demonstrated markedly decreased lung levels of prostaglandin E_2, $F_{2\alpha}$ and thromboxane B_2, whereas the high n-6 offspring had higher eicosanoid levels than the regular diet offspring. Offspring of both high n-6 and high n-3 diet dams demonstrated essentially the same superior hyperoxic tolerance compared to regular diet offspring [7-d (>95% O_2) survival rates of 110/115 and 99/109, respectively, *versus* 70/91, $p < 0.01$]. These studies lend further support to the speculation that increasing lung PUFA content may provide the newborn lung with increased ability to scavenge oxygen-free radicals and thus may serve to protect against oxygen toxicity. (*Pediatr Res* 25:399–404, 1989)

Abbreviations

PUFA, polyunsaturated fatty acids
EPA, eicosapentaenoic acid
DHA, docosahexaenoic acid
DSPC, disaturated phosphatidylcholine
SOD, superoxide dismutase
CAT, catalase
GP, glutathione peroxidase

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Common nursery practices consist of a several-day interval between the birth of a very low birth wt oxygen-requiring premature infant and the initial administration of lipid nutrition. Because of the immaturity of the pulmonary antioxidant defense systems in the premature (1) and the possible role lipids might play in protecting the lung from the toxic effects of high O_2, this withholding of lipids from these critically ill neonates might increase their risk of developing the chronic lung disease, bronchopulmonary dysplasia.

In a previous study exploring the protective role of specific lipids on pulmonary O_2 toxicity, we demonstrated that pregnant rats fed a high PUFA diet (safflower oil) produced offspring with increased PUFA in their lung lipids and superior survival in hyperoxia (2). One possible explanation for the protective effect of high PUFA has been proposed by Dormandy (3), who hypothesized that PUFA, located in noncritical, nonmembrane sites, and immediately replaced after their own autooxidation, could serve as avid scavengers of excess O_2-free radicals, function as an antioxidant, and thereby protect cells from O_2 toxicity.

Whereas safflower oil contains PUFA primarily of the n-6 family of fatty acids (*i.e* linoleic acid, 18:2 n-6), menhaden or fish oil, linked epidemiologically with decreased thrombosis-related cardiovascular disease (4, 5), contains PUFA primarily of the n-3 family, with long carbon chains and multiple double bonds (*i.e.* EPA, 20:5 n-3; and DHA, 22:6 n-3).

The long-chain n-3 family fatty acids differ from the n-6 family fatty acids in their preference to serve as substrate for the lipoxygenase and cyclooxygenase enzymes and in the biologic properties of their oxygenated cyclooxygenase and lipoxygenase products (5–8). In addition, n-3 family fatty acids are major PUFA components in the membranes of the central nervous system and retina, with accumulation into these membranes occurring almost exclusively during the last trimester of the human gestation (9–11).

With these facts as background, we undertook the present study to determine whether menhaden oil, providing elevated PUFA of the n-3, rather than the n-6 family, would be associated with superior tolerance to high O_2 exposure in the neonatal rat. In addition, if superior hyperoxic tolerance did result from menhaden oil nutrition, might this protection be related to altered lung lipid PUFA composition, to fish oil's effect on lung eicosanoids, and/or to altered antioxidant enzyme development or induction during hyperoxia.

MATERIALS AND METHODS

Animals, diets: Adult Sprague-Dawley albino female rats (~200 g) were fed one of three diets *ad libitum*: *1*) standard rat Chow (#5001, Ralston-Purina, Co., St. Louis, MO); (5% fat as a mixture of animal and vegetable fats; double bond index = 74);

2) high n-3 family PUFA diet ("fat-free" test diet #15750, United States Biochemical Corp., Cleveland, OH, plus 10% [by wt] menhaden oil; double bond index = 173); *3*) high n-6 family PUFA diet ("fat-free" test diet plus 10% [by wt] safflower oil; double bond index = 162). Diets 1, 2, and 3 were essentially comparable in protein content (23%, 18%, 18%, respectively), carbohydrate content (66%, 52%, 52%), and caloric density (4.0, 3.7, 3.7 cal/g). The comparative composition of the three test diets is seen in Table 1. Rats were fed the respective diets for at least 3 wk before breeding and then throughout pregnancy and lactation. Breeding was accomplished by placing male and female animals together overnight, checking for sperm-positive vaginal smears the next morning, and considering the midpoint of the cohabitation period as the onset of pregnancy.

Several litters from the high n-3 PUFA (menhaden oil) diet group and regular diet group were killed prematurely at d 20 or 21 of gestation, after term delivery at 22 d of gestation, or at 1 or 7 d of postnatal age. Premature pups were delivered by hysterotomy with the dam under pentobarbital anesthesia; newborn rats were obtained after normal parturition, either within 6 h of the beginning of delivery of the first pup or after 24 h of life. Fetal, newborn, 1-d- or 7-d-old rat lungs were perfused immediately *in situ* via the pulmonary artery using cold saline. The perfused fetal and newborn lungs were removed, stripped of nonpulmonary tissue, and homogenized in 20 to 30 times their wt of cold saline in a Brinkmann polytron (high speed, 90 s), (Brinkmann Instruments, Inc., Westbury, NY). For preterm or small newborn rats, two to four lungs were pooled and homogenized to obtain adequate tissue for the assays.

Antioxidant enzyme, phospholipid and fatty acid analyses. Aliquots of the lung homogenate were analyzed for antioxidant enzyme activities using standard spectrophotometric assays for SOD (12), CAT (13), and GP (14), and for DNA and protein content (15, 16). Purified enzyme standards (SOD, CAT) and DNA standard were obtained from Sigma Diagnostics (St. Louis, MO) and GP from Boehringer-Mannheim, Houston, TX). Results of antioxidant enzyme analyses were expressed as U of enzyme activity/mg of DNA. For DSPC analysis, a separate aliquot of lung homogenate was subjected to lipid extraction (17), reaction with osmium tetroxide (18), and assay for inorganic phosphorus using the method of Morrison (19). A known quantity of ^{14}C-dipalmitoyl-phosphatidylcholine (New England Nuclear, North Billerica, MA) was added before lipid extraction, and aliquots were counted at each step to estimate and correct for sequential losses. DSPC was expressed as mg/g wet lung wt.

Table 1. *Partial fatty acid content of three experimental diets*

Fatty acid (%)	Regular	Safflower	Menhaden
16:0 (palmitic)	20	6	17
18:2n-6 (linoleic)	27	81	2
18:3n-3 (linoleic)	3	1	2
20:4n-6 (arachidonic)	–	–	2
20:5n-3 (EPA)	1	–	17
22:6n-3 (DHA)	1	–	8
n-6/n-3*	5	101	0.1
Double bond index†	74	162	173
Supplier	Ralston-Purina	Beatrice Foods	ICN

* n-6, n-3: each represents a family of structurally related fatty acids based on the position of the first double bond from the methyl end. n-6/n-3 is the ratio of the total n-6/n-3 family of fatty acids in diet.

† Double bond index = the sum of [the number of unsaturated double bonds in each specific fatty acid multiplied by the percentage of each specific fatty acid].

Lungs from 1-d-old rat offspring from regular diet, high n-3 PUFA (menhaden) diet, and high n-6 PUFA (safflower) diet were perfused with iced saline as described and frozen in liquid nitrogen for fatty acid analysis. The frozen tissue was homogenized and aliquoted for protein, and total lipids were extracted using the procedure of Folch *et al.* (20). Total fatty acids were converted to their respective methyl esters with methanolic HCl and separated and quantitated by capillary column gas-liquid chromatography (21). C17:0 was used as the internal standard. The fatty composition of lung phospholipids (from 7-d-old regular diet and high n-3 diet offspring) was determined after separation of phospholipids from other lipid classes by plate chromatography (22). Protein was assayed according to the method of Lowry *et al.* (23).

Nursing female rats maintained on either regular rat Chow or high n-3 PUFA diet (menhaden oil) were selected at random for milk collection. Rat milk was collected by manual expression from lightly anesthetized dams, frozen at −80°C, and assayed for total lipid fatty acid composition. The frozen samples were thawed in cold water, then rapidly heated to 80°C and held for 1 min to prevent lipase activity as described by Bitman *et al.* (24). Total lipids were extracted using the procedure of Folch *et al.* (20), and the fatty acids were converted to their respective methyl esters with methanolic HCl after addition of C13:0 and C17:0 as internal standards. The fatty acid methyl esters were separated and quantitated by capillary column gas-liquid chromatography. The gas-liquid chromatography equipment and operating conditions were used as described in detail (22), except that the column oven was programmed from 80 to 160°C at 20°/min, then to 200°C at 5°/min.

Exposure to hyperoxia. Multiple litters (9–11 pups) of newborn rats from each of the three diet groups were placed with their mothers into >95% O_2 (or room air) at 6–12 h of life. Details of the continually monitored exposure conditions have been reported earlier (25). Dams from the same diet group were rotated between room air and >95% O_2 litters every 24 h to prevent O_2-induced illness with consequent poor mothering. Weight gain and survival of newborn rats from each group was recorded daily for 7 d. In addition, after 7 d of exposure, random surviving offspring from each diet group were removed from high O_2 or from companion room air-exposed litters, killed and their lungs processed and assayed for DSPC and antioxidant enzymes as described above. We also evaluated in a separate experiment the clinicopathologic condition of pups surviving a 7-d hyperoxic exposure. We devised a scoring system that semi-quantitatively scored the pups in blinded fashion from 0 (normal) to 2 (markedly abnormal) on five parameters of O_2 toxicity: degree of respiratory distress, pleural fluid accumulation, and gross appearance of edema, atelectasis, and lung hemorrhage.

Prostaglandin assays. After 5 d of either >95% O_2 or room air exposure, offspring from the three diet groups were killed, and their lungs were removed and homogenized as described above. The homogenizing buffer for these studies, however, contained acetylsalicylic acid (15 mM). Fresh homogenates were assayed for prostaglandin E_2, prostaglandin $F_{2\alpha}$, and thromboxane B_2, using specific RIA test kits (Seragen, Inc., Cambridge, MA).

Statistics. Statistics were performed using Chi-square analysis for survival data, analysis of variance for fatty acid data and Student's *t* test for antioxidant enzyme and phospholipid data (26).

RESULTS

Growth and biochemical development of offspring. Both the high n-3 PUFA diet and the high n-6 PUFA diet were well accepted by the female rats. Birth wt and fetal and neonatal growth were not different in offspring of the two experimental

diet groups and did not differ from offspring of rats fed regular rat Chow.

The comparative development of the antioxidant enzyme system and the surfactant system in the high n-3 PUFA (menhaden) diet offspring and regular diet offspring can be seen in Table 2. Offspring of the high n-3 PUFA diet did not demonstrate advanced development of the three antioxidant enzymes—SOD, CAT, and GP—or of surfactant compared to the regular diet offspring. In fact, significantly depressed lung GP enzyme activity was found in the term and premature menhaden diet offspring. This finding suggests that a deficient level of selenium may exist in the menhaden oil diet. (Arrangements are underway to have selenium levels in the diets evaluated by other investigators experienced with the analysis procedure).

The partial fatty acid profile of the rat milk from the dams of the three diet groups is seen in Table 3. Whereas the rat milk of the high n-6 (safflower) diet dams had increased linoleic acid (31%) compared to regular diet (16.8%) and menhaden diet milk (1.5%), and very low n-3 family PUFA, the milk of menhaden dams demonstrated, in addition to dramatic decreases in linoleic acid, significant increases in n-3 family PUFA.

When the fatty acid composition of total lung lipid was determined in 1-d-old offspring of the three diet groups (Table 4), the high n-3 PUFA offspring lung lipid was found to have significantly less linoleic acid (18:2 n-6) and arachidonic acid (20:4 n-6) but significantly increased amounts of EPA (20:5 n-3) and DHA (22:6 n-3) and a markedly reduced n-6/n-3 ratio (0.19) compared to regular diet (2.4) and high n-6 PUFA (safflower) diet (37.0) offspring. Conversely, the high n-6 PUFA offspring had increases in linoleic acid and total n-6 family PUFA and significant decreases in total n-3 family PUFA compared to the menhaden and regular diet offspring.

The separation of total lung lipid into phospholipid and triacylglycerol subclasses with subsequent fatty acid analyses (Table 5) revealed similar changes in the 7-d-old high n-3 PUFA (menhaden) *versus* regular diet offspring in each lipid subclass compared to what was seen in the total lung lipid aggregate. However, even more marked differences in the double bond index (282 *versus* 182, menhaden *versus* regular) was found in the triacylglycerol subclass of the lung lipid compared to what was found in the phospholipid subclass (double bond index: 185 *versus* 155, menhaden *versus* regular).

Exposure to hyperoxia. Both the high n-3 PUFA offspring and the high n-6 offspring demonstrated similar superior survival after 7 d of hyperoxia (99 out of 109, for menhaden offspring; 110 out of 115 for safflower offspring) compared to regular diet offspring (70 out of 91), Figure 1. These results represent newborn rat pups from the three diet groups placed in high O_2 within 12 h of birth; neonatal pups placed in hyperoxia after several days of life would be expected to have a somewhat higher 7-d hyperoxic survival rate.

In addition to their improved survival rate in hyperoxia, the 7-d survivors from the menhaden oil diet group demonstrated superior clinical/pathologic status by semi-quantitative (blinded) scoring compared to the regular diet group survivors. The total clinicopathologic score (based on assessing five parameters, each giving a maximal lung injury score of 2.0 and a minimal score of 0) was 1.20 ± 0.54 (n = 10) for the fish oil survivors *versus*

Table 2. *Comparative antioxidant enzyme and surfactant development in offspring of menhaden oil and regular diet rats**

Gestation (d)	U/mg of DNA SOD	CAT	GP	mg/g lung DSPC
20				
Regular (8)	24.4	240	0.514	2.06
Menhaden (6)	23.4	228	0.388†	2.14
21				
Regular (8)	18.6	347	0.676	2.87
Menhaden (8)	18.3	326	0.416	3.13
22				
Regular (6)	25.9	541	1.041	5.20
Menhaden (6)	22.7	492	0.488†	5.50

* Mean values for n of pooled lung samples in parentheses.
† $p < 0.05$ difference between diet groups.

Table 3. *Partial fatty acid pattern of rat milk from high n-3 PUFA diet (menhaden oil), high n-6 PUFA diet (safflower oil), and regular diet rats**

Diet Group	Fatty acid (%) 18:2n-6	20:4n-6	20:5n-3	22:6n-3	n-6/n-3
Regular	16.8	1.6	0.4	0.8	9.6
Menhaden	1.5†	1.3	9.0†	6.8†	0.21†
Safflower	31.0‡	1.3	0.02‡	0.0‡	471‡

* Mean values; all SD ≤1.0; n = 3–4 samples/diet group.
† $p \leq 0.05$ *vs.* regular and safflower.
‡ $p \leq 0.05$ *vs.* regular and menhaden.

Table 5. *Partial fatty acid components of lung phospholipids and triacylglycerols in 7-d-old offspring of high n-3 PUFA (menhaden oil) and regular diet rats**

FA (%)	Phospholipids† Menhaden	Regular	Triacylglycerols Menhaden	Regular
18:2n-6	1.5‡	7.0	1.7‡	14.8
20:4n-6	4.5‡	16.0	1.6‡	4.7
22:5n-3	5.5‡	2.6	14.0‡	5.1
22:6n-3	8.8‡	4.7	25.0‡	9.0
Double bond index	185‡	155	282‡	182

* Mean values; n = 3 samples/diet group.
† Phospholipids represent predominately membrane-associated cell lipid, whereas triacylglycerols are predominantly nonmembrane, intracellular lipid.
‡ Significantly different between diet groups at $p < 0.05$.

Table 4. *Partial fatty acid composition of lung lipids**

Diet group	Fatty acid (%) 18:2n-6	20:4n-6	20:5n-3	22:6n-3	Total n-6	Total n-3	n-6/n-3
Regular	5	12	1	6	22	9	2.4
Menhaden	0.6†	3†	9†	11†	5†	26†	0.19†
Safflower	11‡	14	0.1	0.5‡	37‡	1‡	37‡

* Mean values; all SD <1.0; n = 2–7 samples/diet group.
† $p \leq 0.05$ menhaden *vs.* regular.
‡ $p \leq 0.05$ safflower *vs.* regular.

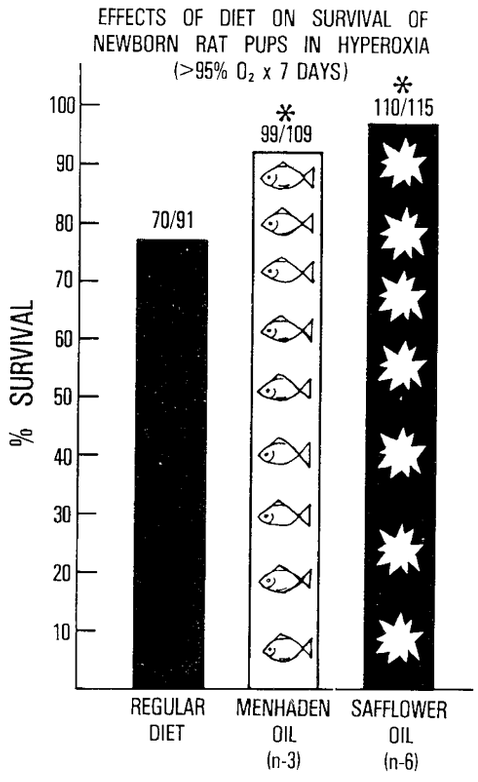

Fig. 1. Percentage survival of offspring of three diet groups after 7 d of hyperoxic exposure (>95% O_2). (*$p < 0.01$ *versus* regular diet group).

2.35 ± 1.53 ($n = 10$) for the regular diet O_2 survivors ($p < 0.05$ between groups).

After 7 d of hyperoxia, both groups of diet pups manifested significantly increased antioxidant enzyme responses to hyperoxia. No statistically significant differences were present in the magnitude of increase of the three antioxidant enzymes and in DSPC in the high n-3 PUFA diet (SOD: ↑19%, CAT: ↑82%, GP: ↑140%, DSPC: 0%) compared to the regular diet offspring (SOD: ↑36%, CAT: ↑55%, GP: ↑95%, DSPC: ↓10%). Results of increases in AOE and DSPC after 7 d of >95% O_2 in high n-6 (safflower) compared to regular diet offspring have been reported previously and were likewise found not to be different in magnitude compared to the regular diet group (2).

Prostaglandins, thromboxane measurements. Lung tissue prostaglandin E_2 and $F_{2\alpha}$ and thromboxane B_2 levels from 4- to 5-d-old offspring from the three diet groups are depicted in Figure 2*A*. High n-3 (menhaden) offspring had comparatively much lower levels of all three eicosanoids than the other diet groups, whereas the high n-6 (safflower) offspring tended to show higher lung eicosanoid levels. When eicosanoid values from the air-exposed animals from the three dietary groups were compared with the values obtained after 5 d of >95% O_2 exposure, the lungs of O_2-exposed pups from the three groups had 2- to 3-fold elevations in all eicosanoids measured, except for a lack of any significant rise in prostaglandin E_2 in the menhaden diet group. After hyperoxic exposure, high n-3 offspring demonstrated obviously lower prostaglandins and thromboxane B_2 levels compared to the regular and high n-6 diet O_2-exposed offspring, whereas high n-6 offspring had significantly increased thromboxane B_2 compared to regular and high n-3 pups (Fig. 2*B*).

DISCUSSION

Fish or marine oil, of which menhaden oil is an example, has received a flurry of recent attention in both the lay and professional press. Epidemiologic investigations have suggested a possible link between a diet high in fish oil and the reduced incidence of thrombosis-related ischemic heart disease found in Greenland Eskimos (4, 5). A number of studies in humans and in a variety of experimental animals have demonstrated that a fish oil diet (containing high levels of n-3 family PUFA) can result in rapid increases in the n-3 PUFA composition of the lung, kidney, spleen, liver, heart, plasma, red blood cells, and macrophages (27–33). Similar modification of the n-3 PUFA composition of cells in culture can be accomplished by changing the n-3 fatty acid content of the culture medium (33). Conversely, deprivation of n-3 family fatty acids to developing animals results in decreases in n-3 PUFA accumulation in brain and retinal membranes (34, 35).

The significance of these n-3 fatty acid changes in tissue lipids relates to changes in cell properties and/or cell functions, such as changes in the activity of membrane-associated cellular enzymes (*i.e.* the activity of membrane-bound adenylate cyclase in rat liver is modulated by the nature of its membrane lipid environment) and membrane fluidity (*i.e.* altering the lipid composition of hepatocytes produces changes in plasma membrane fluidity and increased activity of [Na^+-K^+-dependent] ADP [36, 37]). Specifically relating to n-3 fatty acids, lung microsomes containing increased phospholipid n-3 fatty acids (as a result of menhaden oil diet) demonstrate decreased ability to convert arachidonic acid into thromboxane (27). And importantly, ex-

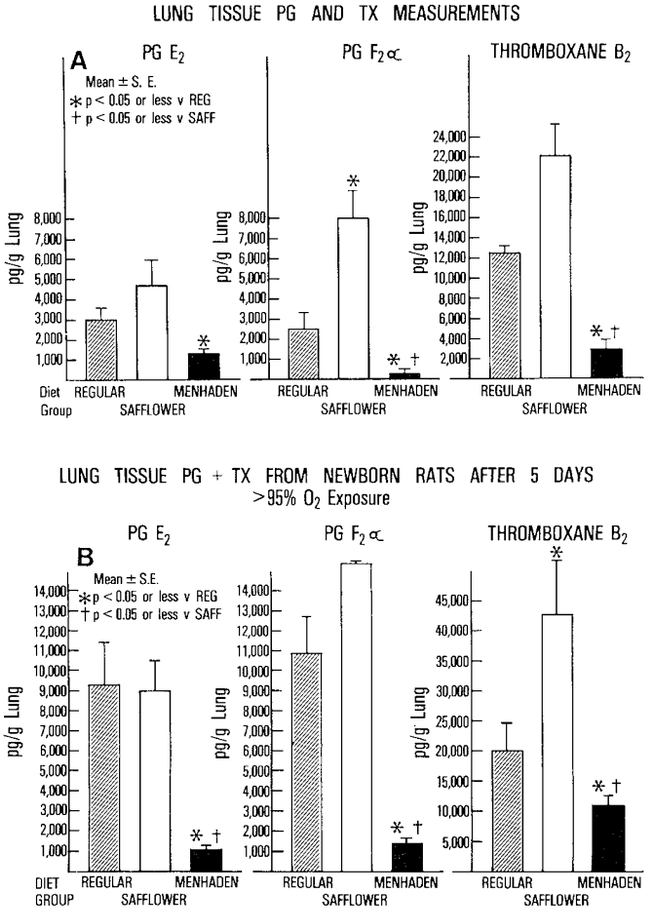

Fig. 2. *A*, lung tissue prostaglandin E_2, $F_{2\alpha}$, and thromboxane B_2 levels in 5-d-old offspring from the three diet groups; n = 3–4 samples/group/prostaglandin assay (†$p < 0.05$ *versus* high n-6 (safflower) offspring; *$p < 0.05$ *versus* regular diet offspring). *B*, lung tissue prostaglandin E_2, $F_{2\alpha}$, and thromboxane B_2 levels in 5-d-old offspring from the three diet groups after 5 d of >95% O_2 exposure; n = 3–4 samples/group/assay (†$p < 0.05$ or less *versus* high n-6 (safflower) offspring; *$p <$ 0.05 or less *versus* regular diet offspring).

perimental animals with deficient accumulation of the n-3 fatty acid DHA (22:6 n-3) in brain and retinal membranes are found to have decreased discrimination learning and visual acuity (34, 35).

Our interest in n-3 PUFA and in menhaden oil stems from our previous observation that offspring of rats fed a high PUFA diet (safflower oil) consistently demonstrated superior tolerance to high O_2 exposure, and this increased resistance to hyperoxia was associated with increased lung PUFA levels (2). We chose menhaden oil to further these investigations for several reasons. First, menhaden oil represents a relatively PUFA-rich dietary oil, with a high double bond index similar to safflower oil. Secondly, the PUFA composition of menhaden oil is, however, markedly different from the PUFA content of safflower oil. Menhaden oil contains increased n-3 family fatty acids (and decreased n-6 family PUFA). Finally, as the hyperoxic protective effect of safflower oil could have been related to an increased capacity for eicosanoid production, a fish oil diet could provide high lung PUFA content yet depress eicosanoid production during hyperoxic exposure (27, 32).

As anticipated from our previous studies (2) and those of other investigators (28), offspring of rats fed modified PUFA diets had modifications in the fatty acid composition of their lung lipid. Specifically, we found that offspring of rats fed a high n-3 PUFA diet in the form of menhaden oil had increased n-3 PUFA in total lung lipids. In addition, in the menhaden-diet offspring, although the increases in n-3 PUFA were observed in both the phospholipid and triacylglycerol fractions of lung lipid, more marked enrichment with high unsaturated n-3 fatty acids was apparent in the triacylglycerol fraction than in the phospholipid fraction (Table 5).

In association with the marked increases in n-3 PUFA in their lung lipid, offspring of menhaden oil diet rats demonstrated increased tolerance to high O_2 exposure compared to regular diet offspring. In fact, the survival rate after 7 d of >95% O_2 found in the menhaden oil offspring was remarkably similar to the hyperoxic survival found in safflower oil offspring both in this and in our previous study (2). The mechanism whereby increases in maternal dietary PUFA (with consequent increases in rat milk PUFA and ultimately in lung tissue PUFA of the offspring) result in protection against the toxic events of high O_2 remains speculative. The high PUFA offspring did not have significantly augmented antioxidant enzyme system or surfactant development nor a greater ability to induce a protective antioxidant enzyme response during hyperoxia. Calculations of dietary intake of vitamins E and A in the three diets studied do not suggest that differences in these vitamins are playing a deciding role in hyperoxic protection in the high PUFA offspring, as menhaden oil diet was found to contain essentially the same amount of vitamin E as regular diet, and only ⅔ the quantity of vitamin A. (Lung tissue analysis of these vitamins in experimental and regular diet offspring is currently in progress.)

In addition, one of our major reasons for choosing the fish oil diet was to explore the role prostaglandins and thromboxanes (derived from arachidonic acid) might play in protecting the neonatal lung from hyperoxic damage. Studies by Hageman *et al.* (38), Smith *et al.* (39) and others found that specific eicosanoid levels were elevated in bronchoalveolar lavage fluid in association with prolonged hyperoxic challenge in rabbits, and that in adult rats, lipoxygenase blockade resulted in decreased mortality and indices of tissue damage during hyperoxic exposure (40). In addition, lipoxygenase products were increased in lavage fluid of infants with bronchopulmonary dysplasia, suggesting a possible etiologic role for these agents in clinical O_2 toxicity (41). In the present study, we found that despite similar hyperoxic tolerance, the menhaden diet offspring had depressed lung levels of three major prostanoids compared to the regular diet offspring, whereas the high n-6 diet offspring demonstrated the highest mean values for the three prostanoids. These disparate prostaglandin and thromboxane levels, especially the large differences noted with O_2 exposure (Fig. 2*B*), associated with similar hyperoxic tolerance between the high n-3 and high n-6 offspring, suggest that elevated lung PUFA is not functioning to protect against O_2 toxicity through a prostaglandin-mediated mechanism.

There is thus reason to believe that increased lung tissue PUFA content itself may be responsible for protection against O_2 toxicity, possibly through the free radical scavenging mechanism proposed by Dormandy (3). Various lines of evidence from the present and previous study support Dormandy's "alternate hypothesis" of a protective effect of high PUFA. First, two qualitatively different PUFA diets (high n-3 PUFA or high n-6 PUFA), but with a similar quantitative degree of polyunsaturation (*i.e.* double bond index of 173 for menhaden oil and 162 for safflower oil), confer essentially similar degrees of protection against hyperoxia. Secondly, both high PUFA diets result in increased unsaturation of the lung lipids; and this increased degree of unsaturation (or double bond index) is more pronounced in the triacylglycerol (nonmembrane) lipid fraction than in the phospholipid (membrane) fraction. And thirdly, the high PUFA diets do not appear to be exerting their protective effect through an antioxidant enzyme or prostanoid mechanism.

The results of this and our previous study deal with newborn rat offspring that manifest a relatively milder pulmonary pathologic response to hyperoxia compared to the human newborn infant. Nonetheless, these studies raise intriguing questions as to whether providing specific PUFA nutrition (perhaps a combination of n-6 and n-3 family fatty acids) to oxygen-requiring very low birth wt premature infants very soon after birth might be advantageous, not only to their nutritional status, but also to their ability to resist the toxic effects of oxygen and ventilator therapy.

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