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HIGH FISH OIL DIET INCREASES OXIDATIVE STRESS POTENTIAL IN MAMMARY GLAND OF SPONTANEOUSLY HYPERTENSIVE RATS

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SUMMARY

1. The purpose of this study was to determine whether high ω-3 (19% menhaden oil, 1% corn oil) or high ω-6 (20% corn oil) fatty acid diets would decrease expression of hypertension in the female spontaneously hypertensive rat (SHR), promote tumourigenesis in the rat 7,12-dimethylbenz[a]anthracene (DMBA) model of mammary cancer or increase the susceptibility of the mammary gland to lipid peroxidation. A group of rats on a 5% corn oil diet served as the low fat control group.

2. We found that the high ω-3 and high ω-6 fatty acid diets did not significantly decrease mean arterial pressure. Marked differences occurred between the effects of ω-3 and ω-6 high fatty acid diets on baseline oxidation, auto-oxidation and iron-ascorbate catalyzed oxidation. The ω-3 diet showed 675% increase in basal oxidation, a 2624% increase in auto-oxidation and a 4244% increase in iron-ascorbate catalyzed oxidation compared to the ω-6 diet in mammary tissue homogenates. Although all rats were given 5 mg DMBA (i.g.), no mammary tumours were observed in any of the dietary groups.

3. We conclude that: (i) high polyunsaturated fatty acid diets do not decrease blood pressure in the female SHR; (ii) high fish oil diet markedly increases oxidative potential in the mammary gland; and (iii) the female SHR is resistant to DMBA-induced tumourigenesis.

Key words: aging, blood pressure, 7,12-dimethylbenz[a]anthracene (DMBA), heart rate, malondialdehyde, omega-3/ω-3, omega-6/ω-6, polyunsaturated fatty acids, thiobarbituric acid reactive substances (TBARS), tumourigenesis.

INTRODUCTION

Peroxidation of polyunsaturated fatty acids (PUFA) is thought to be an important factor in aging of biological membranes in many tissues. The types of fat in the diet determine the relative composition of biomembranes. The ingestion of high PUFA diets changes the unsaturated/ saturated fatty acid ratios in membranes. There are multiple functional physiological consequences of producing membranes of varying degrees of susceptibility to lipid peroxidation, for example, changes in membrane fluidity and the consequent changes in receptor transduction mechanisms (Vliet & Bast 1992). Pathophysiological states in many organs have also been associated with lipid peroxidation. These include cell death, cell aging and mutagenesis (Kehrer 1993).

In the mammary gland alterations in both level and type of dietary fat greatly influence both spontaneous and carcinogen-induced benign and malignant experimental rodent mammary tumours (Bunce & Abou El-Ela, 1990; Welsch *et al.* 1990). Diets rich in PUFA have shown to be more efficacious in promoting mammary tumours than diets rich in saturated fatty acids (Abou El-Ela *et al.* 1987). The ω-6 series is more effective in this regard than the ω-3 series (Bunce & Abou El-Ela 1990). Two ways in which dietary fat can influence the health of the mammary gland are by

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influencing eicosanoid synthesis (Abou El-Ela *et al.* 1988) and by altering membrane lipid composition and therefore susceptibility to oxidative damage. Polyunsaturated fatty acids can oxidize to form reactive oxygen species. The auto-oxidation of PUFA produces various products including malondialdehyde (MDA), a reactive species that can cross-link proteins or DNA (Mukai & Goldstein 1976). The biological consequences of oxidative stress caused by lipid peroxide formation include mutations, chromosomal aberrations, sister chromatid exchanges, carcinogenesis, cytotoxicity and cellular degeneration related to aging (Cerutti 1985). In the area of carcinogenesis, some report a positive correlation between increased lipid peroxidation and breast cancer (Gromadzinska *et al.* 1992). Also, some initiators of mammary tumourigenesis are known to produce oxidative free radicals and initiate lipid peroxidation (Takada *et al.* 1992).

Because ω-6 and ω-3 PUFA have cardiovascular benefits not shared by saturated fatty acids, current dietary guidelines recommend increasing the ratio of PUFA to saturated fatty acids in the diet. Known benefits include antithrombosis (ω-3), decreased hypertriglyceridaemia (ω-6), decreasing low density lipoprotein (LDL)-cholesterol by replacing saturated fatty acids in the diet, increasing high density lipoprotein (HDL) receptor expression by replacing saturated fatty acids in the diet (National Academy of Science Report 1989). Overall, the effects of high PUFA intakes on blood pressure remain controversial in human studies as well as in animal models. Increased dietary PUFA have been reported by some investigators to decrease blood pressure in spontaneously hypertensive rats (SHR; Hoffmann *et al.* 1986; Mtabaji *et al.* 1988; Von Au *et al.* 1988; Bayorh *et al.* 1989; Karanja *et al.* 1989; Brandle & Jacob, 1990; Yin *et al.* 1990; Engler *et al.* 1992); however, others find no significant effects or actual increases in blood pressure (McGreger *et al.* 1981; Codde *et al.* 1989; Croft *et al.* 1988; Kingsley & Snyder 1988; Watanabe *et al.* 1989). Since hypertension is one of the risk factors for developing coronary heart disease, many people are motivated to increase dietary PUFA to achieve the cardiovascular beneficial effects. To date, there are few data available on the cardiovascular benefits of PUFA in female populations, yet the cancer-promoting effects of dietary PUFA (both ω-6 and ω-3) have been well demonstrated in the rat 7,12-dimethylbenz[a]anthracene (DMBA) model of mammary carcinogenesis (Abou El-Ela *et al.* 1988). In human females at risk for both coronary heart disease and mammary cancer there appears to be dichotomy in the risks and benefits associated with high PUFA diets. The type as well as level of fat can increase or decrease certain risk factors. In these studies we used female rats of a strain used most frequently as a model for human essential hypertension, the SHR.

The effects of dietary lipid intake on the blood pressure have been studied by several groups in male SHR (Hoffmann *et al.* 1986; Mtabaji *et al.* 1988; Yin *et al.* 1990; Engler *et al.* 1992), but there are no data available in the literature concerning similar effects in female SHR. All work concerning the effects of high PUFA diets in mammary tumour models has obviously been conducted in female rats, but female rats of the SHR strain have not been previously used as a laboratory model to study mammary tumour promotion. The purposes of the present study were: (i) to evaluate the effects of long-term feeding of high ω-3 and ω-6 fatty acids enriched diets on hypertension in the female SHR; (ii) to assess the relative effects of these diets on promotion of DMBA-induced mammary tumours; and (iii) to determine whether high PUFA diets increased the susceptibility of the mammary gland to lipid peroxidation. For the latter we determined the extent of both spontaneous and iron-ascorbate catalyzed lipid peroxidation *in vitro* in mammary glands to assess the increased potential for oxidative aging caused by high PUFA diets. The data from these high ω-3 and ω-6 fatty acid diet groups were compared with similar data obtained from female SHR fed a low-fat (5% corn oil) diet.

METHODS

Animal housing and dietary regimens

Twenty-one female SHR weighing 95–105 g at 36 days of age were obtained from Charles River Laboratories (Raleigh, NC, USA). Each rat was individually placed in a 10×16 inch plastic cage on Absorb Dri litter and housed in a room held at constant temperature (24°C) and a 12 h light/dark cycle. Free access to tap water was provided at all times. Rats were fed standard Purina rodent chow during a 9 day acclimation period. Rats were then switched to the purified AIN-76 (ICN Biomedicals, Cleveland, OH, USA) standard formula diet containing 5% corn oil for 5 days. At 50 days of age, each animal was gavaged with a single dose of 5 mg of DMBA dissolved in 0.5 mL corn oil. The rats weighed approximately 130 g at this time, and were continued on the AIN-76 diet for one week post-DMBA.

At 57 days of age (1 week post-DMBA) rats were randomly assigned to one of three groups ($n = 7$ for each group) and transferred to the specially formulated

Table 1. Diet composition for three dietary regimens

	Semi-purified diet (AIN-76)	Menhaden oil diet (19% MO + 1% CO)	Corn oil diet (20% CO)
Casein purified high nitrogen (%)	20.0	20.0	20.0
DL-methionine (%)	0.3	0.3	0.3
Sucrose (%)	50.0	30.0	30.0
Corn starch (%)	15.0	20.0	20.0
Alphacel, non-nutritive bulk (%)	5.0	5.0	5.0
Menhaden oil (%)	0	19.0	0
Corn oil (%)	5.0	1.0	20.0
AIN mineral mix (%)	3.5	4.0	4.0
ICN vitamin diet fortification mix (%)	0	1.2	1.2
AIN vitamin[a] mix (%)	1.0	0	0

[a] α-tocopherol was added with the vitamin mix so that each diet contained a total of 157 i.u. of vitamin E/kg of diet.

Table 2. Fatty acid composition of dietary oils

Fatty acid	Structure	Corn oil[a]	Menhaden oil[b]
Myristic acid	14:0	—	8.45
Palmitic acid	16:0	11.2	15.2
Palmitoleic acid	16:1, ω-7	—	11.6
	16:2	—	2.4
	16:3	—	2.0
	16:4	—	1.7
Stearic acid	18:0	2.1	2.7
Oleic acid	18:1, ω-9	25.0	9.5
Linoleic acid	18:2, ω-6	59.9	1.8
γ-Linolenic acid	18:3, ω-6	0.5	—
α-Linolenic acid	18:3, ω-3	—	1.8
	18:4	—	3.5
	20:1	—	2.3
Arachidonic acid	20:4, ω-6	—	2.3
Eicosapentaenoic acid	20:5, ω-3	—	16.0
	22:5	—	3.9
Docosahexaenoic acid	22:6, ω-3	—	10.8
Others	—	0.1	4.4

[a] Analysis was provided by ICN Nutritional Biochemical, Cleveland, OH, USA.

[b] Analysis was provided by Zapata Haynie Corp., Reedville, VA, USA.

diets shown in Table 1 for 16 weeks. 10–20 kg batches of these three diets were prepared by ICN Nutritional Biochemical (Cleveland, OH, USA), cold pressed into jumbo pellets and sealed under nitrogen. The diets were shipped second-day delivery by United Parcel Service at two-week intervals. Upon arrival, 1 kg bags of pellets were placed in Seal-N-Save bags, flushed with nitrogen, sealed and stored frozen at −20°C until used. The control group (AIN) was continued on the low-fat, balanced diet (AIN-76) containing 5% corn oil. The high ω-3 fatty acid diet group (MO) was fed a diet containing 19% menhaden oil and 1% corn oil; the high ω-6 fatty acid diet group (CO) was fed a diet containing 20% corn oil. The 20% CO diet was a standard AIN formulation for high fat diets. The 19% MO + 1% CO diet was a substitution of 19% MO and 1% CO for 20% CO. Alpha-tocopherol (vitamin E) was added with the AIN vitamin mix so that each diet contained a total of 157 i.u. of vitamin E/kg (DL-α-tocopherol powder, 250 i.u./g) in the diet. Fatty acid composition of corn oil and menhaden oil is shown in Table 2. All diets were stored frozen under nitrogen to minimize auto-oxidation during storage. Measured amounts of fresh, frozen diet was placed in the food cups daily and food intake was recorded daily. Uneaten pellets were discarded. All animals were weighed and palpated for presence of mammary tumours weekly. Rats were allowed free access to food and water throughout the study period.

After 16 weeks of this dietary regimen, each rat was again weighed and then anaesthetized with ketamine anaesthesia (10 mg/kg, i.m.) supplemented with pentobarbital (4.5 mg/kg, i.p.). Body temperatures during anaesthesia were maintained at 37 ± 1°C using a servo-controlled warming pad. Rats were fitted with in-dwelling carotid arterial catheters (PE-50 tubing, tapered 1 cm at the arterial end) to monitor direct carotid blood pressure. Analogue signals for mean arterial pressure and heart rate were derived from the pulsatile signals using an Electromedics (Engelwood, CO, USA) small volume displacement pressure transducer and a cardiotachometer, respectively, when the animals had recovered from anaesthesia. The mean arterial blood pressure was recorded using a Gould Model RS 3200 recorder.

Following blood pressure and heart rate measurements, the animals were killed using an overdose of pentobarbital anaesthesia followed by pneumothorax

procedure. Animals were each examined directly for presence of mammary tumours by retraction of ventral skin flaps bilaterally. Mammary glands were then immediately removed. Sections of mammary glands were sent for histopathological examination and remaining samples were frozen using liquid nitrogen. Tissues were stored at −80°C for *in vitro* lipid peroxidation analysis.

In vitro lipid peroxidation assay

Mammary tissues were weighed and homogenized in cold 0.05 mol/L phosphate buffer pH 7.4 (10% w/v) using a Brinkmann Homogenizer ATA 10S (Brinkmann Instruments, Inc., Westbury, NY, USA). Three 500 μL aliquots of the homogenate were immediately transferred into three borosilicate disposable culture tubes. 50 μL of butylated hydroxytoluene (BHT) in absolute ethanol (8% w/v) was immediately added to the first of the three aliquots in order to arrest oxidation. The second aliquot received no BHT and was used to measure auto-oxidation under the conditions of the subsequent incubation. 50 μL ferric chloride (20 μmol/L) and 50 μL ascorbic acid (100 μmol/L) were added to the third aliquot to catalyze lipid peroxidation. Appropriate volume adjustments in the BHT and auto-oxidation tubes were made with double distilled water so that all reaction volumes were equal. The tubes were incubated in a shaking water bath (American Scientific Products, Mcgraw Park, IL, USA) at 37°C for exactly 1 h. At the end of 1 h, 50 μL of BHT was added to both the auto-oxidation and iron-ascorbate catalyzed oxidation reactions to arrest further oxidation. 200 μL duplicates from each of these three incubates were transferred into Pyrex disposable screw cap culture tubes (16 mm × 125 mm with Teflon lined caps).

Thiobarbituric acid reactive substances (TBARS) were measured by using a modification of the method described by others (Ciuffi *et al.* 1992). 0.5 mL of 1% thiobarbituric acid in 0.05 mol/L NaOH was added to each tube, followed by 0.5 mL of 6.85 mol/L HCl. All tubes were capped, vortexed and heated in a boiling water bath for 12 min and cooled to room temperature. Thiobarbituric acid reactive substances were extracted by adding 3.0 mL of *N*-butanol to each tube and vortexing vigorously for 30 s. The tubes were centrifuged at 3000 *g* (Jouan CT4-22 table top swing bucket centrifuge, Jouan Inc., Winchester, VA, USA) for 14 min. Finally, the butanol layer was transferred into semi-micro cuvettes and read at 532 nm on a Beckman DU 660 spectrophotometer (Beckman Instruments, Inc., Fullerton, CA, USA). The equivalent MDA concentration was calculated on the basis of the molar absorptivity constant of the MDA-thiobarbituric acid complex, (1.56×10^5 mol/L). A standard curve for MDA was prepared using 1,1,3,3,-tetraethoxypropane . The curve was linear between 0.01 and 1.5 nmol of MDA. Finally, the protein contents of the homogenates were determined by the Lowry method (Lowry *et al.* 1951). All lipid peroxidation data were expressed as nmol of MDA per mg protein.

Chemicals

1,1,3,3-tetraethoxypropane, ferric chloride, butylated hydroxy toluene, L-ascorbic acid, 2-thiobarbituric acid, 7,12-dimethylbenz[a]anthracene (DMBA) were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

Statistical analysis

Bodyweights, weights of mammary glands, mean arterial pressures and heart rates among the three dietary groups are reported as dietary group mean ± s.e.m. Basal oxidation levels (BHT arrested at time zero), auto-oxidation and iron-ascorbate catalyzed lipid peroxidation in the three dietary regimens were analysed by one-way ANOVA. Whenever *F*-ratios significant at 5% or less were obtained with the ANOVA, Fisher's least significant difference (LSD) procedure was applied to determine the significance of differences between specific groups. The criterion for significance in each comparison was $P \leq 0.05$, with 2/18 degrees of freedom.

RESULTS

Throughout the study period weekly physical examinations detected no palpable mammary tumours. At necropsy, no mammary tumours were found in any of the three dietary groups. Histopathological examinations confirmed no tumours or other abnormalities in the mammary glands of the three dietary groups.

Bodyweights at the end of study, feed efficiency, mean arterial pressures and heart rates are tabulated in Table 3. Data are expressed as mean ± s.e.m. Bodyweights of rats in the MO group were significantly higher than those of the AIN and CO groups at $P \leq 0.05$. Feed efficiency was calculated as reciprocal of total kilocalories consumed throughout the study divided by average bodyweight gain in g. In the AIN-76 group, feed efficiency was found to be 1.008 g/kCal whereas in the CO group it was 1.0 g/kCal. In the MO group, feed efficiency was found to be 1.267 g/kCal. This means that the kCal required to increase

Table 3. Effect of dietary fat type on bodyweight, feed efficiency, mean arterial pressure and heart rate in female SHR

Dietary group	Bodyweights (g)	Feed efficiency† (g/kCal)	Mean arterial pressure (mmHg)	Heart rate (beats/min)
Control (AIN)	240.57 ± 5.47	1.008	194.00 ± 5.89	392.60 ± 7.62
ω-3 fatty acid (MO)	273.86 ± 8.28*	1.267*	186.67 ± 7.04	409.67 ± 18.82
ω-6 fatty acid (CO)	242.43 ± 5.17	0.992	190.29 ± 8.61	416.86 ± 17.03

All data are expressed as mean ± s.e.m.
*Indicates MO *vs* AIN and CO; $P \leq 0.05$; degree of freedom = 2/18.
†Calculated as feed efficiency = 100/(Average kcal consumed throughout study/average bodyweight gain in g).

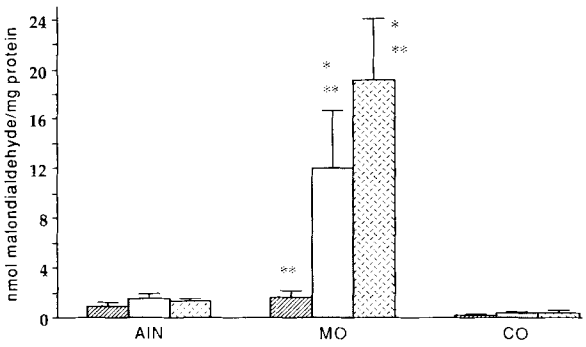

Fig. 1. *In vitro* lipid peroxidation of mammary homogenates from SHR. ▨ = butylated hydroxytoluene (BHT) arrested at time ○; ▩ = No iron but BHT arrested; □ = with iron and BHT arrested. Data from the TBARS assay are expressed as mean nmol malondialdehyde/mg protein ± s.e.m. (n = 7 in each dietary group). AIN = 5% corn oil, low-fat diet; MO = 19% Menhaden oil + 1% corn oil; CO = 20% corn oil. *Indicates $P \leq 0.05$ *vs* AIN; **indicates $P \leq 0.05$ *vs* CO.

weight (feed efficiency) were significantly lower in the MO group and indicates that feed efficiency was greatest in the MO group. There was no significant difference among the dietary groups in either blood pressure or heart rate.

Results of the *in vitro* lipid peroxidation experiment are shown in Fig. 1. In whole homogenates of mammary glands, basal oxidation (BHT arrested at time 0) in the MO group (675%) was significantly higher than the basal oxidation level of the CO group ($P \leq 0.05$) but was not significantly different from the AIN group. *In vitro* lipid auto-oxidation was significantly higher in the MO group compared with the AIN and CO groups (760% and 2624% increases, respectively). Iron-ascorbate catalyzed lipid peroxidation was also significantly higher in the MO group compared to the AIN and CO groups (1436 and 4244% increases, respectively). Per cent increases in lipid peroxidation catalyzed with the iron-ascorbate free radical generating system over the basal oxidation levels (BHT arrested at time 0) were 142% in the AIN group, 184% in the CO group and 1178% in the MO group. In the AIN and CO diets there were no significant differences between their respective basal, spontaneous or iron-ascorbate catalyzed lipid peroxidations. There were also no significant differences between the basal, spontaneous and iron-ascorbate catalyzed peroxidations within either the AIN or the CO group. In the MO group, however, *in vitro* lipid auto-oxidation was significantly higher than basal oxidation, and iron-ascorbate catalyzed oxidation was significantly higher than the extent of spontaneous auto-oxidation.

DISCUSSION

The female SHR was used as the model in this study because human females with major risks for developing cardiovascular disease may try to alter their previous diets to decrease chances of a major cardiovascular health problem. Current recommendations are to lower dietary fat while increasing the ratio of unsaturated fats to saturated fat in the diet (National Academy of Science Report 1989). Since it is easier for most patients to comply with the latter than the former, dietary shifts often result in initiation of high PUFA/high fat diets. A typical American diet is 20% fat, resulting in 40% caloric intake from fat. These high PUFA diets are known to produce other health risks, for example, the promotion of mammary tumours (Bunce & Abou El-Ela 1990; Welsch *et al.* 1990). In this study, we found that feeding of high PUFA diets for 16 weeks did not decrease blood pressure or heart rate compared with a low fat diet. We also found that the high fish oil diet profoundly increased both enzymatic and non-enzymatic catalyzed lipid peroxidation in mammary gland compared with either high ω-6 (CO) or low-fat (AIN) diets, but no dietary regimen promoted mammary tumours in the DMBA-pretreated SHR.

The failure of DMBA to produce tumours in the

SHR model, particularly in the presence of high fat, high calorie diets was not anticipated. In previous experiments our laboratory showed that in the female Sprague-Dawley rat model, the same dosage from the same lot of DMBA initiates a high incidence of tumours which are promoted by similar high PUFA diets during even shorter feeding spans (Abou El-Ela *et al.* 1987; Mehta *et al.* 1993). Thus, the SHR strain appears to be resistant to mammary tumourigenesis under the same experimental conditions in which the Sprague-Dawley strain is very susceptible. Although the decreased susceptibility of the SHR strain has not previously been reported, others have found major genetic variations in susceptibility to DMBA in rats of different strains (Gould & Zang 1991). For example, Copenhagen rats are highly resistant to both spontaneous and induced mammary carcinogenesis, probably due to the presence of a homozygous autosomal dominant mammary carcinoma suppressor (MCS) gene (Wang *et al.* 1991). The Wistar-Kyoto (WKY) strain is also resistant to DMBA-induced mammary carcinoma, while the Wistar-Furth strain is extremely susceptible (Haag *et al.* 1992). The resistance in WKY rats may also be due to the presence of an MCS gene (Haag *et al.* 1992).

When we chose this model, we hypothesized that the SHR strain, although derived from the same original colony of rats as the WKY may have segregated genetically from the WKY in regard to its DMBA sensitivity, just as this strain has obviously segregated from the WKY strain for the genes programming hypertension (Matsumoto *et al.* 1991). We based this hypothesis on three independent studies by others showing that rats of the SHR strain show significantly higher frequencies of chromosomal aberrations in bone marrow cells 12 and 18 h after DMBA treatment compared to WKY (Ueda & Kondo 1984). This bone marrow model is used as an index of DMBA's mutagenic effects. In another study, the SHR strain showed a higher incidence of 3-methylcholanthrene-induced fibrosarcomas compared to control rats (Takeichi 1982). The SHR strain is also extremely susceptible to *N*-2-fluorenylacetamide-induced hepatocarcinogenesis (Ohmori *et al.* 1983). Our DMBA mammary tumour initiation protocol followed by promotion with high fat diets failed to induce any mammary carcinomas in SHR. The possibilities therefore exist that the SHR strain may: (i) possess mammary tumour suppressor genes similar to the WKY strain or (ii) may have some other mechanism of resistance to this dose of DMBA. In summary, the inability of this dose of DMBA to initiate mammary tumourigenesis indicates that the SHR may be another useful strain to study for factors influencing resistance to mammary tumourigenesis.

Another goal of the study was to determine the influence of high PUFA diets on the development of hypertension in female SHR. We found that even after 16 weeks of feeding high ω-3 or high ω-6 diets, blood pressures in both the CO and MO groups were similar to the low-fat AIN group. All rats were very hypertensive and there were also no significant differences in heart rate among three dietary groups. Several reports in the literature have shown that both ω-3 and ω-6 fatty acid-supplemented diets can reduce blood pressure in male SHR. In male SHR, a fish oil diet (18% menhaden oil) was significantly more efficient in attenuating blood pressure development than a corn oil (18%) diet (Karanja *et al.* 1989). Yin *et al.* (1990) have also shown that mean systolic blood pressure in fish oil-fed male SHR was significantly lower than a saturated fat diet. In another study (Bayorh *et al.* 1989) chronic administration of eicosapentaenoic acid subcutaneously for 8 weeks lowered blood pressure in SHR significantly compared to WKY rats. In male SHR, some find that blood pressure decreases after supplementation with fish oil (high ω-3; Hoffmann *et al.* 1986; Von Au *et al.* 1988; Brandle & Jacob 1990), dietary borage oil (Engler *et al.* 1992; Engler 1993) or corn oil (high ω-6; Mtabaji *et al.* 1988).

While it may first appear that our results in female SHR are at variance with the reported antihypertensive effects in male SHR because of the difference in gender, the literature is replete with examples of no effects of high ω-3 or high ω-6 diets found on blood pressure in male rats. McGreger *et al.* (1981) have shown a high ω-6 fatty acid diet on blood pressure in male SHR to have no effect. Some studies, however, show that linoleic acid (ω-6) in the diet prevents salt-induced hypertension in male Wistar rats (Ten-Hoor & Graaf 1978; McDonald *et al.* 1981; Hoffmann *et al.* 1982). Others have found that high ω-3 fatty acids or eicosapentaenoic acid actually cause an increase in blood pressure in salt loaded SHR (Codde *et al.* 1987). Watanabe *et al.* (1989) found that high ω-6 or high ω-3 diets had no effect on the development of hypertension in male SHR. Kingsley and Snyder (1988) found that neither the degree of hypertension nor the plasma lipid profile varied in SHR fed ω-3 or ω-6 diets for 8 months. Croft *et al.* (1988) found a pressor effect of fish oil supplementation during the first week if the animals were salt-loaded. Thereafter, fish oil did not significantly affect blood pressure but thromboxane (a vasoconstrictor) generation was significantly depressed. The literature is clearly not in agreement concerning the effects of either high ω-6 or high ω-3 fatty acid diets on blood pressure. This is not surprising considering the complexity of blood pressure regulation and the multiple systems affected by dietary fat type and level. Gender difference is there-

fore not a logical reason for the absence of an antihypertensive effect in these studies.

A more likely reason for differences between this study and some of the previous investigations that found antihypertensive effects in male SHR is the method of blood pressure recording. The studies in male SHR cited above each employed the indirect tail-cuff method (either plethysmographic or photoelectric) to assess blood pressures (Codde *et al.* 1987; Mtabaji *et al.* 1988; Von Au *et al.* 1988; Brandle & Jacob 1990; Yin *et al.* 1990; Engler *et al.* 1992). That method for systolic blood pressure readings is the most useful and practical method to assess blood pressure in multiple readings over long periods of time because of the difficulties in maintaining patency of chronic indwelling catheters in rats. The tail-cuff method is most successfully performed on restrained (therefore stressed) rats, in a warm environment and sometimes under conditions of light ether anaesthesia. The method has a high degree of error, especially when smaller changes in blood pressure are compared (Bunag & Teravainen 1991). It is possible to get significantly different pressures by the tail-cuff than are obtained by direct arterial blood pressures taken from the same rats (Bunag & Teravainen 1991). The method is also subject to unintentional bias since it involves averaging several representative recordings without benefit of having continuous mean arterial pressure or double-blind analysis. In comparison, direct arterial pressure measurements have a lower degree of experimental error and can be performed in conscious, unrestrained rats. Any effects of diet on thermoregulation, restraint stress or tonus regulation of the caudal artery are therefore eliminated.

The final goal of the present study was to determine whether the high PUFA dietary regimens affected oxidative processes in the mammary gland. We found a major effect of MO on lipid peroxidation in mammary gland that suggests an increased risk/benefit ratio of high ω-3 diets in the SHR. Membrane PUFA are highly susceptible to lipid peroxidation which can occur via both enzymatic and non-enzymatic mechanisms. Enzymatic lipid peroxidation is catalyzed by multiple enzyme systems *in vivo*, for example, lipoxygenase, cyclo-oxygenase. Free ions of transition metals such as iron and copper are known to catalyze the formation of oxygen free radicals and stimulate the non-enzymatic peroxidation of lipids. Under normal circumstances, most iron is bound in transport form (transferrin) or storage form (ferritin; Sevanian & Hochstein 1985), but decompartmentalization of iron occurs in the body. Accelerated rates of stored iron release are associated with certain toxicity states, for example, from hepatocytes in hepatitis; from erythrocytes in haemolytic diseases (Weinberg 1993); from ferritin by agents such as ascorbate (Gutteridge *et al.* 1983); paraquat, adriamycin and alloxan (Reif 1992) and also by constituents of cigarette smoke (Moreno *et al.* 1992). Even sequestered iron in ferritin (Koster & Slee 1986) and transferrin (Britigan & Edekar 1991) can enhance lipid peroxidation in the presence of reducing agents such as ascorbate and glutathione (Dix & Akiens 1993). It has been suggested that the iron-haeme complex of haemoglobin is a Fenton reagent that may accelerate free radical production in the vascular endothelium (Motterlini & McDonald 1993). Ascorbic acid and iron are important in the mediation of non-enzymatic lipid peroxidation in various tissues. Ascorbic acid reduces ferric ions to ferrous which can then catalyze the oxidative breakdown of PUFA (Das 1989). Iron-ascorbate catalyzed lipid peroxidation *in vitro* is therefore a useful index of the susceptibility of biomembranes to oxidative free radical damage (Kehrer 1993).

Although both corn oil and menhaden oil are composed of oxidizable PUFA, our results indicate that susceptibility of mammary homogenates to peroxidation was significantly increased by the menhaden oil diet (high ω-3) but not by the high corn oil diet (high ω-6). The lack of an increase in the 20% corn oil group is in agreement with the work of others who find that 20% corn oil diets do not increase lipid peroxidation in microsomal–mitochondrial membranes of normal mouse mammary tissue as indicated by either levels of conjugated dienes or TBARS (Lane *et al.* 1985). It is also known that high ω-3 fatty acid diets are more oxidizable than high ω-6 diets. Haegele *et al.* (1994) showed that a diet containing 24.6% w/w corn oil has an iodine value of 126, whereas if 19.9% of that diet is replaced with menhaden oil, the iodine value increases to 176. The iodine value is a measure of the degree of fatty acid unsaturation in the diet oils. These workers also find a positive correlation between the iodine value and production of 8-hydroxyguanine, a marker for the extent of oxidative DNA damage in mammary glands. The concentration of 8-hydroxy-2′-deoxyguanosine increases in the mammary gland of Sprague-Dawley rats as the fatty acid unsaturation in the diet increases (Haegele *et al.* 1994). High ω-3 diets have also been shown to increase lipid peroxidation of liver and kidney homogenates (Hu *et al.* 1989; Leibovitz *et al.* 1990), heart homogenates (Donzel *et al.* 1993) and liver and kidney slices (Hu *et al.* 1989; Leibovitz *et al.* 1990). Thus, high ω-3 PUFA diet may significantly increase susceptibility to oxidative aging processes in the mammary gland.

In summary, our results indicated that high ω-3 fatty acid diets markedly increase the susceptibility of the mammary gland to lipid peroxidative stress in the SHR. A high ω-6 diet did not similarly increase

susceptibility of the mammary gland to lipid peroxidation. We found the SHR strain to be resistant to DMBA mammary tumourigenesis under conditions that initiate and promote a high incidence of tumours in Sprague-Dawley rats. More studies are necessary to determine the *in vivo* consequences within the mammary gland of long-term high ω-3 PUFA diet. The results suggest that any oxidative stressor that can initiate lipid peroxidation has the capability of producing greater mammary gland damage in rats predisposed with a high ω-3 fatty acid diet.

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