Dietary fats and cholesterol supplementation effects on aortic and lipid response in rats

Mariane Lutz, Jenny Cortez, and Raúl Vinet

Escuela de Química y Farmacia, Universidad de Valparaíso, Valparaíso, Chile

The experiment was designed to elucidate the effects of feeding four dietary oils (corn, hazelnut, olive, and fish), and cholesterol supplementation on plasma, liver lipids, and aortic smooth muscle response to drugs. Male Sprague Dawley rats were fed semipurified diets containing one of the above oils (15% wt/wt), either with or without cholesterol supplementation (1% wt/wt), for 20 days. Hazelnut oil-fed rats showed the highest plasma total cholesterol level, while animals fed fish oil exhibited the lowest plasma total and high density lipoprotein cholesterol concentrations. Hepatic cholesterol content was not affected by dietary oils. Liver lipids increased when dietary cholesterol was added to any of the oils used. Acetylcholine pD_2 was elevated in fish oil- and hazelnut oil-fed rats, but rats fed all dietary oils showed maximal relaxation. Cholesterol supplementation reduced aortic maximal relaxation caused by acetylcholine. These results indicate that the type of dietary oil and cholesterol intake differentially raise plasma and liver lipid levels and modulate aortic smooth muscle response in the rat.
(J. Nutr. Biochem. 5:446–450, 1994.)

Keywords: dietary oils; cholesterol; lipids; aorta response; rats

Introduction

Dietary fats and endogenous lipids produced by endothelial cells play central roles in the regulation of vascular tone.[1] It has been shown that dietary lipids affect the response of vascular smooth muscle.[2,3] Thus they may contribute to the prevention or development of various diseases.

Neurohumoral agents and mechanical factors stimulate vascular endothelial cells to release vasoactive substances that modify vessel tone. Among them are prostacyclin and nitric oxide (NO), known as endothelium-derived relaxing factor (EDRF), the major physiological regulator of basal blood vessel tone,[4–6] and contracting factors.[7]

The contractile response of aortic smooth muscle is related to the development of cardiovascular disease, affecting thrombus formation and atherogenesis.[8,9] Vascular endothelial cells release EDRF after stimulation by a variety of substances, including polyunsaturated fatty acids (PUFA). It has been demonstrated that PUFA are readily taken up and incorporated into endothelial lipids, where they may be converted to active metabolites.[10] As a result, EDRF relaxes smooth vascular muscle and inhibits platelet aggregation and adhesion.

Several studies show that hypercholesterolemia alters vascular reactivity. This altered response is mediated by an endothelium-dependent relaxation induced by a variety of vasodilator agents, including acethylcholine (ACh), and enhanced contractile response to agents such as norepinephrine, serotonin, or K+ depolarization.[11–13]

Atherosclerosis impairs endothelium-dependent vasodilation, contributing to vasoconstriction and arterial spasm, which may be related to decreased EDRF-NO production or release, among other mechanisms.[12–15] In nutritionally induced atherosclerosis, endothelium-dependent relaxation is markedly reduced, while lyso phosphatidylcholine (LPC) is significantly increased, in the aorta.[16] It has been shown that oxidized low density lipoprotein (ox-LDL) inhibits endothelium-dependent arterial relaxation, with LPC being the main substance produced during oxidative modification, which inhibits the production and/or release of EDRF-NO and promotes the inactivation of EDRF after its release.[17]

Taking into consideration that dietary fats may modulate the response of vascular smooth muscle, the present study was undertaken to examine the effects of the intake of four

This work was supported by the Dirección de Investigación, U. de Valparaíso (Proy. 13/91) and FONDECYT (Proy. 430/92).
Present address for Dr. Vinet is Laboratory of Cell Biology and Genetics, NIDDK, National Institutes of Health, Bethesda, MD USA.
Address reprint requests to Dr. Mariane Lutz at the Escuela de Química y Farmacia, Universidad de Valparaíso, Casilla 92-V, Valparaíso, Chile.
Received October 21, 1993; accepted February 10, 1994.

dietary oils that supply different amounts of fatty acids, on the reactivity of aortic ring segments in the rat. We further examined whether these parameters were affected by cholesterol supplementation. The comparison of all dietary treatments can provide useful information on possible changes of aorta response due to the nature of the fat.

Methods and materials

Animals and diets

Male Sprague Dawley rats, 90 to 110 g initial body weight, (Universidad de Valparaiso Breeding Unit, Valparaiso, Chile) were housed individually in wire-mesh cages in a room regulated for temperature (21 ± 2° C), humidity (45 to 50%), and light/dark cycles (12 hr). Animals were randomly fed, ad libitum, for 20 days, one of four semi-synthetic diets containing 15% (wt/wt) olive oil (OO, Cánepa, Valparaíso, Chile), corn oil (CO, Mazola, Llay-Llay, Chile), hazelnut oil (HO, ACENAT, Santiago, Chile), or fish oil (FO, Pesquera Quintero, Quintero, Chile) as the only lipid source, with or without cholesterol (C) supplementation (1%, wt/wt). The other ingredients of the diets were (wt/wt): casein (20%), dl-methionine (0.3%), mineral mix[18] (4%), vitamin mix[18] (1%), corn starch (20%), cellulose (5%), and sucrose (34.7%) (non-supplemented diet); or sucrose (33.7%) and cholesterol (1%) (supplemented diet). The treatment groups were as follows: CO, CO + C, HO, HO + C, OO, OO + C, FO, and FO + C. The fatty acid composition of dietary oils is shown in *Table 1*. Methylesters of fatty acids[19] were quantified by capillary gas-liquid chromatography in a Hewlett-Packard 5890 Series II model (Palo Alto, CA, USA) equipped with a 25-m HP-FFAP column (0.2 mm I.D. coated with polyethyleneglycol-TPA film), a temperature program, a flame ionization detector, and a model 3396 Series II integrator.

Methods of analysis

After an overnight fast, eight animals per group were exsanguinated from the abdominal aorta under ethylether anesthesia and the liver was removed, washed with cold saline, excised, blotted, weighed, and frozen. Plasma and liver lipid concentrations were determined spectrophotometrically using commercial kits (E. Merck, Darmstadt, Germany) as previously described.[20] Another six animals per group were killed by a blow to the head, and two ring segments of the thoracic aorta (5 mm) from each rat were immediatly mounted on 30-mL organ baths simultaneously as described elsewhere.[17,18] Vascular response was measured on ring strips of aorta by maximal tension (T_{max}) developed isometrically with KCl (70mM) and phenylephrine (Phen) (10^{-7}M), and maximal relaxation (R_{max}) obtained with acetylcholine (ACh) (10^{-5}M).[21,22] In every ACh dose-response curve the dose producing 50% of the R_{max} (ED_{50}) was calculated according to the method of Fleming et al.[23] and was expressed as the percentage of T_{max} obtained by Phen (10^{-7}M) in each ring or pD_2. All drugs were purchased from Sigma Chemicals Co. (St. Louis, MO USA)

Table 1 Fatty acid relative composition of dietary oils

Fatty acid	Olive oil	Corn oil	Hazelnut oil	Fish oil
		(g/100 g)		
14:0	—	—	—	9.1
16:0	12.6	10.4	1.9	26.1
16:1	0.9	—	20.2	10.7
17:0	—	—	—	1.1
18:0	1.7	2.1	0.8	4.8
18:1	74.7	28.8	39.4	19.2
18:2	10.0	52.2	6.9	2.7
18:3	0.3	2.8	2.7	0.3
18:4	—	—	—	3.8
20:0	—	—	1.6	—
20:1	—	1.8	10.5	1.4
22:0	—	2.1	3.3	—
22:1	—	—	11.7	—
20:4 n-6	—	—	—	1.1
20:5 n-3	—	—	—	13.8
22:5 n-3	—	—	—	1.0
22:6 n-3	—	—	—	3.6

Table 2 Food intake, weight gain, and relative liver weight of rats

Dietary fat	Food intake	Weight gain	Relative liver weight
	(g/day)	(g/day)	(g/100 g body wt)
OO	18.0 ± 0.5	6.2 ± 0.2	3.5 ± 0.1
OO+C	19.4 ± 0.9	6.5 ± 0.4	4.2 ± 0.2
CO	16.1 ± 0.6	7.4 ± 0.3	3.6 ± 0.1
CO+C	16.6 ± 0.4	7.3 ± 0.3	4.5 ± 0.1
HO	18.0 ± 0.6	6.9 ± 0.4	3.8 ± 0.2
HO+C	18.6 ± 0.5	7.7 ± 0.4	4.2 ± 0.1
FO	16.7 ± 0.6	6.9 ± 0.3	4.0 ± 0.1
FO+C	15.7 ± 0.6	6.3 ± 0.4	4.4 ± 0.1

Results are mean ± SEM (n = 14). OO, olive oil; CO, corn oil; HO, hazelnut oil; FO, fish oil; C, cholesterol. There was no significant difference in values from rats fed different oils.

Effect of cholesterol supplementation (Student's t test):			
OO/OO+C	NS	NS	$P < 0.001$
CO/CO+C	NS	NS	$P < 0.001$
HO/HO+C	NS	NS	NS
FO/FO+C	NS	NS	NS

Statistics

The data are reported as mean ± SEM. The significance of differences among the various groups studied was calculated using one-way analysis of variance (ANOVA) and Tukey test for multiple comparisons. Values assigned a different superscript letter were significantly different at $P < 0.05$. The values of cholesterol supplemented and nonsupplemented diets were compared using Student's t test.

Results

As shown in *Table 2*, all groups of rats consumed similar amounts of food and there was no significant difference in weight gain among the groups during the 20-day feeding period, although animals fed OO grew at a lower daily rate. In spite of the different trends observed in dietary intake and growth rate, the final body weight of all animals was similar, and none of these parameters was affected by cholesterol supplementation. The type of dietary oil did not affect the percentage of relative body weight of liver (g/100 g body wt). On the other hand, the enrichment of diets with cholesterol elevated liver relative weight in all groups, reaching statistical significance in OO+C and CO+C fed animals. The highest increase in relative liver weight was observed in CO+C fed group.

Table 3 summarizes the concentrations of plasma lipids. Feeding the FO diet significantly reduced plasma total and HDL cholesterol. Total plasma cholesterol level was the lowest in the marine oil-fed group, even after dietary choles-

terol supplementation. On the contrary, the highest plasma cholesterol level was observed in rats fed the HO diet, with and without the addition of cholesterol. There was no difference in the concentration of plasma triglycerides among the groups.

Figure 1 shows the hepatic levels of cholesterol. The concentration of this lipid was similar in the liver of all groups fed non-supplemented diets and increased with cholesterol intake in animals fed vegetable oils. This effect of high vegetable fat diets containing cholesterol is in agreement with previous findings.[20]

The results of the vascular smooth muscle reactivity parameters are shown in *Table 4*. The maximal tension (T_{max}) of aortic ring segments after KCl and Phen-induced contraction was similar in all experimental groups, although animals fed FO did not exhibit significantly lower values. There was a 100% relaxation response (R_{max}) in arteries from animals fed all dietary oils. However, pD_2 was higher in rats fed FO or HO compared with rats fed CO or OO. Dietary cholesterol supplementation decreased R_{max} in animals fed OO + C or CO + C versus the respective non-supplemented groups ($P < 0.05$). Rats fed OO + C also showed an increase of pD_2 compared with the group fed OO ($P < 0.05$).

Discussion

Although the rat is resistant to the development of experimental atherosclerosis, it is a suitable animal model to evaluate the effects of nutrition on the action of pharmacological agents on vascular preparations. In a recent study of the concentration-response curves for n-3, n-6, and n-9 fatty acids on precontracted aortic rings, it was proposed that the fatty acid-induced relaxation of the rat aorta is specific to long chain PUFA of the n-3 and n-6 families.[24] In the current communication, the comparative effect of the dietary intake

Table 3 Plasma lipid concentrations in rats

Diet	Total Cholesterol	HDL	Triacylglycerides
	(mmol/L)		
OO	2.34 ± 0.09[a]	1.93 ± 0.09[a]	1.12 ± 0.16
OO + C	1.84 ± 0.18	1.39 ± 0.12	0.83 ± 0.14
CO	2.46 ± 0.10[a]	1.99 ± 0.11[a]	1.22 ± 0.12
CO + C	2.20 ± 0.16	1.36 ± 0.05	1.14 ± 0.12
HO	3.41 ± 0.32[b]	2.00 ± 0.20[a]	1.40 ± 0.22
HO + C	2.71 ± 0.12	2.33 ± 0.12	1.28 ± 0.09
FO	1.27 ± 0.10[c]	1.11 ± 0.08[c]	1.14 ± 0.17
FO + C	1.61 ± 0.17	1.46 ± 0.14	0.91 ± 0.13

Results are mean ± SEM (n = 8). OO, olive oil; CO, corn oil; HO, hazelnut oil; SO, sardine oil; C, cholesterol. Values assigned different superscript letters were significantly different according to ANOVA and Tukey's test; $P < 0.001$.

Effect of cholesterol supplementation (Student's t test):

OO/OO + C	$P < 0.01$	$P < 0.01$	NS
CO/CO + C	NS	$P < 0.001$	NS
HO/HO + C	NS	NS	NS
FO/FO + C	NS	$P < 0.05$	NS

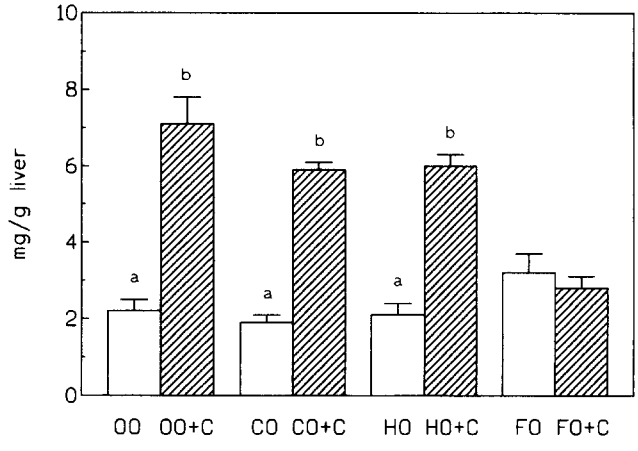

Figure 1 Liver cholesterol level of rats at the end of the 20-day feeding trial (mean ± SEM, n = 8). OO, olive oil; CO, corn oil; HO, hazelnut oil; FO, fish oil; C, cholesterol supplementation. Columns not sharing a common letter differ ($P < 0.001$).

Table 4 Aortic ring segments reactivity parameters

Dietary fat	T_{max} KCl	T_{max} Phen	R_{max}	pD_2
	(mg tension/mg tissue)		(%)	
OO	743.0 ± 59.0	470.8 ± 53.5	100.4 ± 0.7	7.74 ± 0.08[a]
OO + C	667.8 ± 18.0	362.6 ± 42.2	95.5 ± 1.7	8.05 ± 0.11
CO	759.9 ± 52.4	360.3 ± 44.1	100.1 ± 1.2	7.63 ± 0.06[a]
CO + C	840.6 ± 19.8	336.9 ± 50.5	89.3 ± 4.3	7.58 ± 0.08
HO	944.7 ± 108.0	370.0 ± 72.7	100.2 ± 0.9	7.99 ± 0.11[b]
HO + C	826.4 ± 53.4	244.2 ± 22.9	99.9 ± 1.0	7.98 ± 0.14
FO	692.0 ± 45.5	297.4 ± 52.9	100.7 ± 1.0	8.07 ± 0.07[b]
FO + C	686.9 ± 35.7	341.6 ± 43.5	99.9 ± 1.1	7.85 ± 0.07

Results are mean ± SEM (n = 6). OO, olive oil; CO, corn oil; HO, hazelnut oil; FO, fish oil; C, cholesterol. Values assigned different superscript letters were significantly different according to ANOVA and Tukey's test; $P < 0.01$.

Effect of cholesterol supplementation (Student's t test):

OO/OO + C	NS	NS	$P < 0.05$	$P < 0.05$
CO/CO + C	NS	NS	$P < 0.05$	NS
HO/HO + C	NS	NS	NS	NS
FO/FO + C	NS	NS	NS	NS

(for a 20-day period) of four dietary oils with different relative fatty acid composition on vascular reactivity parameters is described.

The supply of fats to the endothelial cell is dependent on the circulating lipids, thus it is of interest to determine the plasma levels of lipids. Oleic acid has been described as hypocholesterolemic in a similar way to PUFA such as linoleic and α-linolenic acids.[25] In the current communication, OO intake exhibited an equal effect on plasma cholesterol levels to CO, rich in linoleic acid. On the other hand, HO is a source of various species of monounsaturated fatty acids (MUFA) besides oleic acid, which could explain why rats fed this oil exhibited significantly higher plasma cholesterol levels than all other experimental groups. FO, which is a source of long chain n-3 PUFA, lowered plasma cholesterol levels as expected[20] *(Table 3)*. The addition of cholesterol to the experimental diets lowered plasma cholesterol levels in vegetable oil-fed animals, reaching statistical significance in the OO + C group. These findings are similar to previous observations and correlate well with the accumulation of cholesterol in the liver.[20,21]

Our results indicate that, in spite of the different dietary supply of fatty acids, maximal tension (T_{max}) and relaxation (R_{max}) of aortic ring segments submitted to the same experimental conditions were not affected by the quality of dietary fat. However, pD_2 was significantly higher in the aorta of rats fed FO. This is attributed to the high content of n-3 PUFA in the marine oil,[21] which is in agreement with the enhanced relaxation described in coronary arteries of animals fed diets rich in these fatty acids.[2,3] On the other hand, the higher pD_2 value obtained after HO feeding was an unexpected finding because this oil is very rich in MUFA that would not exert a direct effect on vascular reactivity,[10] as observed after olive oil intake. Taking into consideration that the relaxation induced by ACh is fully dependent on the endothelial activity, pD_2 is not exclusively related with EDRF (NO) activity but is also dependent on the interaction of ACh with its receptors in the endothelial cell, among other factors. Thus, the intake of high amounts of different MUFA could have caused a modification of the membrane proteins associated with the drug-receptor interaction.

Mixed diets are not only a source of fatty acids but contain cholesterol as well. In rabbits, a short-term high cholesterol diet leads to structural and functional vessel wall changes related to high serum cholesterol levels, accompanied by increased reactivity to noradrenaline.[26] The endothelium-dependent relaxation to ACh is impaired in the thoracic aortas from rats with induced atherosclerosis, while cholesterol feeding alone does not impair the relaxing response to ACh, indicating that the impairment is not due to hypercholesterolemia per se.[27] In rabbit aortas, it has been shown that atherosclerosis induces higher production of prostacyclin and EDRF (NO), although its activity may be decreased.[28] In the present communication, it is noteworthy that the highest plasma cholesterol level was observed in rats fed HO, and it was not related to a measurable impairment of aortic relaxation. On the contrary, aortic ring segments of animals fed HO and FO exhibited similar pD_2 values, while the plasma cholesterol levels differed significantly between these two groups. Aortic ring segments from rats fed OO + C or CO + C diets had lower maximal relaxation (R_{max}) to ACh than the respective non-supplemented counterparts, indicating that cholesterol intake affected this response in a manner dependent on the dietary fat intake as a whole.

The present findings indicate that dietary fats (type of oils and cholesterol intake) influence the response of aortic smooth muscle to certain agents, participating in blood vessel changes that may play important roles in the pathogenesis and/or progression of atherosclerotic lesion and vasospasm.

References

1 Chinellato, A., Bachieri, N., Pandolfo, L., Ragazzi, E., Froldi, G., Norido, F., Caparrota, L., and Fassina, G. (1991). Aortic response to relaxing agents in Watanabe heritable hyperlipidemic (WHHL) rabbits of different age. *Atherosclerosis* **89,** 223–230

2 Shimokawa, H. and Vanhoutte, P.M. (1988). Dietary cod liver oil improves endothelium-dependent responses in hypercholesterolemic and atherosclerotic porcine coronary arteries. *Circ. Res.* **78,** 1421–1430

3 Shimokawa, H. and Vanhoutte, P.M. (1989). Dietary w-3 fatty acids and endothelium-dependent relaxation in porcine coronary arteries. *Am. J. Physiol.* **256,** H968–H973

4 Moncada, S., Palmer, R.M.J., and Higgs, E.A. (1991). Nitric oxide: physiology, pathophysiology, and pharmacology. *Pharmacol. Rev.* **43,** 109–142

5 Vane, J.R., Anggard, E.E., and Botting, R.M. (1990). Regulatory functions of the vascular endothelium. *N. Engl. J. Med.* **323,** 27–36

6 Dinerman, J.L., Lowenstein, C.J., and Snyder, S.H. (1993). Molecular mechanisms of nitric oxide regulation. Potential relevance to cardiovascular disease. *Circ. Res.* **73,** 217–222

7 Gryglewski, R.J., Botting, R.M., and Vane, J.R. (1988). Mediators produced by the endothelial cell. *Hypertension* **12,** 530–548

8 Simionescu, M. and Simionescu, N. (1986). Functions of the endothelial cell surface. *Ann. Rev. Physiol.* **48,** 279–293

9 Moncada, S., Palmer, R.M., and Higgs, E.A. (1991). Relationship between prostacyclin and nitric oxide in the thrombotic process. *Thromb. Res. Suppl.* **11,** 3–13

10 Spector, A.A. and Moore, S.A. (1992). Effects of polyunsaturated fatty acids in endothelium. In *Endothelial Cell Dysfunctions,* (N. Simionescu and M. Simionescu eds.), p. 507–524, Plenum Press, New York, NY USA

11 Jayakody, R.L., Sernaratne, M.P.J., Thomson, A.B.R., and Kappagoda, C.T. (1985). Cholesterol feeding impairs endothelial-dependent relaxation of rabbit aorta. *Can. J. Physiol. Pharmacol.* **63,** 1206–1209

12 Forstermann, U., Mugge, A., Alheid, U., Haverich, A., and Frolich, J.C. (1988). Selective attenuation of endothelium-mediated vasodilation in atherosclerotic human coronary arteries. *Circ. Res.* **62,** 185–190

13 Galle, J., Busse, R., and Bassenge, E. (1991). Hypercholesterolemia and atherosclerosis change vascular reactivity in rabbits by different mechanisms. *Arterioscler. Thromb.* **11,** 1712–1718

14 Guerra, R., Jr., Brotherton, A.F.A., Goodwin, P.J., Clark, C.R., Armstrong, M.L., and Harrison, D.G. (1989). Mechanisms of abnormal endothelium-dependent responses in atherosclerosis. *Blood Vess.* **26,** 300–314

15 Lefer, A.M. and Sedar, A.W. (1991). Endothelial alteration in hypercholesterolaemia and atherosclerosis. *Pharmacol. Res.* **23,** 1–12

16 Inoue, N., Hirata, K., Yamada, M., Hamamori, Y., Matsuda, Y., Akita, H., and Yokoyama, M. (1992). Lysophosphatidylcholine inhibits bradikynin-induced phosphatidylinositide hydrolisis and calcium transients in cultured bovine aortic endothelial cells. *Circ. Res.* **71,** 1410–1421

17 Matsuda, Y., Hirata, K., Inoue, N., Suematsu, M., Kawashima, S., Akita, H., and Yokoyama, M. (1993). High density lipoprotein reverses inhibitory effect of oxidized low density lipoprotein on endothelium-dependent arterial relaxation. *Circ. Res.* **72,** 1103–1109

18 American Institute of Nutrition (1977). Report of the American Institute of Nutrition ad hoc Committee on Standards for Nutritional Studies. *J. Nutr.* **107,** 1340–1348

19 Morrison, W.R. and Smith, L.M. (1964). Preparation of fatty acid methylesters and dimethylacetals from lipids with boron fluoride methanol. *J. Lipid Res.* **5,** 600–608

20 Lutz, M., Ahumada, G., and Ruiz, G. (1993). Plasma lipids and histochemistry of liver in rats fed sea lion or corn oil, with or without cholesterol supplementation. *Fd. Chem. Toxicol.* **31,** 345–350

21 Lutz, M., Topasio, A., Acuña, P., and Vinet, R. (1993). Comparative effects of sardine and olive oils intake and cholesterol supplementation on serum, liver lipids and vascular smooth muscle reactivity in rats. In *Omega-3 Fatty Acids: Metabolism and Biological Effects,* (C.A. Drevon, I. Baksaas, and H.E. Krokan, eds), p. 183–190, Birkhauser, Verlag, Basel, Switzerland

22 Vinet, R., Brieva, C., Pinardi, G., and Penna, M. (1991). Influence of extracellular Ca++ on the modulation of alpha-adrenergic-induced contractions in rat aorta. *Gen. Pharmacol.* **22,** 365–369

23 Fleming, W.W., Westfall, D.P., de la Lande, I.S., and Jellet, L.B. (1972). Log normal distribution of equieffective doses of norepinephrine and acetylcholine in several tissues. *J. Pharmac. Exp. Ther.* **181,** 339–345

24 Engler, M.B. (1992). Effects of omega-3, omega-6 and omega-9 fatty acids on vascular smooth muscle tone. *Eur. J. Pharmacol.* **215,** 325–328

25 Chan, J.K., Bruce, V.M., and McDonald, B.E. (1991). Dietary α-linolenic acid is as effective as oleic acid and linoleic acid in lowering blood cholesterol in normolipidemic men. *Am. J. Clin. Nutr.* **53,** 1230–1234

26 Ludwig, M., Stumpe, K.O., Sauer, A., Kolloch, R., Goertz, U., and Vetter, H. (1991). Short-term dietary cholesterol in early life induces persistent increases in arterial wall thickness and vascular reactivity to noradrenaline. *J. Hyper.* **9,** S136–S137 (Suppl 6)

27 Kitagawa, S., Yamaguchi, Y., Kunitomo, M., Imaizumi, N., and Fujiwara, M. (1992). Impairment of endothelium-dependent relaxation in aorta from rats with arteriosclerosis induced by excess vitamin D and a high-cholesterol diet. *Jap. J. Pharmacol.* **59,** 339–347

28 Brunkwall, J., Mattsson, E., and Bergqvist, D. (1992). Diet-induced atherosclerosis in rabbits alters vascular prostacycin release. *Eicosanoids* **5,** 197–202