Effect of Dietary Fish Oil on the Rate of Very Low Density Lipoprotein Triacylglycerol Formation and on the Metabolism of Chylomicrons

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The mechanism by which ω3 fatty acids lower plasma triacylglycerol levels was investigated. Rats were fed fish oil, olive oil (10% fat by weight) or a nonpurified diet (4% fat by weight) for 15 days. Lipoprotein lipase was inhibited by intra-arterial administration of Triton WR 1339 to estimate hepatic triacylglycerol output. Rats fed the olive oil diet showed a higher rate of triacylglycerol formation than rats fed the ω3 fatty acid diet or the low-fat diet. All three groups showed identical rates of removal from plasma of intraarterially administered artificial chylomicrons that had simultaneously been labeled with cholesteryl [1-^{14}C]oleate and [9,10(n)-^{3}H]triolein. Liver radioactivity and total fat content were lowest in rats fed the fish oil diet, indicating that ω3 fatty acids were preferentially metabolized in liver. Chylomicrons obtained from donor rats fed either fish oil containing [^{14}C]cholesterol or olive oil containing [^{3}H]cholesterol were removed at similar rates when infused together intraarterially into recipient animals. A slower formation of plasma very low density lipoprotein triacylglycerols in rats fed fish oil is probably due to a faster rate of oxidation of the fatty acid chains in the liver resulting in decreased plasma triacylglycerol concentrations.
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The role that dietary ω3 eicosapentaenoic and docosahexaenoic acids of marine oils play in the prevention of coronary heart disease has been studied extensively (1,2). Fish oils are known to lower plasma triacylglycerol (TG) levels and very low density lipoprotein (VLDL) levels both in humans (3-5) and in experimental animals (6), although the mechanisms that are involved are not clearly understood.

Fish oil has been shown to retard *in vivo* hepatic production of VLDL triacylglycerol in humans (5,7-10) and in roosters (11), as well as in perfused liver (12,13) and in cultured hepatocytes (14-16). Less evidence is available in support of the enhancing effect of fish oil on the removal of plasma VLDL (7,9). Fish oil can also influence chylomicron metabolism, although similar intestinal absorption rates for eicosapentaenoic acid and oleic acid have been reported (17). However, the results of chylomicron metabolism studies are not entirely conclusive. When analyzed between 25 and 90 min, the disappearance rate of eicosapentaenoic acid enriched chylomicrons, that had been infused in the bloodstream of rats, was slower than that of oleic acid enriched particles. At 10 min, no difference in turnover rates was observed between the two types of chylomicrons (18). Chylomicron clearance rates in perfused rat hearts were similar for particles made from ω3 fatty acid with those made from oleic acid (19).

We set out to determine whether the fats affected metabolism because the particles differed in fatty acid composition or because the dietary fats by themselves modified lipoprotein lipase activity and the splanchnic organ receptors for chylomicron particles. In our study, native chylomicrons from animals fed fish oil and [^{14}C]cholesterol, or olive oil and [^{3}H]cholesterol, were injected into rats fed a nonpurified low-fat diet. Also, the plasma disappearance rates of intra-arterially infused artificial chylomicrons simultaneously labeled with cholesteryl [1-^{14}C]oleate and [9,10(n)-^{3}H]triolein were compared in rats fed fish oil, olive oil and low fat. Triacylglycerol mass and radioactivity were measured in the liver and spleen of the animals at the end of the artificial chylomicron infusion experiment. Finally, the effect of the different diets on hepatic secretion of triacylglycerol and on the metabolism of chylomicrons was examined after inhibition of the enzyme lipoprotein lipase by Triton WR 1339.

MATERIALS AND METHODS

Experimental protocol. Adult male Wistar rats, weighing approximately 230 g, were divided into three experimental groups of 10 animals each. The animals were fed for 15 days (% by weight) 10% fish oil, 10% olive oil, or a nonpurified diet containing 4% fat (Control, provided by Novilab, São Paulo, Brazil). Both fat enriched diets contained (% by weight): fat (10%), casein (10%), cellulose (3.5%), saline mixture (3.5%), vitamin supplement (1.0%), choline bitartrate (0.2%), D,L-methionine (0.3%), and starch (71.5%).

Body weights (mean ± SD) of rats in experimental groups (initial and final, respectively) were: controls (195 ± 19 and 244 ± 33), fish oil (250 ± 22 and 264 ± 28), and olive oil (235 ± 58 and 268 ± 38).

Commercially available fish oil (MaxEPA, Seven Seas Ltd., Marfleet, England) contained 18.6% eicosapentaenoic acid, 12.1% docosahexaenoic acid, 15.2% oleic acid, 22.95% saturated fatty acids and 31.3% unidentified unsaturated fatty acids. Olive oil was composed of 77.5% oleic, 7.6% linoleic, 2.3% stearic, 10% palmitic, and 0.6% unidentified fatty acids. After fasting overnight, rats were treated with Triton WR 1339 (Tyloxapol, Sigma Chemical Co., St. Louis, MO; 600 mg/kg body weight), which was administered through an intra-arterial catheter, to evaluate hepatic production of triacylglycerols (20).

Rats were infused intra-arterially with chylomicrons that had been simultaneously labeled with cholesteryl [1-^{14}C]oleate and [9,10(nn)-^{3}H]triolein (21,22) (NEN Research Products, Boston, MA). To follow the effect of various diets on the rate of chylomicron removal from plasma, a chylomicron-like emulsion was used which consisted of (% by weight) 2% cholesterol, 23% phosphatidyl-

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Abbreviations: EDTA, ethylenediaminetetraacetic acid; FCR, fractional catabolic rate; HDL, high density lipoproteins; LDL, low density lipoproteins; TG, triacylglycerol; VLDL, very low density lipoproteins.

choline (Lipid Products, Surrey, U.K.), 6% cholesteryl oleate and 69% triolein (NuChek Prep, Inc., Elysian, MN). Lipids were dispensed from stock solutions into vials and radioactive materials were added. After evaporation of solvents under a nitrogen stream, the vials were placed overnight in a vacuum desiccator at 4°C to remove residual solvent. Lipids were emulsified by sonication in 8 mL of 2.785 M NaCl solution (density = 1.100 g/mL), at approximately 55°C using a Branson Cell Disruptor (Branson Ultrasonics Corp., Danbury, CT) and a 1-cm probe at a continuous output of 70–80 W. Crude emulsions were purified by ultracentrifugation using an SW-41 rotor (Beckman ultracentrifuge, model L7-55, Beckman Instruments, Palo Alto, CA) at 12,000 rpm (density gradient 1.100–1.006) at 20°C for 15 min. After the first run, the coarse lipid that floated to the top was removed by aspiration and replaced with a corresponding volume of fresh 1.006 g/mL solution. The chylomicron-like emulsion was again ultracentrifuged at 36,000 rpm for 25 min. The emulsion particles floating to the top were then aspirated and used for the analyses and metabolic studies. The lipid composition of the emulsions was determined by standard procedures. Cholesterol was analyzed by the enzymatic Chod-Pap method (Boehringer Mannheim, Mannheim, Germany). Triacylglycerols were measured using the enzymatic Biodiagnostica kit (São Paulo, Brazil). Phospholipids were assayed by the Bartlett method (23). Chylomicron composition was (% by weight): cholesterol, 1.9%; phospholipids, 10.4%; cholesteryl ester, 11.2%; and triacylglycerol 76.5%.

Approximately 159 μg of chylomicron triacylglycerol was infused into each rat together with cholesteryl (1-^{14}C)oleate (0.34 μCi) and [9,10(n)-^{3}H]triolein (1.17 μCi). Blood samples were drawn sequentially, and 10 min later the liver and spleen were rapidly removed and analyzed for triacylglycerol total fat and radioactivity levels.

Native chylomicrons drawn from the pooled intestinal lymph of donor rats that had been fed fish oil and [^{14}C]-cholesterol, or olive oil and [^{3}H]cholesterol, were pooled and pulse infused intra-arterially into recipient rats fed the non-purified diet to measure the rates of disappearance of both chylomicron species from plasma. Each rat received 8.45 mg of chylomicron triacylglycerols from each dietary source containing ^{14}C (0.43 μCi) and ^{3}H (0.93 μCi). In these experiments adult male Wistar rats were subjected to intestinal lymph duct catheterization under pentobarbital anesthesia (5 mg/100 g of body weight) and restrained in Bollman-type metabolic cages. A single dose of fish oil or olive oil containing radioactive cholesterol was administered to each rat by gavage 24 hr later. The intestinal lymph was collected on ice in 0.01% sodium ethylenediaminetetraacetic acid (EDTA) over a period of 24 hr while the animals drank 5% glucose solution in saline *ad libitum*. For native chylomicron separation, the lymph was submitted to ultracentrifugation at 24,500 rpm at 20°C (density = 1.006 g/mL) using an SW 41 rotor (Beckman ultracentrifuge model L3-50, Beckman Instruments). Chylomicrons were harvested with a Pasteur pipette and were used within 24 hr.

Plasma analyses. Blood was drawn after carotid catheterization. In the Triton WR 1339 experiment, blood was sequentially drawn over a 90 min period and triacylglycerols were measured enzymatically (Biodiagnostica). Triacylglycerols secreted by the liver were calculated by linear regression of the plasma triacylglycerol concentration data on the ordinate as related to time (on the abscissa) according to the equation $y = a + bx$, where b is the angular coefficient of the minimum square straight line, expressed in mg/100 mL/min.

In the artificial chylomicron infusion experiments, blood was drawn at 2, 4, 6, 8 and 10 min and plasma (100 μL) was extracted with chloroform/methanol (2:1, v/v). Radioactivity in the triacylglycerol and cholesteryl ester fractions was measured as previously described (21). In the natural chylomicron infusion experiment, ^{14}C and ^{3}H radioactivities were measured in blood samples drawn as described above; plasma aliquots were counted using a toluene-phosphor solution and a Beckman LS-100 beta scintillation counter (Beckman Instruments, Inc.) (24).

The fractional removal rate for each isotope in the plasma was computed from a monoexponential curve fitting after plotting the plasma radioactivity log values (on the ordinate) against time in minutes (on the abscissa). A monoexponential least square line as drawn ($y = a \cdot e^{bx}$), where b represents the angular coefficient which is the fractional removal rate expressed in minutes (21).

Tissue analyses. Aliquots of liver and spleen (1g) were extracted with 30 mL of a solution of chloroform/methanol (2:1, v/v). After filtration and addition of water (7 mL), the upper water phase was discarded. A solution of chloroform/methanol/water (3:48:47, v/v/v; 4 mL) was then added, and the water phase discarded. The solvents were evaporated under a stream of nitrogen. The extracted lipids were purified by thin-layer chromatography (TLC) on silica gel using the solvent system hexane/diethyl ether/acetic acid (70:30:1, v/v/v). The cholesteryl ester and triacylglycerol bands were identified in an iodine chamber and eluted with diethyl ether. Radioactivity was determined in a toluene phosphor scintillation solution. An aliquot of the extracted tissue fat was used for the gravimetric measurement of total fat and triacylglycerols using the enzymatic kit (Biodiagnostica; Clinical Chem. Industry, São Paulo, Brazil).

RESULTS

Triacylglycerol and cholesterol concentrations in plasma after 15 days on each diet are shown in Table 1. Although plasma lipids were measured in the fasting state, the plasma triacylglycerol level was much higher in the olive oil group than in the fish oil and control groups. Plasma cholesterol concentration was slightly, but significantly, higher in the fish oil fed rats than in the olive oil fed and control animals. Thus fish oil reduced plasma triacylglycerol concentration, but increased the plasma cholesterol level, in agreement with results reported by others for humans and animals (25,26).

Figure 1 shows the increase in plasma triacylglycerol level with time after intraarterial administration of Triton WR 1339. Formation of liver triacylglycerol was significantly lower in controls and in fish oil fed rats than in olive oil fed animals. Fractional removal rates of the artificial chylomicrons are shown in Table 2. The plasma fractional catabolic rate (FCR) of [9,10(n)-^{3}H]triolein measures the triacylglycerol lipolysis and the particle removal rates altogether, while the FCR of cholesteryl [1-^{14}C]oleate measures the particle removal rate only. The FCR of both radioactive moieties did not differ among the three ex-

TABLE 1

Plasma Levels of Triacylglycerols and Cholesterol in Rats After 15 Days of Dietary Treatment[a]

Experimental groups	Triacylglycerols	Cholesterol
Controls (n = 7)	94.3 ± 19.6	56.3 ± 9.4
Fish oil (n = 9)	98.3 ± 34.3	75.2 ± 14.3
Olive oil (n = 7)	170.4 ± 48.1	62.7 ± 11.8

[a]Mean mg/dL ± SD. Statistical comparisons between groups by Student's t-test was as follows:

Controls × fish oil	NS	$p < 0.005$
Controls × olive oil	$p < 0.005$	NS
Fish oil × olive oil	$p < 0.005$	$p < 0.05$

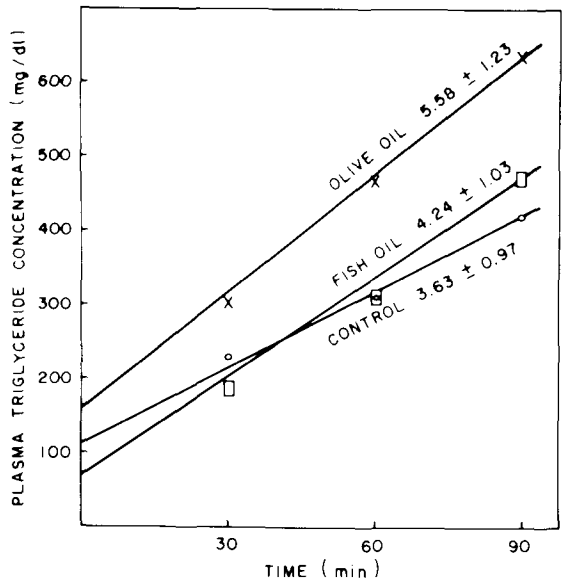

FIG. 1. Plasma triacylglycerol secretion rate. Variation of plasma triacylglycerol concentration after intra-arterial pulse infusion of Triton WR-1339. Control group: circles (n = 7); olive oil group: crosses (n =); fish oil group: squares (n = 9). Statistical comparisons: controls × olive oil ($p < 0.01$); control × fish oil (p = not significant); olive oil × fish oil ($p < 0.05$). For calculation of secretion rates, see text.

TABLE 2

Fractional Catabolic Rates of Artificial Chylomicrons Doubly Labeled with Cholesteryl [1-^{14}C] oleate [^{14}C-CE] and [9,10(n)-^{3}H] triolein [^{3}H TG] Pulse Infused Intra-Arterially[a]

Experimental groups	^{14}C-CE	^{3}H-TG
Controls (n = 10)	0.23 ± 0.09	0.36 ± 0.14
Fish oil (n = 10)	0.21 ± 0.04	0.34 ± 0.11
Olive oil (n = 10)	0.21 ± 0.04	0.28 ± 0.07

[a]Mean min^{-1} ± SD. Differences between experimental groups by Student's t-test were not significant.

perimental dietary groups. Prolonged periods of feeding the diets did not interfere with the rate of metabolism of the artificial chylomicrons in plasma.

After the simultaneous intra-arterial injection into control rats, the fractional removal rate of the native chylomicrons containing radioactive cholesterol, labeled either with ^{3}H or ^{14}C (min; mean ± SD), was 0.07 ± 0.03 for those made from fish oil (labeled with [^{14}C]cholesterol) and 0.08 ± 0.03 for those made from olive oil (labeled with [^{3}H]cholesterol). The liver radioactive contents, as measured after artificial chylomicron administration, were different among the experimental groups (Table 3). Control rats had the highest liver cholesteryl [1-^{14}C]oleate content, while the fish oil fed group had the lowest. The liver content of [9,10(n)-^{3}H]triolein was lower in fish oil and olive oil fed rats than in control rats. Lower levels of [^{3}H]triacylglycerols were also present in the spleen of control rats as compared to the fish oil and olive oil fed groups. However, it is difficult to interpret the spleen results in view of the extremely low radioactivity values as compared to the liver data. More total fat and triacylglycerol were present in the liver of the olive oil fed group than in the fish oil fed rats; the control livers showed the lowest values (Tables 4 and 5).

DISCUSSION

Our data show that lower liver triacylglycerol output is the major plasma triacylglycerol lowering mechanism of dietary fish oil, which is in agreement with previous reports (7-11, 13-16).

Feeding different fats modified the chylomicron composition, but may also have affected the number of liver chylomicron receptors or lipoprotein lipase activity. These changes may thus have affected the rate of chylomicron metabolism. In order to distinguish between these two alternatives, native chylomicrons containing radioactive cholesterol were obtained from the intestinal lymph of olive oil and of fish oil fed rats and simultaneously infused intra-arterially into recipient rats that had been on a non-purified diet. The metabolism in plasma of chylomicron particles made of olive oil was indistinguishable from that of chylomicron particles made of fish oil, as their FCR were identical. Similar results were suggested in a previous study where infused rat mesenteric lymph chylomicrons had been enriched with either [^{14}C]oleic acid or [^{14}C]eicosapentaenoic acids (18).

Artificial chylomicrons were removed from the plasma at similar rates independent of the diet. Therefore, liver receptors for chylomicrons and the activity of enzymes (lipoprotein lipase) that delipidated the large particles were not influenced by the regular feeding of different types of fats.

The liver is the major organ that metabolizes chylomicron remnants (27,28); therefore after a 10-min period, similar fractions of the injected radioactive artificial chylomicrons should have been found in the liver with all three experimental dietary groups. However, the liver radioactivity values were much higher in the low fat (control) rats than in the other two groups. The liver of fish oil fed rats contained the smallest radioactive concentration of the three groups (Table 3) and much less total fat (Table 4) or triacylglycerol mass than the olive oil

TABLE 3

Liver Radioactivity 10 Minutes After Pulse Arterial Infusion of Artificial Chylomicrons Doubly Labeled with Cholesteryl [1-^{14}C]oleate and [9,10(n) — ^{3}H] Triolein[a]

Experimental groups[b]	Liver		Spleen	
	^{14}CpCE	^{3}H-TG	^{14}C-CE	^{3}H-TG
Controls (n = 10)	22.9 ± 11.4	16.5 ± 8.8	1.1 ± 0.5	0.2 ± 0.1
Fish oil (n = 10)	9.8 ± 1.5	8.0 ± 2.1	1.6 ± 0.8	0.3 ± 0.2
Olive oil (n = 10)	11.7 ± 1.2	9.4 ± 1.3	1.3 ± 0.8	0.4 ± 0.1

[a]Values are expressed as percent of the radioactive dose administered.

[b]Statistical comparisons between groups by Student's t-test were as follows:

Control × fish oil	$p < 0.01$	$p < 0.01$	NS	$p < 0.05$
Control × olive oil	$p < 0.01$	$p < 0.01$	NS	$p < 0.01$
Fish oil × olive oil	$p < 0.01$	NS	NS	NS

TABLE 4

Total Fat in Liver and Spleen (mean ± SD) as Measured at the End of the Artificial Chylomicron Infusion

Experimental groups[a]	Liver		Spleen	
	Total (mg)	mg/g	Total (mg)	mg/g
Controls (n = 10)	277.7 ± 66.1	32.9 ± 7.0	24.0 ± 4.6	35.0 ± 3.2
Fish oil (n = 10)	372.7 ± 54.5	33.8 ± 2.6	14.1 ± 2.9	24.9 ± 3.7
Olive oil (n = 10)	489.3 ± 120.1	43.1 ± 7.8	17.7 ± 4.4	23.5 ± 2.0

[a]Statistical comparisons between groups (mg/g) by Student's t-test were as follows:

Controls × fish oil	NS	$p < 0.001$
Controls × olive oil	NS	$p < 0.001$
Fish oil × olive oil	$p < 0.001$	NS

TABLE 5

Liver Triacylglycerol Mass (mean ± SD) as Measured at the End of the Artificial Chylomicron Infusion

Experimental groups[a]	Total (mg)	mg/g Liver weight
Controls (n = 10)	37.0 ± 8.3	4.3 ± 0.6
Fish oil (n = 10)	45.7 ± 11.5	4.1 ± 0.9
Olive oil (n = 10)	136.7 ± 36.6	12.2 ± 4.1

[a]Statistical comparisons between groups by Student's t-test were as follows:

Controls × fish oil	$p < 0.05$	NS
Controls × olive oil	$p < 0.001$	$p < 0.001$
Fish oil × olive oil	$p < 0.001$	$p < 0.001$

fed rats when values were expressed per unit of liver weight (Table 5).

The present experiments demonstrate that rats fed dietary fish oil rather than olive oil have a slower VLDL-TG production in liver, a normal plasma catabolic rate of radioactive chylomicrons, a decreased liver concentration of the infused radioactive chylomicrons, and a smaller liver concentration of total fat and of triacylglycerol. The data above are in agreement with a slower production of VLDL-TG in the presence of a normal entrance into the liver of the fat dietary origin. If the exit of fat from the liver were impaired while the local synthesis rate of triacylglycerol were maintained, liver fat would have been stored during fish oil feeding. However, the liver fat content was remarkably small in the fish oil fed group compared to that in the olive oil fed group. Therefore, fish oil must stimulate fatty acid oxidation, impair triacylglycerol synthesis, or both.

Previous studies have shown that in the liver ω3 fatty acids are preferentially oxidized as compared to acid of the ω6 series (13,29). Finding little radioactive triacylglycerol in the liver indicates that chylomicrons taken up are more rapidly metabolized into products that are no longer fatty acids; however, slower synthesis of triacylglycerols cannot be ruled out. Dietary fish oil has been found to reduce the activity of certain liver enzymes, such as diacylglycerol hydrolase (30), diacylglycerol acyltransferase (31), phosphatidate phosphohydrolase (29,30), and acetyl-CoA carboxylase (32). Faster oxidation of fish oil derived fatty acids in liver has also been demonstrated in earlier studies (13,33).

Plasma cholesterol concentration was higher in the fish oil group than in the olive oil fed rats (Table 1). Since the fish oil brand used in our study (MaxEPA) contained 4.6 mg cholesterol/g, and the animals consumed approximately 20 g of diet/day, their daily cholesterol intake was approximately 9–10 mg. This intake may have increased the plasma cholesterol concentration in rats (34). In past studies with normal subjects, fish oil treatment did not influence plasma cholesterol concentrations (35,36), but

increased low density lipoprotein (LDL) cholesterol levels (37). Studies with hypercholesterolemic individuals have shown reduced plasma cholesterol concentrations after fish oil treatment (25). The number of LDL receptors was decreased and the number of high density lipoprotein (HDL) receptors was increased in rats fed fish oil as compared to animals fed sunflower oil (38). In rats fed a nonpurified diet, the largest share of plasma cholesterol was found in HDL. Further studies are needed to explain whether and how dietary fish oil influences LDL and HDL metabolism both in humans and in animals.

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REFERENCES

1. Cahlil, P.D., Sarris, G.E., Cooper, A.D., Wood, P.D., Kosek, J.C., Mitchell, R.S., and Miller, D.C. (1988) *J. Vasc. Surg. 7*, 108–118.
2. Davis, R.H., Bridenstine, R.T., Vesselinovitch, D., and Wissler, R.W. (1987) *Arteriosclerosis 7*, 441–449.
3. David, J.S.K., De Pace, N., Noval, J., and Reichle, F.A. (1987) *Arteriosclerosis 7*, 512 (Abstract).
4. Demke, D.M., Peters, G.R., Linet, O.I., Metzler, C.M., and Klott, K.A. (1988) *Atherosclerosis 70*, 73–80.
5. Harris, W.S., Connor, W.E., Illingworth, D.R., Rothrock, D.W., and Foster, D.M. (1990) *J. Lipid Res. 31*, 1549–1558.
6. Rich, S., Miller, J.F., Davis, H., Shanks, P., Charous, S., Glaqoc, S., and Lands, W.E.M. (1987) *Arteriosclerosis 7*, 527 (Abstract).
7. Nestel, P.J., Connor, W.E., Reardon, M.F., Connor, S., Wong, S., and Boston, R. (1984) *J. Clin. Invest. 74*, 82–89.
8. Harris, W.S. (1989) *J. Lipid Res. 30*, 785–807.
9. Sanders, T.A.B., Sullivan, D.R., Reeve, J., and Thompson, G.R. (1985) *Arteriosclerosis 5*, 459–465.
10. Wong, S.H., and Marsh, J.B. (1988) *Circulation (Supplement II) 78*, 485 (Abstract).
11. Daggy, B., Arost, C., and Bensadoun, A. (1987) *Biochim. Biophys. Acta 920*, 293–300.
12. Parks, J.S., Johnson, F.L., Wilson, M.D., and Rudel, L.L. (1990) *J. Lipid Res. 31*, 455–466.
13. Wong, S.H., Nestel, P.J., Trimble, R.P., Storer, G.B., Illman, R.J., and Topping, D.L. (1984) *Biochim. Biophys. Acta 792*, 103–109.
14. Wong, S., and Nestel, P.J. (1987) *Atherosclerosis 64*, 139–146.
15. Rustan, A.C., Nossen, J.O., Cristiansen, E.N., and Drevon, C.A. (1988) *J. Lipid Res. 29*, 1417–1426.
16. Nossen, J.O., Rustan, A.C., Gloppestad, S.H., Malbakken, S., and Drevon, C.A. (1986) *Biochim. Biophys. Acta 879*, 56–65.
17. Chen, I.S., Subramanian, S., Cassidy, M.M., Sheppard, A.J., and Vahouny, G.V. (1985) *J. Nutr. 115*, 219–225.
18. Chen, I.S., Le, T., Subramanian, S., Cassidy, M.M., Sheppard, A.J., and Vahouny, G.V. (1987) *Lipids 22*, 318–321.
19. Chen, I.S., Sheppard, A.L., Cassidy, M.M., and Vahouny, G.V. (1987) *Atherosclerosis 65*, 193–198.
20. Steiner, G., Haynes, F.J., Yoshino, G., and Vranic, M. (1984) *Am. J. Physiol. 246*, 187–192.
21. Redgrave, T.G., and Maranhão, R.C. (1985) *Biochim. Biophys. Acta 835*, 104–112.
22. Oliveira, H.C.F., Hirata, M.H., Redgrave, T.G., and Maranhão, R.C. (1988) *Biochim. Biophys. Acta 958*, 211–217.
23. Bartlett, G.R. (1959) *J. Biol. Chem. 234*, 466–468.
24. Hirata, M.H., Oliveira, H.C.F., Quintão, E.C.R., Redgrave, T.G., and Maranhao, R.C. (1987) *Biochim. Biophys. Acta 917*, 344–346.
25. Singer, P., Berger, I., Luck, K., Taube, C., Naumann, E., and Godicke, W. (1986) *Atherosclerosis 62*, 259–265.
26. Rogers, K.A., and Adelstein, R. (1990) *Am. J. Pathol. 137*, 945–951.
27. Brown, M.S., Kovanen, P.T., and Goldstein, J.L. (1981) *Science 212*, 628–635.
28. Redgrave, T.G. (1983) in *Gastrointestinal Physiology IV. International Review of Physiology* (Young, J.A., ed.) Vol. 28, pp. 103–130, United-Park Press, Baltimore.
29. Wong, S.H., and Marsh, J.B. (1988) *Metabolism 37*, 1177–1181.
30. Marsh, J.B., Topping, D.L., and Nestel, P.J. (1987) *Biochim. Biophys. Acta 922*, 239–243.
31. Rustan, A.C., Nossen, J.O., Christiansen, E.N., and Drevon, C.A. (1988) *J. Lipid Res. 29*, 1417–1426.
32. Iritani, N., Inogychi, K., Endo, M., Fukada, E., and Morita, M. (1980) *Biochim. Biophys. Acta 210*, 483–486.
33. Hagve, T.A., and Christophersen, B.O. (1988) *Scand. J. Clin. Lab. Invest. 48*, 813–816.
34. Ho, K.J., and Taylor, C.B. (1968) *Arch. Pathol. 86*, 585–596.
35. Rogers, S., James, K.S., Butland, B.K., Etherington, M.D., O'Brien, J.R., and Jones, J.G. (1987) *Atherosclerosis 63*, 137–143.
36. Sanders, T.A.B., Vickers, M., and Haines, A.P., (1981) *Clin. Sci. 61*, 317–324.
37. Leaf, A., and Weber, P. (1988) *N. Engl. J. Med. 318*, 549–557.
38. Roach, P.D., Kambouris, A.M., Trimble, R.P., Topping, D.L., and Nestel, P. (1987) *FEBS Lett. 222*, 159–162.
39. Lasser, N.L., Roheim, P.S., Edelstein, D., and Eder, A.A. (1973) *J. Lipid Res. 14*, 1–8.

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