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The effects of dietary fish oil on hepatic high density and low density lipoprotein receptor activities in the rat

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Rats were fed either a standard ration diet or that diet supplemented with 8% by wt of a marine fish oil or safflower oil. After 10 days, plasma triacylglycerols, total cholesterol, high density lipoprotein (HDL) cholesterol, hepatic cholesterol and fatty acid synthesis and hepatic low density lipoprotein (LDL) receptor activity were significantly depressed while HDL receptor activity was significantly increased in rats fed fish oil. Fish oil-induced effects on cholesterol metabolism in the rat therefore include reciprocal changes in the activities of hepatic LDL and HDL receptors.

LDL receptor; HDL receptor; Cholesterol synthesis; Fatty acid synthesis; (Fish oil, Safflower oil)

1. INTRODUCTION

Dietary long-chain fatty acids of marine fish oils are highly effective in lowering plasma triacylglycerol levels in man [1] and other species including the rat [2]. This fall reflects a substantial reduction in the hepatic production of very low density lipoprotein (VLDL) [3] caused by enhanced fatty acid oxidation, depressed lipogenesis and decreased secretion of apolipoprotein B (apo B) [4–6].

Despite substantial reductions in VLDL secretion, the effect of dietary fish oil on plasma cholesterol in man has been inconsistent. Some studies have shown a fall in plasma low density lipoprotein (LDL) levels as well as an attenuation of the rise in plasma cholesterol with cholesterol feeding while others have reported no change or a rise in LDL cholesterol and apo B [1,7]. Also, feeding menhaden oil to rabbits results in a doubling of the plasma and LDL cholesterol concentrations despite a 77% reduction in hepatic HMG-CoA reductase (EC 1.1.1.34) activity [8].

Clearly there is uncertainty as to the effects of dietary fish oils on the handling of LDL cholesterol by the liver and the present experiments were designed to investigate the effects of such oils on hepatic cholesterol synthesis and hepatic LDL receptor activity in the rat. Because cholesterol transport occurs mainly in HDL in the rat we have also examined the effects of fish oils on hepatic HDL receptor activity.

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2. MATERIALS AND METHODS

Male Hooded Wistar rats (220–240 g) were kept in wire-mesh cages under conditions of controlled heating (21 ± 1°C) and lighting (10:00–22:00 h) in a room of low background noise. Three groups, each comprising 6 rats, were fed ad libitum either a standard commercial laboratory ration or that diet supplemented with 8% by wt of either a marine fish oil (San-omega) or safflower oil [2]. After 10 days the rats were exsanguinated for determination of plasma lipids and portions of liver were taken for measurement of LDL and

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HDL receptor activities and rates of cholesterol and fatty acid synthesis.

Hepatic cholesterol and fatty acid synthesis was measured following the intraperitoneal injection of 20 mCi ^3H_2O at 10:00 h, 60 min prior to anesthesia [9]. Plasma triacylglycerols and cholesterol concentrations were measured as in [2].

The LDL receptor activity was quantified as described [10]. Samples of solubilized hepatic membrane proteins (8 μg in 20 μl) were spotted onto nitrocellulose using a dot-blot apparatus and the nitrocellulose was incubated with colloidal gold-LDL conjugates using normal human LDL (*d* 1.025–1.050 g/ml). The coloration of the receptor gold-LDL complex was amplified by incubation with a silver stain and the receptor activity was quantified by scanning with an LKB 2202 Ultrascan laser densitometer linked to a computing integrator. Nonspecific LDL binding was measured in parallel incubations in the presence of a 25-fold excess of unlabeled LDL and this was subtracted from total gold-LDL binding (in absence of unlabeled LDL) to provide specific LDL receptor activity expressed as integrator peak height in mm. We have previously demonstrated the specificity and the high sensitivity of this technique which has the capacity to quantify LDL receptor activity even in normal rat liver [10].

The dot-blot method was also used to quantify the binding of human HDL_3 (*d* 1.125–1.210 g/ml) to the solubilized hepatic membrane proteins. As for LDL, binding of colloidal gold-HDL_3 conjugates was assayed in the absence and in the presence of a 25-fold excess of unlabeled HDL_3 to determine specific binding. The validation of this technique is being reported elsewhere (Kambouris, A.M. et al., in preparation): it is specific for HDL_3 in that it is not displaceable by LDL, is not Ca^{2+}-dependent and readily detects up-regulation of HDL_3 binding to solubilized membrane proteins from Hep G2 cells preincubated with cholesterol. Furthermore, the intensity (laser densitometry) of the silver-intensified colloidal gold colour was linearly related to the amount of solubilized liver membrane protein applied to nitrocellulose.

Statistical evaluation of all data was by the analysis of variance and a value of $P < 0.05$ was taken as the criterion of significance.

3. RESULTS

In keeping with previous observations [1,2,7], plasma triacylglycerols, plasma cholesterol as well as HDL cholesterol [11] were significantly lower with fish oil feeding (table 1).

Table 2 shows the mean specific binding activities of LDL and HDL_3 receptors in the three dietary groups. The fish oil diet exerted opposing effects on the two receptor activities, down-regulating the LDL receptor and up-regulating the

Table 1

Effects of dietary fish and safflower oils on the plasma lipid and HDL cholesterol concentrations

Diet (n = 6 for each diet)	Plasma triacylglycerol	Plasma cholesterol (μmol/ml; mean ± SE)	HDL cholesterol
Chow	1.35 ± 0.15	2.76 ± 0.09	1.32 ± 0.03
Chow + 8% fish oil	0.64 ± 0.04[a]	1.82 ± 0.07[a]	1.15 ± 0.04[a]
Chow + 8% safflower oil	1.37 ± 0.14[c]	2.95 ± 0.11[b]	1.57 ± 0.06[b]

[a] Significantly ($P < 0.05$) different from chow and chow + safflower oil
[b] Significantly ($P < 0.05$) different from chow and chow + fish oil
[c] Significantly ($P < 0.05$) different from chow + fish oil

Table 2

Effects of dietary fish and safflower oils on hepatic LDL and HDL receptor activities

Diet (n = 6 for each diet)	Integrator peak height (mm) (mean ± SE)	
	LDL receptor	HDL receptor
Chow	16.7 ± 2.2	39.4 ± 6.4
Chow + 8% fish oil	10.6 ± 1.3[a]	67.4 ± 2.3[b]
Chow + 8% safflower oil	13.1 ± 1.3	43.8 ± 6.3[c]

[a] Significantly ($P < 0.05$) different from chow
[b] Significantly ($P < 0.05$) different from chow and chow + safflower oil
[c] Significantly ($P < 0.05$) different from chow + fish oil

Quantified as specific LDL or HDL_3 binding. Solubilized liver membranes were dot-blotted on nitrocellulose and incubated with colloidal gold conjugates of either human LDL or human HDL_3. After amplification with silver staining the blots were scanned by laser beam densitometry

HDL_3 receptor by 37 and 71%, respectively, relative to the standard ration diet. Although its effects were not statistically significant, safflower oil also reduced the LDL receptor activity and increased the HDL receptor activity.

Cholesterol synthesis was reduced in livers from rats fed either oils although fish oils had the greater effect (table 3). Of particular interest is the linear correlation observed between the LDL receptor activity and cholesterol synthesis (fig.1). Hepatic fatty acid synthesis was also decreased substantially by both oils (table 3).

Table 3

Effects of dietary fish and safflower oils on hepatic cholesterol and fatty acid synthesis

Diet (n = 6 for each diet)	Cholesterol synthesis (nmol/g per h)	Fatty acid synthesis (μmol/g per h)
Chow	408 ± 38	2.66 ± 0.45
Chow + 8% fish oil	189 ± 25[b]	1.54 ± 0.10[a]
Chow + 8% safflower oil	262 ± 17[c]	1.74 ± 0.15[a]

[a] Significantly ($P < 0.05$) different from chow
[b] Significantly ($P < 0.05$) different from chow and chow + safflower oil
[c] Significantly ($P < 0.05$) different from chow and chow + fish oil

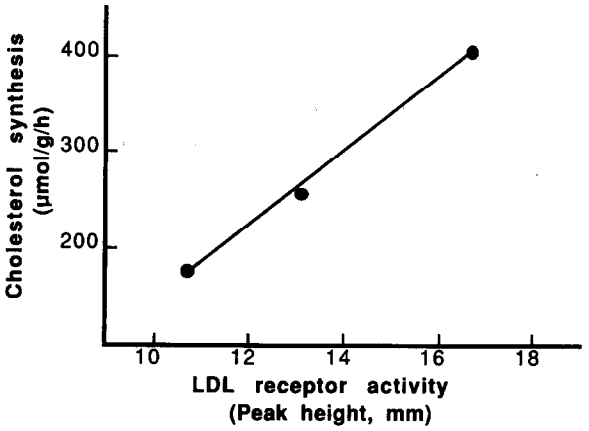

Fig.1. Linear correlation of hepatic LDL receptor activity with hepatic cholesterol synthesis. Values of hepatic cholesterol synthesis from table 3 are plotted vs values of hepatic LDL receptor activity from table 2.

4. DISCUSSION

The major finding of this study is the significant effect of dietary fish oil on two lipoprotein receptors of rat liver. This occurred in association with the inhibition of hepatic cholesterol and fatty acid synthesis as well as the lowering of the concentrations of plasma triacylglycerols, total cholesterol and HDL cholesterol. It may also be calculated that fish oil lowered both VLDL and LDL cholesterol. The question arises as to whether the changes in lipoprotein cholesterol concentrations resulted from diminished hepatic secretion or increased uptake of lipoproteins from the plasma.

The hepatic secretion of VLDL components, including cholesterol, is strikingly reduced by fish oil feeding [3] reflecting in part their reduced synthesis (table 3) [4–6]. Since LDL is at least partly derived from VLDL in the rat [12], a fall in LDL concentration is not unexpected. However, the seemingly paradoxical 37% reduction in rat hepatic LDL receptor activity (table 2) was not totally unexpected either. We have previously demonstrated that eicosapentaenoic acid, one of the major $n-3$ fatty acids of fish oils, completely abolishes the specific binding of ^{125}I-LDL to Hep G2 cells [6]. The present in vivo effects of fish oils are therefore

entirely consistent with our earlier in vitro observations. Because the hepatic LDL receptor is responsible for more than 70% of LDL removal in the rat [13] and may also be involved in the uptake of its immediate precursor [14], intermediate density lipoprotein (IDL), the LDL could rise when the receptor is down-regulated. However, in our fish oil-fed rats, the lower production of LDL cholesterol rather than its reduced uptake from the plasma was obviously the predominant factor affecting the LDL cholesterol concentration.

Our finding that fish oils reduce hepatic LDL receptor activity may however explain some of the effects observed in other species. In humans [1,7] and in rabbits [8] fed fish oil, LDL cholesterol can actually increase. A down-regulation of the LDL receptor rather than a reduced production of LDL cholesterol may well be the predominant factor in these cases.

Whereas LDL receptor activity is coordinated with changes in HMG-CoA reductase activity in cultured cells [15], it is not a general finding in rat liver [13]. Dietary oil feeding appears to be one circumstance under which such a coordinate effect is observable (fig.1). The observed reduction in cholesterol synthesis (table 3) is probably due to the inhibition of HMG-CoA reductase activity, an effect seen with fish oil in the rabbit [8].

In contrast to our earlier findings with the perfused rat liver [4], both oils inhibited hepatic fatty acid synthesis to the same extent (table 3). This lack of difference probably reflects the higher plasma free fatty acid concentrations found with safflower oil feeding [2]. The greater direct inhibitory effects of these fatty acids [16] are likely to compensate for the greater adaptive down-regulating effects [4] of fish oil on the hepatic enzymes involved in fatty acid synthesis.

For the rat in the post-absorptive state, HDL carries about 6-times as much of the plasma cholesterol [17] and probably delivers about 3-times as much cholesterol to the liver as does LDL [13]. The present finding that dietary fish oil stimulated hepatic HDL receptor activity (table 2) by 71% is therefore biologically significant. It is likely to have at least contributed to the lowering in circulating HDL cholesterol (table 1). Since more cholesterol than protein is removed through the interaction of HDL with binding sites on the liver [18], an increase in HDL receptor activity is expected to increase substantially the hepatic uptake of cholesterol.

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REFERENCES

[1] Herold, P.M. and Kinsella, J.E. (1986) Am. J. Clin. Nutr. 43, 566–598.
[2] Illman, R.J., Trimble, R.P., Storer, G.B., Topping, D.L. and Oliver, J.R. (1986) Atherosclerosis 59, 313–321.
[3] Nestel, P.J., Connor, W.E., Reardon, M.F., Connor, S., Wong, S. and Boston, R. (1984) J. Clin. Invest. 74, 82–89.
[4] 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.
[5] Illingworth, D.R., Harris, W.S. and Connor, W.E. (1984) Arteriosclerosis 4, 270–275.
[6] Wong, S. and Nestel, P.J. (1987) Atherosclerosis 64, 139–146.
[7] Nestel, P.J. (1987) Am. J. Clin. Nutr. 45, 1161–1167.
[8] Field, F.J., Albright, E.J. and Mathur, S.N. (1987) J. Lipid Res. 28, 50–58.
[9] Topping, D.L., Trimble, R.P. and Storer, G.B. (1987) Biochim. Biophys. Acta 927, 423–428.
[10] Roach, P.D., Zollinger, M. and Noël, S.P. (1987) J. Lipid Res., in press.
[11] Parks, J.S., Martin, J.A., Sonbert, B.L. and Bullock, B.C. (1987) Arteriosclerosis 7, 71–79.
[12] Sparks, C.E., Rader, D.J. and Marsh, J.B. (1983) J. Lipid Res. 24, 156–166.
[13] Koelz, H.R., Sherrill, B.C., Turley, S.D. and Dietschy, J.M. (1982) J. Biol. Chem. 257, 8061–8072.
[14] Roach, P.D. and Noël, S.P. (1985) J. Lipid Res. 26, 713–720.
[15] Goldstein, J.L. and Brown, M.S. (1977) Annu. Rev. Biochem. 46, 897–930.
[16] Mayes, P.A. and Topping, D.L. (1974) Biochem. J. 140, 111–114.
[17] Weisgraber, K.H. and Mahley, R.W. (1983) Handb. Electrophor. 4, 103–132.
[18] Arbeeny, C.M., Rifici, V.A. and Eder, H.A. (1987) Biochim. Biophys. Acta 917, 9–17.