Scand J Clin Lab Invest 1988; 48: 813–816

Mechanisms for the serum lipid-lowering effect of n-3 fatty acids

T.-A. HAGVE & B. O. CHRISTOPHERSEN

Institute of Clinical Biochemistry, University of Oslo, Rikshospitalet, Oslo, Norway

Hagve T-A, Christophersen BO. Mechanisms for the serum lipid-lowering effect of n-3 fatty acids. Scand J Clin Lab Invest 1988; 48: 813–816.

Both epidemiological and experimental studies have demonstrated that a high content of n-3 fatty acids in the diet lowers serum lipid concentration. However, the mechanism for this effect is unclear. In this present study it has been shown that labelled linolenic acid (18:3,n-3) is oxidized to a larger extent than linoleic acid (18:2,n-6) in isolated rat hepatocytes. Conversely, the incorporation of linolenic acid and the desaturated/chain-elongated products in VLDL-triacylglycerol is decreased compared with linoleic acid. Dietary n-3 fatty acids have probably a depressing effect on both hepatic triacylglycerol synthesis and on secretion of VLDL. The finding that n-3 fatty acids are transported from the liver as ketone bodies to a larger extent than n-6 fatty acids may thus explain that a high intake of n-3 fatty acids is not accompanied with hepatic steatosis.

Key words: FABP; hepatocytes; n-3 fatty acids; triacylglycerol; VLDL

Tor-Arne Hagve, Institute of Clinical Biochemistry, Rikshospitalet, 0027 Oslo 1, Norway

During the last 10–15 years it has been well established that dietary n-3 fatty acids have a preventive effect on the development of obstructive coronary heart disease [1–3]. This beneficial effect of n-3 fatty acids is probably due to both an effect on platelet aggregation and on the level of blood lipids. As for the inhibiting effect of n-3 fatty acids on platelet aggregation, it has been proposed that eicosapentaenoic acid (20:5,n-3) is a precursor for thromboxanes and prostacyclines of the 3-series which lower platelet aggregability [4–7]. Dietary n-3 fatty acids, especially eicosapentaenoic acid (20:5,n-3) may also be antithrombotic by competitively inhibiting the enzyme cyclooxogenase, thus depressing the production of thromboxane A_2, which stimulates platelet aggregation [5, 6].

The understanding of the blood–lipid lowering effect of n-3 fatty acids [8–10] is even more diffuse. The reduction in the concentration of plasma very low density lipoprotein (VLDL)-triacylglycerol may either occur from lower rates of synthesis of the triacylglycerol or apo B moieties of VLDL, from lower secretion of VLDL-triacylglycerol, or may result from an increased rate of clearance from the plasma. In light of the first possibility it has been shown by tracer kinetic studies in man that dietary n-3 fatty acids inhibit the formation of VLDL-triacylglycerol [11], LDL [12] and VLDL-apolipoproteins [10]. In studies with cultured rat hepatocytes, eicosapentaenoic acid reduced the secretion of VLDL by depressing the synthesis of triacylglycerol from labelled glycerol [13].

It has also been demonstrated in studies with rats that eicosapentaenoic acid, compared with linoleic acid, reduce the activity of the enzyme acetyl-CoA carboxylase, thus reducing the

supply of fatty acids for triacylglycerol synthesis [14].

In several works [15, 16] it has been proposed that n-3 fatty acids accelerate triacylglycerol clearance rates, possibly by increasing lipoprotein lipase activity.

In 1985 Beynen and Katan [17] suggested, as an hypothesis, that replacement of saturated by polyunsaturated fatty acids in the diet may lower VLDL concentration because the liver preferentially converts polyunsaturated fatty acids into ketone bodies instead of into lipoprotein-triacylglycerol.

The working hypothesis of the present work was that n-3 fatty acids are an even better substrate for ketogenesis than other polyunsaturated fatty acids.

MATERIALS AND METHODS

[1-^{14}C]Linoleic acid was from The Radiochemical Centre, Amersham, UK and [1-^{14}C]linolenic acid was from New England Nuclear, Boston, Mass., USA. The specific activity of labelled fatty acid was 7 mCi/mmol. (+)Lactate, essential fatty-acid free bovine serum albumin, N-2-hydroxyethylpiperazin-N-2 ethanesulphonic acid (Hepes), collagenase type 1 and unlabelled fatty acids were from Sigma Chemicals (St. Louis, Mo., USA). Mature male rats of the Wistar strain were from Møllegaard Laboratory (Denmark). In order to increase fatty acid esterification, the animals were fed a semisynthetic diet deficient in essential fatty acids [18] with 15 wt.% hydrogenated coconut oil for at least 60 days.

Parenchymal liver cells were prepared and purified according to Seglen [19]. About 100–300×10^6 cells were obtained from each liver, and 90–95% were viable, as measured by resistance to uptake of trypan blue.

Cells were incubated with 200 nmol of ^{14}C-labelled fatty acid at 37 °C for 120 min in an oxygenated suspension medium [20] with 1.5% (w/v) bovine serum albumine, 5 mmol/l glucose and 10 mmol/l (+)lactate. The concentration of cells in the preparation was approximately 6×10^6 cells/ml, and 1 ml of this suspension (in a total volume of 2 ml) was used.

The extraction of lipids from the total incubation suspension and the measurement of radioactive acid soluble products (as a measure of the rate of β-oxidation) and of radioactive CO_2 were performed as described by Christiansen [21].

The lipids were separated on silicic acid thin-layer plates (Stahl H+) (hexane-diethylether-glacial acetic acid, 80:20:1,v/v/v).

Very low density lipoprotein fractions from the incubation medium were separated by centrifugation for 16 h at 4 °C using 115,000 ***g***. The layer of VLDL (d=1.006) floated on top of a separation layer of saline (d=1.006).

The solutions used for lipid extraction and thin-layer chromatography contained 2,6-di-tert-butyl-p-cresol (50 mg/l) as an antioxidant and the lipid extracts were stored under nitrogen gas in the dark at −20 °C to prevent peroxidation of unsaturated fatty acids. The cellular protein was determined according to the method of Lowry *et al.* [22].

RESULTS

Figure 1 shows that when [^{14}C]linoleic acid (18:2,n-6) was added to the incubations, significantly more labelled fatty acids were recovered in the VLDL-fraction (20% of total labelled fatty acids) than with [^{14}C]linolenic acid (18:3,n-3) as substrate (8%). Conversely, more linolenic acid was oxidized to acid-soluble products than was linoleic acid.

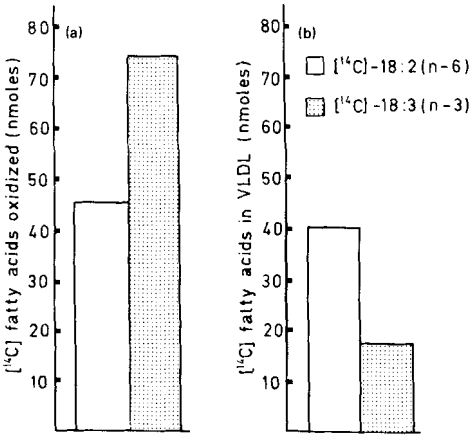

FIG. 1. Oxidation and incorporation into VLDL of labelled fatty acids in hepatocytes incubated with [^{14}C]linoleic acid or [^{14}C]linolenic acid. The incubation conditions were as described in the text. 0.1 mmol/l of labelled fatty acid was incubated with hepatocytes (25.3–27.8 mg protein) for 120 min. The results are expressed as nmol of labelled fatty acids recovered as acid soluble products or in VLDL. Mean of two parallel incubations from three different livers is given.

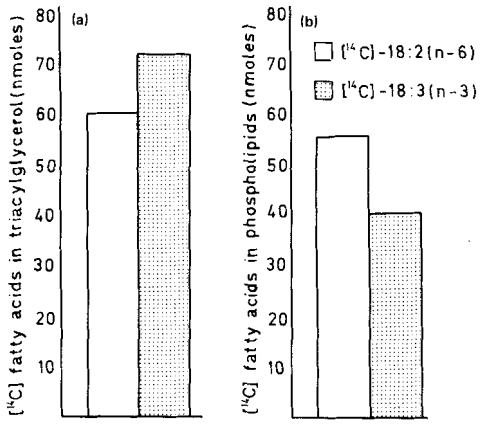

FIG. 2. Esterification of labelled fatty acids in hepatocytes incubated with [^{14}C]linoleic acid or [^{14}C]linolenic acid. The incubation conditions were as described in the text and in the legend to Fig. 1. The results are expressed as nmol of labelled fatty acid incorporated in triacylglycerol or phospholipid fractions. Mean of two parallel incubations from three different livers is given.

Linoleic acid and the desaturated/chain-elongated products were to a larger extent than linolenic acid incorporated in phospholipids (29% and 20%, respectively) (Fig. 2). In contrast, linolenic acid was under the present conditions a better substrate for triacylglycerol synthesis (Fig. 2).

DISCUSSION

The present work indicates that n-3 fatty acids are more easily converted to ketone bodies than n-6 fatty acids, with a concomitant decrease in VLDL-triacylglycerol secretion. The finding that linolenic acid (18:3,n-3) is more easily incorporated in cellular triacylglycerol than linoleic acid (18:2,n-6) may suggest that the fatty acid specificity is in secretion of VLDL and not in triacylglycerol synthesis.

In accordance with these results, Bergseth *et al.* [23] have reported that dietary fish oil accelerates hepatic ketogenesis. Similar results were also found by Wong *et al.* [24] reporting diminished lipogenesis and increased fatty acid oxidation in perfused livers of fish oil fed rats compared with rats receiving safflower oil. It was suggested by the same authors that fish oil feeding induced the rate of peroxisomal β-oxidation. This is probably not correct since the hypothesis is partly based on earlier works [25, 26] with partially hydrogenated fish oil, which contains no n-3 fatty acids [27]. However, it is striking that peroxisomal proliferation is a characteristic finding in livers from rats fed clofibrate [28], a potent plasma triacylglycerol lowering agent. It is thus interesting that clofibrate, like fish oil, stimulates fatty acid oxidation and ketogenesis in rats [29], thereby decreasing the flux of fatty acids into VLDL-triacylglycerol secretion.

In a recent study Strum Odin *et al.* [30] have shown that n-3 fatty acids compared with n-6 fatty acids, added to the culture medium of rat hepatocytes, decrease the synthesis of triacylglycerol with a concomitant increase in diacylglycerol formation. This finding is strikingly similar to the previously demonstrated effect of α-bromopalmitate [31] on diacylglycerol/triacylglycerol metabolism in a system with isolated hepatocytes. α-Bromopalmitate is an efficient inhibitor of the binding of fatty acids or acyl-CoA' to cytosolic fatty acid binding protein [32]. Cytosolic fatty acid binding proteins seem to be involved in the partitioning of fatty acids between the different intracellular metabolic pathways, favouring triacylglycerol synthesis. It is thus tempting to speculate that the serum lipid-lowering effect of n-3 fatty acids is mediated through a competitive inhibition of acyl-CoA binding to fatty acid binding protein, thus depressing triacylglycerol synthesis.

The lipid-lowering effect of n-3 fatty acids probably reflects a depression of hepatic triacylglycerol synthesis and of VLDL secretion, as well as long-term adapted increase in oxidation of long-chain fatty acids. The present study indicates that n-3 fatty acids are oxidized, and transported from the liver as ketone bodies, to a larger extent than n-6 fatty acids. The latter observation may explain that high intake of n-3 fatty acids is not accompanied with hepatic steatosis.

ACKNOWLEDGMENT

We thank Dr Magny S. Thomassen for valuable comments during the preparation of this manuscript.

REFERENCES

1 Dyerberg J, Bang OH, Stoffersen E, Moncada S, Vane J. Eicosapentaenoic acid in prevention of

thrombosis and atherosclerosis. Lancet 1978; ii: 117–9.
2 Hirai A, Hamazaki T, Terano T. Eicosapentaenoic acid and platelet function in Japanese. Lancet 1980; ii: 1132–3.
3 Kromhout D, Bosschieter EB, Coulander CDL. The inverse relation between fish consumption and 20-year mortality from coronary heart disease. N Engl J Med 1985; 312: 1205–9.
4 Hornstra G, Haddeman E, Ten Hoor F. Fish oils, prostaglandins and arterial thrombosis. Lancet 1979; 2: 1080.
5 Needleman P, Ratz A, Minkes MS, Ferendelli JA, Sprecher H. Triene prostaglandins: prostacycline and thromboxane biosynthesis and unique biological properties. Proc Natl Acad Sci 1979; 76: 944–8.
6 Whitaker MO, Wyche A, Fitzpatrick F, Sun F, Needleman P. Triene prostaglandins: Prostaglandin D and icosapentaenoic acid as potential antithrombotic substances. Proc Natl Acad Sci USA 1979; 76: 5919–23.
7 Fisher S, Weber PC. Prostaglandin I_3 is formed in vivo in man after dietary eicosapentaenoic acid. Nature 1984; 307: 165–8.
8 Von Lossonczy TO, Ruiter A, Bronsgeest-Shoute HC, Van Gent CM, Hermus RJJ. The effect of a fish diet on serum lipids in healthy human subjects. Am J Clin Nutr 1978; 31: 1340–6.
9 Harris NS, Connor WE, McMurphy MP. The comparative reduction of the plasma lipids and lipoproteins by dietary polyunsaturated fats: Salmon oil versus vegetable oils. Metabolism 1983; 32: 179–84.
10 Nestel PJ, Connor WE, Reardon MF, Connor S, Wong SH, Boston R. Suppression by diets rich in fish oil of Very Low Density Lipoprotein production in man. J Clin Invest 1984; 74: 82–9.
11 Harris WS, Connor WE, Illingworth RD, Foster DM. The mechanism of the hypotriglyceridemic effect of dietary omega-3 fatty acids in man. Clin Res 1984; 32: 560A.
12 Illingworth DR, Harris WS, Connor WE. Inhibition of low density lipoprotein synthesis by dietary omega-3 fatty acids in humans. Arteriosclerosis 1984; 4: 270–5.
13 Nossen JØ, Rustan AC, Gloppestad SH, Målbakken S, Drevon CA. Eicosapentaenoic acid inhibits synthesis and secretion of triacylglycerols by cultured rat hepatocytes. Biochim Biophys Acta 1986; 879: 56–65.
14 Iritani N, Inoguchi K, Endo M, Fukuda E, Moreta M. Identification of shellfish fatty acids and their effects on lipogenic enzymes. Biochim Biophys Acta 1980; 618: 378–82.
15 Pawar S, Tidwell HC. Effect of ingestion of unsaturated fat on lipolytic activity in rat tissues. J Lip Res 1968; 9: 334–6.
16 Daggy B, Arost C, Bensadoun A. Dietary fish oil decreases VLDL production rates. Biochim Biophys 1987; 920: 293–300.
17 Beynen AC, Katan MB. Why do polyunsaturated fatty acids lower serum cholesterol? Am J Clin Nutr 1985; 42: 560–3.
18 Thomassen MS, Strøm E, Christiansen EN, Norum KR. Effect of marine oil and rapeseed oil in composition of fatty acids in lipoprotein triacylglycerols from rat blood plasma and liver perfusate. Lipids 1979; 14: 58–65.
19 Seglen PO. Preparation of rat liver cells. 3. Enzymatic requirements for tissue dispension. Exp Cell Res 1973; 82: 391–8.
20 Nordby G, Berg T, Nilsson M, Norum KR. Secretion of lechithine:cholesterol acyltransferase from isolated rat hepatocytes. Biochim Biophys Acta 1976; 450: 69–77.
21 Christiansen R. Regulation of palmitate metabolism by carnitine and glucagone in hepatocytes isolated from fasted and carbohydrate-fed rats. Biochim Biophys Acta 1977; 448: 249–62.
22 Lowry OH, Roseborough NJ, Farr AL, Randall RJ. Protein measurements with the folin phenol reagent. Biol Chem 1951; 193: 265–75.
23 Bergseth S, Christiansen EN, Bremer J. The effect of feeding fish oils, vegetable oils and clofibrate on the ketogenesis from long chain fatty acids in hepatocytes. Lipids 1986; 21: 508–14.
24 Wong SH, Nestel PJ, Trimble RP, Storer GB, Illman RJ, Topping DL. The adaptive effects of dietary fish and safflower oil on lipid and lipoprotein metabolism in perfused rat liver. Biochim Biophys Acta 1984; 792: 103–9.
25 Osmundsen H, Neat CE, Borrebaek B. Fatty acid products of peroxisomal β-oxidation. Int J Biochem 1980; 12: 625–30.
26 Neat CE, Thomassen MS, Osmundsen H. Effects of high fat diets on hepatic fatty acid oxidation in the rat. Isolation of rat liver peroxisomes by vertical rotor centrifugation by using a self-generating, isoosmotic, Percoll gradient. Biochem J 1981; 196: 149–59.
27 Hagve TA, Christophersen BO. Effect of dietary fats on arachidonic acid and eicosapentaenoic acid biosynthesis and conversion to C_{22} fatty acids in isolated liver cells. Biochim Biophys Acta 1984; 796: 205–17.
28 Lazarow PB, Shio H, Leroy-Hoyet M. Specificity of the action of hypolipemic drugs: increase of peroxisomal β-oxidation largely dissociated from hepatomegaly and peroxisome proliferation in the rat. J Lip Res 1982; 23: 317–26.
29 Ide T, Oku H, Sugano M. The reciprocal response to clofibrate in ketogenesis and triglyceride and cholesterol secretion in isolated rat liver. Metabolism 1982; 31: 1065–72.
30 Strum-Odin R, Adkins-Finke B, Blake WL, Phinney SD, Clarke SD. Modification of fatty acid composition of membrane phospholipid in hepatocyte monolayer with n-3, n-6 and n-9 fatty acids and its relationship to triacylglycerol production. Biochim Biophys Acta 1987; 921: 378–91.
31 Hagve TA, Christophersen BO. In vitro effects of α-bromopalmitate on metabolism of essential fatty acids studied in isolated rat hepatocytes; sex differences. Biochim Biophys Acta 1987; 917: 333–6.
32 Ockner RK, Manning JA. Fatty acid binding protein. Role in esterification of absorbed long chain fatty acid in rat intestine. J Clin Invest 1976; 58: 632–41.

Received 11 November 1988
Accepted 16 June 1988