Metabolic Basis for the Cholesterol-Lowering Action of Dietary Polyunsaturated Fatty Acids*

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Replacement of dietary saturated by polyunsaturated fatty acids is a very effective way to lower serum cholesterol in man, but the mechanism of this effect is unclear. Several mechanisms of action have been proposed to explain the hypocholesterolemic effect of polyunsaturated fatty acids, but there is considerable controversy. On the basis of literature data we propose that replacement of saturated by polyunsaturated fatty acids in the diet may lower serum very low-density and low-density lipoprotein concentrations because the liver preferentially converts polyunsaturated fatty acids into ketone bodies instead of into very low-density lipoprotein triglycerides. Thus unlike saturated fatty acids, polyunsaturated fatty acids are transported to the tissues for oxidation without leaving a trail of lipoprotein remnants in the form of low-density lipoproteins.

Stoffwechselgrundlage der cholesterinsenkenden Wirkung der mit der Nahrung aufgenommenen mehrfach ungesättigten Fettsäuren

Der Ersatz gesättigter Speisefette durch mehrfach ungesättigte Fettsäuren ist eine sehr effektive Möglichkeit, um beim Menschen das Serumcholesterin herabzusenken, aber der Mechanismus dieser Wirkung ist unklar. Es sind mehrere Mechanismen vorgeschlagen worden, um den hypocholesterinämischen Effekt der mehrfach ungesättigten Fettsäuren zu erklären, aber es besteht eine beträchtliche Meinungsverschiedenheit. Ausgehend von Daten aus der Literatur schlagen wir vor, daß der Ersatz gesättigter durch mehrfach ungesättigte Fettsäuren in der Ernährung die very low-density-Lipoprotein-Konzentrationen und die low-density-Lipoprotein-Konzentration im Serum herabsetzen kann, weil die Leber vorzugsweise mehrfach ungesättigte Fettsäuren in Ketokörper umwandelt anstatt in very low-density-Lipoprotein-Triglyceride. Daher werden im Gegensatz zu gesättigten Fettsäuren mehrfach ungesättigte Fettsäuren zur Oxidation in die Gewebe transportiert, ohne in Form von low-density-Lipoproteinen eine Spur von Lipoproteinresten zu hinterlassen.

Introduction

In man, the replacement of saturated by polyunsaturated fatty acids is the single most powerful dietary intervention to lower serum cholesterol concentrations. It is generally recommended to replace dietary saturated fatty acids at least partly by polyunsaturated fatty acids in order to decrease the risk for coronary heart disease. As a result, the

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consumption of high-linoleic oils and margarines has shown a steady increase over the last 20 years in the USA and several countries of Western Europe. In spite of this, the mechanism by which linoleic acid decreases serum cholesterol remains unsolved.

Several mechanisms of action have been proposed to explain the hypocholesterolemic effect of polyunsaturated fatty acids. Mechanisms proposed involve the enhancement of the excretion of bile acids[1], an increased lipoprotein catabolism through altered structure[2], or inhibition of cholesterol absorption[3]. However, none of these proposals have been accepted unanimously. We have recently put forward[4] the suggestion that replacement of saturated by polyunsaturated fatty acids in the diet may lower serum very-low density (VLDL) and low-density lipoprotein (LDL) concentrations because the liver preferentially converts polyunsaturated fatty acids into ketone bodies instead of into VLDL-triglycerides. Thus, unlike saturated fatty acids, polyunsaturated fatty acids are transported to the tissues for oxidation without leaving a trail of lipoprotein remnants in the form of LDL, the main carrier of cholesterol in serum. Here we review the evidence for our hypothesis.

VLDL production

After a fat-containing meal, part of the dietary fatty acids is stored in adipose tissue, from which they are later mobilized and transported as plasma free fatty acids bound to albumin. The liver uses such fatty acids for the production of triglycerides, which are then exported in VLDL. In the plasma, VLDL rapidly loses its triglycerides and is converted into LDL.

Diets rich in polyunsaturated fatty acids lower the concentration of plasma triglycerides[5], and of VLDL, the main carrier of triglycerides. There is evidence that polyunsaturated fatty acids, in contrast to saturated fatty acids, are chanelled away from the hepatic production of VLDL-triglycerides. *Nestel* and *Barter*[6] infused ^{3}H-labelled palmitic acid (a prototypical saturated fatty acid) and ^{14}C-labelled linoleic acid into human subjects. The turnover rates of both fatty acids were determined as well as the rate of label incorporation into plasma triglycerides. Plasma palmitate was incorporated into plasma triglycerides about twice as efficiently as linoleate was. *Chait* and coworkers[7] performed a similar study, and also found that palmitate molecules disappearing from the free palmitate pool were incorporated into VLDL-triglycerides with significantly greater efficiency than free linoleate molecules. The *in-vivo* studies with humans are nicely complemented by studies with perfused rat livers. In two sets of experiments[8,9] the sum of triglycerides accumulated plus triglycerides excreted after 4 hours, and corrected for the fatty-acid free control, was consistently higher for livers perfused with palmitic acid than for linoleic acid (8.6 versus 6.2, and 11.8 versus 6.3 µmoles/g liver, respectively). Thus both the human and rat studies suggest that the saturated fatty acid is preferentially incorporated into VLDL-triglycerides. This implies that dietary polyunsaturated fatty acids, when compared to saturated fatty acids, decrease the rate of secretion of VLDL into plasma.

Cortese et al.[10] have determined the turnover of VLDL apoprotein B in subjects consuming diets containing 40 % of energy as fat, which were either high in saturated (P/S ratio = 0.12) or polyunsaturated fatty acids (P/S ratio = 3.8). When subjects were transferred from the low-P/S to the high-P/S diet VLDL turnover dropped by 31 %. Thus polyunsaturated fatty acids may depress VLDL synthesis. The turnover of LDL apoprotein B showed a 23 % decrease when saturated fatty acids were replaced by polyunsaturated fatty acids[10]. *Turner* et al.[11] and *Shepherd* et al.[12] reported that LDL apoprotein B synthesis was reduced by 9 and 5 %, respectively, on a high-P/S diet compared with a low-P/S diet. Thus, depressed VLDL synthesis may be responsible for the observed reduction of the rate of LDL synthesis in subjects on diets rich in linoleic acid. This would cause a drop in both the concentration of VLDL-triglycerides and of LDL-cholesterol in plasma.

Ketone body synthesis

The hypothesis that polyunsaturated fatty acids induce a decrease in VLDL synthesis and, as a consequence, in LDL production, is attractive; but if part of dietary polyunsaturated fatty acids do not go into VLDL triglyceride formation, then where do they go? Accumulation in the body cannot be the explanation. Subjects eating about 41 g of linoleic acid/day for 5 yr accumulated an average 2 kg of linoleic acid, or 2.7 % of the cumulative intake, into their body fat in the first 3 yr, adding little more after that[13]. Thus polyunsaturates, just like all other fatty acids must end up being oxidized in the various tissues of the body. But how do they get there?

Kohout et al.[9] showed that the output of ketone bodies by the perfused rat liver is significantly enhanced if linoleic acid is the substrate compared to when palmitic acid is the substrate. *Nestel* and *Steinberg*[14] showed that rat liver slices incubated with equal concentrations of linoleic acid or palmitic acid preferentially channelled palmitic acid into the pathway of triglyceride synthesis, and linoleic acid into β-oxidation. Thus polyunsaturated fatty acids may converted by the liver into ketone bodies rather than being incorporated into triglycerides and secreted as VLDL. This could imply that, other things being equal, fasting blood concentrations of ketone bodies are higher on diets rich in polyunsaturated fatty acids than on high-saturated fat diets.

Böhle and coworkers[15,16] have determined the concentration of ketone bodies (or rather of acetone) in the blood of healthy human subjects after an oral load of various fats. A mixture of soybean oil and safflower oil caused a more pronounced hyperketonemia than similar amounts of either olive oil or butter. Thus polyunsaturated fatty acids may give rise to more ketone bodies than do monounsaturated fatty acids or short-chain saturated fatty acids. This is in agreement with our hypothesis. Unfortunately, in the experiments of *Böhle* et al.[15,16] fats with high amounts of long-chain saturated fatty acids, such as palmitic acid, were not used. In order to test our hypothesis high-linoleic fats (e.g. soybean oil, safflower oil, maize oil, sunflowerseed oil) should be compared with high-palmitic fats (e.g. palm oil).

In rats, polyunsaturated fatty acids fed in the form of corn, sesame and groundnut oil have been shown to produce lower plasma concentrations of ketone bodies than saturated fatty acids given in the form of coconut fat and beef tallow[17,18]. This would contradict the reasoning above. However, in these experiments the rats were fed essentially cholesterol-free diets. The intact rat fed such a diet is an unsuitable model for the action of various fatty acids on lipoprotein metabolism in man, because under such conditions dietary polyunsaturated fatty acids in the form of corn oil cause *higher* serum cholesterol and triglyceride levels than saturated fatty acids given as coconut fat[19,20]. The reason for this surprising but reproducible anomaly is unknown. The hypocholesterolemic effect of polyunsaturated fatty acids seen in humans is only found in rats when high-cholesterol diets are used[21,22]. It

would be interesting to know the effects of different fats in high-cholesterol diets on plasma ketone bodies in rats.

Linoleic acid may be oxidized to propionyl-CoA [23] as well as to acetyl-CoA. The latter intermediate will be converted into the ketone bodies, acetoacetate and β-hydroxybutyrate. Propionyl-CoA will be converted into oxaloacetate, which will steer acetyl-CoA into the citric acid cycle instead of into the ketogenic pathway. In the fasting state, when dietary long-chain fatty acids reach the liver after having been stored temporarily in the adipose tissue, the hepatic pathways of β-oxidation and gluconeogenesis are activated. Thus linoleic acid, after having been converted first into propionate and then into oxaloacetate, is finally converted to glucose. In keeping with this suggestion, the output of glucose by the perfused rat liver was significantly enhanced if linoleic acid was infused compared to palmitic acid [9]. However, from these experiments [9] it cannot be concluded whether the glucose carbon had been derived from linoleic acid or from endogenous sources such as glycogen or glucogenic amino acids. In any case, if we postulate that part of the dietary polyunsaturated fatty acids do not go into the pathway of esterification and formation of very low density lipoproteins, then these molecules have to reach their final destination, i. e. oxidation to CO_2 and H_2O in peripheral tissues via some other way, which could be either the ketogenic or the gluconeogenic pathway, or both.

Fatty acid profiles of plasma free fatty acids and triglycerides

As outlined above, there is evidence which suggests that the linoleic acid component of plasma free fatty acids after being taken up by the liver is channelled away from the route of esterification and enters the pathway of β-oxidation. This could imply that in the fasting state plasma triglycerides are relatively poor in linoleic acid when compared to the pool of free fatty acids. In fact, published data indicate otherwise. The relative percentage of linoleic acid appears to be higher in the triglycerides than in the free fatty acids of fasting human plasma [25–27]. It could be argued that, even in the fasting state, the fatty acid composition of triglycerides circulating in the plasma is not determined solely by the fatty acid composition of the triglycerides secreted by the liver. Nevertheless, our hypothesis is not supported by comparing the fatty acid profiles of plasma free fatty acids and triglycerides.

Conclusion

We propose that polyunsaturated fatty acids (as compared to saturated fatty acids) are converted by the liver into ketone bodies rather than being incorporated into triglycerides and exported as VLDL, and then secreted into the bloodstream. Since cholesterol is required as a structural component of the VLDL surface, polyunsaturated fatty acids would also decrease VLDL cholesterol output by the liver. The cholesterol esters in the VLDL core are probably acquired from high-density lipoproteins in plasma, in exchange for VLDL triglycerides [24]. A decrease in the flux of VLDL from the liver would also lower the amount of cholesterol esters acquired by VLDL, which in turn would lower the flux of cholesterol esters into the LDL pool. Thus, the carbons of polyunsaturated fatty acids are transported to muscle in the form of ketone bodies, unlike saturated fatty acids, which are transferred from the liver to the periphery as VLDL triglycerides, and in the process leave a trail of LDL. Obviously, our hypothesis needs to be tested in well-controlled experiments, preferably with human subjects.

Literature

[1] *P. J. Neste, N. Havenstein, T. W. Scott* and *L. J. Cook*, Aust. N. Z. J. Med. **4**, 497 [1974].
[2] *N. Spritz* and *M. A. Mishkel*, J. Clin. Invest. **48**, 78 [1969].
[3] *P. D. S. Wood, Y. L. Lee* and *L. W. Kinsell*, Fed. Proc. **26**, 261 [1967].
[4] *A. C. Beynen* and *M. B. Katan*, Am. J. Clin. Nutr. **42**, 560 [1985].
[5] *E. H. Ahrens Jr., J. Hirsch, W. Insull, T. T. Tsaltas, R. Blomstrand* and *M. L. Peterson*, Lancet **1**, 943 [1957].
[6] *P. J. Nestel* and *P. Barter*, Clin. Sci. **40**, 345 [1971].
[7] *A. Chait, A. Onitri, A. Nicoll, E. Rabaya, J. Davies* and *B. Lewis*, Atherosclerosis **20**, 347 [1974].
[8] *H. G. Wilcox, G. D. Dunn* and *M. Heimberg*, Biochim. Biophys. Acta **398**, 39 [1975].
[9] *M. Kohout, B. Kohoutova* and *M. Heimberg*, J. Biol. Chem. **246**, 5067 [1971].
[10] *C. Cortese, Y. Levy, E. D. Janus, P. R. Turner, S. N. Rao, N. E. Miller* and *B. Lewis*, Eur. J. Clin. Invest. **13**, 79 [1983].
[11] *J. D. Turner, N.-A. Le* and *W. V. Brown*, Am. J. Physiol. **241**, E57 [1981].
[12] *J. Shepherd, C. J. Packard, S. M. Grundy, D. Yeshurun, A. M. Gotto Jr.* and *O. D. Taunton*, J. Lipid Res. **21**, 91 [1980].
[13] *S. Dayton, S. Hashimoto, W. Dixon* and *M. L. Pearce*, J. Lipid Res. **7**, 103 [1966].
[14] *P. J. Nestel* and *D. Steinberg*, J. Lipid Res. **4**, 461 [1963].
[15] *W. Schrade, R. Biegler* and *E. Böhle*, Fette · Seifen · Anstrichmittel **61**, 10 [1959].
[16] *E. Böhle*, Münch. Med. Wschr. **106**, 1165 [1964].
[17] *D. M. Bramankar* and *M. C. Nath*, Proc. Soc. Exp. Biol. Med. **112**, 670 [1963].
[18] *L. H. Chung* and *J. Dupont*, Lipids **3**, 545 [1968].
[19] *D. Kritchevsky, S. A. Tepper, G. Bises* and *D. M. Klurfeld*, Nutr. Res. **3**, 229 [1983].
[20] *A. C. Beynen* and *M. B. Katan*, Nutr. Rep. Int. **29**, 1369 [1984].
[21] *R. B. Alfin-Slater*, J. Dairy Sci. **50**, 781 [1967].
[22] *B. C. O'Brien, C. L. Skutches, G. R. Henderson* and *R. Reiser*, J. Nutr. **107**, 1444 [1977].
[23] *P. Budowski*, Am. J. Clin. Nutr. **44**, 155 [1986].
[24] *T. Chajek* and *C. J. Fielding*, Proc. Natl. Acad. Sci. USA **75**, 3445 [1978].
[25] *D. S. Goodman* and *T. Shiratori*, J. Lipid Res. **5**, 307 [1964].
[26] *T. O. von Lossonczy, A. Ruiter, H. C. Bronsgeest-Schoute, C. M. van Gent* and *R. J. J. Hermus*, Am. J. Clin. Nutr. **31**, 1340 [1978].
[27] *J. Jurand* and *M. F. Oliver*, Atherosclerosis **11**, 125 [1970].

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