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THE EFFECTS OF CHOLESTEROL FEEDING ON THE MEMBRANES OF LIVER CELLS AND ON THE CHOLESTEROL METABOLISM IN THE RAT

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Abstract—1. Feeding of rats with a 2% cholesterol diet for 6 weeks increased the serum cholesterol concentration. The activity of lecithin cholesterol acyltransferase was also increased during the feeding time.

2. The activities of aspartate aminotransferase and alanine aminotransferase remained on a constant level during the experiment on rats having cholesterol in their diet. Omitting cholesterol from the diet enhanced the activities of both enzymes and the increase in alanine aminotransferase activity was more pronounced.

3. The activity of alkaline phosphatase was on higher level during the whole experiment in the rats having cholesterol in the diet than in those fed a cholesterol-free diet.

4. Present data suggest that excluding cholesterol from the diet labilizes the membranes of hepatocytes and facilitates the release of aspartate and alanine aminotransferases in the blood.

INTRODUCTION

Cholesterol is a precursor for the biosynthesis of numerous hormones and bile acids. It is also an integral component of membrane structure (Singer & Nicholson, 1972; Masoro, 1977; Lodish & Rothman, 1979). Part of cholesterol is from dietary sources and part of it synthesized in the liver and intestinal cells. Even though cholesterol turnover is well balanced in the body, large amounts of exogenous cholesterol are still able to exceed the regulatory capacity. Dietary cholesterol and other lipids have been shown to markedly affect the metabolism of foreign compounds in the liver (Paine & McLean, 1973; Tsai & Dyer, 1973; Hietanen *et al.*, 1975; Laitinen, 1976; Lang *et al.*, 1976; Hietanen *et al.*, 1978), and this is supposed to be mediated via modification of the structure of endoplasmic reticulum (Papahadjopoulos *et al.*, 1973; Laitinen *et al.*, 1975; Laitinen, 1976).

The present study was made in order to discover the effects of dietary cholesterol on the activities of aspartate aminotransferase (ASAT, EC 2.6.1.1), alanine aminotransferase (ALAT, EC 2.6.1.2) and alkaline phosphatase (ALP, EC 3.1.3.1), enzymes which are commonly used to monitor the condition of liver. ASAT and ALAT are known to reflect the membrane destruction of hepatocytes and ALP mainly monitors the possibility of biliary obstruction. We also analyzed the effect of dietary cholesterol on the cholesterol and high density lipoprotein (HDL-) cholesterol concentrations and on the activity of lecithin cholesterol acyltransferase (LCAT, EC 2.3.1.43), which is one of the key enzymes in cholesterol metabolism. The LCAT enzyme catalyzes the transport of fatty acids from lecithin to cholesterol facilitating the production of cholesterol esters in HDL biosynthesis (Glomset & Norum, 1973).

MATERIALS AND METHODS

The experimental animals

Forty adult male rats (*Rattus norvegicus*) weighing 350–480 g were used. The animals were balanced for 2 weeks with a cholesterol-free diet (ICN Nutritional Biochemical Company, Cleveland, Ohio, U.S.A.). After this period the animals were divided into 8 experimental groups and 4 groups were started on a 2% cholesterol diet (ICN); the remaining 4 groups continued on a cholesterol-free diet. After rats had been fed the cholesterol diet for 2 days, 1, 2 or 6 weeks the blood samples were taken, simultaneously with decapitation, from groups fed cholesterol-free and 2% cholesterol diets.

Preparation of blood samples

After sampling the blood was allowed to clot and the serum was separated by centrifugation at 2100 *g* for 20 min. The sera were removed into clean plastic tubes and were placed immediately at −70°C until analyzed.

Analytical procedures

The cholesterol concentration was analyzed enzymatically using cholesterol oxide reaction (Boehringer Mannheim GmbH., Mannheim, West Germany). The HDL-cholesterol was analyzed as above after removing low density and very low density lipoprotein fractions with precipitation by adding into 200 μl of serum 10 μl 2.0 mol/l $MgCl_2$ and 10 μl dextranesulphate solution (20 g/l, mol. wt 500,000). The analysis were carried out with IL Multistat III Microcentrifugal analyzer (Instrumentation Laboratory Inc., Lexington, U.S.A.).

The activities of aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase were analyzed

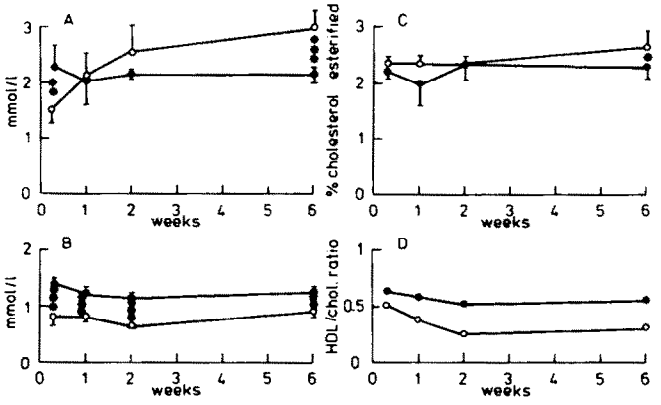

Fig. 1. Figure 1A represents the cholesterol concentration of the serum after feeding rats cholesterol-free and 2% cholesterol diets. The solid circles represent groups having the cholesterol-free diet and the open circles represent those having 2% cholesterol diet. Figure 1B represents HDL-cholesterol concentration, 1C represents the percentage of cholesterol esterified in the reaction mixture by LCAT enzyme and Fig. 1D expresses the HDL-cholesterol/cholesterol ratios. Means and SEs of the means are expressed. The statistical significance of the results was calculated by Student's t-test: $*2P < 0.05$. $**2P < 0.01$. $***2P < 0.001$.

mainly as recommended by The Scandinavian Society for Clinical Chemistry (1974). Because the analyses were carried out in the microlitre range with IL Multistat III Microcentrifugal analyzer a slight modification of the technique had to be made. The sera were preincubated in a higher buffer concentration and at the beginning of the analysis the buffer concentration was adjusted to the one in the recommended method. This modification did not affect the obtained enzyme activity, which was confirmed by comparing the values to those found by an OLLI 3000 Analyzer (Kone Ltd, Espoo, Finland) and with methods according to the Scandinavian Recommendation (1974).

The LCAT activity was determined according to Alcindor *et al.* (1977), after delipoproteinization of the serum samples with Intralipid®, dextran sulphate and calcium chloride. The substrate was prepared from pooled human serum. In this pooled serum the LCAT activity was inhibited by heating and the equilibration of tritiated cholesterol to HDL fraction was obtained by overnight incubation at +5° C.

RESULTS

The serum cholesterol concentration increased markedly during 2% cholesterol feeding, being 36% higher after 6 weeks (Fig. 1A). The HDL-cholesterol concentration was already lower after 2 days feeding with cholesterol and it remained markedly lower during the whole 6-week experimental period than in those fed the cholesterol-free diet (Fig. 1B). When the HDL-cholesterol/cholesterol ratios were calculated the proportion of HDL-cholesterol from the total cholesterol decreased for a 2-week period and then remained on a stabilized level (Fig. 1D). The lecithin cholesterol acyltransferase activity did not change during the first 2 weeks, but after 6 weeks the activity was on a higher level in the cholesterol-fed animals than in cholesterol-free groups. The ASAT, ALAT and ALP activities were analyzed after feeding animals with a cholesterol diet for 1, 2 and 6 weeks. The ASAT activity was approximately the same during the first 2 weeks, but later on during the experiment the activity increased by about 75% in the rats having the cholesterol-free diet (Fig. 2A). The ALAT activity was already on a higher level after 1 week feeding in the cholesterol-free group when compared to the group having 2% cholesterol in the diet (Fig. 1B). During the experiment the ALAT activity increased very markedly, being almost twice as high at the end as in the beginning, in the animals having the cholesterol-free

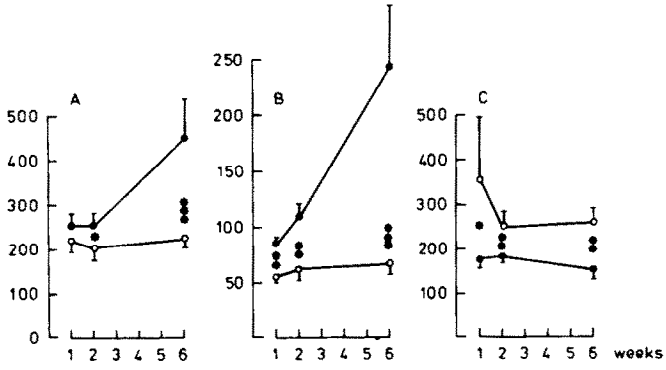

Fig. 2. Figure 2A represents the ASAT activity in sera of rats having cholesterol-free and 2% cholesterol diets. Figure 2B represents the ALAT activity and Fig. 2C represents the ALP activity. The unit for ASAT and ALAT is μmol NADH consumed in 1 min and the unit for ALP is μmol p-nitrophenylphosphate hydrolyzed in 1 min. For other explanations see legend to Fig. 1.

diet. In the animals having 2% cholesterol in their diet the activity remained on a same level during the whole experiment (Fig. 2B). The alkaline phosphatase activity was higher already after 1 week feeding with cholesterol (Fig. 2C). This difference was found during the whole 6-week experimental period.

DISCUSSION

The effective cholesterol feeding increased cholesterol concentration in the blood. Possibly, rats tried to eliminate excessive cholesterol by increasing acyl ester formation. Even though the difference at the beginning of the experiment was such that animals having the cholesterol-free diet showed higher serum cholesterol concentration, the cholesterol concentration at the end of the experiment was more than one-third higher in the cholesterol-fed group. The excess cholesterol was transported in the blood into lipoprotein fractions other than high density lipoprotein, which is known to remove cholesterol from peripheral tissues. This was very clear when HDL-cholesterol values were compared to the total cholesterol concentration.

The important role of cholesterol for the hepatic metabolism has been shown earlier by ourselves and others when the role of cholesterol for the metabolic activities of hepatic endoplasmic reticulum were analyzed (Papahadjopoulos *et al.*, 1973; Hietanen *et al.*, 1975; Laitinen, 1976; Lang *et al.*, 1976). Also it has been shown that changes in the metabolic activity of endoplasmic reticulum are mediated via changes in the interactions between membrane components (Laitinen *et al.*, 1975; Laitinen, 1976; Laitinen, 1977; Land, 1978). In the present study we analyzed ASAT and ALAT activities, in order to find out the possibility of these membrane effects in the activity of enzymes, which are commonly known to express destruction of the hepatocytes. The activity of alkaline phosphatase is also known to react on hepatobiliary disease, especially on biliary obstruction.

In our earlier studies we found that cholesterol in some way increases the metabolic activity of hepatic endoplasmic reticulum (Laitinen, 1976; Lang *et al.*, 1976; Hietanen *et al.*, 1978). In the present study it is obvious that feeding of animals with a cholesterol-free diet for a long time makes the membranes of hepatocytes labile and the release of marker enzymes is increased (Fig. 2). The ALAT activity is known to be a more sensitive parameter in testing the condition of liver, and this was also true in this study (Figs 2A & B). However, the increase of cholesterol in the diet prevents the lesion of hepatocytes.

On the basis of present results the dietary cholesterol has an important role in modifying membrane structure of hepatocytes. The cholesterol feeding increases serum cholesterol level and catabolism. The excess cholesterol is carried in other lipoprotein fractions rather than high density lipoproteins.

REFERENCES

Alcindor L. G., Melin B., Benhmou G. & Piot M. C. (1977) Dosage de la lecithine-cholesterol acyltransferase. Interet de la precipitation des -lipoproteins par le sulfate de dextrane. *Clinica chim. Acta* **81,** 177–182.

Glomset J. A. & Norum K. R. (1973) The metabolic role of lecithin: cholesterol acyltransferase: perspectives from pathology. *Adv. Lipid Res.* **11,** 1–65.

Hietanen E., Laitinen M., Vainio H. & Hänninen O. (1975) Dietary fats and properties of endoplasmic reticulum: II. Dietary lipid induced changes in activities of drug metabolizing enzymes in liver and duodenum of rat. *Lipids* **10,** 467–472.

Hietanen E., Hänninen O., Laitinen M. & Lang M. (1978) Dietary cholesterol induced enhancement of hepatic biotransformation rate in male rats. *Pharmacology* **17,** 163–172.

Laitinen M. (1976) Enhancement of hepatic drug metabolism with dietary cholesterol in the rat. *Acta pharmac. tox.* **39,** 241–249.

Laitinen M. (1978) Dietary lipids on the control of drug metabolizing activity in the rat. Academic dissertation, University of Kuopio, Finland.

Laitinen M., Hietanen E., Vainio H. & Hänninen O. (1975) Dietary fats and properties of endoplasmic reticulum—I. Dietary lipid induced changes in the composition of microsomal membranes in the liver and gastroduodenal mucosa of rat. *Lipids* **10,** 461–466.

Lang M. (1978) Interaction of fluorescent probes with rat liver microsomes and drug metabolizing enzymes. Academic dissertation, University of Kuopio, Finland.

Lang M., Laitinen M., Hietanen E. & Vainio H. (1976) Modification of microsomal membrane components and induction of hepatic drug biotransformation in rats on a high cholesterol diet. *Acta pharmac. tox.* **39,** 273–288.

Lodish H. F. & Rothman J. E. (1979) The assembly of all membranes. *Scient. Am.* **1,** 38–53.

Masoro E. J. (1977) Lipids and lipid metabolism. *A. Rev. Physiol.* **39,** 301–321.

Paine A. J. & McLean A. E. M. (1973) The effect of dietary protein and fat on the activity of aryl hydrocarbon hydroxylase in rat liver, kidney and lung. *Biochem. Pharmac.* **22,** 2875–2880.

Papahadjopoulos D., Cowden M. & Kimleberg H. (1975) Role of cholesterol in membranes. Effects of phospholipid–protein interactions, membrane permeability and enzymatic activity. *Biochim. biophys. Acta* **330,** 8–26.

Singer S. J. & Nicholson C. L. (1972) The fluid mosaic model of the structure of cell membranes. *Science* **175,** 720–731.

The Committee on Enzymes of the Scandinavian Society for Clinical Chemistry and Clinical Physiology (1974) Recommended methods for the determination of four enzymes in blood. *Scand. J. clin. Lab. Invest.* **33,** 291–306.

Tsai A. C. & Dyer I. A. (1973) Liver cholesterol concentration in relation to hepatic cholesterogenesis and activity of certain liver enzymes in rats. *J. Nutr.* **103,** 1119–1125.