Physiol. Res. 41: 263–266, 1992

High Sensitivity of PHHC Rat to Dietary Cholesterol

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Received January 24, 1992
Accepted April 29, 1992

Summary

The Prague Hereditary Hypercholesterolaemic (PHHC) rat is a strain of the Wistar rat very sensitive to dietary cholesterol.The dynamics of changes in serum and liver lipids and lecithin : cholesterol acyltransferase (LCAT) were studied immediatelly after the switch to a high cholesterol diet. Immediate cumulation of free and esterified cholesterol in the liver after the increase in alimentary cholesterol intake is supposed to be the regulating step leading to a subsequent increase in serum cholesterol concentration. Activity of LCAT was negatively correlated to the concentration of free cholesterol in the liver, very early after the cholesterol diet was introduced, a possibility of a down regulation of enzyme synthesis similarly to the regulation of synthesis of cholesterol in hepatocytes was observed.

Key words

Rat – Cholesterol metabolism – Hypercholesterolaemia

Introduction

The strong correlation between elevated plasma cholesterol levels and coronary heart disease has led to several metabolic studies that have related plasma cholesterol levels to dietary cholesterol and the type of dietary fat (Keys *et al.* 1957, 1965).

Some animal models and experimental designs have been used to study experimental diet-induced hypercholesterolaemia (Poledne *et al.* 1989). The Prague Hereditary Hypercholesterolaemic (PHHC) strain was obtained in our laboratory by cross-breeding of Wistar rats (Poledne 1986).

In comparison with Wistar rats, PHHC rats are characterized by their increased basal cholesterolaemia (>2 mmol/l) with a prominent response to dietary cholesterol load.

In the PHHC rat, administration of a diet containing 1-2 % cholesterol for several weeks leads to hypercholesterolaemia (Poledne 1986.).

The gradual increase in dietary cholesterol intake producing an increase in cholesterol concentration in the liver and cholesterolaemia leads to decreased *de novo* cholesterol synthesis in the liver in both PHHC and other Wistar rats. However, the feedback regulation was shifted to a high intracellular concentration in the PHHC rat (Poledne 1986), producing a fivefold rise in cholesterol level compared with the value found in normal rats.

The aim of this study was to follow up the time course of changes in lipoprotein metabolism parametres in the serum and in the liver immediately after a change in dietary choleterol intake.

The activity of lecithin:cholesterol acyl-transferase (LCAT) was measured at the same time using two methods.

Materials and Methods

Animals and diets

Adult female PHHC rats (220–250 g), divided into four groups of 5 animals, were used in this experiment. The first three groups were assigned to dietary cholesterol for one, five and eleven days and the fourth group was taken as a control and received standard rat chow diet (Velaz Prague). The dietary cholesterol mixture used in this study contained a standard diet supplemented by 2 % cholesterol dissolved in 5 % beef tallow. Under the one-day feeding protocol, rats had received the cholesterol diet one day before, early in the morning.In all the groups feeding was finished by midnight and the rats fasted for 8 hours before they were sacrificed by decapitation. Cooled serum and the liver were used for the experiment.

Table 1

Lipid concentrations and cholesterol esterification parameters in the serum

No. of days	0	1	5	11
No. of animals	5	5	5	5
TC mmol/l	2.95 ±0.44	3.58 ±0.52	11.70 ±1.43	12.84 ±2.61
FC mmol/l	0.79 ±0.11	0.74 ±0.08	1.81 ±0.24	2.12 ±0.61
TG mmol/l	1.08 ±0.23	1.05 ±0.40	0.96 ±0.36	0.80 ±0.32
CE	2.15 ±0.33	2.84 ±0.47	9.89 ±0.13	10.72 ±2.00
FER(%/h)	12.11 ±0.57	9.58 ±0.75	4.34 ±1.35	3.63 ±1.97
MER μmol/l	196.6 ±14.05	70.60 ±3.67	77.5 ±3.67	71.4 ±33.4
$LCAT_H$ μmol/l/h	77.9 ±9.04	55.60 ±2.40	65.00 ±3.54	80.30 ±11.37

FC – free cholesterol, TG – triacylglycerol, CE – cholesterol ester, FER – fractional esterification rate, MER – molar esterification rate, LCAT – lecithin : cholesterol acyltransferase.

Total cholesterol (TC) and triacylglycerols (TG) in the serum were determined by enzymatic Bio-Test, LACHEMA cholesterol AM(250) and TG 20 AM kits, respectively. Free cholesterol (FC) was determined using a Boehringer Mannheim kit. TC in the liver was determined by the method of Abel (1955), after extracting total fat by the method of Folch (1957).

The activity of LCAT was determined using the method of autologous substrate (Dobiášová *et al.* 1978). The results were expressed as the fractional esterification rate of cholesterol (FER), i.e. the % of free cholesterol esterified per hour, and the molar esterification rate of cholesterol (MER).

To eliminate the influence of free cholesterol availability for esterification, the method of homologous substrate (Dobiášová *et al.* 1986) was employed. The results obtained using this method (LCAT) reflect the serum enzyme concentrations.

Results

After one day on a cholesterol diet, shifts in serum TC and FC were not significant in comparison with the control group (Tab. 1). After 5 days there was a significant increase in serum TC in rats provided a cholesterol diet ($p<0.001$) in comparison to the control group. A high level of serum FC was observed after five days on the cholesterol diet ($p<0.01$). The values of serum TG have decreased gradually in comparison to the control group up to the end of the experiment,but no significant results were obtained.

Unlike the plasma pool of cholesterol, there was a three fold increase in TC in the liver (Tab. 2), ($p<0.001$), a doubling in FC ($p<0.05$), a three fold increase in TG ($p<0.001$) and a 1.5 fold increase in total fat concentration ($p<0.05$) after only one day of cholesterol diet feeding in comparison with control rats. After five days on cholesterol feeding, TC increased 5 times ($p<0.001$), PC remained at the same level as FC concentration after one day, and TG doubled ($p<0.01$).

After 11 days on a cholesterol diet, total cholesterol in the liver rose 6 times ($p<0.01$), FC 3 times ($p<0.01$), TG nearly 3 times ($p<0.001$) and total fat 2.5 times ($p<0.01$).

As far as cholesterol esterification in the serum is concerned (Tab. 1), FER tended to decline up to day 5 to stabilize thereafter. MER decreased by about a fourth on the first day of the experiment, but there were no other statistically significant changes in MER compared to base line values. LCAT activity measured by the method of homologous substrate

Table 2

Lipids in the liver

No. of days	0	1	5	11
No. of animals	5	5	5	5
TC (mg/g)	6.30 ±1.56	16.66 ±5.10	34.52 ±2.17	38.22 ±3.22
FC (mg/g)	3.43 ±0.22	6.56 ±1.53	5.57 ±1.02	8.04 ±0.48
CE	2.87 ±1.63	10.06 ±4.03	28.75 ±2.13	30.18 ±3.23
TG (mg/g)	13.62 ±4.34	37.52 ±2.59	22.74 ±2.02	30.84 ±1.85
Total fat (mg/g)	50.60 ±8.96	78.80 ±21.44	83.60 ±6.35	121.20 ±20.92
Liver Weight (g)	6.30 ±0.65	5.76 ±0.34	6.64 ±0.38	5.98 ±0.96

Statistical significance of these results was determined using Student's t-test.
TC – total cholesterol, FC – free cholesterol, CE – cholesterol ester.

($LCAT_H$) decreased in the same manner. Later, it gradually increased to reach its previous level at the end of the experiment. A negative correlation between $LCAT_H$ and FC concentration in the liver was found during the first 5 days of the experiment ($r = -0.729$, $p<0.01$), but the correlation was lost when the data from the whole experiment were analysed ($r = -0.111$, $p>0.05$). Correlation was calculated by the program called KORELA.

Discussion

Already after one day feeding of a cholesterol diet, the cholesterol was cumulated in the liver without a significant change of cholesterol concentration in the plasma compartment. The most striking accumulation of dietary cholesterol in the liver after the first day was probably due to the increase in alimentary cholesterol inflow by cholesterol remnant particles (Davignon *et al.* 1988) and the rate of its esterification, as cholesterol ester level in the liver tended to rise faster than that of free cholesterol.

After 5 days on a cholesterol diet, the liver CE level continued to rise, whereby the liver FC concentration remained unchanged to play a key role in the regulation of intracellular cholesterol metabolism.

Besides, the plasma cholesterol level also increased. At the end of the experiment the cholesterolaemia and CE concentration in the liver did not change, and the rise in the liver FC can be explained as a result of adaptation of liver to permanent dietary cholesterol intake.

When analysing the changes in LCAT activity after the dietary cholesterol load, it seems that the decrease in the rate of cholesterol esterification in the serum (MER) could be explained as a result of a decline in enzyme levels ($LCAT_H$). The fast normalization of MER in spite of relatively low enzyme levels may be explained by increased availability of FC for esterification in the sera with elevated cholesterol concentrations.

The negative correlation between $LCAT_H$ and liver FC during the first 5 days of the experiment could indicate that the serum enzyme concentration is, by some, as yet unknown mechanism, regulated by FC concentration in the liver similarly to the regulation of cholesterol synthesis. Considering the short half-life of enzymes in female rats, namely about 7 hours (Soler-Argilaga *et al.* 1977), it is possible, that the dramatic increase in liver FC at the very begining of the experiment would immediately inhibit production of the enzyme in the liver. The loss of the correlation between liver FC and $LCAT_H$ at the end of the experiment could be explained as a result of adaptation of the liver to a permanently high dietary cholesterol intake. Unfortunately, our results are very limited in terms of experiments performed, and the hypothesis that LCAT concentration and activity are regulated by intracellular FC concentration remains to be verified in future research.

We conclude that PHHC rats are very sensitive to dietary cholesterol and the regulation of cholesterol metabolism appeared after only one day of feeding of a high cholesterol diet. Dietary cholesterol is cumulated immediately in the liver and consequently, increased cholesterolaemia appeared.

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