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The effect of cholesterol-enriched diet on the activity of some lysosomal enzymes in the liver and blood plasma of rabbits

Summary

White New Zealand male rabbits (n = 12) were fed by high-cholesterol diet for 7 weeks. The activity of some lysosomal enzymes in blood plasma and in the liver was determined. The cholesterol-enriched diet resulted a significant of AP, LL, BGAL and BGLU activity up increase in the blood plasma to 131%, 186%, 308% and 184% as compared with those in control group of animals (n = 10). In the liver a significant increase of EL activity to 189%, NAG to 172% and BGAL to 196% was observed. The activity of LAP and LL decreased significantly to 76% and 60% of the control level, respectively.

Key Words: lysosomal enzymes, cholesterol, rabbits

Zusammenfassung

Titel der Arbeit: **Einfluss der mit Cholesterin ergänzten Futtermitteln auf die Aktivität von einigen lysosomalen Enzymen in der Kaninchenleber**

Die männlichen Kaninchen der Rasse Weisse Neuseeländer wurden 7 Wochen lang mit einer Cholesterinfuttermitteln gefüttert. Die Aktivität von folgenden lysosomalen Enzymen im Blutplasma und in der Leber wurde bestimmt: säure Phosphatase (AP), Alanin-Aminopeptidase (AAP), Leuzin-Aminopeptidase (LAP), β -D-Glukouronidase (BGRD), β -D-Galaktosidase (BGAL), β -D-Glukosidase (BGLU), N-azetyl- β -D-Gluukoaminidase (NAG), lysosomale Lipase (LL) und lysosomale Esterase (EL). Die Fütterung mit Cholesterin bewirkte eine signifikante Erhöhung der Aktivität von AP, LL, BGAL und BGLU im Blutspiegel und eine Abnahme der Aktivität von EL und NAG im Vergleich mit der Kontrolltiergruppe. In der Leber der Kaninchen, die mit Cholesterin-Futter gefüttert wurden, konnte eine Abnahme der Aktivität von LAP und LL beobachtet werden.

Schlüsselwörter: Lysosomale Enzyme, Cholesterin, Kaninchen

Cholesterol-enriched diets increase cholesterol level in body tissues, first of all in the liver (JONNALAGADA et al., 1993; KONECKA et al., 1996) and may cause pathological changes in the liver structure. Cholesterol-fed animals display fibrosis and strongly enlarged gray-white coloured liver (BEYNEN et al., 1986; KONECKA and JEZERSKI, 1997; SIMONSEN et al., 1995) have found that prolonged intake of large amounts of dietary cholesterol had a significant effect on metabolic processes in the liver of rabbits. A decrease of phosphoglucomutase, phospho-fructokinase, pyruvate kinase and lactate dehydrogenase activity was observed.

The lysosomes are organelles within the cytoplasm of eukariotic cells and a main site of protein, lipoprotein and glycoprotein degradation by intralysosomal hydrolases (GLAUMANN, 1984). It seems interesting to estimate the changes of the activity of some model typical lysosomal enzymes in the liver and blood plasma of rabbits in response to a high-cholesterol diet.

Material and Methods

The experiment was carried out on White New Zealand male rabbits maintained at the Institute of Genetics and Animal Breeding, Polish Academy of Sciences in Jastrzębiec. Twenty three male rabbits were used. The animals were kept in individual wire cages (60 x 60 x 32 cm) in a ventilated room under a natural photoperiod at 14⁰-18⁰ C.

The rabbits were 3 months old and average body weight was 3.65 ± 0.02 kg at the beginning of the experiment. The experiment lasted 7 weeks. During that time, the experimental rabbits (n = 12) were offered 150 g of standard feed daily (18% crude protein, 4.2% bone-meat meal, 13% crude fiber) mixed with one egg yolk and 1.5 g (1%) cholesterol (pure in substantia-SIGMA) except for Saturday and Sunday when they received standard feed only. The egg yolk was used as a binding agent for pure cholesterol in substantia in order to glue the pulverized cholesterol to the pelted standard feed and to distribute it evenly in the feed. Control animals (n = 10) received the same amount of feed without supplementary cholesterol and egg yolk. Water was available ad libitum. Cholesterol content in the diet for the control group constituted 98 ± 7.2 (SEM) mg/ 100 g dry feed. Both control and experimental rabbits were offered 7350 g feed during the experimental period. The unconsumed feed was weighed every day. Thus the actual amount of feed consumed during the experimental period was 6872 g ± 158 in control and 6681 g ± 208 in experimental group. Since the experimental group on Saturday and Sunday received standard feed without any supplementary cholesterol and egg yolk, the total calculated average amount of cholesterol during the experimental period was 6.7 g in control and 63.4 g in experimental group.

At the end of the experiment, blood samples from ear veins were collected into heparinized tubes. Before blood sampling, the rabbits fasted during 24 hrs. Blood samples were centrifuged at 700g for 15 min. at 0-4⁰ C. Next the animals of both groups were slaughtered through interrupting of spinal cord. Slices of the liver were minced and immersed in 0.1 M. phosphate buffer pH 7.0, at 4⁰ C, at ratios of 1 g of tissue/ 6 ml of buffer, homogenized in a teflon homogenizer at 200 rotations/min. The homogenates were centrifuged 8 min. at 700 g at 0-4⁰ C. A precipitate was discarded and the supernatant was centrifuged again at 10.000 g for 20 min. using a Servall centrifuge. The precipitate was dissolved in 5 ml 0.1 M phosphate buffer pH 6.0 with 0.1% Triton X-100 and centrifuged at 700 g for 3 min. at +4⁰ C. Supernatant was subjected for determination of the activities of lysosomal enzymes.

In blood plasma and in the liver homogenates the activity of the following enzymes was determined: acid phosphatase (AP, EC 1.1.3.2); alanine aminopeptidase (AAP, EC 3.4.1.2); leucine aminopeptidase (LAP, EC 3.4.1.1); β-D-glucuronidase (BGRD, EC 3.2.2.31); β-D-galactosidase (BGAL, EC 3.2.1.23); β-D-glucosidase (BGAL, EC 3.2.1.21); N-acetyl-β-D-glucosaminidase (NAG, EC 3.2.1.30); lysosomal lipase (LL, EC 3.1.1.3) and lysosomal esterase (EL, EC 3.1.1.2).

The activity of BGRD, NAG, BGAL, BGLU and AP was determined spectrophotometrically as 4-nitrophenyl derivatives at 420 nm according to BARRETT'S micromethod (1977). The activity of LAP and AAP was determined spectrophotometrically as Fast Blue BB salt (4-benzoylamino-2, 5-diethoxybenzenediazinium chloride) derivatives at 540 nm according to the method of PFLEIDERER and CELLIERS (1963) and PFLEIDERER et al. (1964) for leucine and alanine aminopeptidases, respectively. The activity of LL was determined

spectrophotometrically at 530 nm and EL at 405 nm according to the method of MAIN (1960). Spectrophotometer Spectronic 601 was used. The protein concentration in blood plasma was determined according to the "biuret" method (KRAWCZYŃSKI and OSIŃSKI, 1967), in the liver homogenates by LOWRY'S method (LOWRY et al., 1951). The activity of lysosomal hydrolases was expressed in nmol/mg of protein/hour. Substrates for enzyme and protein determinations were purchased from SIGMA.

The results obtained were analysed by one-way analysis of variance (ANOVA) with feeding regime as a factor.

This experiment has been realized according to the permit issue of the Institute Ethic Commission for Animal Research.

Results

The activity of AP, LL, BGAL and BGLU in blood plasma increased significantly in the rabbits fed cholesterol-enriched diet up to 131.3%, 186.0%, 308.1% and 184.2% of control value, respectively. On the contrary, the activity of EL, NAG and BGRD in blood plasma decreased to 47.0%, 71.6% and 78.7% of the control level (Table 1).

Table 1

The activity of lysosomal enzymes in blood plasma of male rabbits fed cholesterol enriched diet (LSMean \pm SEM, nmol/mg protein/hour)

Enzyme	Control group	Experimen. group	% of control	P
AAP	16.7 \pm 0.86	15.2 \pm 0.87	91	NS
LAP	28.4 \pm 0.92	24.9 \pm 2.09	88	NS
AP	37.9 \pm 4.10	49.7 \pm 3.23	131	***
LL	2.8 \pm 0.09	5.2 \pm 0.40	186	***
EL	4.2 \pm 0.24	1.9 \pm 0.45	47	***
BGRD	3.6 \pm 0.24	2.8 \pm 0.26	79	NS
NAGL	4.4 \pm 0.05	3.2 \pm 0.32	72	***
BGAL	0.4 \pm 0.03	1.1 \pm 0.15	308	***
BGLU	0.2 \pm 0.01	0.4 \pm 0.13	184	***

***p < 0.001; **p < 0.01; NS – non-significant

Table 2

The activity of lysosomal enzymes in the liver of male rabbits fed cholesterol-enriched diet (LSMean \pm SEM, nmol/mg protein/hour)

Enzyme	Control group	Experimen. group	% of control	P
AAP	126.6 \pm 6.92	106.1 \pm 13.83	84	NS
LAP	79.9 \pm 4.49	60.7 \pm 7.52	76	**
AP	945.6 \pm 54.60	945.7 \pm 73.72	100	NS
LL	409.3 \pm 32.10	248.3 \pm 13.82	61	***
EL ^(§)	2.3 \pm 0.37	4.4 \pm 0.36	189	***
BGRD	435.4 \pm 44.08	497.6 \pm 43.25	114	NS
NAGL	290.7 \pm 36.21	501.2 \pm 46.97	172	***
BGAL	9.1 \pm 0.45	17.8 \pm 1.94	196	***
BGLU	415.5 \pm 118.39	437.5 \pm 87.11	106	NS

***p < 0.001; **p < 0.01; NS – non-significant; (§) – activity of EL was expressed as μ mol/mg/hour;

Feeding with the cholesterol-enriched diet increased significantly of BGAL, NAG and EL activity in the liver to 196.4%, 172.4%, 189.3% of the control value whereas the

activity of LAP and LL decreased significantly to 76.0% and 60.6%, respectively (Table 2).

Discussion

In our previous study (JEZIERSKI and KONECKA, 1994) we found that a three-fold increase of cholesterol consumption resulting from mixing standard feed with egg yolk only, without free cholesterol in substantia, had no effect on the total plasma cholesterol level in the rabbits and didn't induce the visible atherosclerosis. Therefore in the next experiment we applied cholesterol-enriched diet where the main source of cholesterol was pulverized cholesterol in substantia. This type of diet has increased five - fold the plasma cholesterol and three - fold the liver cholesterol level and induced the marked atherosclerosis as well as has reduced the activity of some glycolytic enzymes (KONECKA et al., 1996; KONECKA and JEZIERSKI, 1997). In the present studies we have applied the latter type of diet to study the effect on lysosomal enzymes in the liver and blood plasma.

We did not find any reports concerning the effects of high-cholesterol diet on the activity of the lysosomal enzymes. It is known, for example, that the liver lysosomal lipase (LL) plays an important role in lipoprotein homeostasis (AVIRAM et al., 1988; SHINOMIYA et al., 1982). The cholesterol diet can modify its activity in the liver and blood plasma (CLAY et al., 1989). We have found that cholesterol diet increased significantly the LL activity in blood plasma of rabbits. Our results are consistent with the results of EBERT et al. (1993) who found a rapid increase of lipase activity in the blood plasma in cholesterol-fed rabbits. On the contrary to their data and the results of WARREN et al. (1991), in our experiment the activity of LL in the liver decreased significantly. HANDA et al. (1994) observed a similar effect of cholesterol diet on lipolysis in the rat liver. On the other hand, a longer time (7 weeks) of high-cholesterol diet in the present experiment compared to 4 weeks in the studies of WARREN et al. (1991) may be related with reduction of the lipase activity in the liver of rabbits.

It is possible that cholesterol may act as lysosomotropic agent. Our experiment has shown that long-term cholesterol diet may influence significantly on the activity of estimated lysosomal hydrolases in the liver and blood plasma of rabbits. These changes were selective, i.e. not all hydrolases reacted similarly. While the consumption of the cholesterol-enriched diet did not exert in the liver the marked effects on the activity of AAP, AP, BGRD and BGLU an increase of the activity of EL, NAG and BGAL and a decrease of LL and LAP activity was observed. It may be suggested that feeding high-cholesterol diet causes an intralysosomal destroy in the hepatocytes presumably by labilization of membrane compartment.

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