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Influence of cholestyramine and sunflower oil on the content of plasma bile salts and lipoprotein composition in alloxan induced diabetes in rabbits

Wpływ cholestyraminy i oleju słonecznikowego na zawartość soli żółciowych i skład lipoprotein w osoczu krwi królików z cukrzycą alloksanową

K e y w o r d s: alloxan-induced diabetes, lipoproteins, cholestyramine, bile salts, triacylglycerides, cholesterol.
S ł o w a k l u c z o w e: cukrzyca alloksanowa, lipoproteiny, cholestyramina, sole żółciowe, trójglicerydy, cholesterol.

INTRODUCTION

Diabetes mellitus has a detrimental effect on lipid metabolism and plasma lipoproteins (Lp) (3, 7, 15). These alterations are a consequence of a deregulation of adipoinsular axis in which a dual hormonal, insulin and leptin, feedback loop is involved. Insulin is adipogenic and stimulates secretion of leptin, which in turn increases energy expenditure and suppresses insulin secretion. Hyperlipidemia caused by an increase of cholesterol (CH) and triacylgliceroles (TG) has been found in untreated or poorly controlled diabetic state (23). The hypertriacylglyceridemia of diabetes has been shown to be attributed to a reduction in the clearance rate of plasma TG which was associated with impaired lipoprotein lipase activity caused by a deficiency in insulin action. As a result of diabetes, at least in some cases decreased fatty acid oxidation may occur (19). Diabetes is thought to be associated with cholestasis, with alteration in the profile of synthesised bile salts which involves increases in cholic acid and a reduction of chenodeoxycholic acid synthesis, with increased bile acid pool size (7). The glycine-taurine ratio of the conjugated bile acids is increased in the diabetes as the percentage concentration of secondary bile acids (deoxycholic and lithocholic acids). Cholestyramine (Cy) effectively captures bile salts in the intestine and prevents their normal entherohepatic circulation (6, 8, 12) leading to lower levels of plasma cholesterol. Exaggerated bile acid synthesis rate in the liver under the influence of Cy may change the capture of lipoprotein remnants from the plasma. Because

a high fat diet reduces insulin sensitivity in peripheral cells, we hypothesised that it also suppresses beneficial effects of Cy on plasma lipid content in diabetic rabbits.

The purpose of this study was to examine the influence of cholestyramine along or with addition of sunflower oil on plasma lipid response of diabetic rabbits. We compared the composition of lipoprotein (Lp) fractions and their CH and TG contents in both healthy control rabbits with those consuming Cy alone or with sunflower oil and in diabetic control rabbits with those diabetic rabbits consuming Cy alone or Cy with sunflower oil (SFO) as well. To assess the influence of Cy on plasma bile salts (B.S.) content in normal and diabetic rabbits we also analysed total bile salts in the plasma of these animals.

MATERIAL AND METHODS

Experimental procedure. Experiments were performed on 24 (10 female, 14 male) New Zealand white rabbits weighing 2.5-3.0 kg. The experiment was divided into three separate periods: during the first 7 day's period the animals were adapted to the standard diet. Before the proper experiments, the animals were divided into two groups: one healthy and the other with induced diabetes mellitus after 48 h from intravenous injection of alloxan (120 mg/kg b.w.). Induction of diabetes mellitus was checked by the measurement of the plasma level of glucose. In the second period, which lasted also 7 days, the animals of both groups were fed *ad libitum* on three different diets as follows: standard diet – group I, standard diet plus 5% (wt/wt) Cy – group II, standard diet plus 5% (wt/wt) Cy plus 5% (wt/wt) sunflower oil – group III. At the end of this period, the first bleeding was performed. During the third period, which also lasted 7 days, all groups consumed the same experimental diets and then the second bleeding was performed.

Analytical methods. Plasma lipoproteins were sequentially isolated by ultracentrifugation from 1 ml plasma at the density limits of 1.006, 1.019, 1.063 and 1.21 g/ml using UP-65 centrifuge (42,000 rpm, 4°C, 24 h)(1, 10) Into some samples Sudan Black B was added to visualised VLDL, LDL, HDL fraction. Triacylglycerides and cholesterol in the plasma and in the fractions of lipoproteins were measured by reagent kits (Boehringer Mannheim GmbH).

Total bile acid concentration in the plasma was determined with an enzymatic kit (Enzabile; Nycomed Pharma, Oslo, Norway) (2, 24).

RESULTS

The picture of lipoprotein in the rabbit exhibits clearly defined three fractions of which the VLDL fraction was homogeneous but the LDL and HDL were heterogeneous (Fig. 1). A lighter fraction of LDL in the rabbit comprises two separate bands including LDL_1 and LDL_2. In the group treated with Cy both fractions of VLDL and LDL_s are reduced and turn into heavier particles (Fig. 1). In the case of diabetic rabbits the fractions of LDL_1 became so light that it was impossible to separate them from VLDL fraction. Also HDL_2 fraction was appreciably lighter than those in normal animals (Fig. 1).

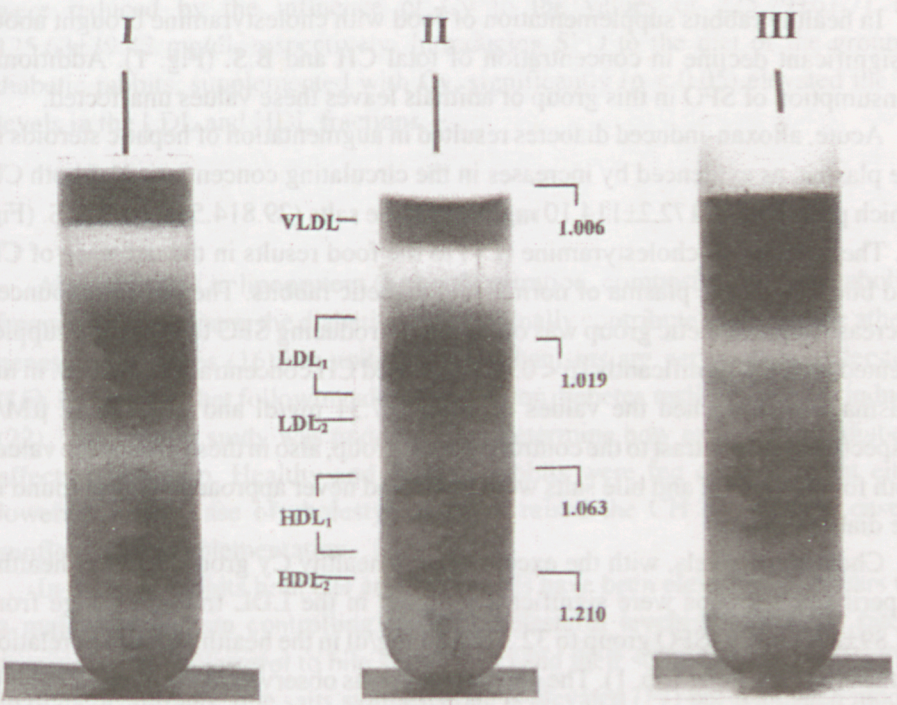

Fig. 1. Lipoprotein fractions in rabbits: I – healthy, II – healthy + Cy + SFO, III – DM + Cy + SFO (1.006 – 1.21 values of KBr gradients)

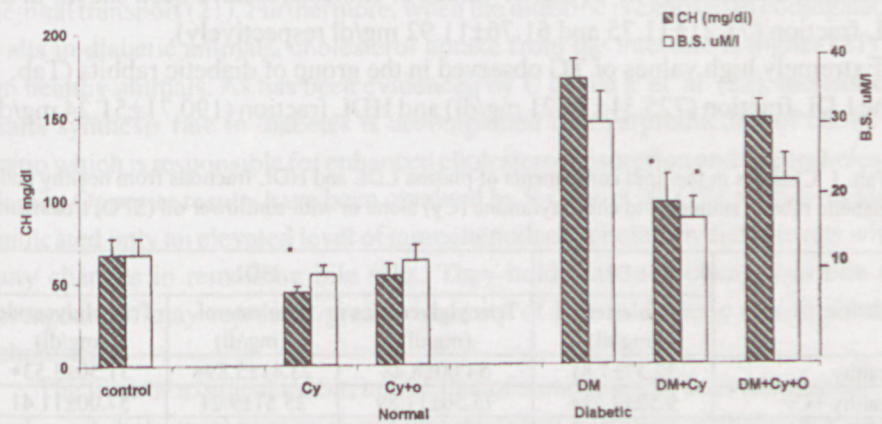

Fig. 2. Plasma bite salts (B.S.) and cholesterol (CH) levels in normal and diabetic (DM) rabbits. Values are means + SEM for 4 animals per group; * $P < 0.05$

In healthy rabbits supplementation of food with cholestyramine brought about a significant decline in concentration of total CH and B.S. (Fig. 1). Additional consumption of SFO in this group of animals leaves these values unaffected.

Acute, alloxan-induced diabetes resulted in augmentation of hepatic steroids in the plasma, as evidenced by increases in the circulating concentration of both CH which peaked up to 172.2±114.10 mg/dl, and bile salts (29.814.56 mM/l) B.S. (Fig. 2). The addition of cholestyramine (Cy) to the food results in the decrease of CH and bile salts in the plasma of normal and diabetic rabbits. The most pronounced decrease in the diabetic group was observed. Introducing SFO to the group supplemented with Cy significantly ($p < 0.05$) increased CH concentration and B.S. in the plasma which reached the values of 148.25±7.31 mg/dl and 22.62±6.12 μM/l, respectively. In contrast to the control diabetic group, also in these groups the values both for cholesterol and bile salts were lower and never approached those found in the diabetic group.

Cholesterol levels, with the exception of a healthy Cy group, in other healthy experimental groups were significantly higher in the LDL fraction (range from 28.89±8.93 in Cy+SFO group to 32.37±7.81 mg/dl in the healthy group) in relation to the HDL fraction (Tab. 1). The phenomenon was observed in the diabetic rabbits but values for cholesterol concentrations were almost doubled in relation to the healthy groups. Apart from the diabetic Cy supplemented group in both the diabetic control rabbits maintained on standard diet and those supplemented with Cy but consuming additionally SFO, higher values of cholesterol have been shown in the LDL fraction (71.21±11.75 and 61.76±11.92 mg/dl respectively).

Extremely high values of TG observed in the group of diabetic rabbits (Tab. 1) in the LDL fraction (725.31±78.21 mg/dl) and HDL fraction (190.71±51.24 mg/dl)

Tab. 1. Changes in the lipid components of plasma LDL and HDL fractions from healthy and diabetic rabbits subjected to cholestyramine (Cy) alone or with sunflower oil (SFO) treatment

	LDL	HDL		
	Cholesterol (mg/dl)	Triacylglycerides (mg/dl)	Cholesterol (mg/dl)	Triacylglycerides (mg/dl)
Healthy	32.37±7.81	54.00±8.48	23.41±5.89*	31.50±3.53*
Healthy +Cy	9.52±2.71*	75.50±11.89	25.51±9.21	57.00±11.41
Healthy+Cy+SFO	28.89±8.93	24.09±9.89*	24.39±5.47*	61.00±22.62
Diabetic	71.21±11.75	725.31± 78.21	56.23±15.27*	190.71±51.24*
Diabetic + Cy	35.27±7.90*	225.79±61.71	51.24±7.83	125.63±39.42*
Diabetic+Cy+SFO	61.76±11.92	525.71± 89.21	41.09±6.23*	211.79±31.70*

Values are means ±SD for 4 rabbits; *$p < 0.05$.

were reduced by the influence of Cy to the values of 225.79±61.71 and 125.63±39.42 mg/dl, respectively. Introducing SFO to the diet of the group of diabetic rabbits, supplemented with Cy, significantly ($p < 0.05$) elevated the TG levels in the LDL and HDL fractions.

DISCUSSION

Abnormalities in lipoprotein (Lp) concentration, composition and metabolism frequently accompany the diabetic state and finally contribute to premature atherogenesis in diabetes (16) but underlying mechanisms are very poorly understood (15). It is known that following alloxan injection diabetes mellitus type I is induced (22). The present study was undertaken to determine how experimental diabetes affects plasma Lp. Healthy and diabetic rabbits were fed on a diet that either lowered, in the case of cholestyramine, or raised the CH level in the case of sunflower oil supplementation.

In diabetic rabbits both CH and B.S. levels have been elevated. It appears that a major mechanism controlling plasma cholesterol levels involves the rate of conversion of cholesterol to bile salts (5, 18) and their subsequent excretion (14). In diabetic animals, bile salts synthesis rate is elevated (17) but with such modification of their composition which favours cholesterol absorption from the gut. High level of B.S. in the plasma of diabetic rabbits was caused by enhanced conversion of CH to B.S. in the liver, increased poll size of B.S. (20) and acceleration of their jejunal transport (21). Furthermore, when the intestine is exposed to conjugated bile salts in diabetic animals, cholesterol uptake from the intestine is higher (21) than in healthy animals. As has been evidenced by U c h i d a et al. (22), increased bile salts synthesis rate in diabetes is accompanied by overproduction of cholic acid ratio which is responsible for enhanced cholesterol absorption and hypercholesterolemia. Opposite results have been obtained by S i o w et al. (17), whose researches indicated only an elevated level of taurochenodeoxycholate in diabetic rats without any changes in remaining bile salts. They hold that such changes in bile acids composition may represent greater capacity of bile in diabetic rats to solubilize cholesterol.

The liver of a typical rabbit, unlike that of some species, does not respond to an enlarged cholesterol pool by increasing the faecal excretion of cholesterol and bile salts. Instead, it responds with a decrease in cholesterol synthesis and a decrease in the synthesis of LDL receptors (18). As a result, there is a rise of plasma LDL. It is known that hepatic binding of LDL is suppressed by high cholesterol content (11), so in diabetic state the reduction in the activity of hepatic LDL receptors is one of the reasons of the enlargement of this fraction in the plasma.

The reduction of cholesterol content in LDL fraction was the most pronounced effect of a non-absorbable bile acid binding resin Cy fed to both groups of rabbits. In this fraction of diabetic rabbits the concentration of CH returned almost to the normal values under the influence of Cy treatment.

The fraction of LDL isolated from diabetic rabbits had an approximately 100% higher lipid content compared with that from non-diabetic rabbits. As a consequence, this fraction became lighter and appeared near the VLDL region when flotation procedure was employed. In the diabetic rabbits the CH level of LDL fraction was doubled in relation to healthy animals. Both in the diabetic and healthy group, Cy exerts considerable lowering effect on the CH level of LDL fraction. In Cy experiments with healthy rabbits, the Cy level of LDL fraction was the most suppressed in comparison to that of LDL from healthy animals untreated with this resin.

The diabetic state exerts the most dramatic, increasing changes on TG concentration in LDL fraction (7). Contrary to CH changes, a decreasing effect of Cy on concentration of TG was observed only in diabetic rabbits. Because simultaneously with the increase of LDL fraction in diabetic rabbits the level of B.S. in the blood increased significantly, these data suggest that the extraction of LDL fraction and B.S. is impaired in diabetic state. Changes in the composition of LDL fractions in diabetic rabbits may be the result of impaired activity of lipoprotein lipase (LPL) caused by a deficiency in insulin action (9). As a LDL fraction in the plasma is the remnant of VLDL fraction rich in TG (10) in the case when LPL activity is suppressed, the LDL fraction is more loaded with TG. In diabetes mellitus, however, some factors other than susceptibility of VLDL to LPL change lipid composition of LDL fraction. As reported in other papers (13, 16), glycation of apoproteins might influence both the composition of diabetic LDL and the clearance of this fraction by the liver, since all apolipoproteins (including apo B of LDL fraction) are non-enzymatically glycated in the circulation.

Other abnormalities of diabetes included a reduced concentration of HDL lipoproteins and loading of this fraction with TG. When HDL cholesterol was compared, both in healthy and diabetic rabbits, the addition of Cy alone or with sunflower oil essentially remains without any effect on CH level in this fraction (Tab. 1). HDL fraction especially in diabetic rabbits contained less TG than LDL fraction. Furthermore, in comparison to the healthy rabbits, the ratio of CH to TG in HDL fraction of diabetic rabbits was low. It has been suggested that hypertriacylglyceridemia would augment the enrichment of HDL with TG and thereby render that lipoprotein more vulnerable to destruction by a lipase. The results support the hypothesis that HDL fraction is reduced in hypertriacylglyceridemic states with diabetes (3). This was due to hypertriacylglyceridemia, which appears to exacerbate

the HDL-lowering effect of cholesteryl ester transfer protein (CETP) by accelerating the enrichment of HDL with a lipid that is susceptible to destruction by lipases (3).

As has been postulated by other authors (4), hypercholesterolemia and atherogenic lipoproteins including LDL remnants cause an increase in the activity of hepatic lipase which was accompanied by reduction in HDL size.

Consistently with our results, Cy treatment of diabetic rabbits effectively reduces LDL levels and brings back the CH level in this fraction almost to the concentration observed in the healthy rabbits. This lowering effect of Cy was attenuated when sunflower oil was introduced to the diet of rabbits treated with Cy.

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STRESZCZENIE

Celem przeprowadzonych badań było określenie wpływu cholestyraminy (Cy), nieresorbowalnej żywicy o własnościach wiązania w jelitach soli żółciowych, podawanej oddzielnie jako 5% dodatek do karmy lub łącznie z olejem słonecznikowym (SFO), na odpowiedź w składzie lipoprotein (Lp) osocza u królików z cukrzycą alloksanową. Badania przeprowadzono na dwóch grupach: 12 zdrowych i 12 cukrzycowych królików rasy nowozelandzkiej podzielonych w obrębie każdej grupy na 3 podgrupy: kontrolną, żywioną z dodatkiem 5% (w/w) Cy i żywioną z dodatkiem 5% Cy oraz 5% SFO (w/w). W osoczu badanych królików oznaczono frakcje Lp metodą ultrawirowania w gradiencie KBr, cholesterol (CH) całkowity i we frakcjach Lp, trójacylglicerole (TG) całkowite i we frakcjach Lp oraz stężenie osoczowych soli żółciowych (B.S.).

U królików cukrzycowych frakcja LDL_1 wskutek obciążenia lipidami przemieszcza się w kierunku lekkiej frakcji VLDL. Podawanie Cy tej grupie królików powoduje zmniejszenie obu frakcji i przesunięcie w kierunku cięższych frakcji. W osoczu królików cukrzycowych istotnie zwiększa się stężenie CH i B.S., których wartości zbliżają się odpowiednio do $172{,}2 \pm 114{,}10$ mg/dl i $29{,}81 \pm 4{,}56$ µM/l. Podawanie Cy zmniejsza stężenie obu oznaczanych sterydów. Zawartość CH we frakcji LDL statystycznie istotnie ($p < 0{,}05$) zwiększa się w cukrzycy, a podanie Cy doprowadza do obniżenia poziomu CH w tej frakcji tylko w przypadku nieuzupełniania pokarmu SFO. Poziom TG zarówno we frakcji LDL, jak i HDL był istotnie ($p < 0{,}05$) wyższy u królików cukrzycowych. Podawanie Cy prowadziło do obniżenia TG w obu frakcjach, a uzupełniające wprowadzenie SFO istotnie ($p < 0{,}05$) zwiększało poziom TG w obu frakcjach.

Przedstawione wyniki wskazują, że podawanie Cy, zmniejszającej pulę krążących soli żółciowych, powoduje nasilenie ich wątrobowej syntezy z cholesterolu i w stanie cukrzycy obniża poziom CH w osoczu krwi. Szczególnie dotyczy to redukcji zawartości CH w aterogennej frakcji LDL. W cukrzycy ma miejsce nasilona wątrobowa synteza B.S., ale o zmienionym profilu, sprzyjającym wchłanianiu jelitowego CH. Podawanie Cy nie tylko obniża zawartość B.S. w krążeniu jelitowo-wątrobowym, ale eliminuje sprzyjający wchłanianiu CH ich skład w stanie cukrzycy.