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Effect of the Oyster Fungus on Glycaemia and Cholesterolaemia in Rats with Insulin-Dependent Diabetes

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Summary

We have investigated the effect of a diet containing of 4 % oyster fungus (*Pleurotus ostreatus*) and 0.1 % cholesterol on glycaemia and hyperlipoproteinaemia in rats with insulin-dependent diabetes (streptozotocin 45 mg/kg). After two months, the rats with diabetes kept on the oyster fungus diet, had a significantly lower basal and postprandial glycaemia, the insulinaemia remained unchanged. The cholesterol concentration was decreased by more than 40 %, the lipoprotein profile was upgraded by the decrease of the cholesterol in both the low density and very low density lipoproteins. The oyster fungus decreased the cholesterol accumulation in the liver and had no significant effects on the levels of serum and liver triacylglycerols.

Key words

Insulin-dependent diabetes - Rat - Oyster fungus - Glycaemia - Cholesterol - Triacylglycerol - Lipoproteins

Introduction

The dietary fibre affects several physiological functions and metabolic processes in the organism. It should be used in rational nutrition because epidemiological studies in developed industrial countries showed a higher occurrence of diverticulosis and colonic carcinoma, heart ischaemia, obesity and diabetes in the population with a lower fibre consumption (Anderson 1980). The high intake of fibre decreases the risk of pathological changes in the gastrointestinal tract. It smoothes the postprandial hyperglycaemia in diabetics, it decreases the level of plasma cholesterol and thus the risk of atherosclerosis (Kritchevsky 1988). At present, the attention is being paid to the fibres in fruit, vegetables, cereals and leguminous plants. Less consideration is given to the fact that the seed-bearing part of higher fungi meets almost ideally the requirements of rational nutrition by its high content of fibre, plant sterols, proteins, microelements and a low level of energy (Ginter and Bobek 1987).

The oyster fungus (*Pleurotus ostreatus*) is a wood rot fungus cultivated industrially on lignocellulose waste substrates in our country. Several tests on hamsters with hyperlipoproteinaemia have shown that the addition of dried seed-bearing parts of this fungus have significantly decreased the level of lipids in the blood and liver (Bobek *et al.* 1989, 1990). Up to now, the mechanism of the hypolipidaemic effect of the oyster fungus is not known.

This knowledge and the well-known action of fibre on postprandial hyperglycaemia has led us to test the effects of the oyster fungus on rats with impaired glycoregulation and hyperlipoproteinaemia supported by intake of exogenous cholesterol.

Material and Methods

Experimental model and nutrition regime

We used male Wistar rats (Dobrá Voda) of 290 ± 10 g body weight, fed a standard laboratory diet. The diabetes was provoked by i.v. dose of streptozotocin (45 $mg.kg^{-1}$ b.w.) in a citrate buffer at pH 4.5. The detailed characteristics of this model were published in a previous report (Chorváthová *et al.* 1981). Together with streptozotocin the animals were given Insulin Interdep (6 $U.kg^{-1}$ b.w.) s.c. at 1300 to 1400 h. Starting on the 8th day of diabetes, the diabetcic animals were fed a semisynthetic diet *ad libitum* (g/100 g), for 8 weeks: starch 60, casein 18, pork fat 10, choline chloride 0.15, Bovine gall 0.4,

cholesterol 0.1, a mixture of minerals and vitamins (Yamashita *et al.* 1980). The control diet contained of 6 % cellulose, while the experimental diet contained only 2 % and 4 % dried oyster fungus (Oyster fungus breeding cooperative farm, Sušice na Hané). After 8 weeks the animals were decapitated in a satiated state (without limitation of the nutrition intake). For the investigation of glucose tolerance, we used other animals with diabetes: animals with the acute diabetes (8 days) before diet administration, and animals fed semisynthetic diet containing 4 % oyster fungus plus 2 % cellulose or 6 % cellulose.

Biochemical methods

The oral glucose tolerance tests were carried out according to Schwartz and Levine (1980). The animals were given glucose in doses of 0.15 g/100 g and oyster fungus and cellulose, respectively, in the amount of 0.2 g/100 g in 5 ml distilled water. The glycaemia levels were determined after 14 hours of fasting and 30, 60, 120 and 180 min after giving glucose with oyster fungus and cellulose, respectively. The levels of blood glucose, triacylglycerols and the total serum cholesterol were determined by BIO-LA Test kits. The concentration of free cholesterol was determined by gas chromatography according to Hindriks *et al.* (1977) and the insulinaemia was determined by kits from Novo (Denmark). The concentration of cholesterol in the Folch extract from the liver was determined according to Lieberman-Burchard (Hořejší *et al.* 1957). Triacylglycerols concentration was determined by the BIO-LA Test kit after the removal of the phospholipids with silica gel. In samples combined from two animals we isolated the lipoproteins at limit densities (Murase and Itakura 1981) by stepwise flotation (Havel *et al.* 1955) using a preparation centrifuge (Beckman, USA). Namely, very low density lipoproteins (VLDL, $d < 1.006$), low density lipoproteins (LDL, $d < 1.063$) and high density lipoproteins (HDL, $d < 1.21$ kg. dm^3) were studied. The cholesterol content in the isolated lipoproteins was determined according to Zlatkis *et al.* (1953). The results were evaluated by Student's t-test.

Results

Before starting with the diet (8 days of diabetes) the animals had hyperglycaemia (16.9 ± 0.6 vs. 5.3 ± 0.07 $mmol.l^{-1}$), hypertriacylglycerolaemia (3.7 ± 0.53 vs. 0.8 ± 0.08 $mmol.l^{-1}$), but normo-cholesterolaemia (1.8 ± 0.11 vs. 1.4 ± 0.11 $mmol.l^{-1}$), when compared with the values before the induction of diabetes. The glucose tolerance, examined by the oral glucose tolerance test after single dose of the oyster fungus and cellulose, respectively, was identical for both groups of animals with diabetes (Fig. 1). The addition of the oyster fungus to the diet had no effect on the tolerance, or on the weight of the animals with diabetes at the end of 8 weeks of observation (324 ± 13 vs. 336 ± 11 g).

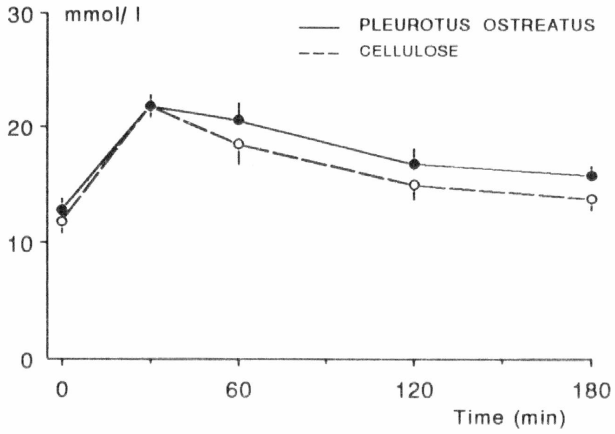

Fig. 1
Glucose tolerance in diabetic rats after sole application of oyster fungus

Glucose and insulin

After two months, the diabetic animals on the oyster fungus diet had significantly lower glycaemia levels postprandially (Table 1), after an overnight fasting and even after a glucose load, than the control group (Fig. 2). The peak of glycaemia was reached at 60 min, while animals receiving cellulose attained maximum at 30 min and their significantly lower glycaemia levels remained unchanged during the whole loading test. The levels of immuno-reactive insulin showed no significant differences between the groups (Table 1).

Table 1
Effect of oyster fungus on glycaemia, insulinaemia and serum and liver lipids in diabetic rats

Group	Control (10)	Oyster fungus (9)
	$mmol.l^{-1}$	
Glucose	2.6 ± 1.38	14.7 ± 2.11[c]
Total cholesterol	10.1 ± 1.41	5.6 ± 0.59[b]
Free cholesterol	1.2 ± 0.24	0.9 ± 0.15
Esterified cholesterol	8.8 ± 1.17	4.6 ± 0.58[c]
Triacylglycerol	4.4 ± 0.99	5.1 ± 0.92
	$pmol.l^{-1}$	
Insulin	184.0 ± 20	156.0 ± 15
Liver	$mmol.kg^{-1}$	
Cholesterol	110.3 ± 14.60	39.3 ± 7.40[d]
Triacylglycerol	38.4 ± 6.91	25.7 ± 2.43

Statistical significance: [a]$p < 0.05$, [b]$p < 0.02$, [c]$p < 0.01$, [d]$p < 0.001$. Number of rats in brackets.

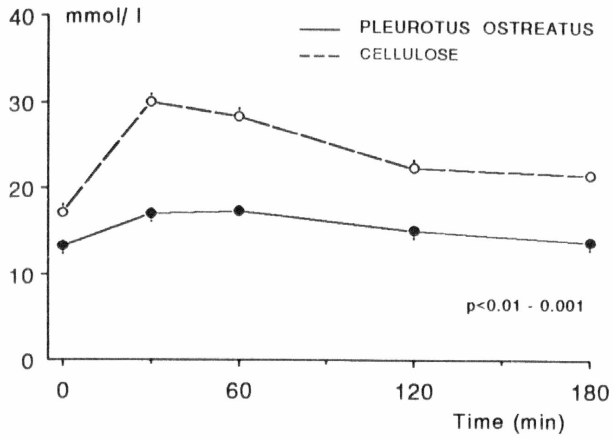

Fig. 2
Glucose tolerance in diabetic rats after a two-month feeding period with oyster fungus

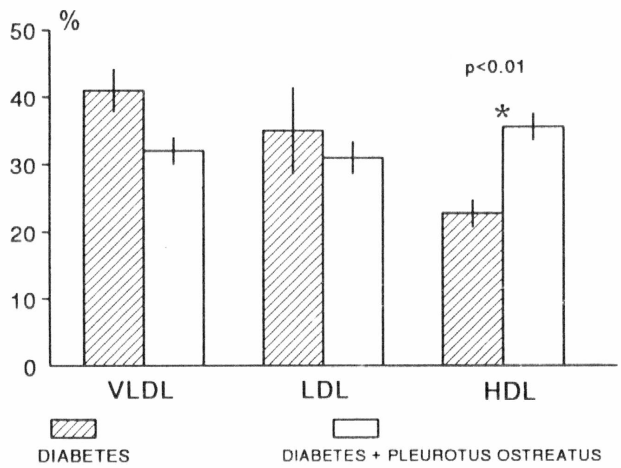

Fig. 3
Effect of oyster fungus on the cholesterol distribution in diabetic rats

Lipids and lipoproteins

The intake of cholesterol exogenous resulted in hypercholesterolaemia in both groups of the diabetic animals. The diabetic animals on the oyster fungus diet had a significantly lower level of both total and esterified cholesterol than the control group (Table 1). The decrease of total cholesterol was due to the lower cholesterol concentration in both the VLDL and LDL. The level of the HDL cholesterol did not change significantly (Table 2). The addition of the oyster fungus also affected the cholesterol distribution in plasma lipoproteins so that amount of cholesterol carried in the HDL fraction was increased (Fig. 3). The oyster fungus reduced the value of the atherogenic index of serum/HDL cholesterol (Table 2) and the cholesterol accumulation in the liver by 60 % (Table 1). The effect on the serum triacylglycerols was not significant, the values in the liver were lower by 30 %, but were not statistically significant.

Table 2
Effect of oyster fungus on the cholesterol concentration in lipoproteins of diabetic rats

Group	Control (6)	Oyster fungus (6)
	mmol.l^{-1}	
VLDL	4.31±0.55	1.98±0.35^{c}
LDL	3.76±0.75	1.70±0.21^{a}
HDL	1.80±0.21	1.98±0.18
Total C/HDL-C	6.08±1.70	2.90±0.33^{a}

Statistical significance: ^{a} $p<0.05$, ^{c} $p<0.01$. Number of rats in brackets.

Discussion

The insulin-dependent diabetes induced by streptozotocin treatment is accompanied by hyperlipoproteinaemia of multifactorial origin. The discrepancy in the control of glucose metabolism results from the toxic damage of the pancreatic β-cells and insulin deficiency (Junod *et al.* 1969). The hypertriacylglycerolaemia. already discernible in the acute period, was due to the discrepancy in VLDL metabolism and chylomicrons, resulting from the deficiency of lipoprotein lipase (Wilson *et al.* 1987). The hypercholesterolaemia, developing even after several weeks of the diabetes, is the result of increased synthesis and/or reabsorption of cholesterol (Nakabou *et al.* 1978, Feingold *et al.* 1984). The exogenic intake of cholesterol can lower the glucose tolerance (Portha *et al.* 1982) and enhance the development of hyperlipoproteinaemia (Saudek and Young 1981).

The results of the present study have shown that the intake of oyster fungus for two months resulted in a decrease of the glycaemia not only basal but even after a glucose load, and had no effect on the insulinaemia of diabetic animals (at the end of the investigation). Similarly, as in hamsters with alimentary hyperlipoproteinaemia (Bobek *et al.* 1989), the oyster fungus also had a hypocholesterolaemic effect. It decreased cholesterolaemia, improved the lipoprotein profile and attenuated the cholesterol accumulation in the liver of diabetic animals. The oyster fungus could have participated in the improvement of glucose tolerance by several mechanisms:. The passage of the stomach and intestine contents might be influenced mainly after acute application (Aro *et al.* 1981). Lower reabsorption and intraluminal diffusion of glucose in the intestinal mucosa should be considered at long-

term intake (Schwartz and Levine 1980, Ebihara and Kiriyama 1982). The insulin secretion and its receptor binding (Eberling *et al.* 1988, Hagander *et al.* 1988), lowering of the secretion of glucagon, GIP and others hormones (Kritchevsky 1988) as well as influencing of the activity of pancreatic enzymes, which limit the use of the substrates resorbed from the food should be also taken into account Kritchevsky 1988). We are not able to judge from our present results which mechanism is able the improve glucose tolerance in long-term feeding of the oyster fungus. It can be supposed that the content and composition of the oyster fungus fibre plays a part in the hypoglycaemic effect, but we cannot exclude the effect of other components of the oyster fungus. According to Kurasawa *et al.* (1982), the oyster fungus has a high content of fibres (47.5 % in the dry substance), 11.6 % of which are cellulose, 27.8 % hemicelluloses, 6.1 % pectins and 2 % lignin. According to our analysis, the oyster fungus (in the dry substance) contains 7.4 % of a basic chitin biopolymer, forming a complex with saccharides (glucans and mannans) in higher fungi, which can act as an ionex (Ginter and Bobek 1987). Up to now, the mechanism of the hypocholesterolaemic effect of the oyster fungus is unknown. We suppose that a complex of substances affects the production of VLDL under conditions of the cholesterol diet, which may also affect cholesterol reabsorption. These considerations are based upon the results of Bobek *et al.* (1990), who showed that the isolated "coarse fibres" of the oyster fungus had a lower hypocholesteroalemic effect than the seed-bearing parts of the whole oyster fungus. As far as the hypocholesterolaemic effect is concerned, there couldbe a synergic participation of the fibres, mainly protein substances (Vahouny *et al.* 1980), indigestible residues of plant proteins (Sugano *et al.* 1988) and plant sterols (Heinemann *et al.* 1991), which could bind bile acids in the gastrointestinal tract, slow down their enterohepatic circulation and thus affect the reabsorption and transformation of cholesterol into bile acids. The knowledge about the favourable effects of the oyster fungus on both glycaemia and cholesterolaemia of diabetic animals will contribute to the understanding of the biological effects of higher fungi and can stimulate their use in human alimentation. The mechanism of these effects requires further investigation.

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