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Oyster mushroom (Pleurotus ostreatus) reduces the production and secretion of very low density lipoproteins in hypercholesterolemic rats

Der Austernpilz (Pleurotus ostreatus) verringert die Produktion und Sekretion der Lipoproteine sehr niedriger Dicente bei hypercholesterolämischen Ratten

Summary Oyster mushroom (*Pleurotus ostreatus*) reduced the production and secretion of nascent very low density lipoproteins in hypercholesterolemic rats.

In male Wistar rats (initial body weight about 70 g) fed a semisynthetic diet with 0.3 % of cholesterol, the addition of 5 % of powdered oyster mushroom (*Pleurotus ostreatus*) to the diet reduced after 8 weeks the level of serum cholesterol (by 36 %) and accumulation of cholesterol and triglycerides in liver (by 51 and 32 %, respectively). The decreased levels of serum cholesterol were caused to the same extent by reduction of cholesterol content in very low density lipoproteins (VLDL) and in low density lipoproteins (LDL) (by 53 and 47 %, respectively). Biosynthesis of all structural lipids of VLDL (phospholipids, cholesterol, triglycerides) in liver and incorporation of *de novo* synthesized lipids into secreted nascent VLDL (measured by simultaneous application of Na-acetate-1-^{14} C and Triton WR 1339 which inhibits peripheral lipolysis) was reduced by application of diet with oyster mushroom.

Zusammenfassung Der Austernpilz (*Pleurotus ostreatus*) verringert die Produktion und Sekretion der VLD-Lipoproteine bei hypercholesterolämischen Ratten.

Nach einer semisynthetischen Diät mit 0,3 % Cholesterol und einem Zusatz von 5 % pulverisiertem Austernpilz (*Pleurotus ostreatus*) bei männlichen der Wistar-Ratten (Anfangsgewicht von ca. 70 Gramm) haben sich nach 8wöchiger Diät der Wert für das Serumcholesterol um 36 % und die Werte für Cholesterol und Triglyzeride in der Leber um 51 bzw. 32 % verringert. Die Reduzierung des Cholesterolgehaltes in VLD-Lipoproteinen und LD-Lipoproteinen (53 und 47 %) hatte in entsprechendem Verhältnis zur Reduzierung des Serumcholesterols beigetragen. Die Biosynthese aller strukturellen Lipide der VLDL (Phospholipide, Cholesterol, Triglyzeride) in der Leber und die Inkorporation der neu synthetisierten Lipide in die sezernierten naszierenden VLDL (gemessen bei freiwerdenden Verabreichung von Na-Ace-tat-1-^{14}C und Triton WR 1339, das die periphere Lipolyse blockiert) wurden durch die Austernpilzdiät verringert.

Key words Oyster mushroom – cholesterol – very low density lipoproteins

Schlüsselwörter Austernpilz – Cholesterol – Lipoproteine sehr niedriger Dichte (VLDL)

Abbreviations *VLDL* = very low density lipoprotein · *LDL* = low density lipoprotein · *HDL* = high density lipoprotein · *TGSR* = triglyceride secretion rate

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Introduction

Based on recent knowledge, it is generally accepted that the reduction of high serum cholesterol levels plays an important role in the prevention of atherosclerosis (16). Discovery of natural substances having hypocholesterolemic activity and the study of their effect on cholesterol metabolism is very important in countries with high incidence of hypercholesterolemias and cardiovascular diseases. Using model hypercholesterolemias of various

etiology, we have found in experimental animals that long-term consumption of oyster mushroom (*Pleurotus ostreatus*, a wood-rotting fungus produced industrially in many countries) exhibits a significant hypocholesterolemic effect (1–4). In these experiments, the decrease of serum cholesterol levels was caused principally by reduction of cholesterol concentration in dominant cholesterol-carrying fraction of lipoproteins – very low density lipoproteins (VLDL) and in their catabolic products – low density lipoproteins (LDL). As a part of the study of the mechanisms of this effect, we investigated the production and secretion of VLDL in rats with profound nutritionally-induced hypercholesterolemia that were fed a diet with addition of 5 % of oyster mushroom.

Material and methods

Male Wistar rats (breeding station Velaz, Czech Republic) weighing 70–75 g were used throughout the study. Animals (n = 40) were bred in standard conditions without adjustment of light regime and with unrestricted access to food and water. One-half the number of animals was fed a semisynthetic diet (20) of following composition (g/100 g): starch 60, casein 18, lard 10, cellulose 6, mineral and vitamin mixtures 4 and 1, respectively, Fel tauri (dried commercial bovine bile) 0.55, choline chloride 0.15 and cholesterol 0.3 (control group). The other half was fed the same diet but with cellulose substituted for 5 % of oyster mushroom powder (mushroom group). This powder was prepared by grinding whole fruiting bodies of oyster mushroom and subsequent drying at temperatures not exceeding 60 °C. Powdered oyster mushroom contained (g/100 g): polysaccharides 65–70 (including 20–25 g of water-soluble, gel-forming beta-1,3 D-glucan with a low degree of polymerization), protein 20–25, lipids 2.2, ash 4.8 and water less than 5. After 8 weeks of feeding, portions (n = 10) of both groups were killed (after 18 h of fasting) by decapitation in light ether anesthesia. Cholesterol concentration was estimated in serum (Oxochrom Chol 250 E, Czech Republic), in chloroform-methanol (2:1) extracts of liver (10) (Bio-La Test-CH, Czech Republic; triglycerides were estimated simultaneously by Bio-La Test-Tg and also in serum by Oxochrom Triacylglycerols T, Czech Republic) and in lipoproteins (21) isolated by sequential flotation (13) on preparative ultra centrifuge (Beckman L8-55, rotor 50.3 Ti) (VLDL, LDL and HDL at $d < 1.006$, $d < 1.063$ and $d < 1.21$ g/ml, respectively). In the other portion (n = 10) of animals of both groups, the distribution of newly synthesized lipids between liver and the secreted VLDL was investigated *in vivo* by treating rats simultaneously with Triton WR 1339 (applied in amount 400 mg/kg of body weight in saline intravenously into tail vein) and Na-acetate-1-^{14}C (2 MBq applied in saline intraperitoneally). After 2 h, the animals were killed by decapitation, VLDL were isolated from serum as described above, and radioactivity in lipids was estimated by liquid scintillation counting (Rackbeta, LKB-Pharmacia) after TLC separation (petrolether/diethylether/acetic acid 90:10:1 as developing system) of chloroform-methanol (2:1) extracts. Incorporation of radiolabeled acetate into liver lipids was determined in the same way. Triton WR 1339 (polymeric p-isooctyl polyoxyethylene phenol) blocks the peripheral catabolism of VLDL (15). Lipid components of VLDL synthesized *de novo* from radiolabeled acetate in liver therefore accumulate in plasma of Triton-treated animals. Estimation of hepatic and plasma VLDL lipids provides information about their production and secretion. We calculated the secretion rate of triglycerides (TGSR [μmol TG/kg/hour = TG(mmol/l] x plasma volume [ml/kg]/time[h]) from accumulation of triglycerides in plasma after blocking lipolysis by Triton injection (15). The results were statistically evaluated by Student's *t*-test.

Results

Oyster mushroom diet did not significantly affect the final body weight of experimental animals. On the other hand, this diet markedly reduced cholesterol diet-induced accumulation of cholesterol content in serum, VLDL, LDL and in liver. Oyster mushroom diet did not significantly affect triglyceride concentrations in serum, but their content in liver was reduced by 32 %. The concentration of cholesterol in HDL increased significantly. The reduction of VLDL cholesterol participated by more than 58 % in the total decrease of serum cholesterol level (Table 1). The incorporation of radiolabeled acetate into both total lipids (measured in total lipid extracts of liver) and individual lipidic components of VLDL was significantly reduced by oyster mushroom diet. Similarly, the incorporation of all newly synthesized lipids into secreted VLDL decreased significantly under the effect of oyster mushroom (Table 2). The rate of triglyceride secretion estimated simultaneously from the accumulation of triglycerides in plasma after inhibition of lipolysis was also markedly reduced by oyster mushroom diet (135 ± 7 vs. 98 ± 5 μmol TG/kg/h, control vs. mushroom group, $p < 0.001$).

Discussion

Cholesterol diet in rat causes a remarkable increase in cholesterol concentration in VLDL (at the expense of HDL, carrying about 60 % of serum cholesterol under physiological conditions). The fact that oyster mushroom diet reduced the contribution of VLDL (and their catabolic end-products, LDL) on serum cholesterol transport

Table 1 The levels of serum cholesterol, its distribution in lipoproteins and the content of cholesterol and triglycerides in liver of rats fed the control and mushroom diets

	Diet	
	Control	Mushroom
Body weight (g)	329 ± 17	320 ± 15
Serum	mmol/l	
Triglycerides	0.96 ± 0.07	0.73 ± 0.09
Cholesterol	5.24 ± 0.60	3.34 ± 0.15
VLDL-cholesterol	2.56 ± 0.32	1.34 ± 0.27[c]
LDL-cholesterol	2.12 ± 0.35	1.03 ± 0.12[c]
HDL-cholesterol	0.64 ± 0.08	0.86 ± 0.04[a]
Liver	mmol/kg	
Triglycerides	65 ± 5	44 ± 4[c]
Cholesterol	250 ± 15	122 ± 12[d]

Values are means ± SEM (for 10 animals in each group)
Superscript marks [a–d] indicate statistical significance (mushroom vs control diet(: $^{a}p < 0.05$, $^{b}p < 0.02$, $^{c}p < 0.01$, $^{d}p < 0.001$

Table 2 Biosynthesis of structural lipids of VLDL in liver and their incorporation into nascent secreted VLDL

	Diet	
	Control	Mushroom
Radioactivity associated with liver (dpm/g liver.10^3)		
Total lipids	58.2 ± 4.1	44.1 ± 2.6[c]
Phospholipids	30.8 ± 2.3	24.5 ± 1.5[a]
Cholesterol	3.5 ± 0.35	2.1 ± 0.21[d]
Triglycerides	12.9 ± 0.9	9.9 ± 0.7[b]
Radioactivity associated with VLDL (dpm/ml serum)		
Total lipids	15243 ± 1065	10038 ± 627[d]
Phospholipids	816 ± 86	554 ± 74[a]
Cholesterol	1476 ± 184	816 ± 95[c]
Triglycerides	11966 ± 986	8321 ± 654[c]

Values are means ± SEM (for 10 animals in each group)
Statistical significance as in Table 1

almost by one-half indicates that the regulation of VLDL level in circulation has a fundamental relevance in the mechanism of hypocholesterolemic effect of oyster mushroom. The results obtained in this study have shown that dietary oyster mushroom reduced the rate of synthesis of all structural lipids in VLDL, the incorporation of *de novo* synthesized lipids into nascent VLDL, as well as the rate of VLDL secretion into the plasmatic pool. It has been already found that the *de novo* synthesized lipids are the preferred source of VLDL assembly and that the synthesis of new lipids (especially of cholesterol and phospholipids) might be a major driving force in lipoprotein assembly and secretion (19, 9). Apparently, inhibition of lipid synthesis and reduced incorporation of lipids into VLDL is an important element of the mechanism of the effect of dietary oyster mushroom on the concentration of VLDL cholesterol. Mevinoline (monacolin K, which is a competitive inhibitor of HMG-CoA reductase, the key enzyme of cholesterol biosynthesis) has been isolated recently from cultures of *Pleurotus ostreatus* (12). The possible participation of this substance in the oyster mushroom-induced reduction of cholesterol biosynthesis is not clarified yet, at least from a quantitative viewpoint.

It has been found that cholesterol in the diet of rats stimulates hepatic secretion of all VLDL-lipids as well as of apo B. The rate of VLDL secretion is determined by changes in cholesterol pool in liver (11). In agreement with this findings, Vance and Vance (19) concluded that the process of secretion of hepatic very low density lipoproteins appears to be regulated by the supply of lipids. Consequently, reduction of cholesterol content in liver to 50 % of the original value caused by dietary oyster mushroom enables the reduction of VLDL secretion. Reduced cholesterol accumulation in liver induced by oyster mushroom in the diet is evidently the consequence of reduction of cholesterol biosynthesis and absorption (6, 7) as well as of stimulation of cholesterol catabolism to bile acids in liver (8). It is highly probable that especially water-soluble components of fiber matter and undigested fungal protein residues (17), as well as pectin (18) by their interaction with bile acids interfere with micelle formation in intestine and thus reduce the absorption of cholesterol. Enhanced excretion of bile acids restricts their enterohepatal circulation. This in turn stimulates by a feedback mechanism the activity of 7α-hydroxylase, the enzyme catalyzing the rate-limiting step in degradation of cholesterol to bile acids (14). It is interesting to note that oyster mushroom diet stimulated by a hitherto unknown mechanism the fractional turnover of VLDL-triglycerides (5), although the activity of lipoprotein lipase (the key enzyme of VLDL degradation) was not affected by oyster mushroom (unpublished results). It is surprising in this respect that although oyster mushroom reduced the synthesis of all lipids (including triglycerides) and their incorporation into VLDL, levels of serum triglycerides were not significantly affected in contrast to cholesterol levels. This finding is in agreement with the results of our previous experiments.

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