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Oyster mushroom *(Pleurotus ostreatus)* reduces the activity of 3-hydroxy-3-methylglutaryl CoA reductase in rat liver microsomes

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Abstract. The effect of dried oyster mushroom (*Pleurotus ostreatus*) on cholesterol (C) content in serum, in lipoproteins and in liver, and on the activity of 3-hydroxy-3-methylglutaryl CoA (HMG-CoA) reductase in liver microsomes, was studied in male rats (strain Wistar, initial body weight 75 g) fed on low-cholesterol (9 mg/100 g) and high-cholesterol (0.3%) diets. Addition of 5% oyster mushroom to both diets reduced significantly the C-content in serum (by 30%), in very-low- and low-density lipoproteins (in a 1:1 ratio to the decrease of total serum C) and in liver (by 50%), as well as the activity of HMG-CoA reductase (by more than 30%).
Key words. Oyster mushroom (*Pleurotus ostreatus*); cholesterol; serum; lipoproteins; liver; HMG-CoA reductase.

The search for natural substances with hypocholesterolemic activity and the study of their mechanisms of activity is extremely important in countries with unfavourable statistics for hypercholesterolemia and with a high incidence of cardiovascular disease. Oyster mushroom is a wood-rotting fungus that is produced industrially on lignocellulose substrates in Slovakia as well as in other countries. Addition of 2–5% of dried fruiting bodies of this fungus to a diet with a high cholesterol (C) content effectively reduced development of hypercholesterolemia and accumulation of C in the liver in both hamsters and rats[1,2]. Reduction of cholesterolemia after consuming oyster mushroom was in both cases caused by a decrease C content in primary lipoproteins – very-low-density lipoproteins (VLDL). Since biosynthesis of C (mainly in liver) is also a source of C in primary lipoproteins, we studied the effect of low-C and high-C diets containing 5% of dried oyster mushroom on the activity of the key enzyme of C biosynthesis, 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA reductase)[3], in the rat.

Materials and methods

Male Wistar rats (Breeding Station, Dobrá Voda, Slovakia) were used throughout the experiments. Animals (initial body weight 75 g) were bred in standard conditions without regulation of the light regime and with unrestricted access to food and water. In experiment I, the animals (n = 20) were fed for 8 weeks on a semisynthetic diet[4] of the following composition (g/100 g): starch, 60, casein 18, pork fat 10, cellulose 6, mineral mixture 4, vitamine mixture 1, Fel tauri (commercially available dried bovine bile) 0.55, and choline chloride 0.15 (low-C diet; final C content 9 mg/100 g of the diet). In experiment II, the animals (n = 20) were fed on the same diet with the addition of 0.3% C (high-C diet). In both experiments, one half of the animals were used as control (control group), while cellulose was replaced by 5% of dried oyster mushroom powder in the diet of the other half of animals (mushroom group). At the end of the experiment, animals were decapitated under light ether narcosis after 18 h of fasting. Serum C level was estimated by an enzymatic method (Bio-La Test AM 250, Lachema, Czech Republic) in a chloroform-methanol (2:1) extract[5] of liver; in very-low-density lipoproteins (VLDL, $d < 1.006$), in low-density lipoproteins (LDL, $d < 1.063$), and in high-density lipoproteins (HDL, $d < 1.21$ g/ml), prepared by sequential flotation[6] using a preparative ultracentrifuge[7]. The activity of HMG-CoA reductase in liver microsomes (isolated according to Stange et al.[8]) was quantified as production of ^{14}C-mevalonate from HMG-CoA(3-^{14}C) after incubation with $NADP^+$, glucose-6-phosphate and glucose-6-phosphate dehydrogenase[9]. Protein content in microsomes was determined by the method of Bradford[10]. Results were statistically evaluated by Student's *t*-test.

Results

The presence of oyster mushroom in the diet did not significantly affect the body weights of experimental animals (Exp. I: 302 ± 11 vs 295 ± 12 g; Exp. II: 301 ± 8 vs 296 ± 10 g; mean ± SEM, control vs mushroom group). Animals fed on a high-C diet had 1.7-fold higher cholesterolemia and an 8-fold higher C content in liver when compared to animals fed on the low-C diet. With both diets, the addition of oyster mushroom reduced cholesterolemia by 27–33% and C content in liver by more than 50%. Oyster mushroom-induced reduction of serum C levels was distributed approxi-

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Table. Effect of oyster mushroom on cholesterol level in serum, liver and lipoproteins and on HMG-CoA reductase activity.

Experiment Group	I: low-cholesterol diet		II: high-cholesterol diet	
	control	mushroom	control	mushroom
Cholesterol				
Serum ($mmol.l^{-1}$)	3.00 ± 0.20^{d}	2.20 ± 0.09^{d}	5.12 ± 0.55	3.44 ± 0.16^{b}
Liver ($mmol.kg^{-1}$)	30 ± 3	11 ± 1^{e}	241 ± 12	116 ± 11^{e}
VLDL ($mmol.l^{-1}$)	0.94 ± 0.18	0.26 ± 0.03^{c}	2.56 ± 0.32	1.34 ± 0.27^{b}
LDL ($mmol.l^{-1}$)	0.58 ± 0.11	0.36 ± 0.03	2.12 ± 0.35	1.03 ± 0.12^{b}
HDL ($mmol.l^{-1}$)	1.26 ± 0.04	1.55 ± 0.08^{b}	0.64 ± 0.08	0.86 ± 0.04^{a}
HMG-CoA reductase (pmol/min/mg protein)	237 ± 20	160 ± 16^{c}	137 ± 16	86 ± 9^{b}

Values are means $\pm$ SEM ($n = 10$ in each group).
Statistical significance (control vs mushroom group): $^{a}p < 0.05$, $^{b}p < 0.02$, $^{c}p < 0.01$, $^{d}p < 0.002$, $^{e}p < 0.001$.

mately in a 1:1 ratio between VLDL and LDL in animals fed on both diets. The concentration of HDL-C increased slightly, but significantly, in the presence of oyster mushroom in both diets. The high-C diet reduced the activity of liver HMG-CoA reductase to almost one half of the values in animals on the low-C diet. Feeding on oyster mushroom reduced the activity of this enzyme in both diets ,by more than 30% compared to control animals (table).

Discussion

Our previous results have shown that consuming oyster mushroom reduced absorption of C significantly (by 14%)[11]. We suppose that especially water-soluble, gel-forming components of the fibrous matter in the mushrooms (beta-1, 3-D-glucan and pectin, constituting 15–20 and 5% of dry weight, respectively) are involved in bile acid binding, and thus inhibit the formation of micelles, which is required for C resorption. In addition, these substances can stimulate catabolism of C by restricting the re-entry of bile acids into the liver[12,13]. This supports our recent finding that the diet containing oyster mushroom increases the activity of cholesterol 7α-hydroxylase (a key enzyme in cholesterol catabolism) and excretion of bile acids[14]. Reduction of HMG-CoA activity found in rat liver affected by oyster mushroom can explain decreased cholesterol biosynthesis in liver (estimated from ^{14}C-acetate incorporation in vivo) in rats fed an oyster mushroom diet[15]. Reduced absorption and biosynthesis of cholesterol is the prerequisite for decreased formation of cholesterol-rich VLDL (regulated by substrate availability[16]), which significantly contributes to the hypocholesterolemic effect of oyster mushroom diets in rat.

Results obtained in our laboratory also indicate that oyster mushroom contains factor(s) interfering with cholesterol biosynthesis. This assumption was recently confirmed by the finding that fungi of the basidiomycetous genus *Pleurotus*, especially the species *P. ostreatus*, *P. saca* and *P. sapidus* (with the common name oyster mushroom) are a newly-discovered and promising source of the hypocholesterolemic agent mevinolin (monacolin K, lovastatin)[17]. Similar substances were isolated from several species of lower fungi (e.g. *Penicillium citricum*, *Monascus ruber*)[18] and they represent the most important pharmaceuticals with hypocholesterolemic effect. Crude methanol extracts and purified inhibitor from three different species (*P. ostreatus*, *P. saca* and *P. sapidus*) were tested on solubilized microsomal HMG-CoA reductase from Chinese hamster ovary cells. The identity of the inhibitor was confirmed by thin-layer chromatography, high-pressure liquid chromatography and mass spectroscopy[19]. Mevinolin competitively inhibits HMG-CoA reductase activity, and increases the expression of liver LDL receptors[20,21]. This is in agreement with our finding of increased fractional LDL turnover and decreased LDL-cholesterol level in rats fed on a diet containing *P. ostreatus*[22]. On the other hand, administration of mevinolin to mice[23], hamsters or rabbits[21] resulted in a compensatory increase of HMG-CoA activity in liver. A similar effect was observed after administration of compactin (ML-236 B, mevinolin analog) to rats[24]. In contrast to our results, the latter compound reduced the activity of cholesterol 7α-hydroxylase, and excretion of steroids[24]. Mevinolin is probably only one of several factors in oyster mushroom that interfere in a complex way with regulation of cholesterol metabolism on the level of absorption, biosynthesis and catabolism, as well as on the level of transport in all cholesterol-carrying lipoproteins in plasma.

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