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Effect of Oyster Fungus (Pleurotus ostreatus) on Serum and Liver Lipids of Syrian Hamsters with a Chronic Alcohol Intake

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Summary

The authors studied the effect of oyster fungus (*Pleurotus ostreatus*) (2 % dried fruiting bodies in a standard diet) on the serum and liver lipids of growing male Syrian hamsters with a chronic alcohol intake (a 15 % aqueous solution). After eight weeks' alcohol intake there was an increase in their serum cholesterol, triacylglycerol (TG) and phospholipid (PL) concentration, 40 – 60 % of which was accounted for by an increase in the very low density lipoprotein (VLDL) concentration. The proportion of VLDL in the lipoprotein pool rose by almost 15 %, whereas the proportion of high density lipoproteins (HDL) fell. The simultaneous administration of the fungus in the diet reduced the cholesterol level below the value in the control animals not given any alcohol. Both the serum TG and the VLDL concentration fell by 30 %, but neither the chemical composition and concentration of the HDL nor the cholesterol concentration were affected. The addition of the fungus to the diet completely abolished the increase induced in the liver cholesterol and TG concentration by the chronic intake of alcohol.

Key words:
Syrian hamster – Alcohol – Serum – Lipoproteins – Lipids – Liver

Introduction

Attempts to improve the unfavourable trend of the incidence of hypercholesterolaemia in our population (Píša and Hořejší 1988, Škodová *et al.* 1990) stimulate the search for natural substances capable of lowering the blood lipid level ever-topical in the sphere of nutrition. We demonstrated in a number of studies that the addition of a small amount of oyster fungus (*Pleurotus ostreatus*) to a high fat-cholesterol diet inhibited the progressive accumulation of lipids in the serum and liver of various experimental animals (Bobek *et al.* 1989, 1990, 1990a,b). The mechanism of the positive effect of the fungus on an alimentarily induced increase in blood and liver lipid levels has not yet been explained. Our recent results indicated that the hypolipaemic effect of the fungus, apart from the traditionally accepted effect of fibre on lipid resorption, is also mediated by further (probably low molecular weight) substances. This is borne out by the finding of a hypolipaemically exceptionally active substance – eritadenin – in the related

Japanese fungus shii-take (*Lentinus edodes*) (Rokujo *et al.* 1970). From the results described below, it is evident that oyster fungus contains a substance (or substances) which might interfere with lipid metabolism outside the resorption phase, as indicated by its positive effect on lipid accumulation induced in the blood and liver by chronic alcohol intake.

Material and Methods

For the experiments we used growing male Syrian hamsters (Velaz, Prague) with an initial body weight of about 120 g. The animals had free access to standard chow diet (DOS-2B, Velaz, Prague), in which 30 % of the energy is accounted for by proteins, 12 % by fats and 58 % by glycides. The only source of liquids for the third of the animals was tap water (Control, C) and for two thirds it was 15 % aqueous ethanol solution. Some of these animals acted as the "alcohol intake controls" (AC) and the others had food supplemented by the addition of 2 % dried, minced oyster fungus (AF). After eight weeks the animals were deprived of food (but not liquids) for 18 hours and were then decapitated. Cholesterol (Bio-La-Test); Abell *et al.* 1952), triacylglycerols (TG) (Bio-La-Test) and phospholipids (PL) (Bio-La-Test) were determined in their blood serum and in chloroform-methanol (2:1) extract of their liver (Folch *et al.* 1957). Using pooled serum samples from two animals (containing 10^{-3} mol Na_2.EDTA/l) and sequential flotation (Havel *et al.* 1955) on a L8-55 preparative ultracentrifuge (Beckman, USA; rotor 50.3 Ti; 36 000 rpm / 5 °C / 18 h; in isolation of HDL 48 h), we isolated very low density lipoproteins (in kg/m^3) VLDL, d 1.006), low density lipoproteins (LDL, d < 1.063) and high density lipoproteins (HDL, d < 1.21). The cholesterol content (Zlatkis *et al.* 1953), TG content (Carlson 1963), PL content (by a modification of the Bio-La-Test) and protein content (Lowry *et al.* 1951) of the lipoproteins were determined; lecithin:cholesterol acyltransferase (cholesterol kinetics LCAT TEST, Institute for Research on the Development and Utilization of Radioisotopes, Prague) was also determined in the serum. The results were evaluated statistically by Student's t-test.

Table 1

Effect of oyster fungus on serum and liver lipids in the Syrian hamster

	CONTROL (without alcohol) (C)	ALCOHOL CONTROL (AC)	ALCOHOL FUNGUS (AF)
n	11	13	12
SERUM (mmol/l)			
Cholesterol	3.59 ± 0.07^{b}	3.88 ± 0.09^{b}	3.16 ± 0.14^{e}
Triacylglycerols	2.34 ± 0.14^{e}	6.08 ± 0.45^{e}	4.15 ± 0.35^{c}
Phospholipids	39.04 ± 1.06^{b}	48.35 ± 1.05^{e}	46.35 ± 2.41
LIVER (mmol/kg)			
Cholesterol	7.42 ± 0.17	9.81 ± 0.61^{c}	6.80 ± 0.42^{e}
Triacylglycerols	10.80 ± 0.39	16.10 ± 1.77^{b}	10.87 ± 0.50^{b}

Mean values ± SEM; a,b,c,d,e – statistical significances: P<0.05, 0.02, 0.01, 0.002, 0.001 (in the first column C vs AF, in the second C vs AC, in the third AC vs AF)

Results

Neither chronic alcohol intake nor the simultaneous consumption of a diet containing 2 % oyster fungus had a significant effect on the hamsters' final body mass after an 8 weeks' experiment (C: 181 ± 7 g, AC: 198 ± 6 g, AF: 194 ± 5 g). The fungus likewise did not affect mean alcohol consumption (about 8 – 10 ml/animal/day), which represented 17 % of the animals' total energy intake.

Table 2
Effect of oyster fungus on the protein and lipid content of Syrian hamster lipoproteins

Lipoprotein (mmol/l)	CONTROL (without alcohol) (C)	ALCOHOL CONTROL (AC)	ALCOHOL FUNGUS (AF)
n	6	6	6
VLDL			
Proteins +	9.04 ± 1.06	35.35 ± 0.66^{e}	36.50 ± 5.88
Cholesterol	0.58 ± 0.08	0.98 ± 0.08^{c}	0.66 ± 0.08^{b}
Triacylglycerols	1.91 ± 0.22^{a}	4.30 ± 0.42^{e}	2.95 ± 0.35^{a}
Phospholipids	3.16 ± 0.36^{e}	6.85 ± 1.33^{a}	10.38 ± 0.81^{a}
LDL			
Proteins	23.21 ± 1.04	25.15 ± 2.01	27.38 ± 1.28
Cholesterol	0.71 ± 0.07	0.76 ± 0.07	0.61 ± 0.08
Triacylglycerols	0.39 ± 0.06^{a}	1.21 ± 0.22^{c}	0.98 ± 0.22
Phospholipids	5.82 ± 0.26	7.02 ± 0.44^{a}	7.77 ± 1.25
HDL			
Proteins	129.0 ± 4.74	113.2 ± 5.52	121.0 ± 6.10
Cholesterol	2.16 ± 0.04	1.97 ± 0.06^{a}	1.84 ± 0.17
Triacylglycerols	0.04 ± 0.01^{c}	0.22 ± 0.03^{e}	0.15 ± 0.03
Phospholipids	29.48 ± 0.39^{e}	29.42 ± 0.93	25.03 ± 0.78^{c}

Mean values ± SEM ; a–e – Statistical significances (as in Tab. 1); + – Data in mg/100 ml

Alcohol consumption led to a significant increase in the serum cholesterol concentration, to an almost threefold increase in the TG concentration and to 20 % increase in the PL concentration. A decisive role in the above alcohol-induced increase of serum lipids was played by VLDL, in which the proportion of cholesterol rose by 70 % and TG and PL were more than doubled. LDL and HDL changes were not very marked, except for a more than twofold increase in the TG content of these lipoproteins and a mild decrease in HDL-cholesterol. Alcohol consumption raised the protein content of VLDL almost fourfold. Addition of the fungus to the diet reduced the serum cholesterol level below the value in the controls given no alcohol and lowered the serum TG concentration by 30 %. Conversely, the serum

PL concentration rose under the influence of the fungus. The fungus-induced decrease in serum cholesterol and TG levels was largely determined by a decreased concentration of these lipids in the VLDL (Tab. 1 and 2).

Alcohol caused the VLDL concentration (expressed as the sum of the protein and lipid content of the lipoproteins) to double and markedly raised the LDL concentration. The result was a significant increase in the concentration of the lipoprotein pool (expressed as the sum of the VLDL, LDL and HDL concentrations). The proportion of VLDL in lipid transport rose noticeably, whereas that of HDL fell. Addition of the fungus to the diet lowered the concentration of VLDL and of the lipoprotein pool (nonsignificantly), but did not affect the chemical composition of the HDL or the HDL and HDL-cholesterol concentration (Tab. 3).

Table 3

Effect of oyster fungus on the concentration of the various lipoprotein classes and their proportion in the lipoprotein pool of the hamster

Lipoprotein (mg/100 ml)	CONTROL (without alcohol) (C)	ALCOHOL CONTROL (AC)	ALCOHOL FUNGUS (AF)
n	6	6	6
VLDL+	208 ± 23^{d}	454 ± 33^{d}	353 ± 25^{a}
%	33.3 ± 2.1	47.2 ± 3.8^{c}	44.0 ± 1.6
LDL	103 ± 6	182 ± 24^{b}	160 ± 27
%	16.7 ± 1.1	19.4 ± 0.8	19.7 ± 1.5
HDL	307 ± 8	300 ± 4	283 ± 21
%	50.0 ± 1.4^{e}	33.4 ± 3.8^{c}	36.3 ± 2.3
Lipoprotein pool*	618 ± 30	935 ± 75^{c}	796 ± 96

Mean values ± SEM; a–e – Statistical significances (as in Tab. 1)
*+ Sum of protein and lipid concentrations; * Sum of concentrations of various classes of lipoproteins*

Chronic alcohol intake raised the cholesterol and TG content of hamster liver by 30 % and 49 % respectively. Administration of the fungus in the diet completely abolished the accumulation of lipids in the liver (Tab. 1). Neither alcohol nor simultaneous addition of the fungus to the diet affected lecithin:cholesterol acyltransferase activity (data not shown).

Discussion

In recent experiments we showed that oyster fungus effectively inhibited lipid accumulation induced alimentarily in hamster serum and liver by a high fat-cholesterol diet (Bobek *et al.* 1989, 1990). The decrease in serum lipids was largely due to a drop in the VLDL concentration. The fungus also had the same effect in rats hereditarily hypersensitive to alimentary cholesterol (Bobek *et al.* 1990b). At the same time, the fungus did not affect lipoprotein lipase, the key enzyme in the degradation of VLDL. We therefore assumed that the hypolipaemic effect of the fungus was mediated by a decrease in VLDL production after the resorption of cholesterol and other lipids had been reduced by a complex of several substances in the fungus, e.g. fibre (Vahouny *et al.* 1980), indigestible plant protein residues (Sugano *et al.* 1988), plant sterols (Ikeda *et al.* 1988), chitin (Zemek *et al.* 1987), etc. Further experiments showed indirectly, however, that a substantial portion of the hypolipaemically active substances took effect independently of the macromolecular fibre complex and hence were unconnected with the phase of lipid resorption (Bobek *et al.* 1990a, 1991). This hypothesis is supported by our findings that the fungus effectively slowed down the development of hyperlipaemia as well as cholesterol and TG accumulation induced endogenously in the liver of hamsters with a chronic alcohol intake. The finding of the hypolipaemically exceptionally active substance eritadenin in an aqueous-alcohol extract of the related mushroom shii-take (*Lentinus edodes*) (Rokujo *et al.* 1970) is in agreement with this. Aqueous-alcoholic extracts of several wood-rotting fungi (and the serum of humans who had eaten these fungi) displayed antiatherogenic and antisclerotic activity in human aortic intima cell culture (Ryong *et al.* 1989).

Chronic alcohol intake is known to inhibit the oxidation of fatty acids (Lieber 1971) and to stimulate their synthesis and esterification to TG, PL and cholesterol esters in the liver (Baraona and Lieber 1979), resulting in their accumulation in that organ. Alcohol stimulates the incorporation of TG into the VLDL in the liver and their secretion into the blood stream (Savolainen *et al.* 1986, Taskinen *et al.* 1987), where, in the presence of unchanged or only slightly raised lipoprotein lipase activity, they can accumulate (Taskinen *et al.* 1987). Our present results do not tell us with which phase of the metabolic processes leading to hyperlipaemia and lipid accumulation in the liver the fungus interferes, but it is probably the common base of these processes – the mechanism of increased fatty acid deposits in the liver – that is affected.

The above results indicate that the utilization of oyster fungus, which is grown commercially in Czechoslovakia, could potentiate the effects of hypolipaemic and antisclerotic diets, especially as it is known that the abuse of alcohol belongs to the first and second order risks for the development of atherosclerosis.

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