

Effects of high-fat and *Chlorella vulgaris* feeding on changes in lipid metabolism in mice

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The aim of this study was to test the hypothesis that green alga *Chlorella vulgaris* is able to decrease lipaemia in mice fed a high-fat diet. The anti-lipidemic effect of dried, powdered *Chlorella vulgaris* was investigated using male CD1 mice. Mice were divided into four groups and were fed a standard and a high-fat diet with or without *Chlorella* supplementation (1%, w/w). A ten-week load of high-fat diet remarkably increased serum total cholesterol and triglycerides, causing risk factors for development of atherosclerosis. The cholesterol and triglyceride contents in the liver were higher after HF feeding. In the *Chlorella* group that was administered a high-fat diet containing powdered green alga *Chlorella vulgaris*, the increase of total cholesterol and triglyceride in the serum and also in the liver was significantly inhibited. No significant difference was observed in high-density lipoprotein (HDL). *Chlorella vulgaris*, when used as a food supplement, improved the total cholesterol/HDL-cholesterol ratio. The lipid metabolism in animals on standard diet with *Chlorella* supplementation was not significantly affected indicating that endogenous metabolism remained unaffected.

Key words: *Chlorella vulgaris*, lipid metabolism, liver, high-fat diet, mice.

Introduction

Diet is an important factor controlling serum lipids and consequently the occurrence of coronary heart disease (CHD) (GRUNDY & DENKE, 1990).

The typical human diet in the western world contains a relative high proportion of fat (30–40% energy) especially saturated fatty acids and cholesterol. Diets supplemented with either saturated fats or cholesterol lead to both hypercholesterolemia and hypertriglyceridemia concurrent with hepatic steatosis (KOK et al., 1998). Dyslipidemia,

characterised by abnormally elevated plasma triacylglycerol and cholesterol concentrations, is an established risk factor in the development of CHD.

Excessive intake of a high-fat diet leads to changes in lipid metabolism. It is well established that the liver regulates plasma levels of cholesterol and triglyceride by secretion and transport of these lipids in the lipoproteins (FUNGWE et al., 1993).

Modification of the nutritional regime and application of natural substances with hypocholesterolemic activity are two important approaches

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to the prevention and treatment of CHD (GOULD et al., 1995). The central features of the recommended changes in diet include restricting intake of total fat, saturated fatty acids, cholesterol, and energy (for maintenance of desirable body weight) (DAVIDSON et al., 1998).

Feeding some dietary supplements lowers plasma cholesterol concentration (KENDLER, 1997; HARA et al., 1998). The mechanisms are still not fully understood. Plant foods are good source of dietary fibre, thus a variety of plant foods should be studied to find out their cholesterol lowering effect. Addition of a blue-green alga *Nostoc commune* was effective in depressing the elevated serum cholesterol caused by feeding an atherogenic diet (HORI et al., 1994). Previous studies indicated that addition of green alga *Chlorella vulgaris* into diets modulated lipid metabolism in rabbits and rats (SANO & TANAKA, 1987; SANO et al., 1988).

We tested the hypothesis that green alga is able to decrease hyperlipidemia in mice fed a high-fat diet composed of 40% lipids, namely lard. *Chlorella vulgaris* was chosen as a dietary supplement because of its documented efficacy in the animals (SANO & TANAKA, 1987; SANO et al., 1988), and because of its easy incorporation into a variety of foods.

Material and methods

Animals and diet

Male CD1 mice, 6-8 weeks old were purchased from Anlab s.r.o. Praha. The animals were housed in plastic cages in a conditioned room at $23 \pm 1^\circ\text{C}$ with the illumination from 7 AM to 7 PM. All animals were allowed to adapt to the environment for at least 2 weeks prior to dietary treatment and were provided free access to food and water throughout the experiment. The initial body weight of the animals was approximately 20 g. Weight gain was monitored and the 24-h food intake was recorded every week. The duration of experimental treatment was 10 weeks.

Male mice were divided into four groups. Group 1 was fed a standard diet (STD), group 2 received 1% *Chlorella vulgaris* (supplied by the Institute of Microbiology, Academy of Sciences of the Czech Republic, Třeboň, Czech Republic) in the standard diet (STCh), group 3 was fed a high-fat diet (HF), group 4 received 1% *Chlorella vulgaris* in the high-fat diet (HFCh) (for composition of the diets see FÁBRY, 1959). The standard laboratory diet contained only 5% fat, whereas the high-fat diet consisted of 40% fat corresponding the proportion of fat in a human Western-style high-fat diet. Two different dietary fats were used as a lipid source. Plant margarine was added to the standard diet. Lard as a rich source of saturated fatty acids and cholesterol was used in HF diet. The fatty acid composition of dietary fats is given in Table 1.

Table 1. Fatty acid composition of dietary fats in the standard diet and in the high-fat diet.^a

Fatty acid content normalised to 100 per cent	Margarine	Lard
16:0	9.5	29
18:0	15	14
18:1	62	47
18:2	10	9
18:3	2.5	1
ΣSFA	24.5	43
ΣMUFA	62	47
ΣPUFA	12.5	10

^a Fatty acids are denoted by the number of carbons: the number of double bonds. SFA – saturated fatty acids, MUFA – monounsaturated fatty acids, PUFA – polyunsaturated fatty acids.

At the end of the feeding period, all animals were killed after the 12-hr fast and samples for analyses were collected.

Sample collection and analytical procedures

Mice were anaesthetized with diethylether and blood was collected from each mouse from the carotis (a. carotis). Obtained blood samples were transferred to a test tube and the serum separated by centrifugation. The murine liver was excised, weighted and homogenized in chloroform:methanol (2:1). Samples were obtained from the same lobule of each liver. HDL was separated from other lipoprotein fractions by precipitation and centrifugation (BACHORIK & ALBERT, 1986) and cholesterol concentration binding in HDL was measured using Lachema kits (Czech Republic). Serum cholesterol and triacylglycerol concentrations were determined using kits coupling enzymatic reaction and spectrophotometric detection of reaction end products (Lachema Brno, Czech Republic). Kits of the same provenance were used for hepatic lipid analysis after chloroform:methanol (2:1) extraction according to FOLCH et al. (1957). Alanine aminotransferase (ALT, EC 2.6.1.2.) activity in the serum was estimated by Bio-La Test kit (Czech Republic).

Statistical analysis

Results are expressed as the mean $\pm$ S.D. of ten mice. Statistical differences between groups were evaluated by analysis of variance (ANOVA) and Kruskal-Wallis analysis. The level of significance was set at $P < 0.05$.

Results

Body and liver weights, food intake

There were no significant differences in body and liver weights among the experimental groups on the completion of the treatment (Tab. 2). Throughout the treatment, daily food intake was

Table 2. Effect of HF and *Chlorella* feeding on body and liver weights and food intake in mouse male.^a

Diet	STD	STCh	HF	HFCh
Body weight (g)	36.82 ± 8.93	37.31 ± 4.00	38.88 ± 2.37	39.71 ± 2.68
Liver weight (g)	1.44 ± 0.16	1.52 ± 0.13	1.38 ± 0.25	1.33 ± 0.14
Liver weight (g/100 g body wt)	3.60 ± 0.36	3.80 ± 0.28	3.59 ± 0.50	3.51 ± 0.33
Food intake (g/day)	6.19 ± 0.74	5.82 ± 0.45	3.79 ± 0.40 ^a	4.09 ± 0.83 ^a

^a Results are the mean ± S.D. of ten mice. The STD group corresponds to mice fed the standard diet. The STCh group corresponds to STD-fed mice given *Chlorella vulgaris* supplemented. The HF group corresponds to animals given the high-fat diet. The HFCh group corresponds to HF-fed mice given *Chlorella vulgaris* supplemented. Significant differences are indicated as follows: ^a significantly different from STD ($P < 0.05$).

Table 3. Effect of HF and *Chlorella* feeding on serum lipids and serum alanine aminotransferase activity.^a

Diet	STD	STCh	HF	HFCh
Triglycerides (mmol/L)	1.60 ± 0.50	1.26 ± 0.35	1.81 ± 0.48	1.12 ± 0.37 ^{a,b}
Total cholesterol (mmol/L)	3.14 ± 0.57	3.06 ± 0.62	3.81 ± 0.42 ^a	3.44 ± 0.44
Free cholesterol (mmol/L)	0.66 ± 0.10	0.74 ± 0.17	0.61 ± 0.14	0.72 ± 0.13
HDL-cholesterol (mmol/L)	1.90 ± 0.34	1.83 ± 0.29	1.58 ± 0.26	1.62 ± 0.13
Total cholesterol/HDL-cholesterol ratio	1.67 ± 0.26	1.68 ± 0.25	2.45 ± 0.30 ^a	2.07 ± 0.21 ^{a,b}
ALT activity (μkat/L)	0.47 ± 0.10	0.58 ± 0.16	0.86 ± 0.20 ^a	0.76 ± 0.14 ^a

^aResults are the mean ± S.D. of ten mice. The STD group corresponds to mice fed the standard diet. The STCh group corresponds to STD-fed mice given *Chlorella vulgaris* supplemented. The HF group corresponds to animals given the high-fat diet. The HFCh group corresponds to HF-fed mice given *Chlorella vulgaris* supplemented. Significant differences are indicated as follows: ^asignificantly different from STD ($P < 0.05$); ^bsignificantly different from HF ($P < 0.05$).

Table 4. Effect of HF and *Chlorella* feeding on liver lipid contents.^a

Diet	STD	STCh	HF	HFCh
Triglycerides (mmol/kg)	39.01 ± 5.76	34.13 ± 6.46	50.62 ± 7.20 ^a	37.43 ± 6.97 ^b
Total cholesterol (mmol/kg)	12.95 ± 2.63	11.13 ± 2.71	33.66 ± 6.36 ^a	28.57 ± 2.66 ^{a,b}

^aResults are the mean ± S.D. of ten mice. The STD group corresponds to mice fed the standard diet. The STCh group corresponds to STD-fed mice given *Chlorella vulgaris* supplemented. The HF group corresponds to animals given the high-fat diet. The HFCh group corresponds to HF-fed mice given *Chlorella vulgaris* supplemented. Significant differences are indicated as follows: ^asignificantly different from STD ($P < 0.05$); ^bsignificantly different from HF ($P < 0.05$).

significantly higher in mice fed the standard diet than in mice receiving the high-fat diet with or without *Chlorella* (Tab. 2). However, the daily energy intake, following the mean calorific value of each diet, was not statistically different in the groups (92.87 ± 10.58 (STD), 91.93 ± 7.11(STCh), 82.30 ± 11.11 (HF), 86.55 ± 10.47 (HFCh) kJ/day).

Serum and liver lipid contents

At the time of sacrifice, the serum cholesterol concentration was significantly higher in HF mice than in STD animals (Tab. 3). HF feeding signif-

icantly increased cholesterol concentration in the liver, compared to STD diet (Tab. 4).

In the high-fat diet *Chlorella vulgaris* supplementation did not evade the increase in cholesterol levels in the serum (Tab. 3). On the other hand, the hepatic cholesterol concentration was decreased after *Chlorella* feeding (Tab. 4).

At the time of sacrifice, no differences in triglyceride levels appeared in the serum of HF-fed mice compared to controls (STD) (Tab. 3). Liver triglyceride level in HF mice was significantly higher than in STD animals (Tab. 4).

The *Chlorella* supplementation in the HF diet

significantly decreased triglyceride concentration in the serum, as compared to mice fed the HF diet alone or the STD diet (Tab. 3). The same tendency was obtained in the liver. *Chlorella* feeding significantly lowered the hepatic triglyceride level in HFCh-fed mice compared to HF (Tab. 4).

There were no differences in the concentration of serum free cholesterol and HDL-cholesterol among the groups (Tab. 3). There were no statistically significant changes in lipid levels between STD and STCh animals (Tabs 3,4).

In order to obtain an *in vivo* estimation of possible liver tissue damage, serum ALT was measured. The serum ALT activity was significantly higher in the animals fed the HF diet than in those fed the standard diet (Tab. 3). Addition of powdered *Chlorella* to HF diet slightly reduced the ALT activity in the serum and the ALT activity remained increased in HFCh mice compared to STD animals, unlike in STCh mice (Tab. 3).

Discussion

The mouse has become suitable and the most used animal model to evaluate the effects of nutrition on the changes in the lipid profile (SHIH et al., 1995). The present study extends the hypolipidemic effect of *Chlorella vulgaris* in mice fed a diet enriched in lard, providing a large amount of saturated fatty acids and cholesterol. Two phenomena are discussed: the effect of HF feeding and the effect of *Chlorella* feeding on lipid profile.

Influence of the HF diet on lipid profile

Dietary lipids are able to affect lipoprotein metabolism in a significant way, thereby modifying the risk of cardiovascular disease (HORNSTRA et al., 1998). Together, cholesterol and saturated fat appeared to promote the more atherogenic lipoprotein profile (RUDEL, 1997). Lard is rich in saturated fatty acids, which are known to raise total cholesterol levels (GRUNDY & DENKE, 1990), and it is also a source of cholesterol (15% lard provides 1% cholesterol). Cholesterol feeding increases plasma concentration of total and esterified cholesterol, in a dose-dependent manner (NISHINA et al., 1990). The effect of saturated fatty acids and cholesterol on change of cholesterol metabolism may be explained by change of LDL-receptor activity (SPADY et al., 1993) or by an increase in the production of apoB-100 by the liver (HAYES & KHOSLA, 1992). In this study, total cholesterol concentration value was higher in mice fed the HF diet. This phenomenon occurring in HF-fed mice is most probably due to the com-

bination of the effects of using the fat source.

The HF diet induced a significant accumulation of triglyceride and cholesterol in the liver, as shown previously in several studies (LUTZ et al., 1994; MURAKAMI et al., 1999). High-fat diets containing cholesterol cause lipid accumulation in the liver, and dietary uptake of cholesterol causes hepatomegaly in rats associated with an overload of this lipid in hepatocytes (LUTZ et al., 1993). This imbalance stimulates cholesterol esterification and biliary secretion in order to maintain intracellular homeostasis (CHAUTAN et al., 1990). The increased hepatic esterified cholesterol pool competes with triglycerides for secretion as a component of the VLDL hydrophobic core. Consequently, triglycerides accumulate within the hepatocyte as cytoplasmic droplets (KOK et al., 1998).

The different fatty acid source is considered to cause an alteration of cell membranes leading to an increase in membrane permeability, inactivation of membrane-bound enzymes and eventually cell damage (IZUSHI & OGATA, 1990). Administration of HF diet to mice caused liver injury as demonstrated by an elevated serum level of ALT, and not even *Chlorella* supplement in HF diet evaded the increase in the serum ALT activity.

Effect of the Chlorella vulgaris supplementation in HF diet on lipid profile

A hypolipidemic effect of *Chlorella vulgaris* has been previously reported in rabbits and rats (SANO & TANAKA, 1987; SANO et al., 1988).

In the present study, mice fed the HFCh had lower serum levels of all the lipids measured compared to HF, except of HDL-cholesterol.

The hypolipidemic effect could be due to an impaired fat absorption (KOIDE, 1998) or an interruption of the enterohepatic bile acid circulation (HARA et al., 1998) causing non-digestible compounds. *Chlorella* probably decreased cholesterol absorption in the intestine as described in previous study (SANO et al., 1988). Feeding with *Chlorella* inhibited the absorption of exogenous steroids and promoted turnover of bile acids in the liver to suppress the increase of serum cholesterol level caused by administration of a high-fat diet in rats. Blocking cholesterol absorption from jejunum effectively decreases plasma cholesterol (HAN et al., 1999) and thus reduces the risk of developing atherosclerosis.

In mice, in contrast to humans, cholesterol is mainly transported in the plasma as esterified cholesterol inside HDL. In our study, the ratio of total cholesterol/HDL-cholesterol was signifi-

cantly decreased suggesting that the proportion of cholesterol carried in the HDL fraction may be raised by addition of the powdered *Chlorella vulgaris* (Tab. 3). It is expected to be useful for the prevention of atherosclerosis, since animal studies have indicated that serum HDL-cholesterol levels are inversely correlated with atherosclerotic risk (FRUCHART & DURIEZ, 1998).

The lowering effect of *Chlorella vulgaris* on triglyceride levels, which was also observed in this study, could be associated with the inhibition of hepatic fatty acid synthesis and triglyceride production, thus limiting the output of VLDL. On the other hand, the inhibition of key enzymes of fatty acid synthesis and several lipogenic enzymes might be due to HF feeding as a defence system as described in KOK et al. (1996). Further investigation is required.

An extra-hepatic event could contribute to the significant hypotriglyceridemic effect. The enhancing triglyceride-rich lipoprotein catabolism may be due to the increased serum lipoprotein lipase activity (KOK et al., 1998). Our results show that *Chlorella* feeding is able to decrease hypertriglyceridemia in mice associated with the high-fat feeding.

In conclusion, the present study extends the hypolipidaemic effect of *Chlorella vulgaris* in mice. The *Chlorella* feeding appears to be beneficial as a hypolipidemic agent since it contributed to the changes in lipid profiles. If such a protective action on serum lipids is confirmed in humans, it could be interesting for human health and nutrition.

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