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Original Paper

Effect of Long-term Feeding of Shiitake on Age-related Changes in Serum Lipids in Male SD Rats

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Key words: shiitake (Lentinus edodes), serum lipid, mean life span

Abstract

The purpose of the present study was to observe the changes in serum lipids of rats fed a shiitake-containing diet throughout life. The serum triglyceride level of the shiitake diet group tended to be lower than that for the control diet group, but the difference was not significant. The serum phospholipid level was significantly lower in the shiitake diet group than in the control diet group ($p<0.001$) from 4 weeks to 36 weeks. Serum total cholesterol levels were significantly low in the shiitake diet group compared to the control diet group from 4 weeks to 36 weeks. However, the difference gradually disappeared over more than 48 weeks. Our findings suggested that reducing the serum cholesterol level and the phospholipid level beyond 36 weeks after starting a shiitake-containing diet influenced the average life span and the medium survival rate.

Introduction

As serum lipids in rats were examined during the course of feeding ad libitum, the serum total cholesterol was specifically increased.

It is thought that the increases in serum triacylglycerol and total cholesterol cause the development of atherosclerosis [1] and other diseases that shorten the lifetime of individuals. It has been reported that the increase in serum lipids can be attenuated by ingredients contained in specific foods [2-9]. Bobek *et al.*, have reported that the serum cholesterol value declines when shiitake mushrooms were fed to rats [2-5]. However, their experiments involved only a short-term (12-weeks) administration of shiitake mushrooms. That a similar effect would continue upon feeding shiitake to rats for an extended period has not been reported.

The purpose of the present study was to observe the change in serum lipids and the life span of rats fed on a shiitake-containing diet throughout their lifetime.

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Materials and Methods

1. Materials

The rats fed on a shiitake-containing diet were given commercially dried shiitake. Before they were used, the dried shiitake were further dried (80℃) overnight and ground.

2. Animals and diets

Seven-week-old male Sprague-Dawley rats (Japan Clare Co., Japan) were given a commercial diet (MF,Oriental Yeast Co., Tokyo) for 1 week. After acclimation for 1 week, the rats were divided into two groups and given either a shiitake-containing diet (group S; n=8) or a control diet (group C; n=10). The dried shiitake was added to a level of 1.8% in the shiitake-containing diet. The compositions of the experimental diets are shown in Table 1. The rats were allowed free access to food and tap water. The response to the experimental conditions was monitored by weighing daily and by evaluating the mortality rate. The rats were housed two animals per cage in an animal room at a constant temperature with 12-hr light-dark cycles.

Table 1 Compositions of experimental diets

Ingredient	Control diet	Shiitake diet (%)
Casein[1]	20.00	19.65
DL-Methionine[2]	0.30	0.29
Starch[1]	44.60	43.81
Sucrose[1]	23.00	22.59
Mineral Mixture (AIN-76)[1]	4.00	3.93
Vitamin Mixture (AIN-76)[1]	1.00	0.98
Corn oil[1]	5.00	4.91
Cellulose[1]	1.85	1.82
Choline chloride[2]	0.25	0.25
Shiitake		1.77
	100.00	100.00

1) Purchased from Oriental Yeast Industries Co., Ltd (Japan).

2) Purchased from Wako Pure Chemical Industries Co., Ltd (Japan).

3. Measurement of serum lipids

At weeks zero, 4, 12, 24, 36, 48, 60 and 72 of the experimental period, blood was withdrawn from the tail veins under anesthesia with ether. Serum was frozen immediately and stored at −40℃ until the assay. Serum triglyceride, phospholipid and total-cholesterol were measured using the enzyme method [10-12]. HDL-cholesterol and LDL-cholesterol fractions were separated by agarose gel electrophoresis [13].

4. Statistical analysis

The body weight data and the serum lipids data were expressed as means ± standard error of the mean. Significant differences between the means of the groups with or without shiitake mushrooms were determined by the Student's t-test. The life span data were evaluated using the Cox-Mantel test. Differences were considered significant when $p<0.05$.

Results

1. Body weight

The mean body weight of group S tended to be lower than the group C, but the difference was not significant (Fig.1).

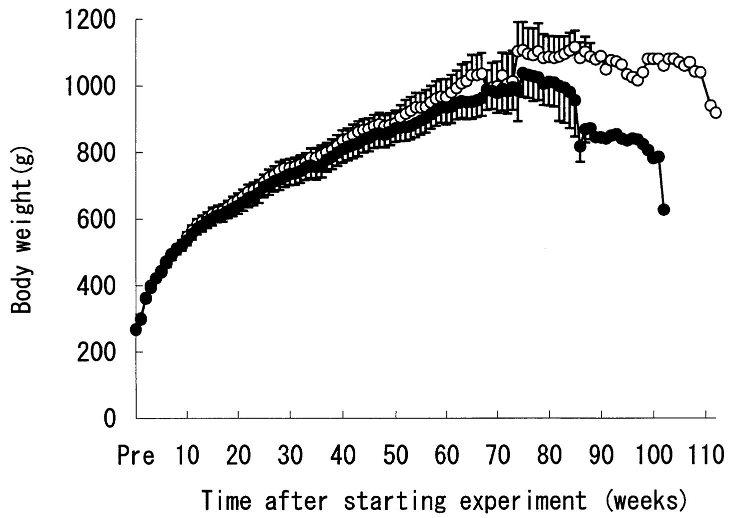

Fig. 1 Effect of shiitake diet on body weight of rats.
Values are means ± SEM. Control diet group (○, n=10), Shiitake diet group (●, n=8).

2. Survival

The average life span of group S was 92±9.5 weeks, and that of group C was 85±19.7 weeks. The median survival rate in group C was 77 weeks and that of group S was 94 weeks. The longest life span in group S was 110 weeks, and that of group C was 120 weeks (Fig.2).

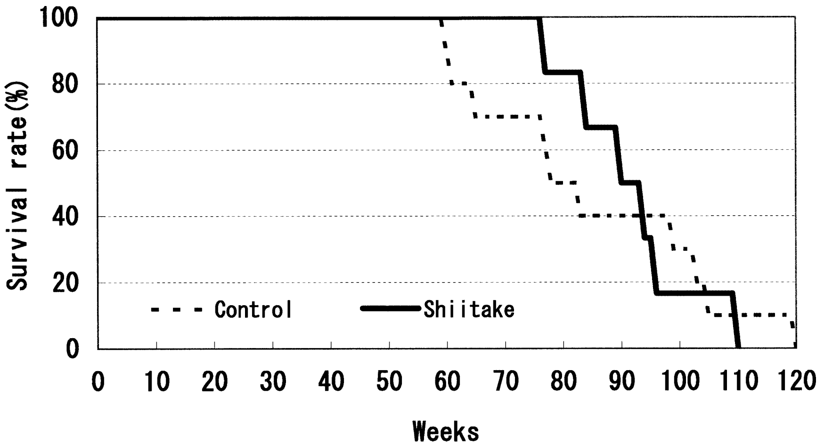

Fig. 2 Effect of shiitake diet on survival rates in rats.
Control diet group (n=10), Shiitake diet group (n=8). Statistical significance was evaluated by Cox-Mantel test.

3. Serum lipids

The serum triglyceride level increased in group C up until 24 weeks and then gradually decreased (Fig.3). The serum triglyceride level of group S was lower than that of group C at 12 and 24 weeks, but the difference was not significant. The serum phospholipid level was significantly lower in group S than in group C (p<0.001) from 4 weeks to 36 weeks (Fig.4). However, no significant difference was seen at more than 48 weeks. Total cholesterol levels were significantly lower in group S than in group C from 4 weeks to 36 weeks (Fig.5), and increased in both groups up until 48 weeks. There was no significant difference in the proportions of cholesterol in the HDL-cholesterol and LDL-cholesterol fractions of group S and group C (Fig.6).

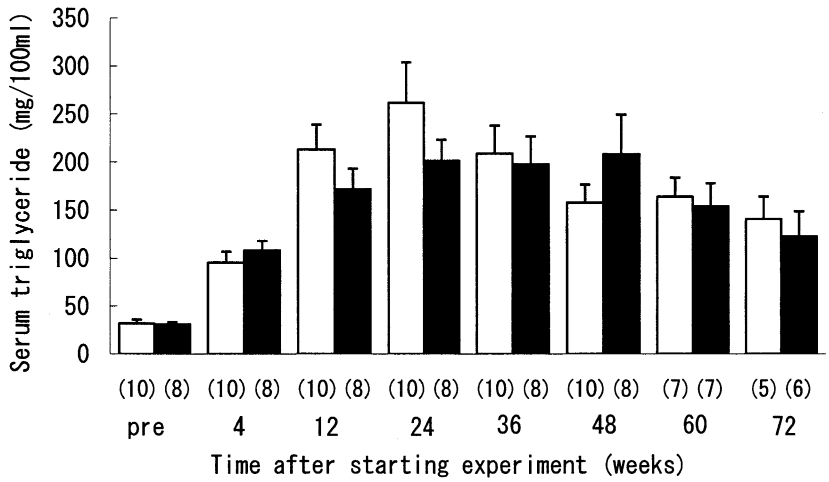

Fig. 3 Effect of shiitake diet on concentration of serum triglyceride in rats.
Values are means ± SEM in both control diet (open columns) and shiitake diet (closed columns). The number of animals used is indicated in parentheses. Statistical significance was evaluated by Student's t-test.

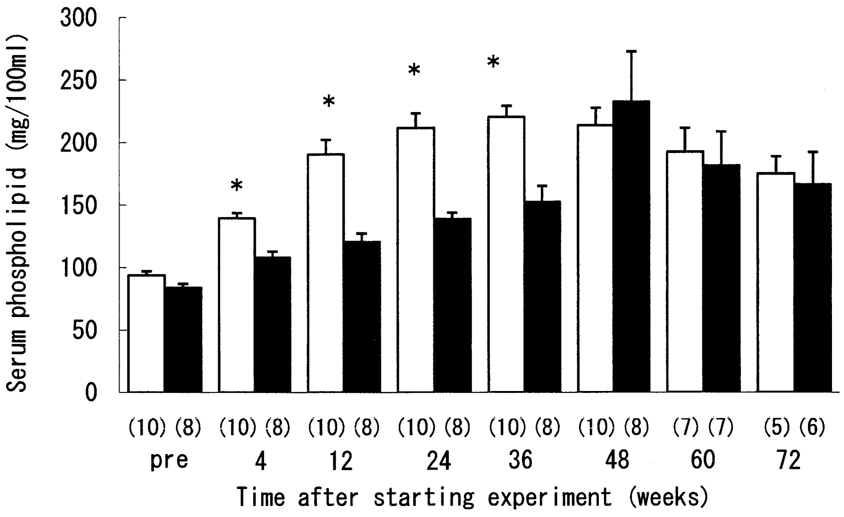

Fig. 4 Effect of shiitake diet on concentrations of serum phospholipids in rats.
Values are means ± SEM in both control diet (open columns) and shiitake diet (closed columns). The number of animals used is indicated in parentheses. Statistical significance was evaluated by Student's t-test, * p<0.001.

Discussion

A high serum cholesterol level is known to be one of the risk factors for atherosclerosis [1]. The hypocholesterolemic effect of the eritadenine in shiitake has been reported by many researchers [7-9]. Shiitake

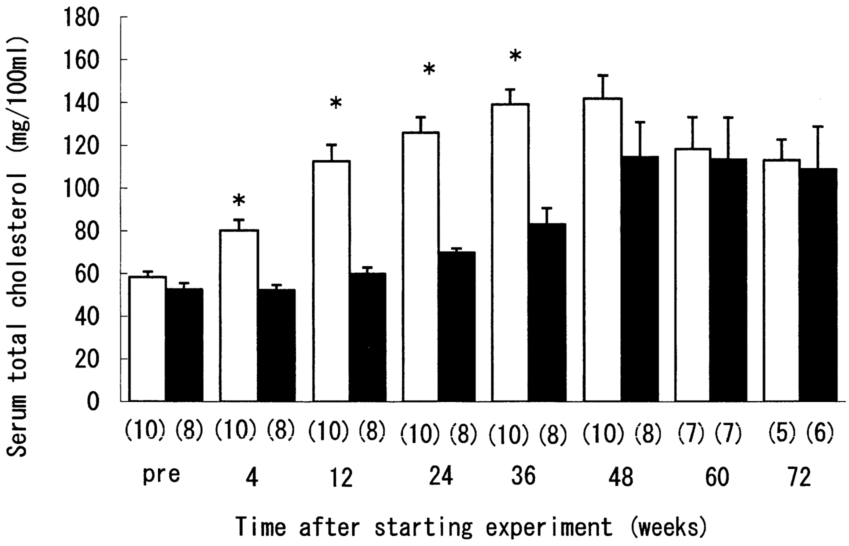

Fig. 5 Effect of shiitake diet on concentration of serum total cholesterol in rats.
Values are means ± SEM in both the control diet (open columns) and shiitake diet (closed columns). The number of animals used is indicated in parentheses. Statistical significance was evaluated by Student's t-test, * $p<0.001$.

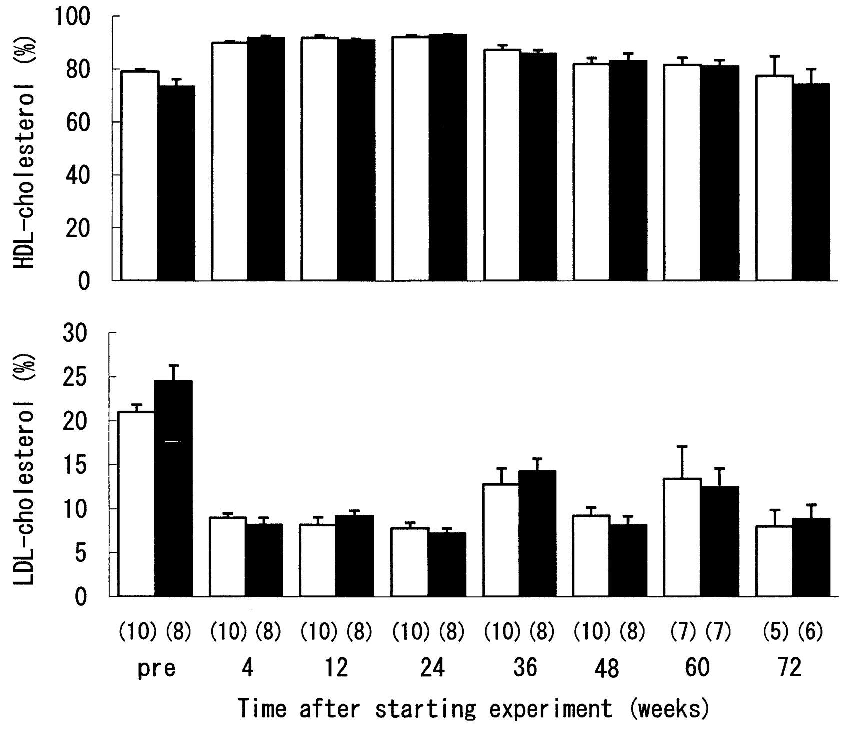

Fig. 6 Effect of shiitake diet on the HDL-cholesterol and LDL-cholesterol fractions in rats.
Values are means ± SEM in both the control diet (open columns) and shiitake diet (closed columns). The number of animals used is indicated in parentheses. Statistical significance was evaluated by Student's t-test.

was a natural material with a hypocholesterolemic effect. However, the long-term influence of shiitake on serum lipids is unknown. In this study we clarified the impact of a shiitake-contained diet on serum lipids of rats throughout life.

The body weight of group S was lower than that of group C. All of the rats died of natural causes.

The median survival rate in group C was 77 weeks, and was 94 weeks for group S. It is suggested that the effects of shiitake potentially extend life span in this study. However, all animals receiving the shiitake diet were dead by week 110, and those receiving the control diet by week 120. Nevertheless, the average life span in group S was 92±9.5 weeks, as compared with 85±19.7 weeks for group C.

The hypocholesterolemic effect of the eritadenine in shiitake has been reported by many researchers [7-9]. Kimoto *et al.* reported that feeding shiitake to rats decreased plasma total cholesterol, free cholesterol and phospholipids slowly[14]. In this study, the serum triglyceride level of group S showed a lower tendency, but there were no statistical differences. And the serum phospholipid level was significantly lower in group S than in group C ($p<0.001$) from 4 weeks to 36 weeks. Serum total cholesterol levels were significantly lower in group S as compared to group C from 4 weeks to 36 weeks. However, no difference in total cholesterol was observed between the two groups at more than 48 weeks.

Takashima *et al.* have reported that the mechanism of the hypocholesterolemic effect of shiitake was based on the balance of cholesterol between tissues and plasma, with a bias toward tissues [15]. On the other hand, Sannoumaru *et al.* reported that shiitake decreases serum and liver cholesterol concentrations, and that cholesterol was excreted as several secondary bile acids and total bile acids in the feces [16]. In this study, we did not measure the cholesterol in liver and feces. However, fatty liver was observed macroscopically in group C and was not observed in group S. It is widely known that the fatty liver involves hypertriglyceridemia. However, no differences in the serum triglyceride levels between the two groups were observed in this study. The total cholesterol levels were significantly lower in group S than in group C. Therefore, these results suggest that shiitake inhibit increases in lipid contents in the liver. However, the serum triglyceride level was not influenced. Previous findings have shown that the HDL fraction is strongly involved in the transport and/or exchange of cholesterol between tissues and plasma [17]. In this study, the HDL and LDL fractions of group C and S were not different. Shiitake may have the effect of controlling the transport or exchange of cholesterol between tissues and plasma. Takashima *et al.* have reported that the effects of eritadenine on the metabolic processes were influenced by cyclic AMP [15].

Our results suggest that group S had fewer early deaths because the shiitake mushrooms restrained these serum phospholipid and total cholesterol levels from 4 to 36 weeks after starting the experiment.

Acknowledgements

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