

Rapid evaluation method for plasma endogenous cholesterol level in rats given a Shiitake fungus powder diet*

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Summary

To evaluate more rapidly the plasma endogenous cholesterol level in rats given diets containing Shiitake fungus powder, two animal experiments were conducted. One was to feed rats on diets for consecutive two weeks and the other to feed rats on diets for three days after two-day fasting. In both experiments, there were found decreases in food intake, lowered increase in body weight and drastically lowered plasma total- and HDL- cholesterols with significancies of $P < 0.01$ when the Shiitake diet was given. This finding indicated that the latter experiment, which takes only a total of five days, can be profitable for rapid evaluation of plasma endogenous cholesterol in rats as animal experiment terms are shortened and expenses for feeding are restrained.

Introduction

In general, when rats are used as an experimental animal for blood cholesterol evaluation, in many of the studies, they are fed diets ordinary containing 0.5-1.5% of cholesterol together with 0.2-0.3% of bile acids or bile salts, because of their ability to regulate its own cholesterol level (1-3). In the investigation of blood cholesterol lowering effects of Shiitake fungus (*Lentinus edodes*), it was done just like so by several investigators (4-8), but a few studies were conducted on the evaluation of plasma endogeneous cholesterol (9-12) where rats have been fed diets for long terms such as 4-10 weeks. Since tissue cholesterol metabolism in mice is known to be markedly enhanced for less than a week after short-term starvation, 48 hours, followed by the ingestion of a repletion diet (1,13-15), to

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shorten test periods, an experimental animal model, where rats were fasted for two days followed by a repletion diet for 3 days, was adopted in the present study. We report here that only five-day feeding was found to be as effective as 2-week consecutive feeding for evaluation of plasma cholesterol level in rats.

Methods

Animal experiments

Male rats of Wistar/ST strain, initial body weights of 90-100g, were purchased commercially. They were housed in individual, suspended, wire-mesh stainless cages in a room maintained at 24°C and 50% of humidity with alternate 12-hour periods of light and dark. The rats were provided diets and water ad libitum. They were divided randomly into four groups of 7-8 animals each. The two groups were given the Shiitake diet containing 10% of Shiitake (*Lentinus edodes*) powder or the control diet, respectively, for consecutive 14 days. The remaining two groups were given the Shiitake diet or the control diet, respectively, for three days after 2-day fasting. The control diet contained the following ingredients, as percentage weights: sucrose, 60; casein, 20; corn oil, 10; mineral mixture, 4; vitamin mixture, 1; cellulose powder, 10. The Shiitake diet was prepared by replacing cellulose powder in the control diet for Shiitake powder. The body weights and food intakes were recorded every day.

Plasma collection and cholesterol analyses

After feedings finished, rats were anesthetized with intraperitoneal injection of sodium pentobarbiturate and bloods were collected with a heart puncture. Immediately, they were centrifuged at 3,000 rpm for 15 minutes and supernatants were collected, being kept at -50°C until cholesterol were analyzed. Total- and HDL- cholesterol in plasma were assayed colorimetrically using the kits that were commercially purchased from Wako Chemical Co. Ltd., Tokyo. The kit for total cholesterol was based on the cholesterol oxidase-DAOS method and that for HDL-cholesterol was on the cholesterol oxidase-heparin-Mn precipitation method. Atherosclerotic index was computed with a formula: $(\text{total cholesterol} - \text{HDL-cholesterol}) / \text{HDL-cholesterol}$.

Results and discussion

To evaluate more rapidly the plasma endogenous cholesterol level, two animal experiments using rats were conducted. The rats, which were fed the 10% Shiitake diet for consecutive two weeks, showed significant decreases in food intake, growth and plasma total- and HDL- cholesterol ($P < 0.01$) compared to the control rats, as seen in Table 1. The significant decrease in plasma endogenous total cholesterol level in rats given the diet

Table 1. Food intake, increase in body weight and plasma endogenous total- and HDL-cholesterol values in rats fed control and Shiitake diets for consecutive 14 days.

Diet	Nos. of rat	Food intake (g/rat/2weeks)	Increase in body weight (g/rat/2weeks)	Plasma cholesterol value (mg/dl)		Total-HDL/HDL
				Total	HDL	
Control	8	212±10	94± 6	83.5±8.4	52.9±7.4	0.63±0.12
Shiitake	8	193±27*	65±17*	52.1±5.0*	31.8±4.5*	0.71±0.21

*Significantly different from the control diet, $P < 0.01$.

containing 10% Shiitake powder in the present study was basically consistent with the results that Kaneda et al. (4) and Kabir et al. (12) had reported where they fed rats on 5 % Shiitake powder diets for 9 or 10 weeks. Table 1 also showed that the HDL-cholesterol in plasma was significantly lowered by the Shiitake diet like as the total cholesterol level, which was the same as the results by Kabir et al. (12). Meanwhile, our data on significantly lowered growth by the Shiitake diet resemble the results by Kabir et al. (12) who had observed significantly lowered growth by feeding Shiitake diets from four weeks on, though they had reported no data on food intakes in the experiment with no addition of cholesterol to the diet.

On the other hand, the rats, which were given the Shiitake diet for three days after two-day fasting, showed significant decreases in food intake, increase in body weight and plasma total- and HDL- cholesterol ($P < 0.01$) compared to the control rats, as seen in Table 2. The atherosclerotic indexes were not changed in all the groups of rats tested here. Thus it is likely that only five-day feeding experiment can be as effective in evaluation for plasma endogenous cholesterol as longer term feeding experiments since our results of the two experiments were coincided. This finding can give us a greater profit, particularly to shorten feeding term and to restrain expenses for diet.

Table 2. Food intake, increase in body weight and plasma endogenous total- and HDL-cholesterol values in rats fed control and Shiitake diets for 3 days after 2-day fasting.

Diet	Nos. of rat	Food intake (g/rat/3 days)	Increase in body weight (g/rat/3 days)	Plasma cholesterol value (mg/dl)		Total-HDL/HDL
				Total	HDL	
Control	7	45.4±3.4	35.2±3.0	84.0±2.9	54.3±4.7	0.56±0.12
Shiitake	7	37.7±3.5*	28.8±5.4*	31.9±3.0*	22.9±4.0*	0.43±0.21

*Significantly different from the control diet, $P < 0.01$.

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