Wheat Flour Lipase Inhibitor Decreases Serum Lipid Levels in Male Rats

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Summary Triacylglycerol is digested and absorbed primarily through the action of lipase in the lingual and pancreatic juices. We used a wheat flour-derived lipase inhibitor to inhibit triacylglycerol absorption from the intestinal tract, and studied the effect on serum lipid concentration. Rats were given free access to high fat diets containing the lipase inhibitor at 0 (control), 0.05, 0.1 and 0.2% levels for 3 weeks, and their serum triacylglycerol, total cholesterol, and HDL-cholesterol concentrations were measured at weeks 1, 2 and 3. The measured values were significantly lower in the groups receiving the lipase inhibitor than in the control group. The degree of decrease in these respects was roughly dose-dependent. Additionally, the inhibitor intake raised the fecal lipid excretion and lowered the hepatic cholesterol level. It was, therefore, assumed that the inhibition of lipase in the digestive tract interfered with lipolysis and thereby with cholesterol absorption.

Key Words lipase inhibitor, triacylglycerol, hypotriglyceridemia, serum lipids

Excess fat intake is the main cause of chronic degenerative diseases, so-called 'adult's diseases,' such as hyperlipidemia, atherosclerosis and obesity (1,2). The relationship between cholesterol or triacylglycerol and atherosclerosis or heart disease has been well established. The serum cholesterol concentration is closely related to the incidence of ischemic heart disease (3,4). The concept of normalcy in the serum cholesterol concentration is somewhat in doubt, while various treatments have been established to lower the serum triacylglycerol level. For example, dietary fiber is believed to improve lipidemia. Lin et al. (5), and Miettinen and

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Tarpila (6) attempted to improve the serum lipid level by the use of pectin, useful to lower the cholesterol concentration but not the triacylglycerol concentration, although pectin served as an inhibitor of pancreatic lipase (7,8). In fact, excess administration of pectin caused morphological abnormalities in the gastric and small intestinal mucosae (9–11). As a side effect, pectin, chitin and chitosan somewhat disturbed intestinal absorption of minerals and other nutrients (12–15). Effective measures for hypolipidemia have not been firmly established.

We took note of the fact that triacylglycerol would not be absorbed as such unless hydrolyzed by lingual or pancreatic lipase. We had already purified a proteinous lipase inhibitor from wheat flour (WFLI) (16). This investigation was designed to examine the inhibitory effects of WFLI on digestion and absorption of dietary fat relating to improvement in the serum lipid levels.

MATERIALS AND METHODS

Chemicals. Lipase of porcine pancreatic juice origin (Type VI-s) was purchased from Sigma Chemical Co. (St. Louis, MO). Other chemicals were purchased from Wako Pure Chemical Industries Ltd. (Osaka). A powdered stock diet 'CE-2' consisting of 25% protein, 4.4% fat, 4.4% fiber and 7% ash was obtained from Japan Clea Inc., Tokyo.

Preparation of WFLI and activity measurement. WFLI was purified according to the method of Tani et al. (16) and its activity was measured by the method of Satouchi et al. (17). The specific activity of WFLI used throughout this experiment was 600 U/mg on a dry weight basis.

Animals and feeding. Male Sprague-Dawley rats weighing about 90 g were purchased from Sankyo Labo Service Co., Tokyo. Rats of 4 groups ($n=7$) were individually housed in metabolism cages which were placed in an environmentally controlled room (22–24°C). A high fat diet was made by adding 100 g of soybean oil to 1 kg of CE-2. Each rat was given free access to both drinking water and 15 g/day of the high fat diet supplemented without (control) or with WFLI (0.05, 0.1 or 0.2% group) for 3 weeks.

Analysis of serum lipids. At stated intervals during the experimental period, the rats not deprived of the diet were bled from the tail vein, and the sera were immediately separated. Total cholesterol was determined by the cholesterol oxidase method (18), triacylglycerol by the glycerol-3-phosphate oxidase (GPO) method (19), and HDL-cholesterol by the heparin-Mn precipitation method (20).

Analysis of fecal lipids. Feces collected at one-week intervals were lyophilized and powdered. Their part (1 g) was extracted with three volumes of a chloroform/methanol (1:1) mixture. The quantity of fecal lipids is expressed as mg/day per 100 g of body weight.

Analysis of hepatic lipids. At the end of the experimental period, the rats were anesthetized with ether and killed by cervical dislocation, and then the livers were removed. Each liver was homogenized with nine volumes of physiological saline.

The total cholesterol and triacylglycerol concentrations were measured as described above.

Statistical analysis. Data are expressed as the $M \pm SD$. Comparison between groups was made by ANOVA, followed by Duncan's multiple range test (21). $p < 0.05$ was taken as the level of significance.

RESULTS

Table 1 summarizes alterations in body weight as well as daily food intake at one-week intervals during the feeding period of 3 weeks. There were no significant differences in food intake or growth among the four groups without and with supplemental WFLI. Weekly changes in the serum triacylglycerol concentration for all the groups are presented in Fig. 1. The serum triacylglycerol concentration at week 3 in the control group was raised to 1.5 times as much as at week 1. Such an increase was depressed when WFLI was given; the degree of increase was reduced by one-half in the 0.05% group and by one-quarter in the 0.2% group.

Weekly changes in the serum total cholesterol concentration are illustrated in Fig. 2. The same degree of total cholesterol level was shown in the control and 0.05% group at week 1. Thereafter, the total cholesterol level tended to decrease in all the groups. The WFLI uptake was also effective in lowering the total cholesterol level; the significant difference ($p < 0.05$) from the control was observed in the 0.2% group at week 1, in the 0.1 and 0.2% groups at week 2 and even in the 0.05% group at week 3.

Weekly changes in the serum HDL-cholesterol concentration are depicted in Fig. 3. At week 3, the three groups receiving WFLI were significantly different from the control group; their relative levels to the control were 81% in the 0.05% group, 77% in the 0.1% group and 80% in the 0.2% group, respectively. Nonetheless, the serum HDL-cholesterol concentration was not so much affected by the WFLI uptake as those of the serum total cholesterol and triacylglycerol.

The effects of WFLI uptake on the fecal lipids collected as specified are shown

Table 1. Body weights and daily food intake at one-week intervals of rats fed with the respective experimental diets.

Group (n = 7)	Week 1		Week 2		Week 3	
	Body weight (g)	Food intake (g/day)	Body weight (g)	Food intake (g/day)	Body weight (g)	Food intake (g/day)
Control	106 $\pm$ 15	9.6 $\pm$ 2.1	134 $\pm$ 9	11.0 $\pm$ 2.8	165 $\pm$ 27	13.1 $\pm$ 2.5
WFLI 0.05%	105 $\pm$ 7	10.1 $\pm$ 1.7	129 $\pm$ 16	12.5 $\pm$ 1.4	177 $\pm$ 8	14.5 $\pm$ 0.7
0.1%	111 $\pm$ 5	10.1 $\pm$ 2.1	140 $\pm$ 10	12.9 $\pm$ 1.5	177 $\pm$ 15	14.2 $\pm$ 1.1
0.2%	109 $\pm$ 4	9.3 $\pm$ 2.1	137 $\pm$ 11	13.9 $\pm$ 1.6	184 $\pm$ 7	14.7 $\pm$ 0.5

Values are the $M \pm SD$ of 7 rats in each group.

Fig. 1. Effect of lipase inhibitor supplementation on serum triacylglycerol concentration in rats. Rats were daily fed 15 g of high fat diets supplemented without (control group) and with WFLI at 0.05, 0.1, 0.2% (w/w) for 3 weeks. Meanwhile, their serum triacylglycerol levels were measured at one-week intervals. Values are the $M \pm SD$ ($n=7$). Superscripts not sharing a common letter are significantly different at $p < 0.05$.

Fig. 2. Effect of lipase inhibitor supplementation on serum total cholesterol concentration in rats. The experimental details were the same as in Fig. 1, except for total cholesterol instead of triacylglycerol.

Fig. 3. Effect of lipase inhibitor supplementation on serum HDL-cholesterol concentration in rats. The experimental details were the same as in Fig. 1, except for total HDL-cholesterol instead of triacylglycerol.

in Fig. 4. The average lipid content in the feces throughout the experimental period in the control group was 1,500 mg/day/100 g of body weight. In the groups receiving WFLI, the fecal lipid excretion was maintained almost at a fixed level from week 1 to week 3, although it was a little elevated compared with that in the control.

The triacylglycerol and total cholesterol contents in the liver at the end of experiment are summarized in Table 2. The hepatic triacylglycerol content was 118 mg/g in the control group. Those in the groups receiving WFLI tended to decrease with increasing amounts of WFLI administration. A similar tendency was observed for the hepatic cholesterol level. The hepatic total cholesterol content was 40.8 mg/g in the control group, and those in the groups receiving WFLI were reduced by 7.2% in the 0.05% group, and by 20.8% in the 0.2% group relative to the level in the control group.

DISCUSSION

The relations of serum cholesterol and/or triacylglycerol levels with chronic degenerative diseases have been well investigated. Various remedies have been proposed for improvement in serum lipids. The use of pectin as a dietary fiber, for example, has been reported to lower the serum cholesterol concentration (5,6). This is accounted for by interfering with the pancreatic lipase action due to wrapping lipid droplets in pectin gels (7,8). The excess uptake of pectin causes morphological abnormalities in the gastric and small intestinal mucosal membranes

Fig. 4. Fecal lipid excretion in rats fed high fat diets supplemented with and without lipase inhibitor. Feces collected at one-week intervals were lyophilized and powdered. Then, they were extracted with chloroform/methanol (1:1). The lipid content is expressed as mg/day/100 g of body weight. Values are the $M \pm SD$ ($n=7$). Superscripts not sharing a common letter are significantly different at $p < 0.05$.

Table 2. Effects of WFLI administration on triacylglycerol and total cholesterol contents in the liver of rats.

Group	Liver (g)	Triacylglycerol (mg/g)	Total cholesterol (mg/g)
Control	5.3 ± 0.1	118 ± 21^a	40.8 ± 6.3^a
WFLI 0.05%	5.4 ± 0.1	111 ± 18^a	37.9 ± 8.1^{ab}
0.1%	5.5 ± 0.1	94 ± 10^b	33.0 ± 5.4^b
0.2%	5.6 ± 0.1	92 ± 16^b	32.3 ± 8.6^b

Values are the $M \pm SD$ of 7 rats in each group. Different superscript letters in the same column denote a significant difference at $p < 0.05$ between groups.

(9–11). Chitosan has a similar cholesterol-lowering action as well. It remains obscure, however, to what extent their long-term ingestion affects the vital function. Alternatively, we noticed that WFLI is effective in lowering the serum triacylglycerol concentration. As a result of examination, the serum triacylglycerol concentration at week 2 was found to be significantly lower in the groups receiving

WFLI than in the control group (Fig. 1). This finding suggests that WFLI inhibits digestion as well as absorption of triacylglycerol in the small intestine. The serum triacylglycerol concentration decreased with increasing dose of WFLI, being at a marginal level throughout the experimental period in the 0.2% WFLI group. Thus, the inhibition of such a lipolytic enzyme is considered as a useful means of treating hyperlipidemia.

The serum cholesterol concentration could be also effectively lowered by WFLI administration (Fig. 2). We assumed that the inhibition of lipase by WFLI in the digestive tract would not only prevent lipolysis but also depress cholesterol absorption. This assumption is supported by a tendency of the serum HDL-cholesterol to decrease (Fig. 3) or the fecal lipid excretion to increase (Fig. 4).

The hepatic triacylglycerol and total cholesterol contents were lower in the groups receiving WFLI than in the control group. Their contents in the liver decreased with increasing WFLI doses (Table 2). The effect seemed roughly dose-dependent.

The risk of chronic degenerative diseases such as hyperlipidemia, obesity and atherosclerosis tends to increase in developed countries. It is a grave matter that chronic degenerative diseases occur even in the younger generation. Although the importance of serum triacylglycerol has been indicated in recent years, an effective means of lowering the serum triacylglycerol concentration other than dietary restriction has not yet been established. In this study, WFLI was used to disturb lipolysis in view of lipid digestion and absorption. In practice, the inhibition of lipase resulted in a significant decrease in the serum lipid level. The appropriate use of WFLI is expected to be effective in the treatment and prevention of chronic degenerative diseases. Undesirable side effects due to WFLI administration are now being investigated.

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