Effects of Oil Heated with Gluten on Weight-Loss Dieting. II.

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Abstract: We previously proposed that oil heated with gluten was suitable for use as a safe oil for weight-loss dieting. In the present paper, the properties of the oil were improved, and the weight-loss effect was compared with that of heated oil. Fresh oil was heated for 10 h at 180°C with or without gluten and filtered using filter paper. A powdered diet (AIN93G; no fat) was mixed with 7 wt% of fresh oil (control) or filtrates of the heated oils described above, and the mixture was fed to male Wistar rats for 12 weeks. The gluten and heated oil groups showed no gross symptoms attributable to the experimental oils but had a slowed body weight increase; a significant difference was found in weight on and after 21 weeks of age as compared to rats consuming the control diet, and fecal excretion was increased as compared to the control group. Serum levels of triacylglycerol, phospholipids, cholesterol, and glucose of the gluten and heated oil groups were significantly lower than those of the control group. High aspartate aminotransferase (AST) levels occurred more frequently in the heated oil group than the gluten group. The number of rats with dark red patches on the surface of the liver, which are indicative of liver damage, was higher in the heated oil group. In conclusion, the weight-reducing effect of the oil heated with gluten was confirmed and improved by removing traces of heated gluten from the oil.

Key words: diet, body weight, gluten, melanoidin, metabolic syndrome

1 INTRODUCTION

The fundamental treatment of the metabolic syndrome lies in dietary modification and exercise. In particular, the most effective strategy involves suppression of total energy intake. However, it is difficult to modify lifelong food habits, and the sensation of repletion, in particular, will not be fulfilled with reduced food intake. The high blood triacylglycerol levels in metabolic syndrome are due to a continuously excessive intake of carbohydrates and lipids. The amount of lipids in foods can be decreased physically, but consumers may not find such products acceptable because lipids greatly increase the savor of foods. When overweight or obese individuals try to lose weight through dieting, they still want to have tasty foods containing sufficient lipids for palatability.

We heated oil with amino acids, gluten, sucrose, or wheat starch at 180°C for 20 h^{1,2)}. Among the oils obtained, only the oil heated with gluten had a mild odor; the others

had a rancid odor. When Wistar rats were fed *ad libitum* a commercial standard diet containing 7 wt% of the oil supernatant, respectively, for 12 weeks, no gross symptoms attributable to the oil were observed. The body weight of the rats fed a diet containing oil heated with gluten (gluten group) increased slowly from the 5th experimental week and was 5% lower in the 11th and 12th weeks, with a significant difference from that of control animals fed a diet containing fresh oil. Although one and two of the eight rats showed slight histological damage in the kidneys and liver, respectively, there was no hematological abnormality in the group. Thus, oil heated with gluten appears to be suitable as a safe oil for people who are dieting to lose weight.

In the present study, an animal experiment similar to the one described above was carried out employing oil filtered after heating with gluten. The filtration step was added because heated gluten remaining in the oil supernatant

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seemed to be responsible for the slight cytotoxicity observed in the previous study. Feces were collected to compare body weight increases with amounts excreted.

2 EXPERIMENTAL

2.1 Materials

2.1.1 Oil

One liter of fresh oil, commercial frying oil, was heated at 180°C for 10 h in a 2-L four-necked flask under stirring with/without 1 wt% gluten from wheat (Nacalai Tesque, Inc., Kyoto), respectively. Oil heated with gluten was filtered over filter paper under a reduced pressure and both employed for an animal experiment.

2.1.2 Diets

A commercial pelleted diet (Labo MR Stock, Nihon Nosan Kohgyo, Japan) and a powdered AIN93G diet without fat (Japan Clea, Tokyo) were purchased. Using a blender, the latter was mixed uniformly with 7 wt% of experimental oils and fresh oil, respectively. The three kinds of diets, thus prepared, were handled and provided as described in our paper¹⁻⁶. All the diets were used after radio-sterilization.

2.1.3 Chemical Analyses

Methods for chemical analyses of oil, peroxide value (POV), carbonyl value (COV), polar compound content (PC), triacylglycerol content (TG), and acid value (AV), were the same as in our previous papers⁵⁻⁹. The fatty acid compositions of the fresh oil analyzed as previously⁵⁻⁹ were as follows: myristic acid 0.1%; palmitic acid 9.4%; palmitoleic acid 0.1%; stearic acid 3.6%; oleic acid 32.6%; linoleic acid 44.1%; and α -linolenic acid 6.3%; eicosenoic acid 0.5%; others 3.3%.

2.2 Animals

Male Wistar rats aged 9 weeks were obtained from Japan SLC, Inc., Shizuoka, Japan, and were housed separately in wire cages at $24 \pm 2^\circ\text{C}$ and humidity $50 \pm 10\%$, with light from 7:00 to 19:00 at Japan SLC, Inc., Animal Experiment Center, Shizuoka, Japan. Animal care and handling were in accordance with the Ethical Agreement Concerning Care and Use of Laboratory Animals for Research and Education, Kobe-Gakuin University.

2.3 Procedure

2.3.1 Animal experiment

Twenty-four animals were maintained on Labo MR Stock for 1 week of adaptation; animals were then divided into three groups (8 rats/ group) by Statlight System, Yukms, Tokyo, Japan in order to make average body weights similar and minimize standard deviation in body weight. Two groups were fed a diet containing 7 wt% oil heated with or without gluten, (gluten group/ heated oil group), and the

third group was fed a diet containing 7 wt% fresh oil (control). All animals were allowed feed and water *ad libitum* throughout the experiment. Autoxidation of oil in the diet was avoided by supplying a fresh diet daily. Body weight, the amount of feed ingested, and fecal amounts were determined weekly; feces were collected by using a metabolism cage. After 12 weeks, a fasting period of 18 h was imposed prior to the administration of anesthesia. Serum was obtained from blood drawn from the abdominal aorta. Livers, kidneys, and retroperitoneal tissue were excised, weighed, and examined.

2.4 Hematological analyses

All the analyses were determined as in our previous papers^{1,2}.

2.5 Histological evaluation

All the methods were the same as those in our previous papers¹.

2.6 Statistical analysis

All the values obtained from animals are revealed as mean $\pm$ SD. Data from 8 animals each for experimental and control groups were analyzed using Student's t-test for unpaired observations and results were considered significant at $p < 0.05$.

3 RESULTS

3.1 Oil analyses

Chemical properties of gluten, heated, and fresh oils were as follows, respectively: PC (%) 21.6, 23.9, 4.2; TG (%) 83.5, 85.0, 98.6; COV 30.6, 35.4, 2.7; POV (mEq/kg) 1.4, 3.6, 0.9; AV 0.1, 0.1, 0.1; Color (Gardner) 8, 6, 2; smell mild, rancid, fresh. After heating for 10 h, PC and COV increased and TG decreased. Peroxides generated during heating seem to have decomposed to corresponding carbonyl compounds, resulting in very low POV. AV stayed unchanged because there was no contact with water during heating. Gluten browned oil due to the amino carbonyl reaction but produced a mild, slightly paint-like odor.

3.2 Growth of animals

All the rats appeared to grow normally; no diarrhea, seborrhea, dermatitis, or excessive hair loss was observed after the administration of any diet. Body weight also increased normally (Fig. 1), but the difference among the three groups became gradually greater after 17 weeks of age, and the weights of the rats in the gluten and heated oil groups were significantly lower than that of the control rats at 21 and 22 weeks of age. Because of age, the amount of food ingestion decreased slowly without any statistical difference among the three groups. This indicates that the

Fig. 1 Growth of Wistar Rats Fed a Diet Containing Oil Heated with/without Gluten.

* $p < 0.05$, significantly different from the value of control (unpaired t-test).

experimental oils did not have adverse effects on the appetite of the rats.

No difference in the weight of liver (means of the three groups from 8.921 g to 9.213 g), left and right kidneys (means of the three groups from 0.989 g to 1.093 g) was detected between groups. However, there were significant decreases ($p < 0.01$) from the value of control in weights of retroperitoneal fat of the experimental groups. Means $\pm$ SD for eight animals were as follows; gluten group 7.816 ± 0.976 , heated oil group 8.425 ± 1.642 , and control 11.064 ± 1.955 . The effect of heated oil should be related directly to the fat weight. However, it was notable that the weight for the gluten group tended to be less than that for the heated oil group in spite of body weight.

3.3 Fecal analysis

Feces from rats in the experimental groups were often bigger and more brownish in color than those from the control animals (Fig. 2). In addition, feces excreted by the

gluten group were sometimes white during the latter half of the experimental period. All the feces collected at 10 weeks of age smelled abnormal, but the rest had a similar light smell, probably because of the basal diet difference. Dry weights of feces accounted for about 80% of the wet weights in all collections, implying that the water contents of feces did not differ during the experimental period (Fig. 3). Values for 10 weeks of age were obtained from feces collected on the 4th day after diet change from Labo MR Stock to the experimental diets. After 11 weeks of age, the fecal quantity of all the rats decreased by 20 to 30% and remained in a low weight range. The highest to lowest weights were those of the heated oil group, gluten group, and control group, respectively, except for the values for 12 weeks of age; after that, weights of feces of the experimental groups, both wet and dry, were significantly higher than that of the control group. This seems to show that the two experimental oils contained portions that were difficult to digest and were excreted vigorously. Although the excreted amount of the gluten group was two-thirds of that of the heated oil group, the body weights of the both groups differed little from each other. Ten-, twelve-, thirteen-, and twenty-one-week old rats of the gluten group showed a significantly higher amount of dry feces than the heated oil group did.

3.4 Hematological analyses

As shown in Table 1, levels of glucose, triacylglycerol, phospholipids, and cholesterol in the gluten and heated oil groups were significantly lower than those of the control group. There were no significant differences in levels of AST, alanine aminotransferase (ALT), insulin, and adiponectin. In particular, the ALT values of all the groups were almost the same. But, the occurrence of AST values higher than maximum AST (118 IU/L) of the control was 1 rat / 8 rats for the gluten group, and 2 rats / 8 rats for the heated oil group.

3.5 Visible changes in livers and kidneys

As shown in our previous paper^{1,10)} the individual degree of damage to the liver is indicated clearly by the number of

Fig. 2 Typical Feces of Rats Fed a Diet Containing Oil Heated with/without Gluten.

Fig. 3 Fecal Weights of Rats Fed a Diet Containing Oil Heated with/without Gluten.

Fecal weights (wet and dry) of gluten and heated oil groups were significantly higher than that of control at $p < 0.05$ on and after 14 weeks old (unpaired t-test). # $p < 0.05$, significantly different from the dry weight of heated oil group (unpaired t-test).

Table 1 Hematological Values in Wistar Rats Fed for 12 Weeks a Diet Containing Oil Heated with/without Gluten.

	Gluten	Heated oil	Control
Glucose (mg/dL)	116.0 $\pm$ 9.9*	114.8 $\pm$ 8.2*	129.9 $\pm$ 14.7
Triacylglycerol (mg/dL)	46.5 $\pm$ 13.8**	46.5 $\pm$ 12.1**	149.6 $\pm$ 38.1
Phospholipids (mg/dL)	107.4 $\pm$ 10.8**	106.4 $\pm$ 12.1**	132.9 $\pm$ 12.0
Free fatty acids (mEq/L)	0.72 $\pm$ 0.07	0.66 $\pm$ 0.08	0.66 $\pm$ 0.11
Cholesterol (mg/dL)	68.9 $\pm$ 7.8**	68.5 $\pm$ 9.2**	95.0 $\pm$ 11.7
AST (IU/L)	95.4 $\pm$ 34.1	100.1 $\pm$ 33.0	87.6 $\pm$ 17.9
ALT (IU/L)	52.5 $\pm$ 11.1	53.3 $\pm$ 9.1	54.4 $\pm$ 23.9
Insulin (ng/mL)	1.75 $\pm$ 0.93	1.85 $\pm$ 0.89	2.09 $\pm$ 1.12
Adiponectine (μ g/mL)	3.77 $\pm$ 0.66	3.83 $\pm$ 0.85	4.30 $\pm$ 0.92

Values are means $\pm$ SD for eight animals.

* $p < 0.05$, significantly different from the value of control (unpaired t-test).

** $p < 0.01$, significantly different from the value of control (unpaired t-test).

dark red patches due to dotted bleeding observed on the surface of the liver¹⁻³⁾ as well as by AST, ALT, and histological microscopic specimens, so microscopic analysis was not performed in the current study. The number of rats with dark red patches per group (8 rats) was 1, 3, and 1, for the gluten group, heated oil group, and control, respectively. A control rat and a rat in the gluten group also had patches, but their frequency and numbers were obviously small. Although the average AST of the heated oil group did not differ significantly from that of the control group, a

clear difference was found in the heated oil group in the number of rats with dark red patches.

No differences in color and size were observed in the kidneys of experimental vs. control rats, and no patches were observed in any group.

4 DISCUSSION

Lopez-Varela *et al.*¹¹⁾ fed growing male Wistar rats a diet

containing sunflower oil used for frying (PC 19.1%). Although food intake was similar in the experimental and control groups, final body weight and body weight gain were significantly lower in the experimental group. The increase of the hepatosomatic index in the experimental group was attributed to ingestion of potentially toxic polar compounds in the used oil. But in the present study, the liver and kidneys of the gluten group were as healthy as those of the control group in spite of feeding oil heated with gluten (PC 21.6%). This supports our previous finding¹⁰ that chemical properties of oils, such as PC, POV, COV, TG, and AV, have little to do with the cytotoxicity of deteriorated oil.

The impairment of the nutritional value of thermally oxidized edible oils has been extensively reported¹², and low fat absorption has generally been attributed to the presence of triacylglycerol polymers¹³. Marquez-Ruiz *et al.*¹⁴ fed Wistar rats *ad libitum* a diet containing 6 to 20 wt% of oil heated at 180°C for 150 h. They collected feces and analyzed the fecal lipids by high-performance size exclusion chromatography. They found that enzymatic hydrolysis was markedly reduced when the dietary triglycerides were of complex structure and that the extent of *in vivo* hydrolysis depended on both the amount and degree of alteration of the dietary oil. In addition, it was found that the reduction of non-altered fatty acid digestibility for the heated oil was mainly attributable to fat hydrolysis impairment, and that difficulties in the hydrolysis of complex glyceridic molecules contributed more to the low fat absorption of heat-oxidized fats than poor absorption of fatty acid polymers did. In contrast¹⁵, the extremely low digestibility coefficient of non-polar dimer fatty acids along with the presence of high free fatty acid levels in non-absorbed lipids suggested difficulties specifically during the absorption process. Thus, oil in which the chemical structure is partly modified by heating has low nutritive value because it is not fully hydrolyzed and absorbed from the intestine. The experimental oils in the present study should also be hydrolyzed and absorbed incompletely, resulting in increased amounts of fecal lipids. The important dietary differences between the gluten and the heated oils were cytotoxicity to the organs, fecal amounts, and possibly the effect on the weight of retroperitoneal fat ($p = 0.19$). We will report the result of fecal analysis and measurements of total lipids, cholesterol, and bile acids in another paper.

In the present study the body weights of the gluten group decreased 5.2%, 6.2%, and 7.3% at 20, 21, and 22 weeks of age, respectively. These decreases were greater than the decreases in the previous study², which were 4.1%, 4.2%, and 4.5%, correspondingly. Although the chemical properties of the oil employed in the present study were less changed by heating than those of the previous one, the weight-reducing effects of the oil were emphasized. This is probably because traces of heated gluten

remaining in the previous oil influenced body weight increase. Possible inhibition of the heated gluten against weight-reducing effects is an important point as much heated protein exists in favorite Japanese fried foods, especially in batter coatings of fried foods^{7,8}. The oil heated with gluten also decreased the weight of fat tissue substantially. The heated oil suppressed the body weight increase of rats more than gluten did, but the same relationship was not seen for the weight of fat tissue. The difference between the oil heated with gluten and the heated oil must relate to the presence of oil-soluble heated gluten and/or melanoidin, although both oils should contain similar amounts of complex structures that are resistant to hydrolysis by lipase. This finding suggests fat weight-reducing effects of the oil-soluble heated gluten and/or melanoidin in addition to the weight-reducing effects of the complex described above.

Fahey *et al.*¹⁶ reported that a diet containing brown pigments separated from cane molasses stimulated the growth of rats, while Lee *et al.*¹⁷ found that diets containing Maillard reaction products formed from a reaction mixture of reducing sugar and protein or amino acid caused diarrhea as well as depressed growth rates. Homma *et al.*¹⁸ fed Wistar rats a diet containing 2% of ¹⁵N-nondialyzable melanoidin. The growth response and the digestibility of protein and lipids in rats fed the experimental diet were not significantly different from those of control animals in the study.

Structure-specific health-promoting effects of newly identified melanoidins by means of their antioxidant and chemopreventive activities have been described¹⁹ in cell culture investigations as well as in animal feeding studies and human trials. Harmful effects of dietary melanoidins have also been investigated¹⁹ in the context of their ability to promote glycation reactions *in vivo*. Papetti *et al.*²⁰ reported that roasted barley yielded strong antioxidant components that were able to efficiently scavenge free radicals in any system used. Borrelli *et al.*^{21,22} also reported that soluble melanoidins from a model system and biscuits exhibited strong antioxidant activity and did not show any cytotoxicity on Caco-2 cells. In our previous paper¹⁰, the odor of fresh oil and oil heated with/without gluten was analyzed by head-space gas chromatography. The height of several peaks assignable to rancid odor of the heated oil decreased in the oil heated with gluten by one third, while fresh oil showed a few very small peaks. The oil heated with gluten had a new peak instead, which was likely to make its mild odor different from the rancid odor of deteriorated oil. This property of gluten against oil or oxidized oil seems to be different from the antioxidant activity of protein described above.

Marquez-Ruiz *et al.*²³ reported that fecal endogenous sterols, particularly cholesterol, increased as dietary oil alteration increased. The reason why white feces were

observed in the gluten group may relate to the presence of increased amounts of steroids in the feces. The amount of feces produced by the experimental groups was obviously greater than that of the control. The increased weight should be due not only to increased amounts of lipids including steroids but also to non-lipid food components. On the other hand, an increase in feces production caused by melanoidin was not found, as reported previously²⁴⁾.

Hematological evaluation showed that levels of triacylglycerols (a major cause of metabolic syndrome), phospholipids, cholesterol, and glucose were decreased significantly by the ingestion of reasonable amounts of the oil heated with gluten. In particular, the level of triacylglycerols decreased to one third of that of the control group, showing that the supply of fatty acids was strictly restricted by the administration of the oil heated with gluten. When compared to the control group, the gluten group had a tendency toward higher AST and toward lower levels of insulin and adiponectine, with non-significant differences. The same pattern was observed in the heated oil group, but in histological evaluation the heated oil was obviously inferior to the oil heated with gluten. The cytotoxicity seems to be attributable to low-molecular-weight substances that are closely related to the rancid odor of deteriorated oil¹⁰⁾.

In conclusion, the weight-reducing effects of the oil heated with gluten was confirmed, and improved by removing traces of heated gluten from the oil. The oil heated with gluten needs to be improved in color, odor, and other properties, but it should be acceptable to people who are eager to lose weight because it would not be different from the oil contained in conventional fried foods in mouth feel, palatability, sensations of fullness, digestion, excretion, usage, stability under air, and other important properties.

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