A Novel Body Weight-loss Promoting Oil Prepared with Vegetable Protein

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Abstract: It has been reported that oil thermally processed with wheat gluten (gluten oil) exhibited safe weight-loss promoting effects in animal experiments. However, as the oil has a high color index, and its chemical properties and smell differ from those of fresh oil, it is uncertain if the oil will find market acceptance. In order to resolve the issue, frying oil was heated with soybean protein under reduced pressure (soybean protein oil), resulting in a product with an appearance, chemical properties and smell comparable to those of fresh oil. This improved oil was mixed (7 wt%) with powdered AIN93G no fat, defined standard diet and fed to 10-week-old Wistar rats *ad libitum*. The experimental rats grew normally, ingesting the same amount as that of the control rats; however, there was a negative correlation between body weight increases and fecal weight increases. After the 12-week feeding period, all the rats were sacrificed to obtain blood and organs. In the experimental group, liver weight, retroperitoneal fat tissue weight and serum triacylglycerol (TG) levels decreased significantly. Aspartate aminotransferase (AST), alanine aminotransferase (ALT), and histological analysis supported the safety of the improved oil. In conclusion, it was found that soybean protein oil inhibited body weight increases without any adverse effects in animal experiments. The oil holds promise as a novel dieting oil that steadily decreases body weight at an appropriate rate.

Key words: soybean protein, weight-loss promoting, fecal weight, serum triacylglycerol level, retroperitoneal fat tissue

1 INTRODUCTION

Metabolic syndrome is prevalent in major countries around the world. It should be highly worthwhile developing an effective body weight-loss promoting oil that is comparable to traditional oils in quality, palatability, sensations from ingestion to excretion, cooking properties, oxidation stability, and availability. We have reported that gluten oil showed safe body weight-loss promoting effects in animal experiments¹⁾. However, as the oil has slightly degraded chemical properties, and a smell different from that of fresh oil, it is uncertain if the oil will find market acceptance. Attempts were made in refining the oil, but this was not possible by ordinary means.

The weight-loss promoting effects are thought to come partly from the polymerized oil contained in the gluten oil (TG content 83.5%), which is minimally hydrolyzed by lipase and easily excreted. However, body weight increases

in the dieting oil group were inhibited to the same extent as those of rats fed oil heated without wheat gluten (heated oil; TG content 85.0%) in spite of the finding that fecal lipids of the former group were significantly lower than those of the latter (Fig. 5 in our previous paper²⁾). As it seems that the effects were not 100% due to the polymerized oil, it would be expected that wheat gluten contributes to the effects. Thus, a revised method for the preparation of dieting oil should be investigated to reduce the formation of polymerized oil, as well as improving its chemical properties and smell. In the present study, soybean protein, frequently used in everyday cooking, was employed as a protein source. Frying oil was heated with the protein under reduced pressure, filtered, and fed to rats to determine its body weight-loss promoting effects.

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2 EXPERIMENTAL

2.1 Materials

2.1.1 Protein

Soybean protein (trial product, Fuji Oil Co., Ltd., Osaka, Japan) was obtained.

2.1.2 Oil

One liter of fresh frying oil (Fuji Oil Co., Ltd., Osaka, Japan) was heated at 180°C and 5 Torr for 10 h in a 2-L four-neck round-bottom flask under stirring with 1wt% soybean protein. Oil heated with protein was filtered over filter paper under reduced pressure (soybean protein oil) and employed for an animal experiment.

2.1.3 Oil Analyses

Peroxide value (POV), carbonyl value (COV), acid value (AV), iodine value (IV) and Lovibond color were measured according to the Japan Oil Chemists' Society's Standard Methods for the Analysis of Fats, Oils, and Related Materials. The content of polar compounds (PC) was analyzed by a PC Tester, 3M, Saint-Ouen l'Aumone, France³). Triacylglycerol contents (TG) were determined by applying samples on Chromarods followed by development in a solvent mixture, hexane/diethyl ether/acetic acid, 50:10:1 v/v/v, and quantification with Iatroscan MK-6s, Iatron Laboratories, Inc., Tokyo, Japan. After esterification of oil with diazomethane for possible free fatty acids and then with sodium methoxide for triacylglycerol, oil was analyzed by Shimadzu GC-2010 gas chromatograph equipped with 60-m SPTM-2340 capillary column of 0.25 mm bore, Supelco, St. Louis, MO, US; sample injection temp. 240°C; column temperature 150°C, then raised at 1.3°C/min to 220°C; split (1:100); carrier gas He 0.5 mL/min; FID.

2.1.4 Diets

A commercial powdered AIN93G diet without fat (Japan Clea, Tokyo, Japan) was purchased. Using a blender, the diet was mixed uniformly with 7 wt% of soybean protein oil and fresh oil, respectively, and 140 g of diet for each rat was sealed in a plastic bag (Diamilon M, Mitsubishi Plastics, Tokyo, Japan) by vacuum sealer (Tospack V-380G, Tosei, Tokyo, Japan), kept in a cold room, and subjected to radio-sterilization of 10 kGy by Kohga Isotope (Shiga, Japan) prior to the animal experiment. When the diet in a bag was used up, the next bag was opened for feeding.

2.2 Animals

Sixteen weanling male Wistar rats aged 3 weeks were obtained from Japan SLC, Inc., Shizuoka, Japan, and were housed separately in aluminum flat cages at 24 ± 2°C and humidity 50 ± 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

Rats were maintained for 7 weeks on commercial pelleted AIN93G, then divided into two groups (8 rats/ group) by Statlight System, Yukms, Tokyo, Japan, so as to make their average body weights similar and minimize standard deviation (SD), housed separately and allowed *ad libitum* water and AIN93G no-fat powder added with 7 wt% soybean protein oil, or 7 wt% fresh oil throughout 12 experimental weeks. Autoxidation of oil in the diet was avoided by supplying a fresh diet daily. Body weight, and the amount of feed ingested in 24 h were determined weekly. Fecal amounts in 24 h were also determined in 10, 13, 16 and 19 weeks of age; feces collected were freeze-dried before weighing. When rats were 22 weeks of age, a fasting period of 18 h was imposed prior to the administration of anesthesia with pentobarbital. Serum was obtained from blood drawn from the abdominal aorta. Livers, kidneys, and retroperitoneal fat tissue were excised, weighed, and examined. Total feed ingestion of each rat was also measured.

2.4 Serum biochemical analyses

Determinations of serum AST, ALT, insulin, glucose (GLC), TG, phospholipids (PL), total cholesterol (T-CHO), and nonesterified fatty acid (NEFA) were made with Autoser A AST, Autoser A ALT (Daiichi-Kagaku-Yakuin Co., Ltd., Tokyo, Japan), Morinaga Insulin Measurement Kit (Morinaga Institute of Biological Science Inc., Tokyo, Japan), Glucose CII-test Wako, Triglyceride E-test Wako, Phospholipid C-test Wako, Cholesterol E-test Wako, and NEFA C-test Wako (Wako Pure Chemical Industries, Osaka, Japan), respectively.

2.5 Histological evaluation

Fixed liver and kidneys were embedded with paraffin, and microscopic specimens were sliced, then subjected to hematoxylin and eosin stain according to conventional methods. Histological evaluation was made at a magnification on 100 and 400 depending on circumstances.

2.6 Statistical analysis

All the values obtained from animals are revealed as mean ± SD. Data from 8 animals were analyzed between the experimental group and control group 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 the oils are shown in Table 1. There was no remarkable difference in any of the variables

Table 1 Chemical Properties of Oil Fed to Rats.

	Soybean protein	Fresh oil
POV (mEq/kg)	0.1	0.1
COV	8.4	3.9
Polar compounds (%)	4.2	4.2
AV	0.1	0.2
TG content (%)	96.9	97.9
IV	124.6	124.0
Lovibond color (R/Y)	1.1/10.0	0.5/5.0
Smell	fresh	fresh

measured. TG content and IV showed almost no polymerization in the experimental oils, while POV and COV should reveal that some peroxidation products were immediately decomposed to carbonyl compounds; AV and polar compound levels show no oxidation/decomposition during preparation. The Lovibond color of soybean protein oil increased slightly, but the difference from fresh oil was difficult to distinguish with the naked eye.

Fatty acid composition of fresh and soybean protein oils obtained was as follows: myristic acid 0.1%, 0.1%; palmitic acid 8.4%, 8.1%; stearic acid 3.5%, 3.4%; oleic acid 33.9%, 34.2%; vaccenic acid (*cis*-11 18:1) 1.9%, 1.9%; linoleic acid 42.4%, 42.6%; α -linolenic acid 7.0%, 6.9%; and others 2.8%, 2.8%. Under the GC conditions employed, neither conjugated linoleic acids eluted immediately after α -linolenic acid nor conjugated linolenic acids eluted more than 13 min after α -linolenic acid were detected (detection limit 0.1%).

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 each diet. Body weight increased normally (Fig. 1), but the difference between the two groups increased gradually and the weight of the soybean oil group was statistically lower than that of control at 16, 18, and 22 weeks of age. Due to age, the amount of food ingested decreased slowly without any statistical difference in the two groups. Thus, the experimental oil did not dampen the appetite of rats. Fecal excretion in the

Fig. 1 Growth of Rats Fed a Diet Containing Oil Heated with Soybean Protein.

* $p < 0.05$, ** $p < 0.01$, significantly different from the value of control (unpaired t-test). Values are expressed as mean $\pm$ SD (n=8).

Table 2 Total Diet Ingested and Food Efficiency Ratio.

	Diet ingested by a rat in 12 weeks (g)	Food efficiency ratio
Soybean protein	1403 $\pm$ 178	0.092 $\pm$ 0.016
Control	1463 $\pm$ 51	0.096 $\pm$ 0.006

Values are expressed as mean $\pm$ SD (n=8).

experimental group was significantly higher than that in the control group.

Table 2 shows the total amount of diet consumed in 12 weeks with no difference among the groups. The food efficiency ratio (body weight increase/total food ingestion) for the experimental group showed a tendency toward low values, but was not statistically different.

3.3 Fecal analysis

The experimental group excreted more feces than the control group in total weight (Fig. 1) and number of parti-

Table 3 Daily Fecal Excretion of Rats Fed a Diet Containing Oil Heated with Soybean Protein.

Age (weeks)	10		13		16		19	
	Particles	Average particle weight (mg)	Particles	Average particle weight (mg)	Particles	Average particle weight (mg)	Particles	Average particle weight (mg)
Soybean protein	5.4 ± 3.4	94 ± 20	8.4 ± 3.5*	95 ± 27	6.1 ± 2.8*	105 ± 23	6.6 ± 2.1*	108 ± 14
Control	4.1 ± 3.0	114 ± 36	4.6 ± 2.4	95 ± 29	3.6 ± 2.3	104 ± 18	3.3 ± 2.0	111 ± 34

* $p < 0.05$, significantly different from the value of control (unpaired t-test). Values are expressed as mean ± SD (n=8).

Fig. 2 Organ Weights of Rats Fed a Diet Containing Oil Heated with Soybean Protein.

* $p < 0.05$, ** $p < 0.01$, significantly different from the value of control (unpaired t-test). Values are expressed as mean $\pm$ SD (n=8).

cles, but the average particle weight was constant regardless of the group (Table 3).

3.4 Organ weights

The weights of organs excised are shown in Fig. 2. No difference in the weights of kidneys was detected between groups. However, significant decreases in the weights of liver and retroperitoneal fat tissue were observed in the experimental group.

3.5 Serum biochemical analyses

As shown in Fig. 3, the TG level of the experimental group was lower than that of control. There were no differences in the remainder of the parameters determined.

Fig. 3 Serum Biochemical Values in Rats Fed a Diet Containing Oil Heated with Soybean Protein.

** $p < 0.01$, significantly different from the value of control (unpaired t-test). Values are expressed as mean $\pm$ SD (n=8).

3.6 Morphological changes in liver and kidneys

There were no observable differences in the color and size of the kidneys in the experimental and control groups, and no patches⁴⁾ were observed on the rat liver surface. Histological observation of the liver and kidneys did not reveal abnormalities in any of the rats⁵⁾.

4 DISCUSSION

In order to remove the effect of the commercial standard diet containing 4% oil⁵⁾ fed to rats from 3-10 weeks of age (adaptation period) in our previous animal experiment¹⁾, AIN93G containing 7% fresh soybean oil was instead given to rats in the present experiment, resulting in an average body weight 20 g more than that in the previous experiment at 10 weeks of age. Therefore, the body weight increases of the control and experimental groups were slowed in the early stage of the experimental period as rats were reaching their maximum body weights. As a result, the dieting effect seemed to be more difficult to observe when compared with our previous experiment¹⁾.

Soybean protein oil showed very little thermal deterioration, such as oxidation and polymerization. The color was slightly yellowish, but seems indistinguishable from fresh oil by ordinary consumers, and no peculiar smell from the soybean protein was detectable (Table 1). The experimental rats fed a diet containing 7 wt% soybean protein oil grew normally without any gross symptoms (Fig. 1). However, body weight increases were slowed from the first week onward and significant differences were found at 16, 18, and 22 weeks of age. There was no difference in daily and total food ingestion and food efficiency ratio between experimental and control groups (Fig. 1, Table 2). The feces of the experimental group appeared normal and individual particle weights were almost constant, but the number of feces was obviously greater than that of the control group (Table 3). The significantly increased fecal amounts resulting from the ingestion of soybean protein oil are in agreement with our previous result¹⁾.

It has been reported that digestive tract motility⁶⁾ is stimulated by hormones such as motilin⁷⁾ and substance P⁸⁾, while somatostatin⁹⁾, gastric inhibitory polypeptide (GIP)¹⁰⁾, and Peptide-YY¹¹⁾ have an inhibitory effect. Motility is delicately controlled by the balance of stimulating and suppressive hormone levels, and therefore, analysis of a particular hormone would not completely elucidate the mechanism for elevated fecal excretion. On the other hand, inhibition of digestive enzyme activity may increase the fecal amount regardless of the motility change. It would be of interest to determine if digestive enzyme activity is inhibited by the dieting oil.

The weights of liver and retroperitoneal fat tissue were low in the experimental group (Fig. 2). This corresponds

well with our previous result¹⁾ and was probably due to insufficient digestion and/or absorption of foods, resulting in decreased nutrient supply to the body and decreased body weight increases.

The experimental group AST and ALT values did not differ from those of the control group (Fig. 3), and histological observation did not reveal any damage to the liver and kidneys, thereby confirming that soybean protein oil lacks cytotoxicity. The TG level of the experimental group was significantly lower than that of the control group, but the remainder of the variables determined were unchanged. This also suggests that the triacylglycerol ingested was not fully digested and/or absorbed. However, levels of PL, T-CHO and NEFA were not at all influenced by the TG change. In our previous result employing gluten oil¹⁾, TG, PL, T-CHO, NEFA, and GLC levels decreased in the experimental group. The difference between the two results may come from the polymerized oil and other coexisting components in the oil heated in the presence of air. Unabsorbed oil should be excreted in feces²⁾, but neither a change in fecal appearance nor a release of unabsorbed oil from the feces was observed¹²⁾.

It is very important to keep in our mind the relation between oil and lipids/energy metabolism. Koch *et al.*¹³⁾ and Eder¹⁴⁾ showed that feeding thermally oxidized oils to rats caused a reduction in the concentration of triacylglycerols and cholesterol in liver and plasma. They explain that the reduction of triacylglycerols may be due to stimulation of hepatic β -oxidation triggered by activation of peroxisome proliferators-activated receptor α (PPAR α) and reduced hepatic *de novo* fatty acid synthesis¹⁵⁻¹⁷⁾. But this is not the case for soybean protein oil which was hardly oxidized.

As conjugated fatty acids were not detected (see 3.1), it was confirmed that the thermal treatment of oil with soybean protein under reduced pressure did not isomerize linoleic and linolenic acids in double bond positions. In addition, conjugated linoleic acid reducing TG levels in the liver and serum^{18,19)} has nothing to do with the present weight loss-promoting effects.

Soybean protein oil showed properties similar to those of fresh frying oil, as described above. During feeding experimentation, the amount of ingested food and calories consumed in rats caged separately were the same. Nevertheless, body weight-loss promoting effects of soybean protein oil were observed. Although gluten oil and heated oil contained 83.5% and 85.0% TG¹⁾, respectively, fecal weights and fecal lipid were higher in the heated oil group^{1,2)}, while body weights were not substantially different from those of the gluten oil group. This shows that the body weight-loss effects are not derived solely from polymerized oil and increases in fecal weight and fecal lipid. Soybean protein oil did not contain much polymerized oil (Table 1), but the soybean protein oil group showed fecal amounts compara-

ble with those of the gluten oil group¹⁾. Thus, it is tantalizing to speculate that soybean protein oil contains some substances inhibiting food digestion and/or absorption. Ideally, the substances should be soluble in oil and effective in very limited quantities. Soybean protein employed in the present study contained various components other than protein, but it has been confirmed^{20,21)} that the body weight-loss promoting effects in excess of those from protein were not expected from amino acids, sucrose, and carbohydrates.

Dieting oils presently marketed around the world contain Salatrim^{®22)}, Caprenin^{®23)}, Olestra^{®24,25)}, Econa^{®26-28)} and Healthy Resetta^{®29-31)}. Salatrim[®], Caprenin[®], and Healthy Resetta[®] are triacylglycerides esterified with long and short (medium) chain fatty acids. Econa[®] is composed mainly of diacylglycerol, and Olestra[®] is sucrose esterified with 6-8 long-chain fatty acids. All the dieting oils, excluding Olestra[®], are subjected to hydrolysis and absorption in the small bowel; Olestra[®] is not hydrolyzed by lipase, but excreted. The soybean protein oil is a simple triacylglycerol, but probably contains a small amount of substances formed from components in soybean protein and oil during heating. In addition to soybean protein, other vegetable proteins can be employed in generating the same body weight-loss effects as those in the present study, such as the use of wheat gluten in our previous paper¹⁾.

In conclusion, healthy rats were fed AIN93G containing a 7% soybean protein oil comparable to fresh oil in appearance, chemical properties, and smell for 12 weeks *ad libitum*. No abnormalities were found in growth, serum biochemical analyses or histological analysis. However, the experimental group body weight increase was obviously decreased, while liver weight, retroperitoneal fat tissue weight and serum triacylglycerol (TG) levels decreased significantly. It is suggested that oil thermally processed with vegetable protein provides safe and effective inhibition of body weight increases and is feasible as a novel weight-loss promoting oil.

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