The Mechanism of Weight-Loss Promoting Effects of Oil Heated with Vegetable Protein

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Abstract: We have previously reported that a soy oil-containing experimental diet (fat-free AIN93G containing oil thermally processed with soybean protein followed by filtration), inhibited body weight increases without any adverse effects when given ad libitum to male Wistar rats for 12 weeks. In the present paper, the mechanism of weight-loss promoting effects was investigated. Fasted 10-week-old rats were fed a slurry composed of AIN93G (fat-free), Cr2O3 (marker), water, and 7 wt% soy oil or fresh oil (control) and sacrificed at 20, 60, 90, 120, 150, 210, 270 or 360 min. The stomach, small intestine, cecum, colon and feces were then collected to determine the distribution of the slurry in the digestive tract. The results indicated that the content was transferred faster from stomach to small intestine in the soy oil group than in the control group. Fecal excretion (derived from a commercial standard diet ingested before slurry administration) in the soy oil group was significantly higher than in the control group. Digestive enzyme activities, lipase, sucrose, and maltose, were not inhibited by soy oil. In addition, feces collected in the 12-week feeding experiment were more in the dry weight and contained higher levels of nitrogen and water in the soy oil group than in the control group, revealing that an increased amount of nutrition was continuously excreted in the former group. The above-described findings suggest that soy oil stimulated peristalsis of the gastrointestinal tract and that colon contents are actively excreted, resulting in safe and steady body weight decreases.

Key words: weight-loss, gastrointestinal tract content transfer, feces, digestive enzyme, soybean protein

1 INTRODUCTION

As the average Japanese ingests a quarter of their oil/fat intake from edible oil, butter and margarine and the rest from meat, fish and so forth1, a reasonable approach to dealing with metabolic syndrome would be through the exploitation of edible oil. It was reported that oil heated with soybean protein under reduced pressure, followed by filtration of the protein (soy oil), accelerated gastrointestinal tract content transfer2. Furthermore, the content in the colon was actively excreted, ending up with remarkable weight-loss even though amounts ingested were constant3. From close observation of the small intestine and colon contents, the feces were found to be derived not from the diet administered in slurry, but from a diet ingested before slurry administration and excreted faster in the soy oil group than in the control group2. Thus, as one of the reasons for the safe and steady weight-loss effects, it was suggested that soy oil in the stomach stimulates gastrointestinal tract peristalsis, resulting in the active excretion of the colon contents3. However, fecal weight increases may be due to incomplete digestion and/or absorption; fecal amounts will increase when digestive enzyme activities, or absorption of the digested diet are inhibited by soy oil.

In the present study, the influence of soy oil on feces, digestive enzymes, and gastrointestinal tract content transfer was examined in detail to clarify the mechanism of weight-loss promoting effects.

2 EXPERIMENTAL

2.1 Materials

2.1.1 Protein

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

Methods for chemical analyses of heated oils, 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, France. 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. Fatty acid composition of fresh and soy oils was determined by GC 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%.

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 soy oil or fresh oil, added with 0.4% chromium (III) oxide and two-fold water to make slurry, kept in a cold room, and subjected to radio-sterilization of 10 kGy by Kohga Isotope (Shiga, Japan) prior to the animal experiment.

2.2 Animals

Eighty weaning male Wistar rats aged 9 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.

2.3 Procedure of animal experiment

Rats were maintained for 1 week on radio-sterilized commercial pelleted diet, Labo MR Stock, Nihon Nosoan Kogyo, Yokohama, Japan, then divided into 16 groups (5 rats/group) by Statlight System, Yukms, Tokyo, Japan, housed separately. After a fasting period of 16 h, rats were administered experimental diet (8 groups) or control diet (8 groups) orally with syringe (1 mL/100 g body weight). At 20, 60, 90, 120, 150, 210, 270 or 360 min after administration, one group fed each diet was sacrificed under anesthesia with pentobarbital and the stomach, small intestine, cecum and colon were excised from the animals; both ends of the small intestine and colon were tied to prevent leakage of the contents. Serum was obtained from blood drawn from the abdominal aorta. All the organs were kept at −30°C until analyzed and feces were freeze-dried before weighing. 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.

2.4 Gastrointestinal tract content transfer

According to the method of Kobayashi et al., gastrointestinal tract and feces were individually minced with medical scissors, added to 3 mL potassium phosphate reagent and mixed well in porcelain crucibles. Then, the sample was removed of water and carbonized until no smoke came out by heating over a gas burner. The crucibles were placed in an electric oven (FUW242PA, Advantec, Tokyo, Japan) and heated at 800°C for 30 min to completely make ash from the sample; green Cr⁶⁺ was oxidized to yellow Cr⁷⁺. When cooled to room temperature, the contents of the crucibles were transferred to 100-mL volumetric flasks and the volume was made up to 100 mL with water. After the sample was allowed to stand overnight, a portion of the water mixture was filtered over filter paper and spectrophotometric absorbance of the filtrate was determined at 370 nm, with distilled water as a blank, to generate Cr⁴⁺ concentration. Percent Cr⁴⁺ in the stomach, small intestine, cecum, colon and feces was calculated for each rat.

2.5 Enzyme activity under existence of soy oil

2.5.1 Lipase activity

Soy oil, 80 mg, was weighed in a 10-mL tube and added a 9-mL TES buffer solution (0.1 M, PH 7) containing 10 mg L-α-phosphatidylcholine (Sigma, St. Louis, MO, USA), 5 mg taurocholic acid sodium salt (Wako Pure Chemical Industries, Osaka, Japan), and NaCl (0.1 M), followed by ultrasonic emulsification for 3 min to make substrate. The enzyme solution (Lipase from porcine pancreas, Sigma, 15 unit) was mixed with 300 µL substrate and incubated at 37°C. After 30 min, the reaction mixture was shaken by a Voltec mixer (Scientific Industries, Inc., Bohemia, NY, USA) with 3 mL chloroform/heptan/methanol, 49:49:2 v/v/v for 10 sec, then centrifuged at 3,000 rpm for 10 min to obtain the lower layer. One mL of cupper reagent (2.98 g triethanol amine, Kishida Kagaku, Osaka, Japan, 2.42 g cupper nitrate (Ⅱ) trihydrate, Wako Pure Chemical Industries, and 0.48 g NaOH were dissolved in 200-mL water followed by 66 g NaCl addition) was added to the layer, shaken for 20 sec by a Voltex mixer, then centrifuged at 3,000 rpm for 10 min to obtain the upper layer. Color reagent (1.5 mL) (0.2 g bathocupuroine, Wako Pure Chemical Industries, and 0.1 g 3-t-Butyl-4-hydroxianisole, ICN-Biomedicals, Costa Mesa, CA, USA, dissolved in 200 mL chloroform) was added to the layer for the colorimetric analysis at 480 µm. The lipase hydrolysis rate was calculated with a calibration curve.
2.5.2 Sucrase and maltase activities

Soy oil (80 mg), 15 mg taurocholic acid sodium salt, and 9 mL of a 56-mM maleic acid buffer solution (pH 6) were taken into a 16-mL tube and sonicated for 2 min to make an oil emulsion, while substrate solutions were made by dissolving 20 mg sucrose and 20 mg maltose in 1 mL water, respectively. Rat small intestinal acetone powder (100 mg, Sigma) was added in 9 mL of a 56-mM maleic acid buffer solution (pH 6.0) and homogenized by a glass homogenizer in an ice bath (4°C), followed by centrifugation at 3,000 rpm for 10 min. The resultant supernatant was used as a crude enzyme solution for sucrase and maltase determination. The enzyme solution (500 μL) and oil emulsion were added to a substrate solution (1 mL), and then incubated at 37°C for 120 min with shaking. The enzyme was deactivated by heating the reaction the reaction mixture at 100°C for 10 min. The resultant mixtures were assayed on glucose concentration with Glucose CII-test Wako, Wako Pure Chemical Industries.

2.6 Fecal nitrogen content

In the previous animal experiment,10 that 10-week old male Wistar rats were fed ad libitum AIN93G no fat powder containing 7% soy oil or fresh oil for 12 weeks, feces excreted for 24 h were collected at 13, 16, 19, 22 weeks of age. Feces were freeze-dried, and ground into fine pieces with a mill mixer MR-280, Yamazen Corporation, Tokyo, Japan. The Kjeltec Auto Sampler System Analyzer, Rose Scientific Ltd. Toronto, Canada, was employed for the nitrogen analysis. The method is described briefly as follows. A fecal sample, 0.4 g, was weighed out in a Kjeltec tube, to which approximately 15 mL concentrated sulfuric acid and approximately 5 g decomposition promoter (copper sulfate/potassium sulfate, 1:9 w/w) was added, and allowed to thermally decompose for 2 h. After the sample was cooled to room temperature, approximately 80 mL water and sodium hydroxide solution were added to the tube, and the contents were subjected to steam-evaporation. The distillate obtained was titrated by 0.05 mol/L sulfuric acid solution with bromocresol green/methyl red solution as detectors.

2.7 Nitrogen content in soy oil

Determination of nitrogen content in soy and fresh oils was carried out by employing 1 g each of oil for the Kjeldahl method described in 2.6.

2.8 Statistical analysis

All the values obtained from animals are revealed as mean±SD. Data from 5 or 8 animals each for two 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 soy and fresh oils are as follows, respectively: POV (mEq/kg) 0.1, 0.1; COV 8.4, 3.9; polar compound content (%) 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. Soy oil appeared slightly yellowish, but other properties were the same as those of fresh oil.

3.2 Gastrointestinal tract content transfer

Body weights of all the rats used in the present study were about 215 g and no difference between groups were observed (Fig. 1). The amount of slurry orally administered to rats was dependent on their body weights, and the rats were sacrificed at fixed points. Changes in organ weights are shown in Fig. 2. The soy oil and control groups stomach weights decreased rapidly; however, no significant differences between the two groups were observed. Small intestine weight showed an increasing tendency in the soy oil group until 120 min as compared to the control group, with a remarkable difference at 60 min. This suggests that the slurry was transferred rapidly in the soy oil group; however, the soy oil group might have ingested more water than the control group as there was free access to water. Cecum and colon weights were almost constant, although increases and decreases were frequent. This is probably due to the balance between content inflow from the small intestine and content outflow, as feces.

Changes in organ Cr⁶⁺ content is shown in Fig. 3. The stomach content declined rapidly until 120 min in both groups, with decreased amounts in the soy oil group, after which the decline slowed down. In contrast, the content of the small intestine elevated rapidly in both groups until 120 min, with an increased amount observed in the soy oil group. The soy oil group content decreased after 120 min,
while the control group content kept increasing until 210 min. This reveals that the slurry was transferred from stomach to small intestine more rapidly in the soy oil group than the control. The content tended to reach the cecum earlier in the soy oil group than in the control group. However, Cr\textsuperscript{6+} was not detected in the feces, which confirms the previous result that the feces collected were derived from a commercial standard diet ingested before slurry administration. 

Fecal weights (dry) excreted between slurry administration and sacrifice, and water content in the feces are shown in Fig. 4. The soy oil group showed increased excretion as compared to control, and significant differences were found at 90, 120, and 360 min. Water content of the control group approached 30\% with the passage of time, but that of the soy oil group (60\%) was almost constant, which was double the value of the control group at 150 min and onwards.

3.3 Enzyme activity in the presence of soy oil
As hydrolysis rates of soy and fresh oils by lipase were the same (Fig. 5), it was found that soy oil did not inhibit lipase activity. Additionally, sucrase and maltase activities were the same in the mixture containing soy oil or fresh oil; the influence of soy oil on the enzymes was judged to be the same as those of fresh oil.

3.4 Fecal nitrogen content
The proportion of fecal nitrogen was significantly lower in the soy oil group after 16 weeks than in the control group (Fig. 6); however, the effect on daily nitrogen content was reversed due to increased fecal weight.

3.5 Nitrogen content in soy and fresh oils
Nitrogen was not detected in both oils under a detection limit of 100 ppm.

4 DISCUSSION
Weight-loss promoting effects in animals fed with a diet containing vegetable or animal protein were reported in some papers. Aoyama et al.\textsuperscript{5, 6} fed fattened Sprague-Dawley
Weight-Loss Promoting Oil

rats energy-restricted, low-fat (5%), and high-protein (35%) casein, soy protein isolate (SPI), or soy protein isolate hydrolysate (SPI-H) diets for 4 weeks at 60% of the level of the energy intake of rodents on laboratory chow, and found that the apparent absorbability of dietary energy and fat was significantly lower in the SPI and SPI-H groups than in the casein group. They concluded that SPI and SPI-H were suitable protein sources in energy-restricted diets for the treatment of obesity. Royle et al. examined the influence of whey protein isolate (WPI) and glycomacropeptide (GMP) on weight gain and body composition by feeding Wistar rats ad libitum for 7 weeks with five semi-purified AIN-based diets differing in protein type. In conclusion, GMP appears to have a significant additional influence when combined with WPI on fat accumulation. WPI alone appears to have the predominant influence accounting for 70% of the overall effect on body-weight gain. In addition, it has been suggested that Ca affects adipocyte metabolism via suppressing 1,25-dihydroxycholecalciferol and decreases fat absorption. Pilvi et al. studied the effect of Ca and milk protein (whey and casein) on body weight in C57BL/6J mice. Final body weight and body fat content were significantly lower in the high-Ca whey group than in the low-Ca casein group. There was a significant difference in fat excretion between the high-Ca whey and low-Ca casein groups, which may partly explain the effect on body weight. In the present study, however, nitrogen was not found in soy oil under the detection limit so that protein and amino acids seem to have no chance to be involved in the weight-loss promoting effects of soy oil.

Minor components in vegetables were also effective for weight-loss. Murase et al. investigated the effects of tea catechins on the development of obesity by measuring

**Fig. 4** Fecal Weight and Water Content in Rats Fed a Diet Containing Oil Heated with Soybean Protein. *p<0.05, **p<0.01, significantly different from the control group. Values are expressed as mean±SD (n=5).

**Fig. 5** The Effect of Oil Heated with Soybean Protein on Lipase, Sucrase and Maltase Activity. Values are expressed as mean±SD (n=5).
body weight, adipose tissue mass and liver fat content in C57BL/6J mice fed diets containing either low-fat, high-fat, or high-fat supplemented with 0.1-0.5% tea catechins for 11 months. Supplementation with tea catechins resulted in a significant reduction of high-fat diet-induced body weight gain, visceral and liver fat accumulation, and the development of hyperinsulinemia and hyperleptinemia. Yoshikawa et al. examined the antiobesity effects of the hot water-soluble extract (SRHW) from the roots of S. reticulata using obese rat models and an in vitro study, and concluded that polyphenolic compounds might be involved in the antiobesity effects of SRHW in rats through inhibition of fat metabolizing enzymes and enhanced lipolysis. Ruzaidi et al. investigated the effect of cocoa extract on serum glucose levels and lipid profiles in streptozotocin-diabetic rats. Cocoa extract (containing 285.6 mg total polyphenol per gram extract) was prepared from fermented and roasted beans by extraction using 80% ethanol. They indicated that cocoa extract might possess potential hypoglycaemic and hypocholesterolemic effects on serum glucose levels and lipid profiles, respectively. Cho et al. fed ovariectomized Sprague-Dawley rats either ethylacetate extract or three types of safflower polyphenolic compounds in a diet containing 0.5% cholesterol for 4 weeks and found that safflower polyphenols had the effect of improving blood lipid status via increasing HDL-cholesterol formation and cholesterol excretion without significant uterotrophic action in estrogen-deficient animals.

We have reported that a soy oil-containing experimental diet (fat-free AIN93G containing oil thermally processed with soybean protein followed by filtration) inhibited body weight increases and increased fecal excretion without any adverse effects when given ad libitum to male Wistar rats for 12 weeks. When thermal processing is conducted in the presence of air, in the preparation of weight-loss promoting oil, the contribution of oxidized polymers in the oil cannot be ignored in assessing its effects. However, the oil prepared under reduced pressure also exhibited over 50% of the effects and increased fecal amounts; therefore, the existence of some unknown weight-loss factors in the oil, other than oxidized polymers, is hypothesized. At present, we are fractionating soy oil to specify weight-loss promoting substances in soy oil.

As the body weight of rats was almost identical (Fig. 1), the percentage of gastrointestinal content (Fig. 3) closely relates to the absolute amount of Cr\textsuperscript{6+}. The slurry was transferred to the small intestine and cecum rapidly in the soy oil group. In general, ingested oil and fat prolongs digestion time by closing the pylorus\textsuperscript{4, 10}, however, the soy oil in the slurry may counteract this effect, enabling rapid transfer.

It was confirmed\textsuperscript{2} that no slurry composed of AIN93G, soy oil, water, and Cr\textsubscript{2}O\textsubscript{3} but commercial standard diet ingested before the experiment was excreted during the experiment, because no Cr\textsuperscript{6+} was detected in the feces (Fig. 3). At 360 min only 10% of Cr\textsuperscript{6+} reached colon. In addition, the soy oil group showed increased fecal amounts immediately after slurry administration, and kept twice the weight as those of the control group (Fig. 4). Fecal appearance of the soy oil group was normal and solid, but its water content was almost constant and about twice as high as that of control; the water content of the control group decreased by degrees probably because of prolonged stay of the feces in the colon. Thus, it was suggested that the colon content volume in the soy oil group was close to four times that of the control group, resulting in accelerated fecal excretion. The soy oil group could not help but excrete the bulky content in the colon. As the factor increasing fecal water content, stimulation of motility\textsuperscript{12} by hormones such as motilin\textsuperscript{17} and substance P\textsuperscript{18}, and inhibition of water absorption in the small intestine and colon\textsuperscript{19, 20} are given. The former makes the content in the small intestine and colon pass through the tract without sufficient absorption of nutrition and water from the content, resulting in feces high in dry weight and water content. When water absorption from the content is inhibited in the small intestine and colon, the fecal water content will increase. But in this case, dry fecal weight should not increase as shown in Fig. 4.

Soy oil did not inhibit the activity of representative digestive enzymes, such as lipase, sucrase, and maltase (Fig. 5). The weight-loss promoting effects of soy oil is probably not attributable to enzyme activity inhibition, but is most probably due to insufficient nutrient absorption because of the rapid transfer of gastrointestinal contents described above. Kobayashi et al. report that an edible chrysanthemum extract showed remarkable enhancement of gas-

**Fig. 6** Fecal Nitrogen Proportion and Content in Rats Fed a Diet Containing Oil Heated with Soybean Protein.

\*p<0.05, **p<0.01, significantly different from the control group. Values are expressed as mean±SD (n=8).

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stestinal transit and strong inhibitory activity against \( \alpha \)-glucosidase in an animal experiment. As soy oil is hydrophobic, its influence on the activity of water-soluble enzymes may be limited.

The fecal nitrogen content of the soy oil group was remarkably higher than that of the control group (Fig. 6). Nitrogen content in urine is far greater than that in feces\(^2\), although it was not determined in the present study. But a large amount of feces seems to mean a large excretion of unabsorbed nutrition according to the report by Unno et al.\(^2\) that male Wistar rats showed reduced body weight gains by feeding a diet containing tea catechin, and excreted increased amounts of feces containing increased amounts of carbohydrate, lipids and protein. The influence of increased amounts of continuous excretion on body weight and serum neutral lipid levels should be substantial\(^3\).

In addition to the weight-loss promoting factors described above, inhibition of nutrient absorption and activated basal metabolism by soy oil might be factors involved in this phenomenon. In using soy oil, a deficiency in oil-soluble vitamins and other micronutrients seems unlikely, because in overweight persons, foods ingested in excess of nutritional needs are digested, and simply transferred rapidly through the tract and excreted.

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