Effects of Oil Thermally Processed with Vegetable Protein on Gastrointestinal Tract Content Transfer

Nagao Totani*, Yurika Araki and Sayuri Tateishi
Faculty of Nutrition, Kobe-Gakuin University (518 Arise, Ikawadani-cho, Nishi-ku, Kobe, 651-2180 JAPAN)

Abstract: It has been reported that oil heated with vegetable protein under reduced pressure, followed by filtration (soy oil), decreased body, liver and retroperitoneal fat tissue weights and serum triacylglycerol levels in Wistar rats. In order to clarify the mechanism of these weight-loss promoting effects, gastrointestinal tract content transfer was traced. Fasted 10-week-old rats were fed a slurry containing AIN93G without fat, Cr2O3 (marker), and 7 wt% soy oil or fresh oil (control) and sacrificed at 20, 60, 120, or 360 min; then, blood, stomach, small intestine, cecum, colon and feces were collected. The results indicated that the content transferred faster from stomach to small intestine in the soy oil group than in the control group. At 60 min after the ingestion of diet, an increased serum triacylglycerol level was found in the soy oil group. In addition, fecal excretion in the soy oil group was significantly higher 120 min after the administration than in the control group, suggesting that soy oil stimulated peristalsis of the colon and that colon contents (food ingested before administration) were actively excreted.

Key words: weight-loss promoting oil, gastrointestinal tract content transfer, soybean protein, stomach, small intestine, feces

1 INTRODUCTION

Many otherwise healthy overweight or obese people worldwide are currently suffering from metabolic syndrome1). To relieve these symptoms, it is essential to re-establish a normal body weight without lowering the quality of life or requiring excessive effort. As one of the countermeasures to cope with metabolic syndrome, edible oil, which is influential through its daily use in cooking, has attracted attention in recent years. Dieting oils presently marketed around the world contain Salatrim®2), Caprenin®3), Olestra®4,5), Econa®6-8) and Healthy Resetta®9-11). 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 soy oil described above is a simple triacylglycerol, but probably contains a small amount of substances formed from components in soybean protein and oil during heating, and reduced body, liver and retroperitoneal fat tissue weights and serum TG levels in a 12–week animal experiment12). The oil did not result in deterioration of appetite but increased normal fecal excretion, leading to the assumption that there was reduced digestion and/or absorption of the diet. In addition, it is also presumed that the substance generating weight–loss promoting effects was not protein or amino acids but trace materials contained in the vegetable protein13).

The underlying mechanism of soy oil weight–loss promoting effects is of interest if digestion enzymes are inhibited or absorption of digested diet is inhibited biologically or by increased gastrointestinal transit. Kobayashi et al. reported that an edible chrysanthemum extract showed remarkable enhancement of gastrointestinal transit, reduction in blood triglyceride level, and strong inhibitory activity against α–glucosidase in an animal experiment14). In the present study, the effects of soy oil on gastrointestinal tract content transfer were investigated as the first step in clarifying its weight–loss promoting mechanism.

* Correspondence to: Nagao Totani, Faculty of Nutrition, Kobe-Gakuin University, 518 Arise, Ikawadani-cho, Nishi-ku, Kobe, 651-2180 JAPAN
E-mail: totani@nutr.kobegakuin.ac.jp
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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 from Fuji Oil Co., Ltd., Japan.

2.1.2 Oil

One liter of fresh oil (Fuji Oil Co., Ltd., Osaka, Japan), composed of soybean oil and rapeseed oil, was heated with 1 wt% soybean protein at 180°C for 10 h in a 2-L four-neck round-bottom flask under reduced pressure, filtered over filter paper (soy oil) and employed for an animal experiment. Fatty acid composition of fresh oil and soy oil 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%. Both oils did not contain conjugated fatty acids, such as conjugated linoleic and linolenic acids at all.

2.1.3 Oil analyses

Methods for chemical analyses of heated oils, peroxide value (POV), carbonyl value (COV), polar compound content (PC), acid value (AV), triacylglycerol content (TG), iodine value (IV), and Lovibond color were the same as in our previous papers[12].

2.1.4 Diets

A commercial powdered AIN93G diet without fat was purchased from Japan Clea, Tokyo, Japan. Using a blender, the diet was mixed uniformly with 7 wt% of experimental oil or fresh oil, added with 0.4% green chromium (III) oxide and two-fold water to make slurry, and subjected to radio-sterilization of 10 kGy by Kohga Isotope (Shiga, Japan) prior to the animal experiment.

2.2 Animals

Forty weanling 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 Animal experiment procedure

Rats were maintained for 1 week on radio-sterilized commercial pelleted diet (Labo MR Stock, Nihon Nosan Kogyo, Yokohama, Japan) and then divided into eight groups (five rats/group) using the Statlight System (Yukms, Tokyo, Japan). After a fasting period of 16 h, rats were administered experimental diet (4 groups) or control diet (4 groups) orally with a syringe (1 mL/100 g body weight). At 20, 60, 120 or 360 min after administration, animals were sacrificed under anesthesia with pentobarbital and the stomach, small intestine, cecum and colon were excised from a representative animal from each group; 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 Serum biochemical analyses

Determinations of serum glucose (GLC), TG and total cholesterol (TCHO) were made with Glucose CII-test Wako, Triglyceride E-test Wako and Cholesterol E-test Wako (Wako Pure Chemical Industries, Osaka, Japan), respectively.

2.5 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[14]. Then, the sample was removed of water and carbonized until no smoke came out by heating over a gas burner. Porcelain crucibles were placed in an electric oven (FUW242PA, Advan tec, Tokyo, Japan) and heated at 800°C for 30 min to completely make ash from the sample; green Cr3+ was oxidized to yellow Cr6+. When cooled to room temperature, the contents of the porcelain 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 potion 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 Cr6+ concentration. Percent Cr6+ in the stomach, small intestine, cecum, colon and feces was calculated for each rat.

2.6 Statistical analysis

All the values obtained from animals are revealed as mean ± SD. Data from 5 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 AND DISCUSSION

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. Thus, soybean protein hardly changed the chemical properties determined and smell.

3.2 Body weight

Table 1 shows body weights of rats shortly before slurry administration. All the rats weighed between 214–217 g therefore, the amount of slurry administered was 2.14–2.17 mL.
Weight-Loss Promoting Oil

### Table 1 Rat Body Weight before Sacrifice.

<table>
<thead>
<tr>
<th></th>
<th>20 min</th>
<th>60 min</th>
<th>120 min</th>
<th>360 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soybean oil</td>
<td>215.4 ± 5.9</td>
<td>214.4 ± 5.4</td>
<td>214.7 ± 5.6</td>
<td>216.7 ± 7.5</td>
</tr>
<tr>
<td>Control</td>
<td>215.4 ± 6.7</td>
<td>215.7 ± 6.9</td>
<td>215.3 ± 5.0</td>
<td>215.7 ± 4.4</td>
</tr>
</tbody>
</table>

![Graph](image1.png)

**Fig. 1** Gastrointestinal Tract Content Transfer in Rats Fed a Diet Containing Oil Heated with Soybean Protein. * p<0.05, significantly different from the value of control (unpaired t-test). Values are expressed as mean ± SD (n=5).

#### 3.3 Gastrointestinal tract content transfer

As shown in Fig. 1, distribution of gastrointestinal content after 20 min was the same in both groups, ca. 80% in the stomach and ca. 20% in the small intestine. Content transfer from the stomach to small intestine was high in the soy oil group at 60 min, and the amount of content in the stomach was significantly lower, and that of the small intestine significantly higher, in the soy oil group at 120 min as compared to the control. At 360 min, 80% of the gastrointestinal content was concentrated in the cecum of both groups and 10% in the small intestine and colon, respectively.

The stomach sends various signals to the gastrointestinal tract and organs depending on the food ingested, and controls digestion and absorption with gastrointestinal hormones and the autonomic nervous system. In general, protein remains in the stomach longer than sugar, and fat/oil even longer, because protein and fat/oil influence the contractions of the stomach; the pyloric valve is controlled by cholecystokinin synthesized by the duodenum according to the fat/oil, protein and amino acids transferred from the stomach. On the other hand, as many have reported that soybean protein contains phenolic compounds exhibiting endocrine effects, it is possible that soy oil also has this effect. Thus, it was presumed that one of the reasons for the acceleration of content transfer from the stomach to small intestine was that minor components of soy oil inhibited the reaction of the duodenum.

In the present study, gastrointestinal content transfer between 120 min and 360 min was not observed. This is an issue that will require our attention in future investigations.

#### 3.4 Serum biochemical analyses

The soy oil group showed a significantly higher TG level at 60 min as compared to control, and reached maximal values at 120 min (Fig. 2). This is probably due to the comparatively high level of content in the small intestine of the soy oil group at the beginning of the experiment (Fig. 1). However, there were no differences in either GLC or TCHO levels between the groups.
At 360 min, 80% of the gastrointestinal content was concentrated in the cecum and 10% in the colon. At 20 min, 60 min and 120 min, brown digestive content, made from food ingested before fasting, is observed in the small intestines of both groups, while green digestive content proceeded gradually; most green digestive content was gone at 360 min. Even at 20 min, the colon of the both group was filled likewise with much digestive content. Thus, it was confirmed that food ingested before fasting stayed in the colon. At 360 min, a portion of green digestive content should have reached the colon, but the green color was not obvious due to the presence of bilirubin and other colored materials from bile.

Figure 3 shows the changes in organ weights over time. The weight of (stomach + content) decreased rapidly and those of (small intestine + content) and (cecum + content) decreased less rapidly. But almost no change was observed in the weight of (colon + content). There was no significant difference in both groups, but the weights of (small intestine, cecum or colon + content) of the soy oil group showed an increasing tendency as compared to the control group. The heavy weight of (small intestine + content), up to 120 min, agrees well with the result in Fig. 1.

3.6 Fecal analysis
Fecal excretion is shown in Fig. 4 (A). As all the fecal particles were not at all green, and from the result in Fig. 1, it is clear that these feces were composed of food ingested before fasting. At 120 min, fecal amounts in the soy oil group were elevated as compared to control, although the slurry had not yet reached the colon. At 120 min, the colon of the soy oil group contained ca. 1% ingested slurry (Fig. 1). Thus, solid material in the slurry (2.15 g × 0.33 × 0.01=0.007 g, assuming that the slurry contained 67% water) was far less than the elevated fecal amounts (more than 100 mg (dry weight) increase).

Fecal water contents of the soy oil group were almost constant, and those of the control group were significantly lower than those of the soy oil group after 60 min on (Fig. 4 (B)), although the feces had already lost some water before collected. Unfortunately we did not determine the amount of water ingested and urine in the present study. However, it seems possible to understand that the soy oil group excreted feces actively while the water content of the control group decreased by degrees probably because of prolonged stay of the feces in the colon. Thus, soy oil accelerated gastrointestinal tract content transfer, resulting in less-absorption of nutrients, the increased fecal amounts and the retained water content in feces. Unknown trace materials in the soy oil may have triggered fecal excretion by influencing peristalsis of the colon. Finally the soy oil will promote body weight-loss gradually without lowering the quality of life or requiring excessive effort.
Weight-Loss Promoting Oil

4. CONCLUSION

As a part of the mechanism for weight-loss promotion by the soy oil ingestion, it was suggested that gastrointestinal tract content transfer was accelerated by the soy oil, followed by lowered nutrient absorption and increased fecal excretion.

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References


