1 Introduction

Harmful effects of thermally oxidized oil are categorized into two major groups. The first is toxicity of oil thermally oxidized and polymerized without frying foodstuffs, which creates harsh pathological responses such as appetite and growth depression, diarrhea, and histological changes in various tissues when fed to animals (1-10). The second group concerns deteriorated oil used for frying potatoes, fish paste, or other foods (11-16). This oil is a complex composed of oxidized oil, polymerized oil, decomposition products, and the products of these substances’ reactions with many substances exuded from fried foodstuffs, such as amino acids, proteins, sugars, other carbohydrates, lipids, blood, and coloring substances (17). Because feeding of oil used for practical frying to animals generally does not lead to harsh pathological responses, toxic responses were studied at the biomembrane level.

Soriguer et al. (18) reported that the risk of hypertension was positively and independently associated with the intake of cooking oil polar compounds (PC). Thus, diseases potentially associated with deteriorated edible oil have been shown to exist extensively. Food manufacturing industries often use one batch of frying oil for extended periods (about 20 h) before replacing it (17). The oil recovered in large quantity from industry after such use is heavily deteriorated, as shown by its properties - Gardner color 11-12, acid value (AV) 2-4, carbonyl value (COV) > 10, peroxide value (POV) > 5,
and PC 20-30% (19-21). Accordingly, some commercial fried foods can contain oil with properties close to those of recovered oil because recovered oil had been used for frying until banned. As ready-made fried foods are increasingly ingested in the westernized world, there is reason to suspect that risks associated with the consumption of deteriorated frying oil may be increasing.

Japan, some of the European nations, and the U.S. have specific regulations against the use of deteriorated frying oils. It is difficult, however, to implement regulations of this type (22).

When healthy people ingest ordinary diets, they should not suffer from stomach ache, diarrhea, seborrhea, excessive hair loss, or other symptoms after consuming ready-made fried foods. Thus, an animal experiment to investigate the effect of deteriorated oil on human health should employ mild feeding conditions reflecting the situation described above. This is why the origin and amount of oil fed to animals ad libitum are especially important factors. The studies described above (1-9, 11-16) mixed 12-20 wt% of thermally oxidized oil in the diet corresponded to oil contents in an ideal human diet. As the optimum oil content in a rat diet is 4-7 wt%, that for humans seems inapplicable to rats. Battino et al. (10) fed Wistar rats initially weighing 80-90 g a diet containing 8 wt% laboratory-heated lightly oxidized oil. The rats, probably 4 weeks old, seem too young for the purpose of the experiment.

In our previous paper (23), SHR/NDmc-cp rats, aged 10 weeks, were fed for 8 weeks a commercial diet (AIN93G, no fat) to which 7 wt% of fresh virgin soybean oil or thermally oxidized mixed oil recovered from food manufacturing companies after use was added. In the present paper, an animal experiment using Wistar rats as a healthy model, performed parallel to that with SHR/NDmc-cp rats, is reported. Comparison of the results obtained from the two types of animals should yield valuable information.

2 Experimental

2⋅1 Materials
2⋅1⋅1 Oil administered

“B grade recovered vegetable oil” (recovered oil: AV 2.1, COV 10.3, POV 16.5 meq/kg, PC 20.3%, and Gardner color 11) was obtained from Miyoshi Oil & Fat Co., Ltd.; this oil is of comparatively good quality among oils recovered from food manufacturing companies. As the reference oil, fresh virgin soybean oil (AV 0.1, COV 4.0, POV 1.9 meq/kg, PC 4.1%, and Gardner color 1) was purchased from Wako Pure Chemical Industries Ltd., Osaka. The fatty acid compositions of both oil and the reason why soybean oil was employed as the reference oil were described in our previous paper (23).

2⋅1⋅2 Diets

Commercial pelleted AIN93G diet (soybean oil content 7%) and powdered AIN93G diet without fat were purchased from Japan Clea, Tokyo. Using a blender (Kenmix Major, Aicoh, Saitama, Japan), the latter was mixed uniformly with 7 wt% recovered oil or 7 wt% soybean oil.

2⋅2 Animals

Male Wistar rats aged 7 weeks were obtained from Japan SLC, Inc., Shizuoka, Japan, and were housed individually in wire cages at 22.5 ± 0.5°C and humidity 55 ± 5%, with light from 8:00 to 20:00 at the Kobe-Gakuin University Animal Experiment Center. The mean body weight was 151.2 ± 2.3 g. 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

Twelve animals were maintained on a pelleted AIN93G diet for 3 weeks of adaptation. The animals were then divided into two groups so as to make average body weights similar and minimize standard deviation in body weight. One group was fed a diet containing 7 wt% recovered oil, and the other was fed a diet containing 7 wt% soybean oil. All animals were allowed feed and water ad libitum throughout the experiment. Body weights, blood pressures (BP-98A, Softron, Tokyo, Japan), and heart rates (BP-98A, Softron, Tokyo, Japan) were determined, and feces were collected weekly. After 8 weeks, 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 according to conventional methods. Livers, kidneys, and adipose tissues were excised, rinsed with cold saline, and weighed, followed by microscopic examinations.
2.4 Hematological Analyses
Concentrations of insulin, and serum triacylglycerol and cholesterol (Lipo Search, a high-sensitivity lipoprotein profiling system) were determined by Skylight Biotech Inc. Japan. Determinations of glucose, free fatty acids, and phospholipids were made with Glucose C II-test wako, NEFA C-test wako, and Phospholipid C-test wako (Wako Pure Chemical Industries, Osaka, Japan) respectively.

2.5 Histological Evaluations of Liver and Kidneys
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 of 100 for liver and 400 for kidneys.

2.6 Statistical Analysis
All the values obtained from animals except fecal analysis are revealed as mean ± SD. Data from 6 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 Growth on Recovered Oil
Animals receiving recovered oil did not exhibit diarrhea, seborrhea, dermatitis, or excessive hair loss during the experiment as in the experiment using SHR/NDmc-cp rats (23). All the rats grew normally, ingesting almost the same amount of the diet, and final body weights of the experimental and control groups after 8 weeks of feeding reached 359.0 ± 20.3 g and 357.6 ± 12.2 g, respectively. Weights of liver, kidney, and adipose tissue were 8.5 ± 0.8 g, 1.9 ± 0.1 g, and 10.1 ± 1.4 g for the experimental group, and 8.4 ± 0.6 g, 2.0 ± 0.1 g, and 10.5 ± 1.4 g for the control group, respectively, all showing no difference between the two groups. Compared with kidney weights of SHR/NDmc-cp rats (2.7 ± 0.1 g for the experimental group and 2.6 ± 0.1 g for the control group) (23), Wistar rats had heavier kidneys, as SHR/NDmc-cp rats weighed nearly twice as much as Wistar rats did.

Figure 1 shows contents of fat and water in feces and excreted amounts of fat and polar compounds per day. Average weights of dried feces per day and per rat were slightly higher in the experimental group than in the control group. Water content of the feces tended to be slightly higher in the control group than in the experi-

Fig. 1 Fecal Analyses in Wistar Rats Fed a Diet Containing 7% Recovered Oil (△, ▲) or Soybean Oil (■, ▼) for 8 Weeks. Points represent means for six animals; as the feces of each group were collected together, the weight of feces from each individual rat was not calculated.
mental group, while contents and weights of fat and weights of polar compounds were higher in the experimental group than in the control group in most cases. As the feces of each group were collected together, the weight of feces from each individual rat was not calculated. Accordingly, standard deviations could not be calculated.

3.2 Hematological Analyses
As Table 1 shows, serum phospholipid level was significantly higher in the experimental group than in the control group. Levels of glucose, triacylglycerol, and cholesterol in the experimental group were slightly higher than those of the control group, but there was no difference between the groups in insulin level.

Triacylglycerol and cholesterol levels in each lipoprotein are shown in Table 2. The high total triacylglycerol level of the experimental group was attributed mainly to high levels of triacylglycerol in VLDL and slightly to those in LDL. The high total cholesterol level of the experimental group was composed of increase in HDL.

3.3 Blood Pressure and Heart Rate
As systolic, diastolic, and average blood pressure of the rats exhibited similar changes during 8 weeks of the experiment, the last pressure measured is shown in Fig. 2 in addition to heart rate. No difference in either measurement was observed.

Table 2 Distribution of Triacylglycerol and Cholesterol in Lipoproteins of Wistar Rats Fed a Diet Containing 7% Recovered Oil or Soybean Oil for 8 Weeks.

<table>
<thead>
<tr>
<th>Lipoprotein</th>
<th>Recovered oil</th>
<th>Soybean oil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total (mg/dL)</td>
<td>106.4 ± 49.2</td>
<td>71.5 ± 28.6</td>
</tr>
<tr>
<td>Chylomicron (mg/dL)</td>
<td>0.2 ± 0.1</td>
<td>0.1 ± 0.1</td>
</tr>
<tr>
<td>VLDL (mg/dL)</td>
<td>92.4 ± 44.8</td>
<td>60.7 ± 26.0</td>
</tr>
<tr>
<td>LDL (mg/dL)</td>
<td>10.7 ± 3.7</td>
<td>8.0 ± 1.9</td>
</tr>
<tr>
<td>HDL (mg/dL)</td>
<td>3.2 ± 1.0</td>
<td>2.7 ± 0.9</td>
</tr>
<tr>
<td>Cholesterol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total (mg/dL)</td>
<td>63.1 ± 10.5</td>
<td>58.8 ± 6.4</td>
</tr>
<tr>
<td>Chylomicron (mg/dL)</td>
<td>0.003 ± 0.01</td>
<td>0.01 ± 0.01</td>
</tr>
<tr>
<td>VLDL (mg/dL)</td>
<td>5.7 ± 2.5</td>
<td>5.3 ± 1.6</td>
</tr>
<tr>
<td>LDL (mg/dL)</td>
<td>7.1 ± 2.5</td>
<td>6.8 ± 0.9</td>
</tr>
<tr>
<td>HDL (mg/dL)</td>
<td>50.3 ± 7.0</td>
<td>46.7 ± 4.4</td>
</tr>
</tbody>
</table>

Values are means ± SD for six animals. * p < 0.05, significantly different from the value of the soybean oil group (unpaired t-test).
3.4 Histological Evaluation

Figure 3 shows no lesions in liver cells of either group; however, some animals in the experimental group showed fatty accumulation in the liver, especially around veins. In the kidneys of the experimental group, many places were not stained due to cell lesions (cellular degeneration). In addition, disappearance of nuclei in the tubular epithelium (necrosis) was observed very frequently in the experimental group.

4 Discussion

The toxicity of thermally oxidized oil appears to be weakened by frying foodstuffs. The authors hypothesize that the toxicity is modified by substances exuded from fried foodstuffs, leading to the presence of less toxic compounds that do not induce gross symptoms in rats. In the present study, which used conditions similar to the ingestion of oil used for frying by humans, the experimental group did not develop harsh pathological responses in response to the administration of oxidized oil. This was the same case as the experiment using SHR/NDmc-cp rats; the animals did not exhibit diarrhea, dermatitis, or excessive hair loss after the administration of the diet containing recovered oil (23).

Wistar rats fed recovered oil did not show any difference in food intake, body weight gain, or weights of the liver, kidney, and adipose tissue, when compared to control group. In the experimental group, content and daily total amounts of fat in feces tended to be high, as did levels of polar compounds, although the result was not so obvious as in SHR/NDmc-cp rats (23). The recovered oil, which may have been difficult to absorb because of the high content of deteriorated oil, was excreted relatively abundantly, but the control group fed fresh virgin oil also excreted a high content and large amount of fecal fat, which contains not only ingested oil unabsorbed at the small intestines but also oil derived from cells from the digestive tract and from intestinal microbial flora. Rats ingested about 1.2 g/d oil (food intake 17 g/d × 7%) but the experimental group excreted only about 10 mg/d more polar compounds than the control group did. Judging from the PC content (20.3% for recovered oil, and 4.1% for soybean oil), it is suspected that substantial portions of PC stayed in the body, even though the ester linkage of PC was hydrolyzed by lipase to generate smaller chemical structures.

Water contents in feces were less in the experimental group than the control group, as in the previous paper using SHR/NDmc-cp rats (23), but the difference was small. As the feces were collected once a day of collection before replenishing the diet in the morning, the water contents obtained should be a little smaller than those of fresh feces, with smaller differences between the two groups. Nevertheless, we thought that the contents obtained could be used for comparison. Wistar rats have heavier kidneys per body weights than SHR/NDmc-cp rats do, as described in 3.1, so the former might be more resistant than the latter to recovered oil.

Hematological analyses gave high levels of glucose, phospholipids, triacylglycerol, and cholesterol in the experimental group, but a statistical difference was found only in phospholipid level. The previous paper using SHR/NDmc-cp rats (23) showed similar but clear results, with statistical differences in levels of glucose, free fatty acids, and triacylglycerol. The present result appears weak in showing the effect of recovered oil, but the simultaneous experiment with SHR/NDmc-cp rats (23) had consistent results, and the two studies together support the conclusion that the ordinary ingestion of used frying oil is risky in animals.

Fig. 3 Typical Histological Observation of Liver (× 100) and Kidney (× 400) from Wistar Rats Fed a Diet Containing 7% Recovered Oil or Soybean Oil for 8 Weeks. Hematoxylin/Eosin Stain.
In Japan, there are many regulations concerning oil contained in ready-made foods, including the Food Sanitation Act Specification Standard, Japanese Agricultural Standard, and the Guidelines of the Ministry of Health, Welfare and Labor and the Ministry of Agriculture and Fishery. However, only two analytical items (AV < 1.2-5 and peroxide value (POV) < 20-30) are employed in them, with thresholds depending on the category of foods. The values of most recovered oils are within the specifications above, but recovered oil had various negative effects on healthy animals, as shown in this study. If the same effects occur in humans, it would be necessary to lower the threshold values of AV and POV and increase the number of analytical items.

Acrylamide may have high potential carcinogenicity and genetic and generative toxicity (24, 25), and acrolein and pyridine, generated in heated oil, are “relatively high-risk-potential” substances. Acrylamide is formed from asparagine and reducing sugar heated at a high temperature, and its formation is closely related to the Maillard reaction (26, 27). Therefore, thermally oxidized oil has many risky properties related to gross symptoms, toxic responses at the biomembrane level, and other toxicity described above.

In conclusion, adult Wistar rats fed for 8 weeks an ordinary diet containing 7 wt% oil recovered from food manufacturing companies after use showed high levels of serum phospholipids, glucose, triacylglycerol, and cholesterol. In addition, epithelial cells of the renal tubules were obviously damaged. Most of the results agreed well with those obtained in experiments with SHR/NDmc-cp rats, which showed statistically significant effects (23). The results suggested that ingestion of the recovered oil altered blood composition and damaged kidneys, resulting in promotion of lifestyle-related diseases.

References

18. F. SORIGUER, G. ROHO-MARTINEZ, M.C. DOBARGANES,
Deteriorated Edible Oil


