Effects of Deteriorated Frying Oil in SHR/NDmc-cp Rats

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Abstract: Male SHR/NDmc-cp rats aged 10 weeks were fed ad libitum a powdered diet (AIN93G; no fat) containing 7 wt% of fresh virgin soybean oil (control) or used frying oil recovered from Japanese food manufacturing companies (recovered oil) for 8 weeks and subjected to anthropometric measurements, hematological analyses, and histological evaluations of liver and kidneys. All of the rats grew well, and no gross symptoms attributable to recovered oil were observed. The experimental group showed a tendency toward higher body weight gain and higher amounts of fecal excretion than the control group in spite of decreased consumption of the diet. In the serum of the experimental group, remarkably high levels of glucose, triacylglycerol, and free fatty acids were detected. Microscopic observations indicated frequent lesions in renal cells and nuclear losses of tubular epithelium in the experimental group. Thus, the high body weight gain seems to be due to water accumulation in the body. It is not clear, however, why recovered oil increased serum glucose level. No consistent effects on blood pressure or heart rate were observed.

Key words: deteriorated edible oil, thermally oxidized oil, frying, SHR/NDmc-cp rat, glucose level, polar compound

1 Introduction

Food manufacturing industries often use one batch of frying oil for extended periods (about 20 h) before replacing it (1, 2). Oil recovered by renderers, and so forth after such use is turbid, dark brown (Gardner color 11-12), and viscous (>75 mPa·s/25 °C), polar compounds (PC) 20-30%, acid value (AV) 2-4) and smells rancid (peroxide value (POV) >5 meq/kg, carbonyl value (COV) >10) or fishy. Some commercial deep-fried foods, which are increasingly consumed these days, can contain oil with properties close to those of recovered oil.

Soriguer et al. (3) reported that the risk of hypertension was positively and independently associated with the intake of cooking oil polar compounds. Thus, potential diseases derived from deteriorated edible oil are common, although average Japanese use one batch of frying oil for homemade deep-fried foods at most four to five times, and thus deterioration of the oil is limited.

Gabriel et al. (4) fed male weanling rats semisynthetic diets containing 15 wt% of dietary fats for 28 days. Alexander et al. (5) provided male weanling rats with diets containing 15 wt% of either laboratory-heated corn oil or commercial-pressure deep-fry peanut oil for 28 days. A majority of the animals in groups fed the heated fats exhibited various gross symptoms attributable to heated fat toxicity, such as seborrhea, diarrhea, and polyuria, in addition to excessive hair loss.

Izaki et al. (6) fed 5-week-old male COBS-Fisher rats for 13 weeks a diet that contained 15 wt% of thermally oxidized rapeseed oils used by a manufacturer

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of fried fish paste in a conventional manner. In groups given thermally oxidized oil, relative weight of liver and kidney, thiobarbituric acid reactive substances in the liver, and reduced glutathione content were increased significantly in proportion to the degree of deterioration of the oil, but the authors did not observe diarrhea, seborrhea, or other gross clinical symptoms.

Garrido-Polonio et al. (7) compared the effects of two diets containing 15 wt% unused sunflower-seed oil or 15 wt% sunflower-seed oil repeatedly used in frying (PC 27.7%, oligomer content 16.6%) in growing Wistar rats weighing 52 g. Rats fed used sunflower oil had significantly lower weight gain and feed efficiency ratio although gross symptoms were not observed.

Four to seven percent lipid content is recommended in a diet for rats (8, 9), but 15 wt% of oil were administered to very young rats in papers (4-7) described above. Although young rats require relatively high levels of lipids, the oil contents employed in those works appeared too high to allow proper evaluation of the effect of used frying oil. In the present paper, male SHR/NDmc-cp rats, a spontaneously hypertensive, obese, diabetic, and nephritic model, aged 10 weeks (corresponding to human youth), were fed for a longer period a commercial diet (no fat), to which was added 7 wt% thermally oxidized oil recovered from food manufacturing companies after use.

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%, Gardner color 11) was obtained from Miyoshi Oil & Fat Co., Ltd. Fresh virgin soybean oil (AV 0.1, COV 4.0, POV 1.9 meq/kg, PC 4.1%, Gardner color 1) was purchased from Wako Pure Chemical Industries Ltd., Osaka as the reference oil because soybean oil is used most frequently as a lipid source for rat diets.

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 soybean oil.

2·1·3 Chemical analyses

COV, AV, POV, and Gardner 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 PC in the oil was analyzed by a PC Tester (3M, USA).

The fatty acid compositions of the soybean oil and the recovered oil analyzed using a Shimadzu GC-14A gas chromatograph equipped with a 3.1-m glass column of 3.2-mm bore packed with Shimadzu Shnichrom E71 5%/Shimalite 80-100 were as follows, respectively: myristic acid 0.1% and 0.1%; palmitic acid 11.6% and 9.6%; palmitoleic acid 0.1% and 0.4%; stearic acid 4.1% and 3.5%; oleic acid 25.7% and 41.7%; linoleic acid 51.7% and 36.5%; and α-linolenic acid 6.7% and 7.1%; eicosenoic acid 0% and 0.9%; others 0% and 0.2%.

2·2 Animals

Male SHR/NDmc-cp rats aged 7 weeks were obtained from Japan SLC, Inc., Shizuoka, Japan, and were housed separately 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 195.1 ± 13.4 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 diet, AIN93G (oil content 7%), for 3 weeks of adaptation; 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 weight, blood pressure (BP-98A, Softron, Tokyo, Japan), and heart rate (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. Blood drawn from the abdominal aorta was transferred to a centrifuge tube and allowed to stand at room temperature for 30 min. Serum was obtained from coagulated blood by centrifugation at 3,000 rpm for 15 min at 4°C. Liver and adipose tissues
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were excised, rinsed with cold saline, and weighed, followed by microscopic examination.

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 Livers and Kidneys
Fixed livers 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 magnifications of 100 for livers 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
Rats did not exhibit diarrhea, seborrhea, dermatitis, or excessive hair loss after the administration of the diet containing recovered oil. Although it is not known whether our rats had a preference, the experimental group ingested less than the control group did from the first week of the experiment to the final day. However, body weights of the experimental group were always higher than those of the control group, and t-values between the two groups decreased gradually down to 0.3 in the 8th week. Longer experimental periods might yield a statistically significant difference of p<0.05.

Fig. 1 Changes in Body Weight, Food Ingestion, Fecal Weight, Fat/Water/Polar Compound Contents in Feces, and Excreted Fat in SHR/ND mc-cp Rats Fed a Diet Containing 7% Recovered Oil (▲,▲) or Soybean Oil (■,■) for 8 Weeks. Each point of body weight and food ingestion represents mean ± SD for six animals. Points relating to fecal analyses 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.
The average weight of dried feces per day and per rat of the experimental group was a little higher than that of the control group from the second experimental week on. However, the water content of the feces was higher in the control group, while the contents of fat and polar compounds were higher in the experimental group. 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. The weights of liver and kidney were 24.7 ± 4.4 g and 2.7 ± 0.1 g for the experimental group, and 24.5 ± 1.8 g and 2.6 ± 0.1 g for the control group, showing no difference between the two. The weight of adipose tissue was higher in the control group (31.5 ± 5.9 g) than in the experimental group (28.6 ± 4.1 g).

### Table 1  Hematological Values in SHR/NDmc-cp Rats Fed a Diet Containing 7% Recovered Oil or Soybean Oil for 8 Weeks.

<table>
<thead>
<tr>
<th></th>
<th>Recovered oil</th>
<th>Soybean oil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mg/dL)</td>
<td>203.8 ± 31.5*</td>
<td>152.5 ± 32.2</td>
</tr>
<tr>
<td>Free fatty acids (mg/dL)</td>
<td>1.09 ± 0.03*</td>
<td>0.98 ± 0.08</td>
</tr>
<tr>
<td>Phospholipids (mg/dL)</td>
<td>284.5 ± 41.3</td>
<td>254.9 ± 43.8</td>
</tr>
<tr>
<td>Triacylglycerol (mg/dL)</td>
<td>344.0 ± 115.3*</td>
<td>195.2 ± 97.1</td>
</tr>
<tr>
<td>Cholesterol (mg/dL)</td>
<td>158.2 ± 37.9</td>
<td>149.5 ± 24.1</td>
</tr>
<tr>
<td>Insulin (ng/mL)</td>
<td>15.7 ± 1.8</td>
<td>15.9 ± 2.3</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).

### Table 2  Distribution of Triacylglycerol and Cholesterol in Lipoproteins of SHR/NDmc-cp Rats Fed a Diet Containing 7% Recovered Oil or Soybean Oil for 8 Weeks.

<table>
<thead>
<tr>
<th></th>
<th>Recovered oil</th>
<th>Soybean oil</th>
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</thead>
<tbody>
<tr>
<td>Cholesterol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total (mg/dL)</td>
<td>344.0 ± 115.3*</td>
<td>195.2 ± 97.1</td>
</tr>
<tr>
<td>Chylomicron (mg/dL)</td>
<td>7.5 ± 2.4*</td>
<td>2.1 ± 1.2</td>
</tr>
<tr>
<td>VLDL (mg/dL)</td>
<td>277.5 ± 102.6*</td>
<td>158.9 ± 80.9</td>
</tr>
<tr>
<td>LDL (mg/dL)</td>
<td>46.6 ± 11.6*</td>
<td>26.9 ± 12.1</td>
</tr>
<tr>
<td>HDL (mg/dL)</td>
<td>12.4 ± 2.6</td>
<td>7.3 ± 3.2</td>
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Triacylglycerol:

<table>
<thead>
<tr>
<th></th>
<th>Recovered oil</th>
<th>Soybean oil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total (mg/dL)</td>
<td>158.2 ± 37.9</td>
<td>149.5 ± 24.1</td>
</tr>
<tr>
<td>Chylomicron (mg/dL)</td>
<td>0.2 ± 0.04*</td>
<td>0.1 ± 0.04</td>
</tr>
<tr>
<td>VLDL (mg/dL)</td>
<td>12.8 ± 4.4</td>
<td>9.6 ± 2.6</td>
</tr>
<tr>
<td>LDL (mg/dL)</td>
<td>37.4 ± 10.4</td>
<td>37.3 ± 8.6</td>
</tr>
<tr>
<td>HDL (mg/dL)</td>
<td>107.7 ± 28.7</td>
<td>102.6 ± 14.6</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).

### 3.2 Hematological Analyses

As Table 1 shows, the levels of serum glucose, free fatty acids, and triacylglycerol were significantly higher in the experimental group than in the control group. Phospholipid and cholesterol levels of 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. A high total triacylglycerol level of the experimental group was attributed...
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mainly to high levels of triacylglycerol in VLDL and LDL, and slightly to those in HDL and chylomicrons. The high total cholesterol level of the experimental group was composed of increases in HDL, and VLDL.

3 Blood Pressure and Heart Rate

As systolic, diastolic, and average blood pressure of rats exhibited similar changes during the 8 weeks of the experiment, the last pressure measured is shown in Fig. 2. The experimental group was expected to develop higher blood pressure than the control group, but the reverse tendency was actually observed. No difference in heart rate was observed.

3.4 Histological Evaluation

Figure 3 shows no lesions in the liver cells of both groups; however, uniformly distributed large fat particles present in livers of the control group were not observed in livers of the experimental group, and the distribution was biased. The difference in fatty acid compositions of oils added in the diets may have influenced amounts of fat particles. The kidneys of both groups had many places 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 recovered oil is a complicated mixture composed mainly of used soybean, rapeseed, palm, corn, sesame oils, etc. In addition the mixture also contains lipids, and other food components exuded from foodstuffs during frying and has been subjected to further heating which induces oxidation, polymerization, and decomposition of oil. Thus, the recovered oil contains a vast variety of oils and other substances. In general, studies on the effect of single oxidized oil employ fresh oil in question as control, but in the present study we did not find reference oil because of the reason described above. As the purpose of this study is to find whether used frying oil as a whole has negative effects resulting

Fig. 3 Typical Histological Observation of Liver (× 100) and Kidney (× 400) from SHR/NDmc-cp Rats Fed a Diet Containing 7% Recovered Oil or Soybean Oil for 8 Weeks. Hematoxylin/Eosin Stain.
In lifestyle-related diseases, control animals must grow healthy on a diet containing proper oil. Soybean oil is used in standard diets for rats including SHR/NDmc-cp rat most frequently so that we employed it as oil for control group. On the other hand, the experimental diet contained high level of oleic acid, and the control diet, high level of linoleic acid. But it seems obvious that the difference did not play a big role in the evaluation of the recovered oil because Naito et al. (10) observed no changes in body weight, food and water intake, and kidney weight when they fed spontaneously hypertensive rats a commercial defatted diet added 10 wt% soybean oil or canola oil respectively, and gave drinking water containing 1% NaCl for 26 weeks.

One of the reasons for the lack of development of gross symptoms in the previous studies (6, 7) seems to be that the experimental oils employed by Gabriel et al. (4) and Alexander et al. (5) were oxidized to a level that does not occur in oils normally consumed in human diets. In general, deterioration of oil is easily perceived by its smell and viscosity, and therefore it is hard to imagine that present-day people would ingest such highly deteriorated edible oil. In addition, development of the gross symptoms caused by deteriorated oil has rarely been reported during the last few decades. Another reason may be that laboratory-heated oils, which do not come into contact with frying foodstuffs, have effects that differ from those of oils used for frying foodstuffs (6, 7).

It was reported that body weight gains of animals were retarded remarkably by the administration of oxidized oil (4, 5, 7). In the present work, the experimental group developed neither diarrhea nor seborrhea and showed a higher tendency toward body weight gain than the control group did, as in the study by Izaki et al. (6). Thus, it seems that little acute toxicity of oxidized oil occurred in the present experimental conditions, which were close to practical ingestion of thermally oxidized oil by humans. However, Izaki et al. (6) reported a slightly increased intake of diet (containing oil used for manufacturing a traditional Japanese fried fish paste called “Satsuma’age”’) in the experimental group. The rats might have had a preference for the diet containing the oil. Also, effects of protein and other substances exuded from fish paste into the oxidized oil cannot be ignored.

In the present study, the experimental group showed tendency to weigh more and excrete more feces than the control group did in spite of lower food ingestion. This may have been due to accumulation of water in the body. Although we did not determine the urine output of the rats, it is easily conjectured from histological evaluation of the kidneys that urination was extremely obstructed by the ingestion of recovered oil. Contrary to our conjecture, Gabriel et al. (4) observed polyurea in rats fed thermally oxidized oil. Low water contents of feces in the experimental group also leads to the body weight gain. In addition, Gabriel et al. (4) reported by histological evaluation that oxidized lard was very injurious to the kidneys.

In the experimental group, the content and daily total amounts of fat in feces were high, as were those of polar compounds. Thus, it appears that deteriorated oil was excreted abundantly every day.

Histological evaluation indicated that the distribution density of fat droplets in the livers from the experimental group was low, but no difference in liver and kidney weights between the groups was found. Adipose tissue in the control group weighed more than that of the experimental group, reflecting differences in feed intake.

Hematological determinations showed that control SHR/NDmc-cp rats had twice as high levels of triacylglycerol, phospholipids, cholesterol, and free fatty acids as Wistar rats did (11, 12), and the experimental group had even higher levels; significantly high in glucose, free fatty acids, and triacylglycerol levels and high in cholesterol and phospholipid levels of the experimental group when compared with the control. However, Garriodo-Polonio et al. (7) reported that Wistar rats fed used frying oil had significantly increased serum cholesterol and phospholipid levels, but that the triacylglycerol level was not changed. They concluded that the rat increased HDL cholesterol as a protective mechanism against the stress induced by the consumption of a diet containing the thermally oxidized oil. The increase of HDL cholesterol agrees well with our result. High serum triacylglycerol, phospholipids, and cholesterol levels in our experiment is possibly due in part to high composition of oleic acid in the recovered oil (10).

Kidney lesions were probably caused by the increased levels of serum glucose damaging the inside wall of the capillaries (13) or by lipid peroxide damaging the kidney and liver, as reported by Nwanguma et al. (14). It still needs to be clarified, however, why serum glucose was increased by ingestion of recovered oil.
oil. As high levels of insulin were secreted in both groups, it is possible that in the experimental group insulin did not possess its full function; for example, components of recovered oil may have inhibited insulin receptors on the surface of cells.

We had thought to verify the study by Soriguer et al. (3) in the present work by showing high blood pressure in SHR/NDmc-cp rats fed recovered oil. Ikeda (15) reported that male SHR/NDmc-cp rats exhibited serum glucose and blood pressure of 308 mg/dL and 196 mmHg, respectively (16 weeks old), when fed it for 8 weeks. Although the AIN93G diet employed in our experiment is classified as a high-carbohydrate diet (16), and the feeding period was long enough for its development, rats did not show high blood pressure. Instead, high serum glucose, free fatty acid, and triacylglycerol levels were observed under our very mild experimental conditions; all of these are important risk factors for lifestyle-related diseases.

In conclusion, adult SHR/NDmc-cp rats fed for 8 weeks a high-carbohydrate diet containing 7 wt% oil recovered from food manufacturing companies after use showed high serum glucose, free fatty acid, and triacylglycerol levels. In addition, epithelial cells of the renal tubules were obviously damaged, and there were many instances of uneven fat droplet distribution in the liver. These findings suggest that ingestion of the recovered oil altered blood composition and damaged the capillaries and kidneys, resulting in promotion of lifestyle-related diseases.

References

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