Liver and Plasma Lipids in Rats Fed Casein Reacted with Oxidized Ethyl Linoleate

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Experiments were performed to estimate the effect of casein reacted with oxidized ethyl linoleate (casein: lipid = 2:1, w/w, at 50°C and RH 80.4% for 14 days) on the liver and plasma lipids of young rats. The results were compared with those of the ad libitum or pair feeding controls fed the unreacted casein.

At a 9% protein level, feeding the reacted casein resulted in depression of growth and enlargement of liver. Content of liver lipids and glycogen increased, whereas that of liver nitrogen and plasma lipids decreased. The increase in liver lipids was attributable to that of triglyceride. Percentage of stearic and arachidonic acids in liver total lipids of rats fed the reacted casein decreased and that of oleic acid increased. The inverse changes were observed in the composition of plasma lipids. Supplementation of 0.5% Lys or 0.5% Lys and 0.3% Met to the reacted casein exhibited no improvement of the growth and liver and plasma lipids. Further addition of 0.3% Thr showed considerable supplementary effects on the liver and plasma lipids. At a 20% protein level, in contrast to the experiments with a low protein level, the content of liver lipids was decreased: this was mainly attributable to the decrease in phospholipid. In liver lecithin, percentage of arachidonic acid decreased, while that of palmitic and linoleic acids increased.

The present and previous experiments indicated that the responses of the liver lipids to the reacted casein or egg albumin markedly differed from each other.

Despite numerous studies involving the interaction between proteins and oxidized lipids, little is known on the changes in the nutritive values of the interacted proteins. Vavrikova et al. have reported that the nutritive value of casein which had been incubated with soya oil at 18–22°C for 3–4 days, was not always impaired by complex formation with oxidation products of the oil. Horigome et al. demonstrated a considerable fall in the biological value of casein reacted with oxidized lipids (peroxide value, 2500) at 55°C for 24 hr in the presence of water.

In the previous papers, we demonstrated a significant deterioration in the nutritive value of casein and egg albumin accompanied with oxidation of ethyl linoleate under a low moisture environment. Thus, the quantity of available lysine, the digestibility and the biological value of these proteins significantly decreased. Additionally, when these reacted proteins were given to young rats at an approximately 10% level, there were apparent depression of the growth and the modification in the composition of the body components.

The present study was focussed on examining the effect of the reacted casein on the hepatic and plasma lipids of rats reared on the low and adequate dietary levels. The effect of supplementation of several amino acids, which has been damaged in the course of the reaction with oxidized ethyl linoleate, was also examined.

† Effect of Oxidized Fats on the Nutritive Value of Proteins. Part V. See References 5~8).
MATERIALS AND METHODS

Preparation of test casein. The 2:1 (w/w) mixture of casein (Merck, Hammarsten) and ethyl linoleate (Tokyo Kasei Kogyo Co., Ltd.; purity, 93%) was kept in a desiccator containing saturated KCl solution at 50°C (relative humidity, RH was 80.4%) under exposure of a fluorescent lamp. Care was taken to assure adequate oxygen supply during the incubation. Following 14-days of incubation, the mixture was repeatedly extracted with diethyl ether and then with acetone until that essentially no lipid materials were detected in the extract. This was served as the reacted casein.

Control casein was prepared by extracting lipids immediately after mixing ethyl linoleate followed by incubating the protein under the same conditions as for the preparation of the reacted casein.

Animal experiments. Male rats of the Wistar strain were housed individually in the metabolic cages in a room maintained at 22 to 24°C.

Experiment I: This was carried out at a 9% protein level. Rats, weighing approximately 70 g, were divided into 5 groups of 5 rats each and the animals fed on diets containing, as a sole source of protein, either the reacted casein (RC) alone or the one supplemented with 0.5% Lys (RCL), 0.5% Lys and 0.3% Met (RCLM) and 0.5% Lys, 0.3% Met and 0.3% Thr (RCLMT). Control group received a control casein diet (CC). The level of Lys in the supplemented diets was so arranged as the same to that in a control diet, 8.1g/16gN. Diets were given by pair feeding on the basis of the amount ingested by the RC group for 14 days.

Experiment II: This was carried out at a 20% protein level. Rats, weighing approximately 95 g, were divided into 3 groups of 6 rats each and given either the reacted casein diet (RC) or the control casein diet, ad libitum (CCA) or by pair feeding (CCP), for 21 days. Composition of diets is shown in Table I. Food intake and body weight of rats were determined at 1:30 p.m. daily.

Analytical procedure. Nitrogen balance test was carried out for 3-day period between the 7th and 12th day after the initiation of experiment as reported previously.6

The animals were fed freely until sacrificing by decapitation at 10:00 to 10:30 a.m. Liver and plasma lipids were extracted and purified by the procedure of Folch et al.9 Total lipids were determined gravimetrically. Lipid phosphorous, cholesterol and triglyceride were determined by the method of Gomori,10 Sperry and Webb11 and Fletcher,12 respectively. Fractionation of lipids by thin-layer chromatography and determination of fatty acid composition by gas-liquid chromatography were performed as described elsewhere.12 Nitrogen was determined by the semi-micro Kjeldahl method. Liver glycogen and plasma glucose were determined according to Seifer et al.14 and Dubois et al.,15 respectively.

RESULTS

1) Body weight gain, food intake and liver weight

Experiment I: Feeding diets containing the reacted casein resulted in a depression of growth, the extent of which being significant in the RC and RCL groups (Table II). The liver weights tended to increase after feeding the reacted casein. Apparent nitrogen retention (4.6±1.0%) and digestibility (79.4±1.3%) of

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Experiment I</th>
<th>Experiment II</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control casein</td>
<td>Reacted casein</td>
</tr>
<tr>
<td>Control casein</td>
<td>9</td>
<td>—</td>
</tr>
<tr>
<td>Reacted casein</td>
<td>—</td>
<td>10&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Corn oil</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Mineral mixture&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Vitamin mixture&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Choline chloride</td>
<td>0.15</td>
<td>0.15</td>
</tr>
<tr>
<td>Cellulose</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Sucrose</td>
<td>78.85</td>
<td>77.85</td>
</tr>
</tbody>
</table>

<sup>a</sup> Casein-ethyl linoleate mixture (2:1, w/w) was incubated at 50°C, RH 80.4% for 14 days and defatted with diethyl ether and acetone.

<sup>b</sup> Nitrogen contents are equal to those of control casein diet in each experiment.

<sup>c</sup> Purchased from the Tanabe Amino Acid Research Foundation.
Oxidized Lipids and Nutritive Value of Casein

TABLE II. BODY WEIGHT GAIN, FOOD INTAKE AND LIVER WEIGHTa,b,c)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Body weight (g)</th>
<th>Food intake</th>
<th>Liver weight</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>Final</td>
<td>Gain</td>
</tr>
<tr>
<td>Control casein (CC)</td>
<td>71±3</td>
<td>80±2</td>
<td>9±2</td>
</tr>
<tr>
<td>Reacted casein (RC)</td>
<td>71±3</td>
<td>68±3</td>
<td>-3±2</td>
</tr>
<tr>
<td>RC+0.5% Lys (RCL)</td>
<td>71±3</td>
<td>67±2</td>
<td>-4±4</td>
</tr>
<tr>
<td>RC+0.5% Lys, 0.3% Met (RCLM)</td>
<td>71±3</td>
<td>73±2</td>
<td>2±3</td>
</tr>
<tr>
<td>RC+0.5% Lys, 0.3% Met, 0.3% Thr (RCLMT)</td>
<td>71±3</td>
<td>75±3</td>
<td>3±2</td>
</tr>
</tbody>
</table>

Experiment I

Control casein pair feeding (CCP) 95±2 | 163±4 | 68±3 | 10±1 | 4.6±0.1 |
| ad libitum (CCA) | 95±2 | 200±4 | 105±3 | 13±1 | 5.6±0.1 |
| Reacted casein (RC) | 95±3 | 134±4 | 39±4 | 10±1 | 4.5±0.1 |

Experiment II

a) Values are the means and SE of 5 and 6 rats in Experiments I and II, respectively.
b), c) Differs significantly from the control casein group at p<0.01 and p<0.05, respectively in Experiment I and from the control groups at p<0.01 in Experiment II.

The body weight gain, food intake and liver weight of rats fed the reacted casein were significantly lower than those of control casein (41.6±2.1% and 90.5±1.3% respectively).

Experiment II: At a 20% protein level, weight gain of rats fed the reacted casein (RC) was also significantly depressed, even compared with that of the pair feeding control. Though there were no differences in the weight of liver and other organs (heart, kidney and lung) between the groups RC and CCP, the adrenal gland appeared to enlarge in the former (30 vs. 20 mg/100 g body wt.). Apparent nitrogen retention and digestibility of the reacted casein were also significantly low.

2) Liver lipids

Experiment I: Table III shows the effect on the liver lipids of the reacted casein with or without supplementation of some amino acids. Feeding the reacted casein caused an increase in the liver lipid content, this being largely, but not exclusively, due to increased deposition of triglyceride. There was a slight decrease in phospholipid. Supplementation of essential amino acids singly or in combination did not appreciably prevent the accumulation of triglyceride and the decrease in phospholipid in the liver.

Experiment II: Rats fed the reacted casein at a 20% level had a lower level of the hepatic total lipids in comparison with that of the ad libitum controls, this being attributable to the decrease in phospholipid. The content of cholesterol was high in the RC group, mainly due to the increase in the esterified form.

3) Liver glycogen and nitrogen content

Both in the Experiments I and II, there was a significant increase in hepatic glycogen (20~44% increase). Nitrogen content was decreased in Experiment II.

4) Concentration of plasma lipids

Experiment I: The concentration of phospholipid, triglyceride and cholesterol in the groups RC, RCL and RCLM were considerably lower than those in the CC group (Table IV). Percentage of cholesterol ester also decreased in these rats. However, addition of Lys, Met and Thr to the reacted casein (RCLMT) essentially restored the plasma lipid levels to the control values. The concentration of blood glucose was decreased in all of rats fed the reacted protein.

Experiment II: As shown in Table IV, the
response of plasma lipids and glucose was similar to that in Experiment I. The reason for the abnormally high levels of glucose in groups CCP and CCA was not apparent.

5) Fatty acid composition of liver and plasma lipids

Experiment I: The fatty acid composition of hepatic total lipids is shown in Table V. Percentage of stearic and arachidonic acids decreased in the RC group. This was balanced by an increase in oleic acid. Though changes were observed in all of the amino acid supplemented groups, the extent of these modification was considerably moderate in the group RCLMT.

In plasma total lipids, percentage of oleic acid was low, whereas that of arachidonic acid was high in the reacted casein groups (Table VII).

Experiment II: There were considerable differences in the fatty acid composition of hepatic total lipids between the RC and CCP groups. Percentage of palmitic and linoleic acids was significantly high in the former (16.4±0.8% vs. 13.1±0.8% and 18.6±0.4%
TABLE V. FATTY ACID COMPOSITION OF LIVER TOTAL LIPIDS (Experiment I)

<table>
<thead>
<tr>
<th>Groupsa)</th>
<th>16:0</th>
<th>16:1</th>
<th>18:0</th>
<th>18:1</th>
<th>18:2</th>
<th>20:4</th>
<th>22:6</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC</td>
<td>24.7±0.8</td>
<td>2.8±0.2</td>
<td>12.7±0.6</td>
<td>23.5±1.6</td>
<td>13.0±0.3</td>
<td>16.6±1.1</td>
<td>4.0±0.5</td>
</tr>
<tr>
<td>RC</td>
<td>25.0±1.3</td>
<td>2.6±0.4</td>
<td>9.7±0.6b)</td>
<td>31.5±1.1b)</td>
<td>14.0±1.1</td>
<td>11.0±0.7b)</td>
<td>2.9±0.2</td>
</tr>
<tr>
<td>RCL</td>
<td>26.2±1.4</td>
<td>2.8±0.5</td>
<td>9.2±0.6b)</td>
<td>34.0±0.9b)</td>
<td>14.1±1.0</td>
<td>8.6±0.3b)</td>
<td>2.0±0.1b)</td>
</tr>
<tr>
<td>RCLM</td>
<td>31.0±1.5b)</td>
<td>4.5±0.6c)</td>
<td>8.7±0.7b)</td>
<td>31.1±1.4b)</td>
<td>10.2±0.9</td>
<td>8.5±0.9b)</td>
<td>1.6±0.3c)</td>
</tr>
<tr>
<td>RCLMT</td>
<td>30.1±1.1b)</td>
<td>3.3±0.2</td>
<td>11.0±0.3</td>
<td>26.5±1.2</td>
<td>11.2±0.9</td>
<td>12.0±1.0c)</td>
<td>2.3±0.4c)</td>
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</table>

a) See Table II.
b), c) Differs significantly from CC at p<0.01 and p<0.05, respectively.

TABLE VI. FATTY ACID COMPOSITION OF LIVER TRIGLYCERIDE AND LECITHIN (Experiment II)

<table>
<thead>
<tr>
<th>Groupsa)</th>
<th>14:0</th>
<th>16:0</th>
<th>16:1</th>
<th>18:0</th>
<th>18:1</th>
<th>18:2</th>
<th>20:4</th>
<th>22:6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triglyceride</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>CCP</td>
<td>1.3±0.1</td>
<td>25.0±0.9</td>
<td>9.2±1.4</td>
<td>2.2±0.3</td>
<td>38.7±1.6</td>
<td>18.2±2.9</td>
<td>4.5±0.3</td>
<td></td>
</tr>
<tr>
<td>CCA</td>
<td>1.6±0.2</td>
<td>26.9±1.0</td>
<td>9.9±0.5</td>
<td>2.0±0.4</td>
<td>38.5±0.5</td>
<td>17.3±1.8</td>
<td>2.9±0.4</td>
<td></td>
</tr>
<tr>
<td>RC</td>
<td>2.0±0.5</td>
<td>26.6±1.9</td>
<td>9.0±1.1</td>
<td>2.4±0.3</td>
<td>35.2±2.1</td>
<td>19.4±2.7</td>
<td>4.4±0.6</td>
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</tr>
<tr>
<td>Lecithin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CCP</td>
<td>0.5±0.1</td>
<td>15.9±2.1</td>
<td>2.8±0.5</td>
<td>17.1±0.8</td>
<td>12.5±0.8</td>
<td>12.5±1.2</td>
<td>32.7±1.5</td>
<td>3.3±0.5</td>
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<tr>
<td>CCA</td>
<td>0.5±0.1</td>
<td>13.1±2.0</td>
<td>2.7±0.2</td>
<td>17.2±1.0</td>
<td>13.4±1.0</td>
<td>15.9±0.3</td>
<td>31.0±0.8</td>
<td>3.2±0.6</td>
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<tr>
<td>RC</td>
<td>0.5±0.1</td>
<td>16.2±1.4</td>
<td>2.9±0.2</td>
<td>17.1±0.4</td>
<td>12.1±0.4</td>
<td>16.7±0.4b)</td>
<td>28.3±0.7c), d1</td>
<td>3.4±0.2</td>
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</tbody>
</table>

a) See Table II.
b), c) Differs significantly from CCP at p<0.01 and p<0.05, respectively.
d) Differs significantly from CCA at p<0.05.

vs. 14.6±0.4%, respectively), whereas that of arachidonic acid was low (21.3±0.4% vs. 25.9±0.9%). However, the composition in the CCA group was apparently comparable with that in the RC group.

Hepatic triglyceride had a similar fatty acid composition between the three dietary groups. In lecithin, percentage of linoleic acid in the RC group was significantly higher than that in the CCP group at the expense of arachidonic acid (Table VI). Similar changes in proportion of linoleic and arachidonic acids could be found in plasma total lipids (Table VII).

6) Carcass analysis (Experiment II)

There were no demonstrable differences in the composition of carcass lipids. However, the fatty acid composition of total lipids from carcass was modified considerably by feeding the reacted casein, the changes being not comparable with those in liver total lipids.

DISCUSSION

The present study shows that casein reacted with oxidized ethyl linoleate has significant influences on the growth and liver components of young rats. Growth depression was evidenced at both the different dietary levels, 9 and 20%.

Supplementation of Lys alone, which is one of the essential amino acids mostly decomposed during the reaction of casein with oxidized ethyl linoleate,5) did not show any improvement of the growth (Table II). Met is the first limiting amino acid of casein at the low dietary level16) and is one of the unstable amino acids to the lipid oxidation.17) Further addition of Met and Thr improved the growth depression
only slightly.

At a 9% protein level, the content of liver lipids of rats fed the reacted casein was significantly increased, this being due mainly to the increase in triglyceride. In contrast, it tended to decrease at a 20% level. However, the decrease in liver phospholipid was commonly seen in these two experiments. Supplementation of Lys to the reacted casein did not prevent the accumulation of liver lipids. It is a well known phenomenon that supplementation of a small quantity of Met to the diets containing low levels of casein as a sole source of proteins results in fatty infiltration in the liver of rats. There was no such a pathological effect when the reacted casein was given, but rather slightly alleviated accumulation of hepatic lipids. The extent of lipid accumulation was considerably reduced by a further addition of Thr, though it was still somewhat high compared with the control value.

On the other hand, the plasma lipid levels of rats fed the reacted casein were considerably low. Addition of Lys and Met did not elevate the levels to the control value. Further supplementation of Thr was apparently effective on the improvement of the decrease due to the reacted casein. Therefore, these data, apart from the growth depression, may indicate that the damage of particular amino acids is partly responsible for the changes in lipid metabolism in the hepato-plasmic system due to feeding the reacted casein. However, since the damage of Thr during the interaction of casein and oxidized ethyl linoleate was relatively moderate in comparison with that of Lys or Met, a concept such as amino acid unbalance or imbalance should also be taken into consideration. From the damage of some amino acids and decreased digestibility of the reacted casein, unbalanced rate of hydrolysis of particular amino acids in the small intestine may also relate to the altered lipid metabolism.

On the total lipids of the liver of Experiment I, percentage of oleic acid increased, whereas that of stearic and arachidonic acids decreased by feeding the reacted casein. It appears that rats on the reacted casein diet accumulate considerably different types of lipids in the liver. In this connection, it is of interest that the changes in the composition of plasma lipids were completely inverse to those of liver. Apparently, one can consider the decreased transport of lipids from liver to the blood stream as a cause of lipid infiltration in the liver. However, since plasma lipids in rats fed the reacted casein had markedly different composition from those of the liver, it is indeed difficult to explain the mechanism by which fatty infiltration occurs simply as a result of blockage of lipid transport from liver to blood stream.

Another major difference due to feeding the
reacted casein is the increase in liver glycogen and decrease in liver nitrogen. An increased liver glycogen has also been reported with rats fed diets deficient in essential amino acid. It appears likely that, as in the case of amino acid imbalance, rats on the reacted casein utilize insufficiently the dietary calories, probably due to inadequate supply of amino acids in the small intestine.

The present and preceding experiments indicate that the responses of the liver lipids of rats to different proteins, casein and egg albumin, which were reacted with oxidized lipids, differ markedly from each other. At a low dietary level, liver lipids accumulate in the former and decrease in the latter in comparison with those of corresponding controls.

REFERENCES