Elaidic Acid and Oleic Acid Have Opposite Effects on Serum Glucose, Insulin and Immunoglobulin Levels and Eicosanoid Production in Streptozotocin-Induced Diabetic Rats

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Abstract: The present study was designed to evaluate the physiological effect of trans fatty acid in the diabetic condition. After feeding the mixture of oleic (cis) or elaidic (trans) acids with safflower oil (3 : 7, wt/wt) at the dietary level of 10% for 2 weeks, rats were treated with streptozotocin and reared on the same diets for an additional week. The dietary level of elaidic acid was 6.4 energy %, which corresponded to the level that causes an increase in plasma low-density lipoprotein cholesterol and a decrease in high-density lipoprotein cholesterol in humans. The following differences were observed in rats fed trans acid in comparison with those fed cis acid; (a) significant reduction of water consumption and serum glucose, and an elevation of serum insulin, (b) a reducing trend of serum and liver lipids, (c) a reducing trend of the proportion of stearic and arachidonic acids in liver phospholipids and (d) a significant reduction of the concentration of serum immunoglobulin G (IgG) and an increase in IgE. Thus, the results indicate that elaidic acid may not further aggravate metabolic disorders caused under the diabetic condition more than the cis counterpart even when consumed at a relatively high level, with the exception of the response of serum immunoglobulins.

Key words: elaidic acid, oleic acid, streptozotocine-diabetes, immunoglobulin, prostaglandin E2, rat

1 Introduction

Recent studies on the physiological function of trans fatty acid have exclusively been concentrated on its adverse effect on the serum cholesterol level and lipoprotein profile (1-3). The epidemiological studies also suggest the possible untoward effect of trans fatty acids on atherogenesis (4,5). In addition to this effect, trans acid interferes with the metabolic pathway converting linoleic acid to arachidonic acid and hence, eicosanoids (6,7). Thus, it is expected that trans acid exerts diverse metabolic influences more than cholesterol issue (7,8). The observation that trans acids do not promote breast and colon carcinogenesis more than the corresponding cis acid, oleic acid (9,10) is in part attributed to its interference with linoleic acid metabolism, though current study suggested a possible detrimental effect of trans fatty acids in breast cancer incidence in womans (11).

Diabetes mellitus is one of the most common diseases in the developed countries, and dietary management is a crucial approach to the treatment of this dis-

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ease. In addition to polyunsaturated fatty acids (PUFA) (12), monounsaturated fat shows clinical and metabolic benefits in Type 2 non-insulin-dependent diabetic mellitus (NIDDM) patients (13,14). Monounsaturated fatty acid appears to have a favorable effect on serum cholesterol levels and lipoprotein profiles (15,16) and exerts a stability against oxidation (17). Since lipid peroxidation is one crucial factor for the pathogenesis of diabetes, it is interesting to know the influence of elaidic acid, the geometrical isomer of oleic acid, on diabetic symptoms. The trans configuration is more resistant against lipid peroxidation than the cis counterpart (18). In this context, Christiansen et al. (19) showed that both trans monounsaturated and saturated fatty acids when fed at a high level induce an increase in postprandial insulinemia in obese patients with NIDDM. One epidemiological study demonstrated that the risk of diabetes is positively associated with the intake of trans acid (20), while it was not the case in other studies (21,22). However, trans acid appears to increase insulin resistance (23-25). More recently, Ibrahim et al. (26) showed that dietary trans acid decreased adipocyte insulin sensitivity to a greater extent than did saturated acid. Thus, the relation between the intake of trans acid and the appearance of NIDDM has not yet been settled, although there are some indications (27).

Based on these considerations, in the present study rats previously fed the diets containing fats differing in the composition of octadecenoic acid, either elaidic acid or oleic acid, were given free access to respective experimental diets for 2 weeks. The experimental diets were prepared according to the recommendation by the American Institute of Nutrition (AIN, 1977) (28) and contained following ingredients, in g/100 g diet: casein, 20; safflower oil, 7; either oleic or elaidic acids (both purity > 99%, Wako Pure Chemicals, Osaka), 3; mineral mixture (AIN76TM) (28), 3.5; vitamin mixture (AIN76TM), 1; choline bitartrate, 0.2; cellulose, 5; corn starch, 15; and sucrose, 45.3. The fatty acid composition of dietary fats is shown in Table 1 and the composition of monoene fatty acid was the sole variable in dietary fats. The energy value of elaidic acid corresponded to 6.4 energy % of the diet. Food intake, water consumption and body weight were recorded every day. After feeding for 2 weeks, rats were food deprived of for 16 h and received intravenous streptozotocin (STZ, Sigma Chemical Co., St. Louis, MO) dissolved in 25 mM citrate buffer at a dose of 50 mg/kg body weight (29). Rats were allowed to continue the respective diets. One week after STZ administration, foods were removed for 5 h (from 0600 to 1100 h) and blood was withdrawn from the abdominal aorta under diethyl ether anesthesia. Liver, kidney, spleen and perirenal adipose tissue were excised immediately.

### 2 Experimental

#### 2.1 Animals and Diets

The animal experiment adhered to the Nakamura Gakuen University guide for the care and use of laboratory animals. Male Sprague-Dawley rats, 4 weeks old, were purchased from Seiwa Experimental Animals, Fukuoka. Rats were housed individually in a room with a controlled temperature and light (20-22°C, lights on 0800 to 2000). After acclimation for 3 days, the animals were divided into two groups of seven rats each and were given free access to respective experimental diets for 2 weeks. The experimental diets were prepared according to the recommendation by the American Institute of Nutrition (AIN, 1977) (28) and contained following ingredients, in g/100 g diet: casein, 20; safflower oil, 7; either oleic or elaidic acids (both purity > 99%, Wako Pure Chemicals, Osaka), 3; mineral mixture (AIN76TM) (28), 3.5; vitamin mixture (AIN76TM), 1; choline bitartrate, 0.2; cellulose, 5; corn starch, 15; and sucrose, 45.3. The fatty acid composition of dietary fats is shown in Table 1 and the composition of monoene fatty acid was the sole variable in dietary fats. The energy value of elaidic acid corresponded to 6.4 energy % of the diet. Food intake, water consumption and body weight were recorded every day. After feeding for 2 weeks, rats were food deprived of for 16 h and received intravenous streptozotocin (STZ, Sigma Chemical Co., St. Louis, MO) dissolved in 25 mM citrate buffer at a dose of 50 mg/kg body weight (29). Rats were allowed to continue the respective diets. One week after STZ administration, foods were removed for 5 h (from 0600 to 1100 h) and blood was withdrawn from the abdominal aorta under diethyl ether anesthesia. Liver, kidney, spleen and perirenal adipose tissue were excised immediately.

#### 2.2 Lipid Analyses

Liver and serum lipids were extracted by the method of Folch et al. (30) and their lipid components were measured as reported elsewhere (31). Liver phospholipids were separated into their components by thin-layer chromatography (32), and their fatty acid compositions were analyzed by gas-liquid chromatography (GC-14A, Shimadzu, Kyoto) using a CP-Sil 88 capillary column (0.25 mm × 50 m, Chrompack, EA-Middleburg, Netherlands) (33,34).

<table>
<thead>
<tr>
<th>Fatty acid composition (g/100 g)</th>
<th>16:0</th>
<th>18:0</th>
<th>t-18:1</th>
<th>c-18:1</th>
<th>18:2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cis</td>
<td>7.1</td>
<td>1.7</td>
<td>42.5</td>
<td>48.4</td>
<td></td>
</tr>
<tr>
<td>Trans</td>
<td>7.1</td>
<td>1.7</td>
<td>31.8</td>
<td>10.9</td>
<td>48.5</td>
</tr>
</tbody>
</table>

* Safflower oil was mixed with either oleic or elaidic acids at the ratio of 7:3, w/w.

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2.3 Measurement of Serum Glucose, Insulin, Leukotriene B4, Prostaglandin E2 and Creatinine

Serum glucose was measured by a kit (Glucose-Test Wako, Wako Pure Chemicals, Osaka) and insulin by a radioimmunoassay kit (Insulin Riabead, Dainabot Co., Tokyo). Serum LTB4 and PGE2 were measured by radioimmunoassay using commercial kits (NEK-037 and NEK-020, NEN Life Science Products, Boston, MA, respectively) (35,36). Serum creatinine was measured by a commercial kit (Creatinine-Test Wako).

2.4 T-lymphocyte Population Analysis

Spleen lymphocytes were isolated by Lympholyte-Rat (Cedarlane, Hornby, Canada) and the proportion of cells staining positive for CD4⁺ and CD8⁺ were analyzed by flowcytometry (Epics Plofile II, Coulter Electronics Ltd., Beds) (37).

2.5 Measurement of Serum Immunoglobulins

Serum immunoglobulins were measured by the sandwich ELISA method as reported previously (38,39).

2.6 Statistical Analysis

Data were analyzed by Student’s t-test before and after the STZ treatment and within the diet group. Values in the text are means ± SE.

3 Results

3.1 Growth Performance and Tissue Weight

There were no differences in food intake, water consumption and weight gain between the two groups of rats until they received STZ (Table 2). After treatment with STZ, however, rats in the cis acid group consumed significantly more water, by 140% and gained less weight than those in the trans acid group, although food intake was increased by STZ treatment to a similar extent in both groups. Among tissues weighed, relative weight of kidney was significantly higher in rats fed cis acid than in those fed trans acid, but no difference was observed in the weight of liver, spleen and adipose tissue.

3.2 Serum Chemistries and Liver Lipids

The concentration of serum insulin was significantly lower while that of serum glucose was significantly higher in rats fed cis acid than in those fed trans acid (Table 3). No difference was found in the serum creatinine level between groups. The concentration of serum and liver lipids tended to be higher in the cis acid group than in the trans acid group, and the difference in the concentration of serum cholesterol and liver triglyceride was significant.

3.3 Fatty Acid Composition of Liver Phospholipids

In all phospholipid classes except for cardiolipin, trans acid compared to cis acid significantly reduced the proportion of stearic acid and this was compensated by the incorporation of the trans isomer (Table 4). Essentially no trans fatty acid was incorporated in cardiolipin and the proportion of stearic acid in this phospholipid was not different between the two groups. The stearic acid level in cardiolipin was negligibly low as compared with other classes of phospholipids. There was a difference in the extent of incorporation of elaidic acid among phospholipids, and it was in the order of phosphatidylethanolamine = phosphatidylcholine > phosphatidylinositol > phosphatidylserine. After feeding trans acid, a trend toward a reduction of arachidonic acid and an increase in linoleic acid was observed. Con-

### Table 2 Effects of Elaidic Acid on Body Weight Gain, Food Intake, Water Consumption and Tissue Weight.

<table>
<thead>
<tr>
<th>Group</th>
<th>Initial body weight (g)</th>
<th>Body weight gain (g/day)</th>
<th>Food intake (g/day)</th>
<th>Water consumption (ml/day)</th>
<th>STZ injection</th>
<th>Relative tissue weight (g/100 g body weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
<td>Before</td>
<td>After</td>
<td>Before</td>
<td>Kidney*</td>
</tr>
<tr>
<td>Cis</td>
<td>117 ± 1</td>
<td>6.5 ± 0.1</td>
<td>1.3 ± 0.3</td>
<td>15.6 ± 0.3</td>
<td>21.4 ± 1.0</td>
<td>19.8 ± 1.8</td>
</tr>
<tr>
<td></td>
<td>19.8 ± 1.8</td>
<td>139 ± 32*</td>
<td>5.16 ± 0.22</td>
<td>121 ± 0.04*</td>
<td>25.6 ± 0.01</td>
<td>0.25 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>0.70 ± 0.06</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trans</td>
<td>113 ± 3</td>
<td>6.9 ± 0.2</td>
<td>3.0 ± 0.7</td>
<td>15.2 ± 1.0</td>
<td>19.7 ± 1.0</td>
<td>18.2 ± 1.1</td>
</tr>
<tr>
<td></td>
<td>98.3 ± 21.2</td>
<td>5.68 ± 0.15</td>
<td>1.05 ± 0.06</td>
<td>0.27 ± 0.01</td>
<td>0.87 ± 0.06</td>
<td></td>
</tr>
</tbody>
</table>

*Significantly different from the trans group at p < 0.05.

Table 3  Effects of Dietary Fats on Serum Chemistries and Liver Lipids a.

<table>
<thead>
<tr>
<th>Chemistry</th>
<th>Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cis</td>
</tr>
<tr>
<td>Serum</td>
<td></td>
</tr>
<tr>
<td>Insulin (mU/mL)</td>
<td>16.8 ± 4.3*</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>29.1 ± 0.8*</td>
</tr>
<tr>
<td>Creatinine (mmol/L)</td>
<td>63.0 ± 1.8</td>
</tr>
<tr>
<td>Cholesterol (mmol/L)</td>
<td>2.87 ± 0.18*</td>
</tr>
<tr>
<td>Triglyceride (mmol/L)</td>
<td>5.73 ± 1.09</td>
</tr>
<tr>
<td>Phospholipid (mmol/L)</td>
<td>3.31 ± 0.17</td>
</tr>
<tr>
<td>Liver</td>
<td></td>
</tr>
<tr>
<td>Cholesterol (mmol/g)</td>
<td>9.79 ± 0.48</td>
</tr>
<tr>
<td>Triglyceride (mmol/g)</td>
<td>47.0 ± 5.3*</td>
</tr>
<tr>
<td>Phospholipid (mmol/g)</td>
<td>47.2 ± 1.6</td>
</tr>
</tbody>
</table>

Values are means ± SE of 7 rats. Significantly different from the *trans* group at *p < 0.05.

sequently, the desaturation index for linoleic acid, (20:3n-6 + 20:4n-6)/18:2n-6, tended to be lower in rats fed *trans* acid compared to those fed *cis* acid, the difference was significant in phosphatidylcholine and phosphatidylinositol. In addition, the proportion of docosahexaenoic acid decreased significantly by *trans* acid in two major phospholipids, phosphatidylcholine and phosphatidylethanolamine.

### 3.4 Serum Leukotriene B4 (LTB4) and Prostaglandin E2 (PGE2)

The concentration of serum LTB4 was comparable between the two groups (113 ± 4 and 123 ± 5 μg/L for the *cis* and *trans* groups, respectively). There was no significant difference in the serum PGE2 level due to a large individual variation in the *cis* group, although it was approximately one-third in rats fed *trans* acid as compared with those fed *cis* acid (6.37 ± 2.22 and 2.16 ± 0.37 μg/L for the *cis* and *trans* groups, respectively).

### 3.5 Serum Immunoglobulin Levels

The concentration of serum IgG was significantly higher and that of serum IgE was significantly lower in rats fed *cis* acid than in those fed *trans* acid (Table 5). No differences between groups were observed in serum IgA and IgM levels.

### 3.6 Splenic T-cell Population

T-cell population was dependent on the type of dietary fats (Table 6). The proportion of cells staining positive for CD4+ was significantly higher in rats fed *trans* acid compared to those fed *cis* acid. Since the pro-

Table 4  Effects of Elaidic Acid on Fatty Acid Composition of Liver Phospholipids a.

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Phosphatidylinositol</th>
<th>Phosphatidylserine</th>
<th>Cardiolipin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cis</td>
<td>Trans</td>
<td>Cis</td>
</tr>
<tr>
<td>(mol/100 mol)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14:0</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>16:0</td>
<td>17.1 ± 0.4</td>
<td>20.0 ± 1.3</td>
<td>13.8 ± 0.4</td>
</tr>
<tr>
<td>18:0</td>
<td>26.0 ± 0.7**</td>
<td>10.9 ± 0.6</td>
<td>29.1 ± 0.6**</td>
</tr>
<tr>
<td>t-18:1</td>
<td>-</td>
<td>16.7 ± 1.1</td>
<td>-</td>
</tr>
<tr>
<td>c-18:1</td>
<td>7.3 ± 0.3**</td>
<td>5.1 ± 0.5</td>
<td>6.8 ± 0.3**</td>
</tr>
<tr>
<td>18:2n-6</td>
<td>11.4 ± 0.4**</td>
<td>14.1 ± 0.5</td>
<td>6.4 ± 0.3</td>
</tr>
<tr>
<td>20:3n-3</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>20:4n-6</td>
<td>29.8 ± 0.5**</td>
<td>26.4 ± 0.3</td>
<td>29.4 ± 0.4</td>
</tr>
<tr>
<td>22:5n-3</td>
<td>0.6 ± 0.1</td>
<td>0.6 ± 0.1</td>
<td>1.5 ± 0.1</td>
</tr>
<tr>
<td>22:6n-3</td>
<td>2.1 ± 0.2</td>
<td>2.2 ± 0.2</td>
<td>4.3 ± 0.3</td>
</tr>
<tr>
<td><strong>index b</strong></td>
<td>2.6 ± 0.1**</td>
<td>1.9 ± 0.1</td>
<td>4.7 ± 0.3</td>
</tr>
</tbody>
</table>

Values are means ± SE of 7 rats. Significantly different from the corresponding elaidic acid group at *p < 0.05 and **p < 0.01.

b (20:3n-6 + 20:4n-6)/18:2n-6.
portion of CD8+ did not differ between groups, the trans acid group showed a significantly higher CD4+/CD8+ ratio in relation to the cis acid group.

### 4 Discussion

In our preliminary experiments, STZ (Wako Pure Chemicals, Osaka) was administered at the level recommended by Junod et al. (29), 65 mg/kg body weight, to rats which previously received either cis or trans acid diets under the same dietary condition with that of the present study. Five out of 6 rats died 4 days after administration and one 5 days after in the oleic acid group, while in the elaidic acid group 2 out of 6 rats died 4 days after. When the dose level was reduced to 60 mg/kg under the same dietary manipulation, 4 and 2 out of 6 rats died 3 and 4 days after STZ, respectively, in the oleic acid group, while each 2 rats in the elaidic acid group died in the same time interval. The dose was further reduced to 50 mg/kg and it was found that 8 and 6 out of 8 rats died 2 days after administration in the cis and trans acid groups, respectively. Then, the STZ preparation from other supplier (Sigma Chemical Co., St. Louis, MO) was given at a level of 50 mg/kg, and one rat deceased 2 and 3 days after the STZ treatment in the cis and trans groups, respectively. Although the cause of this phenomenon is unclear, it is suggested that rats previously fed elaidic acid at least has tolerance against the drug more than those fed oleic acid.

Greater water consumption, higher serum glucose level, and lower serum insulin level in rats fed cis acid in relation to trans acid may indicate more severe diabetic state in the former group of rats. Additionally, there was a tendency of increased concentrations of serum and liver lipids in the cis group, suggesting greater disturbance of lipid metabolism in this group due to STZ. In NIDDM patients, it has been reported that a high-monounsaturated-fat diet is more effective than a high-complex-carbohydrate diet in reducing blood glucose levels and improving the overall lipoprotein profile (40). Christiansen et al. (19) showed in obese patients with NIDDM trans and cis acids are equally glycemic but insulin and C-peptide responses were significantly greater following the trans acid diet. Thus, it is conceivable that trans monounsaturated fatty acid has a propensity that is different from the cis counterpart in its effect on diabetic situation, although the direct comparison may not be allowed between humans and rats. In this context, it has been shown that trans acid affects insulin receptors by decreasing the number and increasing the affinity, an effect similar to that observed with saturated acid (41), probably through a change in fatty acid composition in the cell membrane. Phospholipid model membranes containing trans acid had a higher membrane cholesterol affinity and lower receptor activation than their cis analogues (42). These changes in membrane structure and function may contribute to metabolic change due to trans acid.

The mechanism by which trans acid reduces the concentration of serum glucose and increases that of insulin, in relation to the cis counterpart, is not apparent at present, but it seems unlikely that the increase in serum insulin by trans acid is caused by increased insulin resistance in the peripheral tissues, since the serum glucose was lowered. Therefore, there is a possibility that trans acid elevates or does not reduce a glucose receptor (Glut 4) activity in relation to cis acid. It is known that the type of dietary fat differently influences fasting blood insulin and glucose levels (43). The difference in the fatty acid composition of membrane phospholipids between the cis and trans groups may cause such a difference (44). In this context, the less oxidizable propensity of elaidic acid compared with oleic acid may also be involved in the different response (18). The insulin secretion also is under regulation of
dietary fat, and monoene fatty acid improves glycemic control (14,40,45). Whatever the mechanism is concerned, these results indicate that elaidic acid may mitigate the diabetic state more than oleic acid in this animal model. Although the extent of decrease in the antilipolytic effect of insulin and insulin-stimulated glucose transport were greater in rats fed trans acid than in those fed saturated acid, and the decrease was not prevented or reduced with linoleic acid (26).

Consistent with our previous observations with normal rats (46,47), there was a difference in the extent of incorporation of trans acid in individual phospholipids even in diabetic rats. Although an enough amount of linoleic acid was supplemented, trans acid interfered with the metabolism of linoleic acid to arachidonic acid, and this interference was more marked in the diabetic rats than in normal rats (46,47). This metabolic pathway is known to be repressed under diabetic situation (7). Reflecting the reducing effect of trans acid on the arachidonic acid level, the concentration of serum PGE2 tended to be lower in rats fed elaidic acid, though not significant because of a large deviation of this parameter in rats fed oleic acid. Insulin stimulates Δ6 desaturase activity (7), the key enzymatic step leading linoleic acid to arachidonic acid, and trans acid elevated the serum insulin level in relation to cis acid. However, the desaturation index was rather lower in rats fed trans acid than in those fed cis acid, suggesting a considerable interfering effect of trans acid on PUFA metabolism. No influence was seen in the concentration of serum LTβs, but it is frequently reported that the response of eicosanoid production is type-specific (29,47) and the magnitude of response to STZ treatment also is different (48).

In rats oleic acid is shown to be immunosuppressive in relation to linoleic acid (49,50). The analysis of splenic T-lymphocyte population showed an increased proportion of CD4+ positive cells and consequently the CD4+CD8+ ratio in rats fed elaidic acid, suggesting the stimulation of immunoglobulin production (51). However, the results of serum immunoglobulin levels were not necessarily reflected in the change in T-cell subsets, and the level of IgG decreased significantly, and that of IgE increased significantly after feeding trans acid. Although the spleen plays a crucial role in the production of immunoglobulin on a whole body basis, it may be possible that the splenic production of immunoglobulin is not totally reflected on the serum level. When normal rats were received the diets same to those given currently, there was no difference in the concentration of individual serum immunoglobulins between the cis and trans groups, respectively (47). The concentrations of serum IgG and IgE observed in the previous study were similar to those in the cis acid group in the present study. Thus, the under the diabetic condition, as compared with the normal state, it is plausible that the difference in the geometry of octadecenoic acid induces a different response to the serum immunoglobulins. Although the mechanism is unknown, this observation deserves further study.

5 Conclusion

In conclusion, elaidic acid, in comparison with oleic acid, even given at a relatively high level, approximately 6 energy %, did not aggravate the pathogenesis of diabetic condition caused by STZ, in so far as the glucose level is concerned. Since trans acid has been shown to exert untoward effects on cholesterol distribution in plasma lipoproteins at the dietary level above 4 to 5 energy % (16,51,52), this observation seems to be worth mentioning. However, the metabolic effects of oleic acid and elaidic acid on diabetic parameters were complex and not necessarily unitary. In this context, it seems interesting to know how elaidic acid modifies the established STZ diabetes.

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Trans Fatty Acid and Diabetes in Rats


