Effects of α-Linolenic Acid-rich Diacylglycerol on Diet-induced Obesity in Mice

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Abstract: In the present study, we have investigated the effects on body fat of α-linolenic acid-rich diacylglycerol (ALA-DAG), which is a combination of the 1,3-DAG structure and n-3 fatty acid. C57BL/6J mice were fed diets of high fat and sucrose (30 wt %, 13 wt %, respectively, HF group) without or with ALA-DAG added to 1,2,4 wt % for 4 weeks. We noted significant gains in body weight and visceral fat in the mice in the HF group as compared to a group fed an ordinary diet containing 5 wt % fat (LF group). Addition of ALA-DAG resulted in a significant restraint of both body weight and visceral fat weight gain. Furthermore, addition of ALA-DAG to 2wt% and 4wt% significantly reduced the plasma leptin level. When high fat and sucrose diet was continued for 20 weeks, leptin and insulin levels significantly increased as compared to the LF group in addition to the body weight and body fat gains. By replacing 3 wt% of the lipid in the high fat and sucrose diet with 3 wt% of ALA-DAG, leptin and insulin concentrations significantly decreased in addition to body weight and visceral fat loss. These results indicate the anti-obesity function of ALA-DAG, and also suggest the effectiveness of using ALA-DAG in the prevention and treatment of life-style related diseases in which obesity is a risk factor.


Key words: diacylglycerol, α-linolenic acid, obesity, C57BL/6J

1 Introduction

Obesity, especially the visceral fat accumulation, has been identified as a risk factor leading to life-style related diseases, such as diabetes, hyperlipidemia, high blood pressure, arteriosclerosis and so on (1-3). Therefore, many studies have been reported that focus on food ingredients (4) and medicinal drugs (4-8) for the prevention and reduction of obesity.

We have studied the nutritional characteristics of diacylglycerol (DAG), which exists widely in natural oils and fats mainly in 1,3-isomorph. In studies comparing DAG with triacylglycerol (TAG), DAG has been shown to decrease the concentration of triacylglycerol in the blood (9). DAG can restrain increases in triacylglycerol in the blood after lipid loading (10), and it has a lower accumulation rate than TAG in the body fats of both animals (11) and humans (12,13). Since the fatty acid components of both DAG and TAG are almost equivalent to each other, the functions of DAG described above are considered to be due to the 1,3-DAG structure.

Also, the n-3 polyunsaturated fatty acids (PUFAs) in foods, such as eicosapentanoic acid (EPA), docosahexanoic acid (DHA), α-linolenic acid (ALA) have been

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reported to have various physiological functions. These include the ability to restrain platelet aggregation (14-16), an anti-arteriosclerosis effect (17-19), an anti-high blood pressure effect (20-22), an anti-inflammatory effect (23-25), an immunity control effect (26), an inhibitory action for carcinoma (27), a function within the nervous system (28,29), a activity for retinal function (30, 31) and so on. The n-3 PUFAs also affect lipid metabolism, and the investigation results show that they have the effects of decreasing lipid concentration in blood (32-40), and the reduction of body weight and body fat weight (41-46).

In this study, we focused on α-linolenic acid-rich diacylglycerol, ALA-DAG, which consists of the 1,3-DAG structure and fatty acids containing 60.8% ALA. We investigated their effects on the body fat metabolism of mice fed ALA-DAG rich diet in the feeding periods of 4 weeks and 20 weeks.

2 Materials and Methods

2.1 Animals and Feeding Methods

Animal experiments were carried out with the approval and control of the Animals Control Committee and the Animals Ethical Committee in Kao Corporation.

We used C57BL/6J mice (7 weeks old, male, Clea Japan, INC., Tokyo) and kept them under the following environmental conditions; a temperature of 23±2°C, humidity of 55±10% and lighting time between 7:00-19:00. After keeping the mice under these conditions for 7 days for acclimatization, we measured their body weights and divided them into groups with similar average weights (n=5/group). They were allowed free access to food and water. We used Roden CAFÉ (Oriental Yeast CO., LTD, Tokyo) for their diet, and the feeders were replaced with a new one every other day. We measured the amount of food consumed per 24 hours and calculated the energy intake for each of the test groups (n=5/cage/group) once a week in Experiment 1 and once in 4 weeks in Experiment 2. The duration was 4 weeks for Experiment 1, and 20 weeks for Experiment 2.

2.2 Test Materials and Food Ingredients

ALA-DAG was prepared from perilla oil in the presence of immobilized lipase as described by Hug-Jensen et al. (47). The fatty acid composition of the test oils, such as ALA-DAG, and the safflower/rapeseed oil mixture (SR-oil) are shown in Table 1.

SR-oil contains mainly oleic acid, 29.1%, and linoleic acid, 57.8%, as its fatty acid components. On the other hand, ALA-DAG contains 60.8% of α-linolenic acid in the total fatty acids. The DAG content and TAG content in the ALA-DAG oil are 85.2% and 14.1% respectively, and the ratio of the 1,3-DAG and the 1,2-DAG is 70:30. Lard, sucrose, casein, cellulose, mineral mixture, vitamin mixture and α-potato starch were purchased.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Fatty Acid Composition of Test Oils (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fatty Acid</td>
</tr>
<tr>
<td>---------</td>
<td>------------</td>
</tr>
<tr>
<td>16:0</td>
<td>6.0</td>
</tr>
<tr>
<td>18:0</td>
<td>2.2</td>
</tr>
<tr>
<td>18:1</td>
<td>29.1</td>
</tr>
<tr>
<td>18:2</td>
<td>57.8</td>
</tr>
<tr>
<td>18:3</td>
<td>2.5</td>
</tr>
<tr>
<td>20:0</td>
<td>0.4</td>
</tr>
<tr>
<td>20:1</td>
<td>0.6</td>
</tr>
<tr>
<td>22:0</td>
<td>0.3</td>
</tr>
<tr>
<td>22:1</td>
<td>0.2</td>
</tr>
<tr>
<td>Others</td>
<td>0.9</td>
</tr>
</tbody>
</table>

Composition of acylglycerol

- Monoacylglycerol  n.d.  0.7
- Diacylglycerol  1.1  88.2
- Triacylglycerol  97.2  14.1

*SR-oil : safflower oil:rapeseed oil = 70:30
*ALA-DAG : α-linolenic acid-rich diacylglycerol
from Oriental Yeast Co. Ltd, and the safflower oil and the rapeseed oil were purchased from The Nissin Oil Mills, Ltd.

2.3 Measurements of Body Weight, Visceral Fat Weight, Liver Weight and Blood Sampling

During the experiments, body weights were measured every week. At the end of the experiments, we put the mice under anaesthetic using ether after fasting them for 12 hours, and we then took blood samples from the posterior vena. Serum was used for various biochemical tests. Under the same conditions of anaesthesia, whole blood was drawn to kill the mice. The visceral fats (epididymal fat, mesenteric fat, retroperitoneal fat and perinephric fat) and liver were dissected and weighed.

2.4 Analysis of Serum Samples

Quantitative analyses were carried out for triacylglycerol, total cholesterol, free fatty acid and glucose using an enzymatic method in an automatic analyser (Analyser Super Z818, Nittech Research Co. Tokyo), and quantitative analyses of GOT and GPT were also carried out in the same automatic analyser using a UV method. Leptin and insulin were measured by the ELISA method using both a mouse leptin measuring kit (Morinaga Bioscience Laboratory Co. Kanagawa) and an insulin measuring kit (Morinaga Bioscience Laboratory Co. Kanagawa).

2.5 Experiment 1: Effects of ALA-DAG Ingestion for 4 weeks

The composition and energy values of the various diets are shown in Table 2.

The low fat diet (LF) contains 5% of lipids, while the high fat diet (HF) contains 30% lipids and 13% sucrose. The energy values of the LF and HF diets per 100 g are 399.7 kcal and 522.2 kcal, respectively i.e., the HF diet has about a 30% higher energy than the LF diet. The ALA-DAG-added diets were prepared by addition of 1%, 2% and 4% of ALA-DAG to the basic HF diet. The content was adjusted by adding measured amounts of α-potato starch. All of the diets were packaged into light-shielding bags containing 2 days-worth of food, and were stored at 4°C after nitrogen gas injection.

2.6 Experiment 2: Effects of ALA-DAG Ingestion for 20 weeks

The composition and energy values for these diets are shown in Table 3.

Both the LF and HF diets had the same energy values as in Experiment 1. The ALA-DAG replaced diet was prepared by replacing 10% of the SR-oil in the HF diet with same amount of ALA-DAG. The dietary materials were stored in the same way as used in Experiment 1.

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>LF</th>
<th>HF</th>
<th>HF + ALA-DAG</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>1% ALA-DAG</td>
</tr>
<tr>
<td>ALA-DAG*</td>
<td>—</td>
<td>—</td>
<td>1.0</td>
</tr>
<tr>
<td>SR-oil*</td>
<td>5.0</td>
<td>20.0</td>
<td>20.0</td>
</tr>
<tr>
<td>Lard</td>
<td>—</td>
<td>10.0</td>
<td>13.0</td>
</tr>
<tr>
<td>Sucrose</td>
<td>—</td>
<td>13.0</td>
<td>13.0</td>
</tr>
<tr>
<td>Casein</td>
<td>20.0</td>
<td>20.0</td>
<td>20.0</td>
</tr>
<tr>
<td>Cellulose</td>
<td>4.0</td>
<td>4.0</td>
<td>4.0</td>
</tr>
<tr>
<td>Mineral mixture*</td>
<td>3.5</td>
<td>3.5</td>
<td>3.5</td>
</tr>
<tr>
<td>Vitamin mixture*</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>α-potato starch</td>
<td>66.5</td>
<td>28.5</td>
<td>27.5</td>
</tr>
<tr>
<td>Energy (kcal/100 g)</td>
<td>399.7</td>
<td>522.2</td>
<td>527.1</td>
</tr>
</tbody>
</table>

*LF : Low-fat diet
HF : High-fat diet
*ALA-DAG : α-linolenic acid-rich diacylglycerol
SR-oil : safflower oil:rapeseed oil = 70:30
*Mineral mixture : AIN–76 prescription
*Vitamin mixture : AIN–76 prescription + choline bitartrate (20 g/100 g)
Table 3 Composition of Diets in Experiment 2 (%).  

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>LF&lt;sup&gt;a&lt;/sup&gt;</th>
<th>HF&lt;sup&gt;b&lt;/sup&gt;</th>
<th>HF + ALA-DAG</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALA-DAG&lt;sup&gt;c&lt;/sup&gt;</td>
<td>—</td>
<td>—</td>
<td>3.0</td>
</tr>
<tr>
<td>SR-oil&lt;sup&gt;d&lt;/sup&gt;</td>
<td>5.0</td>
<td>30.0</td>
<td>27.0</td>
</tr>
<tr>
<td>Sucrose</td>
<td>—</td>
<td>13.0</td>
<td>13.0</td>
</tr>
<tr>
<td>Casein</td>
<td>20.0</td>
<td>20.0</td>
<td>20.0</td>
</tr>
<tr>
<td>Cellulose</td>
<td>4.0</td>
<td>4.0</td>
<td>4.0</td>
</tr>
<tr>
<td>Mineral mixture&lt;sup&gt;e&lt;/sup&gt;</td>
<td>3.5</td>
<td>3.5</td>
<td>3.5</td>
</tr>
<tr>
<td>Vitamin mixture&lt;sup&gt;f&lt;/sup&gt;</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>α-potato starch</td>
<td>66.5</td>
<td>28.5</td>
<td>28.5</td>
</tr>
<tr>
<td>Energy (kcal/100 g)</td>
<td>399.7</td>
<td>522.2</td>
<td>522.2</td>
</tr>
</tbody>
</table>

<sup>a</sup>LF: Low-fat diet  
<sup>b</sup>HF: High-fat diet  
<sup>c</sup>ALA-DAG: α-linolenic acid rich diacylglycerol  
<sup>d</sup>SR-oil: safflower oil:rapeseed oil=70:30  
<sup>e</sup>Mineral mixture: AIN-76 prescription  
<sup>f</sup>Vitamin mixture: AIN-76 prescription + choline bitartrate (20 g/100 g)

2.7 Statistical Analysis  
The results were expressed as mean ± standard error. Statistical analysis was carried out by one-way analysis of variance (ANOVA), followed by Fisher’s PLSD test. Statistical significance was defined as p<0.05.

3 Results  
3.1 Experiment 1
3.1.1 Body weight  
No significant difference was observed in the initial body weight within each group (Table 4).

All of the groups showed gains in body weight in the fourth week. The HF group showed a significantly larger body weight gain than the LF group, with a difference in body weight gain of 2.6 g (p<0.001). On the other hand, the ALA-DAG-added groups showed restraint of body weight gain compared with the HF group. All of the ALA-DAG-added groups showed a significant difference in both body weight and body weight gain compared with the HF group (body weight: 1% and 2% added groups; p<0.05, 4% added group; p<0.01, body weight gain: all added groups; p<0.001). Their final body weight was almost equal to that of the LF group (No significant difference from the LF group was observed). The ALA-DAG 4% added group showed the lowest body weight and the lowest body weight gains in the fourth week. The energy intake for all groups during this experiment showed no significant differences.

3.1.2 Visceral fat weight and liver weight  
The visceral fat weight and liver weight are shown in Table 4. Both the total visceral fat weight and each individual part of the visceral fat weight (epididymal fat, mesenteric fat, retroperitoneal fat and perirenal fat) of the HF group were higher than those of the LF group. Significant differences between these groups were observed for total visceral fat weight (p<0.01), epididymal fat weight (p<0.01), mesenteric fat weight (p<0.05) and retroperitoneal fat weight (p<0.001). The ALA-DAG-added groups also displayed significantly lower values than the HF group for total visceral fat weight (1% added group p<0.05, 2% and 4% added groups p<0.01), epididymal fat weight (1% added group p<0.05, 2% and 4% added groups p<0.01), mesenteric fat weight (1% and 2% added groups p<0.05, 4% added groups p<0.01), and retroperitoneal fat weight (1% added group p<0.05, 2% and 4% added groups p<0.001). The visceral fat weight demonstrated a tendency to decrease with linearly increasing additional ALA-DAG content, and the ALA-DAG 4% added group gave the lowest value.

With regard to liver weight, the ALA-DAG 1% added group and the 4% added group showed significantly lower values (p<0.05) than the HF group, but did not show any significant difference from the LF group.

3.1.3 Biochemical blood test results  
Table 5 shows the biochemical blood test results.  
No significant difference in triacylglycerol, total cho-
Table 4  Effects of ALA-DAG on Body weight at 4 weeks in Experiment 1.

<table>
<thead>
<tr>
<th>Dietary treatment</th>
<th>LF*</th>
<th>HFb</th>
<th>HF + ALA-DAGc</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ALA-DAG 1%</td>
<td>ALA-DAG 2%</td>
<td>ALA-DAG 4%</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial</td>
<td>21.6 ± 1.3</td>
<td>21.6 ± 1.3</td>
<td>21.6 ± 1.2</td>
</tr>
<tr>
<td>Final</td>
<td>26.3 ± 1.2*</td>
<td>29.0 ± 1.9</td>
<td>26.4 ± 0.3*</td>
</tr>
<tr>
<td>Gain (4 weeks)</td>
<td>4.8 ± 0.4***</td>
<td>7.4 ± 1.5</td>
<td>4.9 ± 0.7***</td>
</tr>
<tr>
<td>Liver weight (g)</td>
<td>0.92 ± 0.05</td>
<td>0.99 ± 0.07</td>
<td>0.89 ± 0.04*</td>
</tr>
<tr>
<td>Visceral-fat weight (g)</td>
<td>0.96 ± 0.10**</td>
<td>1.46 ± 0.37</td>
<td>1.11 ± 0.18*</td>
</tr>
<tr>
<td>Total</td>
<td>0.49 ± 0.06**</td>
<td>0.76 ± 0.21</td>
<td>0.56 ± 0.12*</td>
</tr>
<tr>
<td>Epididymal</td>
<td>0.32 ± 0.04*</td>
<td>0.41 ± 0.08</td>
<td>0.34 ± 0.03*</td>
</tr>
<tr>
<td>Mesenteric</td>
<td>0.10 ± 0.03***</td>
<td>0.24 ± 0.07</td>
<td>0.16 ± 0.05*</td>
</tr>
<tr>
<td>Retroperitoneal</td>
<td>0.04 ± 0.00</td>
<td>0.05 ± 0.02</td>
<td>0.04 ± 0.00</td>
</tr>
<tr>
<td>Perinephric</td>
<td>55.8 ± 7.1</td>
<td>62.9 ± 4.2</td>
<td>59.4 ± 5.5</td>
</tr>
<tr>
<td>Energy intake</td>
<td>57.2 ± 4.7</td>
<td>57.9 ± 5.1</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SD (n=5)
*LF : Low-fat diet
*HF : High-fat diet
*ALA-DAG : α-linolenic acid-rich diacylglycerol
Significantly different from HF, *p<0.05, **p<0.01, ***p<0.001

Table 5  Effects of ALA-DAG on Serum Metabolic Index at 4 weeks in Experiment 1.

<table>
<thead>
<tr>
<th>Dietary treatment</th>
<th>LF*</th>
<th>HFb</th>
<th>HF + ALA-DAGc</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ALA-DAG 1%</td>
<td>ALA-DAG 2%</td>
<td>ALA-DAG 4%</td>
</tr>
<tr>
<td>Triacylglycerol (mg/100 ml)</td>
<td>113.7 ± 68.2</td>
<td>89.7 ± 31.3</td>
<td>79.9 ± 36.2</td>
</tr>
<tr>
<td>Total cholesterol (mg/100 ml)</td>
<td>128.9 ± 35.2</td>
<td>115.9 ± 10.2</td>
<td>113.5 ± 4.1</td>
</tr>
<tr>
<td>Free fatty acid (mEq/l)</td>
<td>1.38 ± 0.22</td>
<td>1.40 ± 0.34</td>
<td>1.23 ± 0.19</td>
</tr>
<tr>
<td>Leptin (ng/ml)</td>
<td>1.71 ± 0.99</td>
<td>4.45 ± 4.39</td>
<td>2.03 ± 0.91</td>
</tr>
</tbody>
</table>

Values are means ± SD (n=5)
*LF : Low-fat diet
*HF : High-fat diet
*ALA-DAG : α-linolenic acid-rich diacylglycerol
Significantly different from HF, *p<0.05

Lesterol and free fatty acid was found among the LF group, the HF group and the ALA-DAG-added group. The ALA-DAG 2% and 4% added groups showed significant leptin reduction (p<0.05) compared with the HF group.

3.2 Experiment 2

3.2.1 Body weight

No significant differences were observed in the initial body weight among the groups (Table 6).

All of the groups showed gains in body weight at the 20th week. The HF group showed a significantly larger body weight gain than the LF group, with a difference in body weight gain of 8.2 g (p<0.001). In the same way as Experiment 1, the ALA-DAG replacement group showed significant restraint of body weight and body weight gain at the 20th week in comparison with the HF group (body weight: p<0.05, body weight gain
Table 6  Effects of ALA-DAG on Body weight, Liver weight and Visceral–fat weight at 20 weeks in Experiment 2.

<table>
<thead>
<tr>
<th>Dietary treatment</th>
<th>LF*</th>
<th>HF*</th>
<th>HF + ALA-DAG*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial</td>
<td>22.0±0.6</td>
<td>22.5±0.9</td>
<td>22.0±0.7</td>
</tr>
<tr>
<td>Final</td>
<td>27.6±1.7***</td>
<td>36.4±2.3</td>
<td>32.0±3.7*</td>
</tr>
<tr>
<td>Gain (20 weeks)</td>
<td>5.6±1.2***</td>
<td>13.8±1.9</td>
<td>10.0±3.1*</td>
</tr>
<tr>
<td>Liver weight (g)</td>
<td>0.92±0.07***</td>
<td>1.25±0.11</td>
<td>1.16±0.12</td>
</tr>
<tr>
<td>Visceral-fat weight (g)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>1.26±0.38***</td>
<td>3.18±0.62</td>
<td>2.28±0.87</td>
</tr>
<tr>
<td>Epididymal</td>
<td>0.58±0.19***</td>
<td>1.62±0.33</td>
<td>1.19±0.52</td>
</tr>
<tr>
<td>Mesenteric</td>
<td>0.45±0.10***</td>
<td>0.87±0.16</td>
<td>0.63±0.15*</td>
</tr>
<tr>
<td>Retroperitoneal</td>
<td>0.16±0.07***</td>
<td>0.49±0.09</td>
<td>0.34±0.15*</td>
</tr>
<tr>
<td>Perinephric</td>
<td>0.07±0.02**</td>
<td>0.20±0.07</td>
<td>0.12±0.06*</td>
</tr>
<tr>
<td>Energy intake(kcal/cage/day)</td>
<td>57.4±2.8**</td>
<td>68.6±1.3</td>
<td>66.5±4.2</td>
</tr>
</tbody>
</table>

Values are means±SD (n=5)
*LF: Low-fat diet
*HF: High-fat diet
*ALA-DAG: α-linolenic acid-rich diacylglycerol
Significantly different from HF, *p<0.05, **p<0.01, ***p<0.001

Table 7  Effects of ALA-DAG on Serum Metabolic Index at 20 weeks in Experiment 2.

<table>
<thead>
<tr>
<th>Dietary treatment</th>
<th>LF*</th>
<th>HF*</th>
<th>HF + ALA-DAG*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triacylglycerol (mg/100 ml)</td>
<td>77.2±17.2***</td>
<td>35.3±2.9</td>
<td>37.4±16.7</td>
</tr>
<tr>
<td>Total cholesterol (mg/100 ml)</td>
<td>122.0±20.9</td>
<td>132.8±19.9</td>
<td>102.8±8.2*</td>
</tr>
<tr>
<td>Free fatty acid (mEq/l)</td>
<td>1.26±0.26**</td>
<td>0.86±0.08</td>
<td>0.92±0.14</td>
</tr>
<tr>
<td>Leptin (ng/ml)</td>
<td>1.59±1.07***</td>
<td>16.72±7.66</td>
<td>6.75±5.27*</td>
</tr>
<tr>
<td>Glucose (mg/100 ml)</td>
<td>231.3±20.5</td>
<td>281.6±62.5</td>
<td>259.0±61.8</td>
</tr>
<tr>
<td>Insulin (pg/ml)</td>
<td>176.4±74.0**</td>
<td>897.6±505.3</td>
<td>287.3±72.1**</td>
</tr>
<tr>
<td>GOT (IU/l)</td>
<td>53.5±29.8</td>
<td>46.7±13.1</td>
<td>38.4±6.7</td>
</tr>
<tr>
<td>GPT (IU/l)</td>
<td>18.3±24.1</td>
<td>18.6±11.6</td>
<td>10.3±2.0</td>
</tr>
</tbody>
</table>

Values are means±SD (n=5)
*LF: Low-fat diet
*HF: High-fat diet
*ALA-DAG: α-linolenic acid-rich diacylglycerol
Significantly different from HF, *p<0.05, **p<0.01, ***p<0.001

amount: p<0.05). The energy intake during this experiment showed significant differences between the LF group and both of the HF and ALA-DAG replacement groups, but no significant difference was observed between the HF group and the ALA-DAG replacement group.

3.2.2 Visceral fat weight and liver weight
The visceral fat weight and liver weight are shown in Table 6. Both the total visceral fat weight and each element of the total visceral fat weight (epididymal fat, mesenteric fat, retroperitoneal fat and perinephric fat) of the HF group showed higher levels than those of the LF group. Significant differences between the groups were observed for total visceral fat weight (p<0.001), epididymal fat weight (p<0.001), mesenteric fat weight (p<0.001), retroperitoneal fat weight (p<0.001) and
perinephric fat weight (p<0.01). The ALA-DAG replacement group had significantly lower values than the HF group for mesenteric fat weight, retroperitoneal fat weight and perinephric fat weight (All cases p<0.05).

With regard to liver weight, the HF group and the ALA-DAG replacement group showed significantly higher values (p<0.01) than the LF group, but no significant difference was found between the HF group and the ALA-DAG replacement group.

3.2.3 Biochemical blood test results

Table 7 shows the biochemical blood test results.

Triacylglycerol and free fatty acid were found at significantly lower levels for the HF and ALA-DAG replacement group than for the LF group (HF group: p<0.001, p<0.01 respectively, ALA-DAG replacement group: p<0.001, p<0.01 respectively). Total cholesterol, leptin, glucose and insulin determinations for the HF group showed higher values than those for the LF group, with significant differences for leptin and insulin (leptin p<0.001, insulin p<0.01). Total cholesterol, leptin, glucose and insulin determinations for the ALA-DAG replacement group gave lower values than those of the HF group, with significant differences for total cholesterol, leptin and insulin (total cholesterol p<0.05, leptin p<0.05, insulin p<0.01). GOT and GPT showed no significant differences between the LF group, the HF group and the ALA-DAG replacement group.

4 Discussion

In this study, we demonstrated that ALA-DAG, containing more than 60% of ALA as the fatty acid component of diacylglycerols, displays significant restraining functions for body weight gain and visceral fat weight gain for C57BL/6J mice (Table 4, Table 6). We also showed that long-term ingestion of ALA-DAG gives significant restraining functions in total cholesterol, leptin and insulin concentrations in serum (Table 7). In addition to those, any increase in GOT and GPT values, which indicate tissue damage, were not observed (Table 7).

C57BL/6J mice have been used in various investigations into diet-induced obesity and diabetes-model in animals (48-54). These studies showed that a high-fat intake increased in body weight, visceral fat weight and leptin and insulin concentrations in serum than those of a low-fat intake. Our current study shows that the C57 BL/6J mice were suffering from diet-induced obesity.

Studies have also been carried out into the mechanisms of the lipid metabolism acceleration functions of DAG, such as the reduction of triacylglycerol in serum (9,10) and of body fat (11-13). It was found in comparison with TAG that DAG intake releases significantly lower amounts of triacylglycerol and cholesterol in the lymphatic chylomicron after a single oral dose of the lipid emulsion (55). In addition, during a 21-day period of raising rats on a DAG enhanced diet, depending on the concentration of DAG in the diet, a series of β-oxidation enzymes were activated in liver (56). In the present study, ALA-DAG ingestion may exhibit similar phenomena. Furthermore, in this investigation it was expected that in addition to the functions of 1,3-DAG structure, the functionality of ALA is involved, because of the major fatty acid in the DAG is ALA, one of n-3 PUFAs that are known to affect lipid metabolism in mammals.

The functional mechanism of n-3 PUFAs for lipid metabolism has been studied from two points of view, the restraint of lipid synthesis and the acceleration of lipid catabolism. The former study reported restraint for fatty acid synthesis (57,58) and restraint for triacylglycerol synthesis (59-65), and the latter reported β-oxidation acceleration (66-68). Furthermore, investigations on the generation of various lipid metabolism-related genes have been carried out as a possible mechanism of these phenomena, (69-73). However, when total energy intakes are the same, in order to reduce body weight and body fat weight, not only restraint of lipid synthesis and acceleration of lipid metabolism is required, but also some degree of energy loss. Hum et al. (41) reported that when keeping KK-A^t mice on a diet containing 10% of lipids, replacing 30% of the lipid with fish oil induced an observable restraint of epididymal fat weight gain, although restraint of body weight gain was not observed. Also, at the same time, an increase in the uncoupling protein-2 (UCP-2) mRNA in white adipose tissue occurred. Tsuboyama-Kasaoka et al. (42) reported on a comparative study between keeping C57BL/6J mice on a diet consisting of safflower oil and fish oil with a diet that had been replaced with lipid corresponding to 60% of the total energy intake. A significant restraint of body weight gain and white adipose tissue weight gain were observed in the fish oil ingestion group after five months, and also an increase in the UCP-2 mRNA in the liver occurred. Furthermore, recently, we reported up-regulation of UCP-2 mRNA by PPARα activation with n-3 PUFAs in the small intestine (74). These reports suggest the contribution of energy consumption with UCP as a mechanism for energy loss that can generate the obesity restraint
function. In this investigation, under the same conditions of energy intake we also observed the restraint of body weight gain and visceral fat weight gain. Therefore, there is a possibility that UCP up-regulation might occur in various internal organs.

The various physiological activities of n-3 PUFAs described above were obtained mainly from fish oil, DHA and EPA. There are also similar reports for ALA. Ide et al. (75) studied perilla oil containing 56.7% of ALA, and compared it with palm oil and safflower oil when applied to rats. They reported that the perilla oil ingestion group showed significantly lower triacylglycerol and cholesterol concentrations in their serum, and they also showed significantly higher β-oxidation enzyme activity in their livers compared with the palm oil and safflower oil ingestion groups. Ikemoto et al. (43) studied the ingestion of various diets for C57 BL/6J mice, into which various lipids had been placed corresponding to 60% of the total energy intake. They indicated that the perilla oil that contains α-linolenic acid as the main fatty acid has a higher tendency to restrict body weight gain and white adipose tissue weight gain than soyabean oil, which contains oleic acid and linoleic acid as its main fatty acid constituents. Furthermore, Kawada et al. (44) compared linseed oil containing 58.8% of ALA and lard by feeding them to rats, and reported that linseed oil showed significant restraint of abdominal fat gain, and observed an increase in UCP protein in brown adipose tissue. Some studies suggest the possibility that ALA can be converted to EPA and DHA, which can then show some functionality in a body (76-78). On the other hand, other studies show the functions of ALA as a direct ligand to PPARs, which are transcriptional factors corresponding to lipid metabolism control (79, 80). The generation mechanism of these physiological activities is a point to be investigated in the near future.

In our study we observed the restraint of body weight gain and visceral fat weight gain of C57BL/6J mice by ingestion of ALA-DAG. It has been reported that the visceral fat weight has a positive correlation with leptin concentration in the serum and that leptin was endogenously produced from adipose tissues (51,81). This is in agreement with the present study. A relationship between visceral fat weight and insulin resistance also has been obtained previously (82,83). The cholesterol concentration in serum, which is identified as a risk factor for arteriosclerosis (84,85), was reduced in C57 BL/6J mice fed ALA-DAG diet compared with control. The mice are also used as a non-insulin-dependent (type II) diabetes model. In this study, as indicated in Table 7, we observed a trend for restraint of glucose concentration in the serum due to ALA-DAG, though a significant difference was not obtained. Adding ALA-DAG to the diet also tended to show a significant restraining function against increasing insulin concentration in the serum. These results also suggest the possibility that ALA-DAG may be effective against type II diabetes.

In this study, we demonstrated the first dietary studies undertaken to evaluate the nutritional effects of ALA-DAG in mammals, and found an anti-obesity function of ALA-DAG and the reduction of various risk factors related to diseases. The results indicate the possibility of prevention and improvement of obesity which correspond with the various life-style related diseases such as diabetes, hyperlipidemia and arteriosclerosis by the ALA-DAG intake in humans.

References


2079-2086.