Fatty liver in rats induced by excessive intake of a nutritionally adequate liquid diet
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FATTY LIVER IN RATS INDUCED BY EXCESSIVE INTAKE OF A NUTRITIONALLY ADEQUATE LIQUID DIET

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In order to test whether or not overeating of a nutritionally adequate diet with reasonable fat content could result in significant fat accumulation in the liver, male Sprague–Dawley rats were provided with free access to either a nutritionally adequate liquid diet with 35 per cent of calories as fat or a regular diet (controls) for 3 months. After the feeding period, body weight, Lee index, and epididymal adipose tissue weight, were significantly greater in rats fed with the liquid diet than in the controls. Liver weight, hepatic triglyceride levels were also greater in the liquid diet group. Histologically, remarkable fatty infiltration was observed predominantly in periportal areas in rats fed with the liquid diet ad libitum for 3 months. Compared to a large body of the literature concerning diet-induced obesity in experimental animals, information on animal models of fatty liver by dietary manipulations is insufficient. The results of this study clearly indicate that the overeating of a nutritionally adequate diet with reasonable fat content could result in remarkable fat accumulation in the liver in rats.

Keywords: fatty liver, excessive intake, liquid diet.

Introduction

We recently demonstrated by liver function study that about 50 percent of moderately obese male non-drinkers had significant hepatic steatosis. Nutritional assessment of these subjects with hepatic steatosis has disclosed that their fat intake was even lower than that in western society, whereas their caloric intake was in excess. Of the various dietary manipulations currently known to produce obesity in rats, maintenance of rats on a high-fat diet is the most commonly employed. However, the fat content of the conventionally used high-fat diet (about 60 percent of total calories as fat) is higher than that recommended in western society. In contrast to a large body of the literature dealing with obesity experimentally induced by dietary means, information on animal models of diet-induced fatty liver is insufficient. Based on this background, we wished to determine whether free access to nutritionally adequate liquid diet with reasonable fat contents could result in significant hepatic steatosis in rats.

Materials and methods

A total of 16 male Sprague–Dawley rats (Charles River, Japan) were used. Diets used were a standard solid diet, MF (Oriental Yeast Co., Ltd, Tokyo) and a nutritionally adequate liquid diet (Oriental
Table 1. Composition of a liquid diet* (g/l).

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soda-casine</td>
<td>41.4</td>
</tr>
<tr>
<td>L-cystine</td>
<td>0.5</td>
</tr>
<tr>
<td>DL-methionine</td>
<td>0.3</td>
</tr>
<tr>
<td>Sucrose</td>
<td>115.9</td>
</tr>
<tr>
<td>Corn oil</td>
<td>8.5</td>
</tr>
<tr>
<td>Olive oil</td>
<td>28.5</td>
</tr>
<tr>
<td>Vitamin mixture&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.0</td>
</tr>
<tr>
<td>Hergsted salt mixture&lt;sup&gt;c&lt;/sup&gt;</td>
<td>10.0</td>
</tr>
<tr>
<td>DL-α-tocopherol acetate</td>
<td>0.03</td>
</tr>
<tr>
<td>Sodium carrageenan</td>
<td>2.5</td>
</tr>
<tr>
<td>Ethyl linoleate</td>
<td>2.7</td>
</tr>
</tbody>
</table>

*1008 kcal/l (protein 16.7%, fat 35.4%, carbohydrate 47.8%)

<sup>b</sup> Contained/100 g: Vitamin A (500 000iu) 240 mg, V.D<sub>3</sub> (400 000iu) 20 mg, V.B<sub>1</sub> (×10) 145 mg, V.B<sub>2</sub> (×10) 250 mg, V.B<sub>6</sub> (×10) 145 mg, V.B<sub>12</sub> (×100) 50 mg, V.K<sub>3</sub> (×10) 50 mg, Ca-pant, 100 mg, Niacin 100 mg, Biotin (×100) 50 mg, Folic acid (×10) 50 mg, Inositol 500 mg, P-ABA250 mg, CCl<sub>4</sub> 5000 mg, Glucose 92.9198 g

<sup>c</sup> Contained/100 g: CaCO<sub>3</sub> 29.98 g, K<sub>2</sub>HPO<sub>4</sub> 33.73 g, CaHPO<sub>4</sub> 7.5 g, MgSO<sub>4</sub> 10.0 g, NaCl 15.62 g, Fe-citrate 2.67 g, KI 0.09 g, MnSO<sub>4</sub> 0.37 g, CuSO<sub>4</sub> 0.02 g, ZnCl<sub>2</sub> 0.02 g

Yeast Co., Ltd, Tokyo<sup>4</sup>. The standard solid diet, made of (in decreasing order of amount) flour, corn, soybean meal, whitefish meal, yeast, alphalpa meal and soybean oil, had the following composition (w/w); 7.0 percent water, 24.0 percent crude protein, 5.1 percent crude fat, 6.2 percent crude ash, 3.2 percent crude fiber, 54.5 percent nitrogen free extra (more than 90 percent of which is starch). The composition of the liquid diet is shown in Table 1. The liquid diet, prepared basically according to Lieber and DeCarli<sup>5</sup> with some modifications, was offered as a fresh solution each day, and the amount consumed was recorded. Results of preliminary experiments showed that rats served both the liquid diet and drinking water took minimal amount of water and that an amount of the liquid diet consumed by those rats was comparable to that consumed by rats given liquid diet only. Therefore, rats given the liquid diet were not given drinking water in the rest of the experiments. The rats were split into two groups, one received the liquid diet without access to water (n=8) and the other the standard solid diet with free access to water (n=8). Both diets were available ad libitum. All animals were housed individually in screen-bottomed cages in conditions of controlled lighting (12 h light: 12 h dark) and temperature (21±1°C). All animals were weighed every other day. At the end of 12 weeks, animals were killed by decapitation after an overnight fast. Prior to slaughter, the degree of obesity was assessed by using the formula proposed by Lee<sup>6</sup>, Lee Index (LI) = 3√BW/L, where BW = body weight in g and L = nose to anus length in cm. Blood was taken and the liver was quickly removed. A portion of the right lobe of the liver was fixed with 10 percent buffered formalin for histological evaluation and the rest of the liver was kept frozen at -80°C for determination of hepatic triglyceride content. Epididymal adipose tissue was also removed and weighed. Serum triglyceride levels were measured by an autoanalyzer. Total hepatic lipids were extracted<sup>7</sup> and triglyceride contents were determined by the established method<sup>8</sup>

All values were expressed as mean±s.d. The significance of the difference was assessed by the non-paired Student's t-test.

**Results**

A greater rate of weight gain was evident in rats fed the liquid diet. After 12 weeks, the rats given the control diet weighed 556±46 g and those on the liquid diet weighed 730±55 g (Fig. 1). This difference represented a 31 percent increase in the body weight of the rats fed the liquid diet. The greater rise in body weight was associated with a two-fold increase in epididymal adipose tissue weight and a
significant rise in the Lee index; an index which gives degrees of obesity in rats (Table 2). The liver weight was remarkably greater in the liquid diet fed rats than in the control. Macroscopically, the liver in the liquid diet group was somewhat whitish in appearance and, microscopically, remarkable fatty infiltration was observed predominantly in the periportal area (Fig. 2a). Furthermore hepatic triglyceride levels were remarkably greater in the liquid diet fed rats compared with those of the controls (Table 3). During the 12th week on the diet regimens, food intake was measured in these rats. Rats fed the liquid diet had significantly higher caloric intake than rats fed the standard solid diet (161±13 vs 103±17 kcal/day, P<0.001).

![Graph showing mean body weight of rats provided with either a liquid diet or a standard solid diet for 3 months.]

**Fig. 1. Mean body weight of rats provided with either a liquid diet or a standard solid diet for 3 months.**

**Table 2. Body weight, Lee index, and epididymal adipose tissue weight in male rats provided with either a liquid diet or a standard solid diet for 3 months.**

<table>
<thead>
<tr>
<th>Group</th>
<th>Initial body weight (g)</th>
<th>Final body weight (g)</th>
<th>Epididymal adipose tissue weight (g)</th>
<th>Lee index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liquid diet (n=8)</td>
<td>154±5</td>
<td>730±55</td>
<td>28.5±5.7</td>
<td>0.339±0.01</td>
</tr>
<tr>
<td>Standard solid diet (n=8)</td>
<td>157±6</td>
<td>556±46</td>
<td>14.2±4.8</td>
<td>0.316±0.006</td>
</tr>
</tbody>
</table>

*P<0.001.

**Table 3. Liver weight and triglyceride levels in the liver and serum in male rats provided with either a liquid diet or a standard solid diet for 3 months.**

<table>
<thead>
<tr>
<th>Group</th>
<th>Liver weight (g)</th>
<th>Hepatic triglyceride (mg/g liver)</th>
<th>Serum triglyceride (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liquid diet (n=8)</td>
<td>20.7±2.7</td>
<td>54.7±25.6</td>
<td>244±89</td>
</tr>
<tr>
<td>Standard solid diet (n=8)</td>
<td>14.9±2.8</td>
<td>21.6±10.2</td>
<td>98±38</td>
</tr>
</tbody>
</table>

*P<0.005.

*P<0.01.

*P<0.001.
Fig. 2. *Hepatic histology in the rat.* (a) Rat fed with a nutritionally adequate liquid diet *ad libitum* for 3 months (HE, ×80). Remarkable fatty infiltration is noted mainly in periportal area. (b) Rat fed with the standard solid diet *ad libitum* for 3 months (HE, ×80). No significant change is noted.

**Discussion**

Non-alcoholic fatty liver, particularly the one associated with mild or moderate obesity is frequent in Japan. Our nutritional assessment of these patients with non-alcoholic fatty liver has disclosed that their fat intakes are lower than those observed in western populations, but their caloric intakes are in excess. Of the various dietary means currently available to induce obesity in rats, the maintenance of rats on a high-fat diet and the application of the so-called "cafeteria-diets" are the two most commonly employed. The fat content of the high-fat diet used in most studies is around 60 percent, which is somewhat higher than that
observed in western populations. On the other hand, the percentage of fat intake is not easily assessed in the cafeteria-diet model. For this reason, we tested whether or not overeating of a nutritionally adequate diet could result in significant hepatic steatosis in rats; this was found to be the case. The experimental models of obesity other than the ones produced by dietary manipulations include genetically transmitted obesity and obesity induced by bilateral lesions in the ventromedial hypothalamus (VMH). Diet-induced obesity has an advantage over other forms of experimental obesity being that the interpretation of the metabolic changes observed are relatively easy, because they are not modified by other biochemical abnormalities associated with obesity of hypothalamic or genetic origin. Experimental obesity by dietary manipulations has been extensively studied. The use of a liquid diet to induce obesity in rats was first reported by Ingle; he found that the combination of ad-libitum eating of palatable liquid diets and restriction of activity produced marked obesity in rats. It is also well established that the progression of diet-induced obesity is more or less dependent on the percentage of fat in the diet. Compared to the large amount of literature on diet-induced obesity in experimental animals, information on experimental fatty liver by dietary manipulations has been inadequate. The results of this study clearly indicate that overeating of nutritionally adequate diets with reasonable fat contents could lead to significant hepatic steatosis in rats. Although the liquid diet used in this study is one with a reasonable fat content, it is also high in sucrose. Since high-sucrose diets have significant effects on liver fat contents under some experimental conditions, it is possible that the high sucrose content of the liquid diet played some role in the development of fatty liver. It should also be taken into account that diet form (liquid vs solid) may have a significant influence upon growth in rats.

Thus, fatty liver was induced in rats by excessive intake of a nutritionally adequate liquid diet, which could provide an animal model for the fatty liver condition observed in moderately obese non-drinking men.

References