Note

Effects of High-Fat Diet Intake on Glucose Uptake in Central and Peripheral Tissues of Non-Obese Rats

Tatsuhiro MATSUO,1,* Soh IWASHITA,2 Maki KOMURO2 and Masashige SUZUKI2

1 Division of Nutrition & Biochemistry, Sanyo Women’s College, Hatsukaichi 738–8504, Japan
2 Institute of Health & Sport Sciences, University of Tsukuba, Tsukuba 305–8574, Japan
(Received March 3, 1999)

Summary We previously demonstrated that plasma glucose concentration was higher while plasma insulin concentration was lower in rats fed a high-fat diet. In the present study, we examined the effects of high-fat diet on glucose uptake in central and peripheral tissues in non-obese rats. Forty male Sprague-Dawley rats were fed high- or low-fat diets for 4 wk. Body weight and body fat accumulation were not different between the two diet groups after 4 wk. Glucose uptake in the skeletal muscles and adipose tissues, estimated by the 2-deoxy-D-glucose method, was lower in the rats fed the high-fat diet than that in the rats fed the low-fat diet, whereas uptake in the liver and pancreas did not differ between the two groups. Glucose uptake in the hypothalamus and cortex was higher in the high-fat diet group as compared with that in the low-fat diet group. These results suggest that increased plasma glucose levels in rats fed the high-fat diet were caused by a decrease in glucose uptake in the skeletal muscles and adipose tissues. Reduced plasma insulin level in the high fat diet group with no difference in glucose uptake in the pancreas may be due to increased sympathetic activity in the pancreas resulting from the increased glucose uptake in the brain regions involved in autonomic functions.

Key Words glucose, insulin, glucose uptake, high-fat diet, diurnal variation

It is well established that the high level of fat in Western diets is a major factor in the development of insulin resistance and obesity (1–3). The feeding of laboratory animals with high-fat diets has proved to be a useful model of the putative effects of dietary fat in humans, and there is evidence of reduced insulin-mediated

*Present address: Faculty of Agriculture, Kagawa University, Ikenobe, Mikicho, Kagawa 761–0795, Japan.
glucose metabolism in animals fed high-fat diets (4–7). Some studies in animals have suggested that high-fat diets are more likely to induce hyperglycemia than low-fat diets (8), while other studies have failed to show this finding (9–12). In most previous studies, plasma glucose levels were measured in the fasting condition. Since plasma glucose level has a diurnal variation, it seemed to be essential to measure the diurnal rhythm of plasma glucose concentration.

We have previously demonstrated that when rats are fed a high-fat diet (40% of energy as fat) for 4–21 wk, plasma glucose concentrations in preprandial and postprandial conditions are higher despite lower plasma insulin concentrations as compared with a low-fat diet (5% of energy as fat) (13). In this experiment, since body fat accumulation was not observed in rats fed a high-fat diet for 4 wk, the causes of hyperglycemia in the high-fat diet rats might not originate from obesity and insulin resistance.

The plasma glucose level is regulated by glucose uptake in several tissues, mostly skeletal muscles (13). In addition, glucose taken into the pancreatic β-cells stimulates insulin secretion (14). In the present study, to clarify the cause of the diurnal variations of plasma glucose and insulin observed in non-obese rats fed a high-fat diet, we investigated glucose uptake in central and peripheral tissues estimated by the 2-deoxy-D-glucose method.

**Experimental**

**Animal care.** Forty male Sprague-Dawley rats (4 wk old, 80±1 g) were obtained from CLEA Japan (Tokyo). All procedures involving animals were approved by the Experimental Animal Care Committee of the University of Tsukuba. Rats were isoenergetically meal-fed high- or low-fat diets. The composition of each diet has been described previously (15). The high-fat diet provided 40, 35, and 25% of energy as fat, carbohydrate, and protein, respectively. The low-fat diet provided 5, 70, and 25%, respectively. The rats were individually caged at 22±2°C, with lights on from 7 am to 7 pm. Each group of rats was meal-fed the diet at 8:30 to 9:30 am and 8:30 to 9:30 pm and given free access to water for 4 wk. The amount of diet fed to the rats was increased gradually from 191 kJ/d for the first week to 304 kJ/d for the fourth week of the experiment.

**Diurnal rhythm of plasma insulin and glucose levels.** Diurnal variation of plasma insulin and glucose levels was measured in half of the rats in each diet group after 4 wk of feeding the experimental diet. Blood samples (150 μL) were obtained from a tail artery at 2:30 am, 6:30 am, 10:30 am, 2:30 pm, 6:30 pm and 10:30 pm into tubes coated with heparin and NaF for the determination of plasma insulin and glucose levels.

**Oral glucose tolerance test.** Before the end of the experiment, the remaining rats in each dietary group were orally administered 4 mL/kg body weight of a 50% glucose solution at 8:30 am (fasting condition) (16). Blood samples were obtained from a tail artery (0 min), 5, 15, 30, 60, 120 and 180 min after glucose administration, and transferred to tubes precoated with heparin and NaF.
Glucose uptake estimation. After 4 wk of feeding, 10 rats from each dietary group were prepared for this measurement. A nonmetabolizable glucose analogue, 2-[3H]deoxy-D-glucose (50 mCi) (Amersham), was administered at 2:30 pm when plasma glucose levels were considered to be steady-state and plasma samples were obtained at 2, 5, 10, 15, 20, 30, and 45 min after the administration. Forty-five minutes after administration of the glucose analogue, the rats were sacrificed, and brain, liver, pancreas, skeletal muscles and adipose tissues were rapidly removed and stored at −70°C until analysis. An estimate of glucose uptake in several tissues was calculated from the method described by Kraegen et al (17). Briefly, the accumulation of phosphorylated 2-[3H]deoxy-d-glucose was used as an indication of glucose metabolic rate in individual tissues. Phosphorylated 2-[3H]deoxy-d-glucose, which is trapped in most tissues, undergoes negligible further reaction and can be accurately measured (17). From this measurement and a knowledge of plasma glucose and the time course of plasma 2-[3H]deoxy-d-glucose disappearance, the tissue glucose metabolic index (Rg') was calculated. Rg' is calculated using the equation,

$$ Rg' = \frac{C_p C_m'(T)}{\int_0^T C_p \, dt} $$

where $C_p$ is plasma glucose (μmol/mL), $C_p'$ is plasma 2-[3H]deoxy-d-glucose (dpm/mL), and $C_m'$ is tissue phosphorylated 2-[3H]deoxy-d-glucose (dpm/g) (17).

Analyses. Plasma insulin concentration was determined using an enzyme immunoassay kit (Sanko-Junyaku, Japan), and glucose was analyzed enzymatically (Wako Pure Chemical Industries, Japan).

Statistical analysis. Data are expressed as mean ± SE. Statistical analysis of the oral glucose tolerance test and the diurnal variations in plasma insulin and glucose levels were performed by ANOVA with repeated measures and Fisher PLSD test. All other data were analyzed by Student's t-test.

Results and discussion

Both groups of rats gained the same body weight during the 4-wk experimental period (188 ± 5 vs. 181 ± 6 g, mean ± SE [high-fat diet group vs. low-fat diet group]). Total abdominal adipose tissue weight did not differ between the two groups (8.6 ± 0.6 vs. 7.2 ± 0.5 g). The central and peripheral tissue weights did not differ between the two groups (hypothalamus, 99 ± 11 vs. 94 ± 8 mg; cortex, 941 ± 26 vs. 929 ± 23 mg; soleus, 190 ± 11 vs. 170 ± 10 mg; tibialis anterior, 1.1 ± 0.1 vs. 1.0 ± 0.1 g; perirenal adipose tissue, 3.1 ± 0.3 vs. 2.8 ± 0.3 g; interscapular brown adipose tissue, 304 ± 25 vs. 276 ± 21 mg; liver, 11 ± 1 vs. 10 ± 1 g; pancreas, 786 ± 30 vs. 804 ± 32 mg [high-fat diet group vs. low-fat diet group]). These results confirm our previous findings (13, 18, 19).

After 4 wk of high-fat diet feeding, plasma glucose levels were almost all higher, while insulin levels were lower at all times of the day in the rats fed a high-fat diet (Table 1). These results suggest that 4 wk of the high-fat diet induced hyperglycemia,
Table 1. Diurnal variation of plasma insulin and glucose levels in rats fed high- or low-fat diets.

<table>
<thead>
<tr>
<th>Time of day</th>
<th>6:30 am</th>
<th>10:30 am</th>
<th>2:30 pm</th>
<th>6:30 pm</th>
<th>10:30 pm</th>
<th>2:30 am</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Plasma glucose (mg/100 mL)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High fat</td>
<td>133 ± 2</td>
<td>139 ± 4</td>
<td>146 ± 3</td>
<td>137 ± 4</td>
<td>151 ± 2</td>
<td>149 ± 4</td>
</tr>
<tr>
<td>Low fat</td>
<td>128 ± 4</td>
<td>125 ± 2*</td>
<td>124 ± 2*</td>
<td>127 ± 2</td>
<td>140 ± 2*</td>
<td>137 ± 3*</td>
</tr>
</tbody>
</table>

| **Plasma insulin (μU/mL)** |         |          |         |         |          |         |
| High fat    | 2.6 ± 1.0 | 5.9 ± 2.2 | 7.7 ± 2.7 | 5.4 ± 2.1 | 5.7 ± 2.1 | 5.9 ± 2.2 |
| Low fat     | 12.0 ± 4.6* | 15.7 ± 5.9* | 15.9 ± 5.6* | 11.3 ± 4.3* | 14.9 ± 5.6* | 10.3 ± 3.9* |

Values are means ± SE for 10 rats. * Statistically significant differences (p < 0.05) from the rats fed the high-fat diet (ANOVA with repeated measures and Fisher PLSD test).

not hyperinsulinemia, which supports our previous findings (13).

Figure 1 shows the time course of plasma glucose and insulin concentrations during the oral glucose tolerance test. Compared to the results with the low-fat diet feeding group, plasma glucose levels immediately after glucose ingestion were significantly lower (p < 0.05) in the rats fed the high-fat diet, but this reversed after 30–180 min. Plasma insulin levels were lower at all times in the high-fat diet rats.

The glucose metabolic index (Rg') assessed by the 2-deoxy-D-glucose method in the hypothalamus and cortex was higher, but Rg' in the soleus muscle, tibialis anterior, perirenal and brown adipose tissue was lower in the rats fed the high-fat diet (Table 2). In the liver and pancreas, Rg' did not differ between the two groups (Table 2).

From the results of the oral glucose tolerance test, insulin resistance should not be observed in either dietary group because plasma glucose and insulin concentrations recovered to pre-ingestion levels after 120 min. Fasting and postprandial- and glucose-stimulated insulin concentrations were lower in the rats fed the high-fat diet (Table 2 and Fig. 1). These findings suggest that hypoinsulinemia induced by the high-fat diet was caused by decreasing insulin secretion from pancreas.

Glucose taken from the blood is actively oxidized in the skeletal muscles and brown adipose tissue. Therefore, reduced glucose uptake in those tissues in the rats fed a high-fat diet may contribute to the higher plasma glucose level observed in those rats. Kim et al (18) demonstrated that a high-fat diet impairs glucose metabolism in skeletal muscle by reducing the transcription of GLUT 4 via suppression of plasma insulin without affecting gene expression of the insulin receptor. Our findings are consistent with the results of Kim et al.

Insulin secretion is suppressed by sympathetic nerve activation in the pancreas, independently of glucose stimulation (20, 27). Many researchers have suggested that short-term high-fat diet feeding activates the sympathetic nervous system in animals (22). Pancreatic sympathetic activity might have increased in the rats fed the high-fat...
Effects of High-Fat Diet on Tissue Glucose Uptake

Fig. 1. Time course of plasma glucose and insulin concentrations during oral glucose tolerance tests in rats fed high- and low-fat diets. Values are means ± SE for 10 rats. * Statistically significant differences (p < 0.05) from the rats fed the high-fat diet (ANOVA with repeated measures and Fisher PLSD test).

Table 2. Glucose metabolic index (Rg') in central and peripheral tissues.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>High fat</th>
<th>Low fat</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mmol/100 g/min</td>
<td></td>
</tr>
<tr>
<td>Hypothalamus</td>
<td>17.1 ± 1.0</td>
<td>13.1 ± 0.7**</td>
</tr>
<tr>
<td>Cortex</td>
<td>27.0 ± 1.4</td>
<td>22.6 ± 1.4*</td>
</tr>
<tr>
<td>Soleus</td>
<td>32.8 ± 3.2</td>
<td>43.3 ± 2.9*</td>
</tr>
<tr>
<td>Tibialis anterior</td>
<td>8.0 ± 0.9</td>
<td>12.6 ± 1.1**</td>
</tr>
<tr>
<td>Perirenal adipose tissue</td>
<td>1.3 ± 0.2</td>
<td>3.0 ± 0.5*</td>
</tr>
<tr>
<td>Brown adipose tissue</td>
<td>2.6 ± 1.0</td>
<td>17.6 ± 5.9*</td>
</tr>
<tr>
<td>Liver</td>
<td>0.9 ± 0.2</td>
<td>0.8 ± 0.2</td>
</tr>
<tr>
<td>Pancreas</td>
<td>3.8 ± 0.3</td>
<td>4.0 ± 0.5</td>
</tr>
</tbody>
</table>

Values are means ± SE for 10 rats. **,* Statistically significant differences from the rats fed the high-fat diet (**p < 0.01, *p < 0.05, Student’s t-test).
diet, by which the plasma insulin level was decreased by reducing insulin secretion from the pancreas. The mechanism involved in the increase of sympathetic activity after the consumption of a high-fat diet is unclear, but it may be related to activation of the central nervous system. Sympathetic nerve activities in peripheral tissues are regulated by certain regions of the brain stem, especially in the hypothalamus (22). Levin demonstrated that intracarotid glucose injection increased plasma norepinephrine, as an index of the sympathetic nervous system (23). Moreover, Levin reported that diet-induced obese (DIO) rats had reduced 2-deoxyglucose uptake in the brain areas involved in food intake and/or sympathetic activity compared with diet-resistant (DR) rats (24). DIO rats had lower sympathetic activity when compared with DR rats (20, 23, 24). In the present study, glucose uptake in the hypothalamus and cortex was higher in the rats fed the high-fat diet. The ventromedial hypothalamus nucleus is well known to be the main regulatory center in the sympathetic nervous system (22, 24, 25). Although further studies are required to clarify the details, our findings in this experiment suggest that the modulation of glucose metabolism in rat brain regions may be involved in the promotion of higher sympathetic tone by the intake of a high-fat diet.

In conclusion, 4 wk of a high-fat diet induced hyperglycemia without obesity and hyperinsulinemia. Plasma glucose levels were higher in the rats fed the high-fat diet due to decreased glucose uptake in the skeletal muscles and brown adipose tissue as the result of a lower level of plasma insulin. The reduced plasma insulin level in the high-fat diet group may be due to increased sympathetic activity in the pancreas resulting from increased glucose uptake in the brain regions involved in autonomic functions.

REFERENCES

Effects of High-Fat Diet on Tissue Glucose Uptake


