Effect of the Fat/Carbohydrate Ratio in the Diet on Obesity and Oral Glucose Tolerance in C57BL/6J Mice

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Summary To study whether consumed dietary fat has a linear relationship or a threshold with glycemic controls, female C57BL/6J mice were fed different levels of a safflower oil (10, 20, 30, 40, 50, and 60% of total energy) diet ad libitum for 15 wk. Food intake, body weight, parametrial white adipose tissue (WAT) and liver weight were measured, and oral glucose tolerance tests were conducted. Although there was no significant difference in average energy intake, graded increments of safflower oil resulted in graded deterioration of glucose tolerance during 5 and 12-wk feeding, and deterioration of glucose tolerance was more manifested after 12-wk feeding as compared to 5-wk feeding. After 12-wk feeding, a significant deterioration of glucose tolerance was observed in diets of more than 40% fat. Graded increments of body weight and WAT weight were observed, and their weight increases were manifested in diets of more than 30% fat. These data indicated that the amount of dietary fat had an almost linear relationship with glucose tolerance, and significant differences were observed in mice fed diets more of than 40% fat.

Key Words high-fat diet, obesity, diabetes, oral glucose tolerance test, C57BL/6J mice

The rapidly increasing prevalence of obesity in many affluent and rapidly developing countries has suggested that lifestyle changes must have created conditions in which many susceptible individuals can no longer regulate energy balance at an ideal body weight. High-fat diet and low physical activity are now considered as key factors to promote obesity (1). Cross-sectional epidemiological studies have shown a positive correlation between people's body mass index (BMI) and percentage of energy intake from fat (2, 3). Recent large-scale prospective studies support the idea that obesity is a predominant cause of non-insulin dependent diabetes mellitus (NIDDM) (4, 5). About 74% of female and 64% of male NIDDM

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could have been prevented if they had a BMI less than 24 (4,5). However, epidemiologically, it has not been proven that a high-fat diet leads to NIDDM. This may be due to difficulties in assessing dietary intake in population-based studies, differences of individual susceptibility to high-fat diet, and different effects of dietary fat subtypes. Additionally it is not clear whether a high-fat diet has a threshold or a linear relationship with glucose tolerance. This question can only be answered by long-term human intervention studies, but such studies are difficult to perform because of ethical and economical reasons.

In animal studies, it is well established that the replacement of usual, laboratory, high-carbohydrate diets with high-fat diets induces marked obesity (6–8). Furthermore, a positive dose-response relationship has been observed between dietary fat content and body weight in the mouse (9, 10) and between dietary fat and adiposity in rat (11), but it has not yet been demonstrated whether there is a dose-dependent relationship between fat intake and glucose tolerance. Among several mouse lines, the C57BL/6J mouse carries a genetic predisposition to develop intra-abdominal obesity and NIDDM by high-fat diets (12). To understand how relative carbohydrate and fat contents of a diet influence the development of NIDDM in this mouse strain, we conducted an oral glucose tolerance test where mice had free access to several diets in which fat content was varied at the expense of carbohydrate, while other nutritional components remained relatively constant.

METHODS

Animals. Female C57BL/6J mice were obtained from Tokyo Laboratory Animals Science Co. (Tokyo, Japan) at 7 wk of age and fed laboratory chow (CE2, CLEA, Tokyo, Japan) for 1 wk to stabilize the metabolic conditions. We used female mice in this experiment because female mice are known to fight less with each other in a cage than male mice. The mice were maintained at a constant temperature of 22°C with fixed artificial light cycle (12-h light and 12-h dark). They were allowed free access to experimental diets and water.

Diet. The dietary compositions in our experiment are shown in Table 1. The fatty acid composition of safflower oil used in this study has been described previously (13). The ingredients for the purified diets were mixed, formed into a dough with the addition of water, rolled into pellets, wrapped with Saran Wrap, and stored at –20°C until use to minimize fatty acid oxidation. Preliminary feeding trials were conducted and the composition of each diet was adjusted so that the daily intake of calories and the amount of dietary components except fat and carbohydrate were nearly identical. Fresh food was provided to the mice biweekly. Casein, sucrose, starch, vitamin mixture, mineral mixture, and cellulose powder were purchased from Oriental Yeast Co. (Tokyo, Japan); DL-methionine from Wako Co. (Osaka, Japan); and safflower oil from Benibana Food Co. (Tokyo, Japan).

Experimental procedures. Mice were divided into six groups ($n=5$ –6 in each group) with each provided one of a series of synthetic diets containing variable

Table 1. Composition of the experimental diets.

Experimental diet	10% fat	20% fat	30% fat	40% fat	50% fat	60% fat
Safflower oil (%)	4.0	8.0	12.5	18.0	24.5	32.0
Casein (%)	23.7	25.1	26.8	28.7	31.1	33.7
Sucrose (%)	10.0	10.6	11.3	12.1	13.1	14.2
α -Starch (%)	50.0	43.2	35.5	26.3	15.2	2.6
Vitamin mix (%)	1.0	1.1	1.1	1.2	1.3	1.4
Mineral mix (%)	7.0	7.4	7.9	8.5	9.2	9.9
Cellulose powder (%)	4.0	4.2	4.5	4.8	5.2	5.7
DL-Methionine (%)	0.3	0.4	0.4	0.4	0.4	0.5
Fat energy ^a (%)	10.7	20.2	29.7	39.9	50.2	60.4
Carbohydrate energy ^b	62.8	53.3	43.7	33.6	23.2	13.1

^a Percent of fat energy from total energy intake.

^b Percent of carbohydrate energy from total energy intake.

portions of carbohydrates and fat (Table 1). The mice were fed for 15 wk. Energy intake, body weight, and plasma blood insulin were measured during the study. Also oral glucose tolerance tests were conducted after 5 and 12 wk of feeding. After sacrificing the mice, parametrial white adipose tissue (WAT) and liver weight were measured.

Measurement of energy intake. After 9 and 11 wk of feeding, mice which had been housed in shoe box plastic cages with paper chips (alpha dri, Shepherd Specialty Papers Inc., Kalamazoo, MI, USA) were transferred to shoe box cages with wire bottoms. Beneath the wire, newspapers were spread out to collect food spillage. After removing feces on the paper, food spillage on the paper was collected and dried in an oven to evaporate liquid which originated from urine. To accommodate mice to cages with wire bottoms, food intake measurements were started 2 d after transferring the mice to new cages. Food intake measurement was made 4 consecutive days per week, and then mice were returned to the shoe cages with paper chips. The mean food intake per day was estimated by subtracting the food spillage weight from the initial food weight (dry form) in the cage, and this food consumption amount was divided by the number of mice housed in the cage. Thus, the standard error of energy intake was from the variation of daily intake, but not from that of the individual mouse.

Oral glucose tolerance test (OGTT). Five and 12 wk after feeding experimental diets, D-glucose (1 mg/g body weight, 10% (wt/vol) glucose solution) was administered by stomach tube after overnight fasting. Blood samples were obtained by cutting the tail tip before and 30, 60, and 120 min after glucose administration. Blood glucose concentrations were measured, using a TIDEX glucose analyzer (Sankyo, Tokyo, Japan).

To examine the effects of different amounts of glucose loaded to individual mice on glucose tolerance, after 12 wk of feeding, 10% fat-fed mice were given

D-glucose at different concentrations of glucose solutions, 1 and 1.6 mg/g body weight, respectively, and subsequent glucose concentrations were measured.

Insulin tolerance tests. Under the feeding conditions, human insulin (Humulin R, Eli Lilly Japan K.K., Kobe, Japan) was injected intraperitoneally (0.75 mU/g body weight). Blood glucose was measured in samples obtained from the tail tip before and 15, 30, 60, 90, and 120 min after insulin injection.

Statistical analysis. Statistical comparisons of the groups were made by one-way analysis of variance (ANOVA) and each group was compared with the others by Fisher's PLSD test (Statview 4.0 Abacus Concepts, Inc., Berkeley, CA, USA). Comparisons of data from two experimental groups were made by unpaired Student's *t*-test. The glucose and insulin tolerance curve of two groups was compared by repeated-measure analysis (Super ANOVA, Abacus Concepts, Inc., Berkeley, CA, USA). Statistical significance is defined as $p < 0.05$. Values are mean $\pm$ SE.

RESULTS

During the 15-wk feeding period, mice were allowed free access to food. However, since the food intake of high-fat fed mice was lower than that of high-carbohydrate fed mice, the average energy intake of mice fed each diet was not significantly different (Table 2). Graded increments in the fat-to-carbohydrate ratio of the diets induced a graded increase in body weight (Table 2). Body weight increase was larger for 20 to 30% fat diets and 50 to 60% fat diets. Parallel to body weight increase, the wet weight of parametrial white adipose tissue (WAT) also increased in proportion to the fat content in the diet, but liver weight did not differ significantly (Table 2).

After 5 and 12 wk of feeding, graded increments in the fat/carbohydrate ratio of the diets induced a gradual blood glucose increase at 30 and 60 min after oral glucose challenge (Fig. 1). Deterioration of glucose tolerance was more manifested after 12 wk of feeding than 5 wk of feeding. After 12 wk of feeding, a marked increase of fasting glucose concentration was observed for the 60% fat-diet. When the sum of glucose concentrations of each time point was calculated, gradual blood glucose increases by the graded increase of fat content were manifested (Fig. 2). Significant increases of the total glucose were observed in diets of more than 40% fat after 12 wk of feeding.

Since the amount of glucose given orally to mice was on a per body weight basis and not on a per free-fat mass, it is conceivable that glucose intolerance induced by the high-fat diets was due only to the increased amount of glucose load to obese mice. To examine this possibility, an increased amount of glucose was given orally to 10% fat-fed mice at the end of the 12 wk feeding period and oral glucose tolerance tests were made. No significant difference in glycemic control was observed between the mice given glucose at the concentrations of 1.6 and 1.0 mg/g body weight by repeated-measure analysis (Fig. 3). However, the glucose concentration at 30 min after glucose load was slightly higher in the 1.6 mg/g dose than in the

Table 2. Food intake, final body weight, body weight gain, WAT weight, and liver weight in mice fed different amounts of fat.

Experimental diet	10% fat	20% fat	30% fat	40% fat	50% fat	60% fat
Food intake (kJ/mouse/d)	39.0 ± 1.9	36.2 ± 2.0	31.7 ± 2.8	42.3 ± 2.8	32.9 ± 3.5	33.3 ± 3.4
Final body weight (g)	26.4 ± 1.5	29.6 ± 1.9	35.9 ± 1.2***	36.5 ± 1.6***	37.5 ± 1.3***	43.1 ± 1.3***
Body weight gain (g)	8.8 ± 1.7	12.2 ± 1.8	18.4 ± 1.0***	18.9 ± 1.5***	19.9 ± 1.4***	25.5 ± 1.5***
WAT weight (g)	0.8 ± 0.2	1.1 ± 0.2	2.1 ± 0.2***	2.2 ± 0.3***	2.4 ± 0.1***	3.0 ± 0.1***
Liver weight (g)	1.2 ± 0.17	1.1 ± 0.06	1.3 ± 0.06	1.3 ± 0.03	1.3 ± 0.09	1.4 ± 0.05

Mice were sacrificed after 15 wk of feeding, and body weight and liver and wet white adipose tissue (WAT) weight were measured. Energy intake was measured for 8 d. Results are mean ± SE of individual mean values obtained in each of 5–6 mice. Different doses of high-fat diet are compared with 10% fat diet by Fisher's PLSD test, *** $p < 0.001$.

Fig. 1. Oral glucose tolerance test in mice fed with different concentrations of dietary fat. Mice fed with different fat to carbohydrate ratios in Table 1 for 5 and 12 wk were fasted overnight and then given D-glucose (1 mg/g body weight) orally by stomach tube. Blood glucose levels were determined at the times indicated. Each data point represents mean $\pm$ SE of 5–6 mice.

Fig. 2. Total blood glucose concentrations before and during oral glucose tolerance testing. Total blood glucose concentrations before and 30, 60, and 120 min after glucose administration in Fig. 1 was calculated and presented. Statistical differences are shown as * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ compared with a 10% fat diet by ANOVA and Fisher's PLSD test.

1.0 mg/g dose, but it did not reach a significant level ($p = 0.058$). The 1.6-fold amount of glucose was the same absolute dose which was given to obese mice fed the 60% fat diet after 12 wk of feeding.

The insulin effect was evaluated in vivo by insulin tolerance tests in 10 and 60% fat-fed mice after 9 wk of feeding. The initial fall in blood glucose level induced by insulin (0.75 mU/g body weight) was similar in the two groups, but it was not

Fig. 3. Effects of amount of glucose loaded orally on the glucose tolerance test for 10% fat-fed mice. Mice at 7 wk of age were fed 10% fat for 12 wk and given D-glucose at different concentrations of glucose solutions, 1 and 1.6 mg/g body weight, respectively. No significant difference in the glucose tolerance curve was observed between the two groups by repeated-measure analysis.

Fig. 4. Insulin tolerance testing of mice fed 10% and 60% fat for 9 wk. Under the feeding conditions, human insulin was injected intraperitoneally (0.75 mU/g body weight) in mice fed 10% and 60% fat for 9 wk. Blood glucose levels were determined at the times indicated. Each data point represents mean $\pm$ SE of 5 mice. Significant difference in the insulin tolerance curve was observed between the two groups by repeated-measure analysis ($p < 0.01$). Statistical differences of each point are shown as * $p < 0.05$ and ** $p < 0.01$ by Student's *t*-test.

sustained for a longer period in the 60% fat-fed mice as compared to the 10% fat-fed mice (Fig. 4). The effect of insulin was 25% lower in the 60% fat-fed mice than that in the 10% fat-fed mice as assessed by the area under the curve. Thus, 60% fat-fed mice showed greater insulin resistance than 10% fat-fed mice.

DISCUSSION

In this study, graded increments in the fat/carbohydrate ratio of the diets induced not only obesity but also gradual increases in blood glucose concentration after oral glucose challenge, and deterioration of glucose tolerance was manifested by a longer feeding period and higher fat intake. Significant increases in body weight were observed in diets of 30% fat or more, while significant increases in the total glucose following an oral glucose challenge were observed in diets of 40% fat or more after 12 wk of feeding. The parallel changes in deterioration of obesity and glycemic control by fat diets suggest that it is difficult to separate the effects of obesity from those of high-fat diet on glucose tolerance.

In a human study of US citizens, there was a graded increase of prevalence of impaired glucose tolerance (IGT) and NIDDM parallel with body weight increase from 25 y of age (14). However, there is a threshold of body weight gain to increase IGT and NIDDM. When the rate of IGT is plotted according to weight gain expressed as increase in percent desirable weight (PDW) between age 25 y and age at maximum weight, the IGT rate begins to rise after a gain of 20 PDW units, whereas NIDDM has a delay with a rise in the rate of NIDDM not occurring until a weight gain of 40 PDW units above that experienced at age 25 y (14). These data indicate, if we assume that obesity is related to high-fat diet, there may be a threshold of fat intake to induce glucose intolerance. This observation is consistent with the relationship between the different concentrations of high-fat diet and glycemic control in the present mouse study. In terms of relationship between high-fat diet and glycemic controls, Swinburn et al observed that, compared to high-carbohydrate diet (15% of fat from total energy), a high-fat diet (50% of fat from total energy) for 14 d resulted in the deterioration of oral glucose tolerance in obese subjects (15). This finding suggests that a high-fat diet might have an independent effect on glucose tolerance. This may explain the slight difference of high-fat diet effect on obesity and glucose tolerance observed in this mouse study.

In our mouse experiment, although the total energy intake of each group became nearly identical, the high-fat diet resulted in obesity and diabetes. In this type of experiment, we should pay attention to high-fat diet-induced malnutrition due to a shortage of other nutrients. Carbohydrate contents became lower in the high-fat diets. In human studies, Himsworth showed that a low carbohydrate diet (~8% of total energy) is diabetogenic (16). The carbohydrate content in the high-fat diets of this mouse study was 14%, being about two-fold higher than the minimal value of humans. Our previous study indicated that a high fish oil diet, which also contained 14% carbohydrate, showed relatively good glycemic control without body weight increase (13), suggesting that a carbohydrate content of 14% may not be related to obesity and diabetes.

In humans, blood glucose concentrations under fasting and 2-h oral glucose tolerance testing are critical markers of diabetes and are currently used for diagnosis of diabetes (17, 18). The administration of 75 g of glucose was done to all subjects

regardless of body weight, and the criteria for categorizing glucose intolerance was the same for all individuals (19). The fact that these procedures work well, with no evidence that larger people having more skeletal muscles have better glucose tolerance than smaller people, indicate that muscle mass does not usually play a role (20). There is no standard method for oral glucose tolerance testing in rodents. In rodents, when oral glucose tolerance tests are made, the amount of glucose given is on the basis of body weight. As indicated in Fig. 3, normal mice as well as humans have a good capacity to maintain glucose levels in a relatively wide range of loaded glucose.

Oral glucose tolerance tests are affected by many factors, such as absorption rate in the intestine, insulin secretion, glucose uptake in muscle tissues, and glucose production from the liver (21). Since fat feeding caused widespread *in vivo* insulin resistance and obesity in rats, insulin resistance in the liver and skeletal muscle may contribute to high-fat diet-induced hyperglycemia (22–24). Indeed, in the NIDDM model of the mouse, insulin tolerance testing clearly demonstrated that 60% fat-fed mice showed greater insulin resistance than 10% fat-fed mice (Fig. 4). Unfortunately, due to a shortage of blood volume and large variations in the insulin level from individual mice, we were not able to measure insulin concentrations during the oral glucose tolerance test. We speculate that high-safflower oil-fed mice did not increase insulin concentration sufficiently to compensate their insulin resistance. Previous studies on the effects of different dietary oils on insulin secretion indicated that mice fed dietary high-coconut oil and high-palm oil which contained a higher amount of saturated fatty acids showed hyperinsulinemia (13,25), but mice fed dietary polyunsaturated and monounsaturated fatty acids-rich oils did not show hyperinsulinemia under both feeding and fasting conditions irrespective of their hyperglycemia (26). Recent data of insulin secretion in the perfused rat pancreas also indicated that more saturated animal fat is far more potent in enhancing glucose-stimulated insulin secretion (27). In applying this hypothesis to humans, the difference in NIDDM types between Americans and Japanese may be due to the difference in the amount of intake of dietary oil types. Thus, Americans who eat more saturated fat show basal hyperinsulinemia, while Japanese who eat less saturated fat do not. To prove this hypothesis, human intervention studies are necessary but they may be difficult to perform because of ethical reasons.

The present data provide baseline data for a range of possible further studies using C57BL/6J mice and a rationale for using extremely high-fat diets to investigate fat diet-induced obesity and diabetes. These data also indicate that there is an optimal level of dietary fat to prevent obesity and diabetes in mice. This also suggests that it may apply to humans. To estimate the ideal range of the fat/carbohydrate ratio in the diet for humans, development of accurate methods to estimate the mean intake of dietary fat and carbohydrate is necessary for human longitudinal studies.

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