High-Fat Diet–Induced Hyperglycemia and Obesity in Mice: Differential Effects of Dietary Oils

Shinji Ikemoto, Mayumi Takahashi, Nobuyo Tsunoda, Kayo Maruyama, Hiroshige Itakura, and Osamu Ezaki

Mice fed a high-fat diet develop hyperglycemia and obesity. Using non–insulin-dependent diabetes mellitus (NIDDM) model mice, we investigated the effects of seven different dietary oils on glucose metabolism: palm oil, which contains mainly 45% palmitic acid (16:0) and 40% oleic acid (18:1); lard oil, 24% palmitic and 44% oleic acid; rapeseed oil, 59% oleic and 20% linoleic acid (18:2); soybean oil, 24% oleic and 54% linoleic acid; safflower oil, 76% linoleic acid; perilla oil, 58% α-linolenic acid; and tuna fish oil, 7% eicosapentaenoic acid and 23% docosahexaenoic acid. C57BL/6J mice received each as a high-fat diet (60% of total calories) for 19 weeks (n = 6 to 11 per group). After 19 weeks of feeding, body weight induced by the diets was in the following order: soybean > palm ≥ lard ≥ rapeseed ≥ safflower ≥ perilla > fish oil. Glucose levels 30 minutes after a glucose load were highest for safflower oil (≅21.5 mmol/L), modest for rapeseed oil, soybean oil, and lard (≅17.6 mmol/L), mild for perilla, fish, and palm oil (≅13.8 mmol/L), and minimal for high-carbohydrate meals (≅10.4 mmol/L). Only palm oil–fed mice showed fasting hyperinsulinemia ($P < .001$). By stepwise multiple regression analysis, body weight (or white adipose tissue [WAT] weight) and intake of linoleic acid (or n-3/n-6 ratio) were chosen as independent variables to affect glucose tolerance. By univariate analysis, the linoleic acid intake had a positive correlation with blood glucose level ($r = .83$, $P = .02$) but not with obesity ($r = .46$, $P = .30$). These data indicate that (1) fasting blood insulin levels vary among fat subtypes, and a higher fasting blood insulin level in palm oil–fed mice may explain their better glycemic control irrespective of their marked obesity; (2) a favorable glucose response induced by fish oil feeding may be mediated by a decrease of body weight; and (3) obesity and a higher intake of linoleic acid are independent risk factors for dysregulation of glucose tolerance.

IT IS WIDELY ACCEPTED that non–insulin-dependent diabetes mellitus (NIDDM) is caused by a combination of genetic and environmental factors, notably diet and level of physical activity.[1-3] Among the environmental factors, the high-fat content of the typical Western diet is considered a major cause of obesity-associated insulin resistance.[4-6] Epidemiological[4,5] and animal[6] studies made on several environmental factors suggest that the high fat content of the typical Western diet is a major cause of obesity and insulin resistance. Indeed, various metabolic studies have confirmed the view that calories obtained from fat have a greater effect on obesity than energy per se.[7,8] Among several dietary oils, monounsaturated oil[9,10] and fish oil[11] have been proposed as favorable diets for NIDDM patients. In rats made insulin-resistant with a high-fat diet, the resistance can be prevented by the addition of fish oil,[12] with improvement of insulin resistance and, incorporation of highly unsaturated long-chain n-3 fatty acids into the phospholipid component of muscle tissues.[13] Borkman et al[14] have shown that in patients with coronary artery disease, fasting serum insulin concentration (a marker of insulin resistance) is correlated positively with the percentage of 18:2 (linoleic acid) in the phospholipid fraction of muscle, but negatively with individual long-chain polyunsaturated fatty acids such as 20:4 (arachidonic acid), 22:4 (n-6), 22:5 (n-6), and 22:5 (n-3). However, clinical trials using fish oil supplements failed to show a marked improvement in glucose control.[15-18] These data indicate that there are some discrepancies between the insulin resistance at tissue level and the actual blood glucose level. The importance of fatty acid composition of dietary oils seems evident, but it has been difficult to select favorable oils from human studies because of the difficulty involved in long-term study of dietary oil intake. Indeed, we do not know whether a higher intake of linoleic acid or a lower intake of fish oil (or n-3) is responsible for the development of insulin resistance.

Feeding a high-fat diet in certain strains of mice provides a suitable model of NIDDM[19,20] and atherosclerosis.[21] The present studies were designed to clarify the differences in the effects of long-term feeding of commercially available dietary oils on glucose metabolism and obesity using this NIDDM mouse model. In comparison with high-carbohydrate feeding, the effects of seven dietary oils were investigated: palm oil, which contains mainly 45% palmitic acid (16:0) and 40% oleic acid (18:1); lard oil, 24% palmitic and 44% oleic acid; rapeseed oil, 59% oleic and 20% linoleic acid (18:2); soybean oil, 24% oleic and 54% linoleic acid; safflower oil, 76% linoleic acid; perilla oil, 58% α-linolenic acid; and tuna fish oil, 7% eicosapentaenoic acid and 23% docosahexaenoic acid.

MATERIALS AND METHODS

Animals

C57BL/6J female mice were obtained from Tokyo Laboratory Animals Science (Tokyo, Japan) at 7 weeks of age and fed a high-carbohydrate diet (Table 1) for 1 week to accommodate to a new environment. The mice were maintained at a constant temperature of 22°C with a fixed artificial light cycle (12 hours light and 12

From the Division of Clinical Nutrition, National Institute of Health and Nutrition, Tokyo, Japan.

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Address reprint requests to Osamu Ezaki, MD, Division of Clinical Nutrition, National Institute of Health and Nutrition, 1-23-1, Toyama, Shinjuku-ku, Tokyo 162, Japan.

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Table 1. Composition of the High-Carbohydrate Diet and High-Fat Diets

Component	High-Carbohydrate (%)	High-Fat (%)
Oil	4.0	32.0
Casein	23.7	33.1
Sucrose	10.0	17.6
α-Starch	50.0	—
Vitamin mix	1.0	1.4
Mineral mix	7.0	9.8
Cellulose powder	4.0	5.6
DL-Methionine	0.4	0.5
Energy (kcal/100 g)	343.4	489.8
Fat energy		
kcal/100 g	36.8	294.7
%	10.7	60.2

hours dark). The mice were allowed free access to either a high-carbohydrate diet or various high-fat diets.

Diet

The composition of the high-carbohydrate diet and high-fat diets is shown in Table 1. Fatty acid compositions of dietary oils were measured by gas-liquid chromatography and are shown in Table 2. In the high-carbohydrate diet, safflower oil was used as source of fat. Every 2 weeks, ingredients for the purified diets were mixed, formed into a dough with water, rolled into pellets, wrapped with plastic wrap, and stored at −20°C until use to minimize oxidation. These small pellets were given to mice every day. Preliminary feeding trials were conducted, and the composition of the diets was adjusted so that the daily intake of calories and the amount of dietary components except fat and carbohydrate were nearly identical. Casein, sucrose, starch, vitamin mixture, mineral mixture, and cellulose powder were purchased from Oriental Yeast (Tokyo, Japan); palm oil and soybean oil from Fuji Oil (Osaka, Japan); lard oil from Snow Brand Milk Products (Tokyo, Japan); rapeseed oil from Yonezawa Oil (Saitama, Japan); safflower oil from Benibana Food (Tokyo, Japan); perilla oil from Ohta Oil (Aichi, Japan); fish oil from NOF (Tokyo, Japan); and *DL*-methionine from Sigma (St Louis, MO).

Experimental Procedures

Mice were divided into eight groups. The first group was given the high-carbohydrate diet, which on a caloric basis consisted of 63% carbohydrate, 11% fat, and 26% protein. The other seven groups were given various high-fat diets containing 14% carbohydrate, 60% (of several types of) fat, and 26% protein (Table 1). Mice fed each diet for 16 weeks were fasted overnight, and then blood samples were obtained by snipping the tail for insulin, triglyceride, and cholesterol assays. Mice were killed at 19 weeks of feeding, and body weight, body weight gain, and wet white adipose tissue (WAT) weight were measured. Oral glucose tolerance tests were also conducted at 3 and 18 weeks of feeding. After killing the mice with an intraperitoneal injection of pentobarbital (Abbot, North Chicago, IL) 0.05 mg/g body weight, parametrial white adipose tissue and gastrocnemius were excised for measurement of weight and GLUT4 protein level, respectively.

Food Intake Measurements

The number of mice per cage was six to eight. For high-carbohydrate or high–safflower oil feeding, two cages were used; for other oil feeding, one was used. For food intake measurements at 14 weeks of feeding, mice that had been kept in plastic shoe box-type cages with paper chips (Alpha Dri; Shepherd Specialty Papers, Kalamazoo, MI) 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 water originating from urine. To accomodate mice to cages with wire bottoms, food intake measurements were started 2 days after transferring them to new cages. Food intake measurement was made every day for 5 days, and then mice were returned to the cages with paper chips. The mean food intake per day was estimated by subtracting the weight of food spillage from the initial food weight (dry form) in the cage and dividing by the number of mice housed in the cage. Thus, the standard error for food intake shown in Table 3 was from the variation of daily intake, but not from that of the individual mouse.

Oral Glucose Tolerance Test

Eighteen weeks after feeding the experimental diets, D-glucose (1 mg/g body weight) was administered after an overnight fast, by stomach tube. Blood samples were obtained by snipping the tail

Table 2. Fatty Acid Composition (%) of the Dietary Oils

Fatty Acid	Palm	Lard	Rapeseed	Soybean	Safflower	Perilla	Fish
12:0	0.4						
14:0	1.1	1.7					3.0
16:0	44.5	24.0	3.8	9.9	6.8	6.1	18.2
16:1	0.2	2.6	0.2				4.2
18:0	4.2	14.4	1.7	4.1	2.5	1.7	4.9
18:1	39.5	43.9	59.4	23.6	13.8	18.4	18.8
18:2 (n-6)	9.2	9.1	20.2	53.7	75.7	14.3	1.3
18:3 (n-3)	0.2	0.7	7.1	7.1	0.2	58.3	0.8
20:4 (n-6)		0.1					2.0
20:5 (n-3)							6.8
22:6 (n-3)							22.8
Others	0.7	3.5	7.6	1.6	1.0	1.2	17.2
S:M:P ratio	10:8:2	10:12:2	10:95:41	10:16:42	10:14:75	10:24:92	10:10:14
n-6/n-3 ratio	46.1	13.1	2.9	7.6	378.5	0.3	0.1

Abbreviation: S:M:P, saturated, monounsaturated, and polyunsaturated fatty acid.

Table 3. Food Intake, Final Body Weight, Body Weight Gain, Parametrial WAT Weight, and Fasting Insulin, Triglyceride, and Cholesterol Levels

	High-Carbohydrate (n = 11)	High-Fat							
		Palm (n = 6)	Lard (n = 8)	Rapeseed (n = 6)	Soybean (n = 6)	Safflower (n = 9)	Perilla (n = 5-6)	Fish (n = 7)	ANOVA
Food intake (kcal/mouse/d)	7.3 ± 0.5	7.5 ± 0.5	9.1 ± 0.2	7.1 ± 0.3	8.1 ± 0.3	8.6 ± 0.8	6.4 ± 0.4	9.8 ± 0.4†	$F(7,44) = 2.9$ $P = .013$
Final body weight (g)	26.8 ± 0.9	40.2 ± 2.2‡	40.2 ± 2.4‡	38.8 ± 1.4‡	45.5 ± 2.7‡	38.2 ± 2.1‡	33.4 ± 2.8*	25.9 ± 1.7	$F(7,50) = 12.8$ $P < .0001$
Body weight gain (g)	9.5 ± 0.8	23.0 ± 1.9‡	23.1 ± 2.3‡	21.6 ± 1.5‡	28.3 ± 2.9‡	20.9 ± 2.0‡	16.3 ± 2.5*	9.0 ± 1.5	$F(7,50) = 13.9$ $P < .0001$
WAT weight (g)	0.84 ± 0.10	2.47 ± 0.20‡	2.73 ± 0.26‡	3.03 ± 0.25‡	4.14 ± 0.43‡	2.52 ± 0.21‡	1.95 ± 0.40†	0.91 ± 0.22	$F(7,50) = 20.4$ $P < .0001$
Insulin (pmol/L)	84.8 ± 17.9	245.0 ± 51.5‡	93.5 ± 10.3	112.9 ± 27.5	87.2 ± 17.6	107.1 ± 16.5	112.9 ± 22.5	43.1 ± 4.0	$F(7,51) = 2.7$ $P = .020$
Triglyceride (mg/dL)	71.0 ± 6.8	67.6 ± 11.5	51.9 ± 3.3*	40.5 ± 7.2‡	42.1 ± 2.2†	35.1 ± 3.7‡	41.6 ± 4.9‡	35.9 ± 4.1‡	$F(7,51) = 6.0$ $P < .0001$
Total cholesterol (mg/dL)	77.5 ± 3.3	96.8 ± 4.1‡	92.8 ± 2.2‡	68.6 ± 2.5*	74.0 ± 3.5	75.0 ± 3.1	70.4 ± 3.5	73.2 ± 2.7	$F(7,51) = 9.6$ $P < .0001$

NOTE. Results are the mean ± SE of individual mean values obtained in each of 5 to 11 mice. Food intakes were measured for 5 days and are expressed as the mean ± SE intake per day.

*$P < .05$, †$P < .01$, ‡$P < .001$: high-carbohydrate *v* other groups by Fisher's protected least-significant difference test.

before and at 30, 60, and 120 minutes after glucose administration. Blood glucose levels were measured using a TIDEX glucose analyzer (Sankyo, Tokyo, Japan).

Immunoblotting

Crude membrane fractions from skeletal muscle (gastrocnemius) were prepared as described previously.[22] Proteins separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis were electrophoretically transferred to Immobilon (Millipore, Bedford, MA) and immunoblotted with antibodies directed against the C-terminal amino acid sequence of GLUT4 and then ^{125}I-labeled protein A (ICN, Costa Mesa, CA) as described previously.[22] The amount of protein used per gel lane was 60 μg. The amount of GLUT4 was quantified with an image analyzer (BAS 2000; Fuji Film, Tokyo, Japan).

Other Analyses and Methods

Immunoreactive insulin level was measured by radioimmunoassay (RIA) using a rat insulin RIA kit (Incstar, Stillwater, MN). Triglyceride and total cholesterol levels were measured by enzyme assays, determiner LTG and TC555, respectively (Kyowa Medics, Tokyo, Japan). Protein was assayed using the Micro BCA Protein Assay Reagent Kit (Pierce, Rockford, IL).

Statistical Analysis

Statistical comparisons of the groups were made by ANOVA, and each group was compared with the others by Fisher's protected least-significant difference test (Statview 4.0, Abacus Concepts, Berkeley, CA). The glucose tolerance curve of each group was compared by repeated-measures ANOVA (Super ANOVA; Abacus Concepts). Relations between variables were analyzed by a simple correlation and a stepwise multiple regression model (Statview 4.0; Abacus Concepts). Statistical significance is defined as P less than .05; values are the mean ± SE.

RESULTS

After 19 weeks' feeding, body weight obtained with the diets was in the following order: soybean oil > palm oil ≥ lard oil ≥ rapeseed oil ≥ safflower oil > perilla oil > high-carbohydrate diet ≥ fish oil (Table 3). Mice fed a high–soybean oil diet showed a 70% increase in body weight compared with mice fed a high-carbohydrate diet. Parallel to the body weight change, the wet weight of parametrial WAT was in order of soybean oil > rapeseed oil ≥ lard oil ≥ safflower oil ≥ palm oil > perilla oil > fish oil ≥ high-carbohydrate diet (Table 3). Indeed, the correlation between body weight and WAT weight was high ($r = .95$, $P < .0001$, n = 58). It should be noted that mice were allowed free access to food. Since the intake of high-fat diet mice was less than that of high-carbohydrate diet mice, the total calorie intake of both groups became nearly identical. However, the energy intake for fish oil was significantly higher than for carbohydrate or palm, rapeseed, and perilla oils, but the level for perilla oil was less than for lard or safflower and fish oils (Table 3). Other values were not significant.

After 3 weeks' feeding, all high-fat diets resulted in significant increase of blood glucose levels 30, 60, and 120 minutes after an oral glucose challenge compared with the high-carbohydrate diet (Fig 1). However, after 18 weeks of feeding, each dietary oil showed a different response to the glucose challenge. Glucose levels 30 minutes after the glucose load were highest for safflower oil (≅21.5 mmol/L), modest for rapeseed oil, soybean oil, and lard (≅17.6 mmol/L), mild for perilla oil, fish oil, and palm oil (≅13.8 mmol/L), and minimal for a high-carbohydrate diet (≅10.4 mmol/L). Significance was established by repeated-measures ANOVA ($P < .001$, carbohydrate *v* lard oil, rapeseed oil, and safflower oil; $P < .01$, carbohydrate *v* soybean oil; $P < .01$, safflower oil *v* fish oil and palm oil; $P < .05$, carbohydrate *v* perilla oil and fish oil; and $P < .05$, safflower oil *v* lard and perilla oil). In these oral glucose tolerance tests, obese mice received a higher amount of glucose than lean mice, since the amount of glucose given orally was determined on a body-weight basis. However, the contribution of different amounts of glucose to the glucose tolerance curve in each group of mice was minimal, since in the

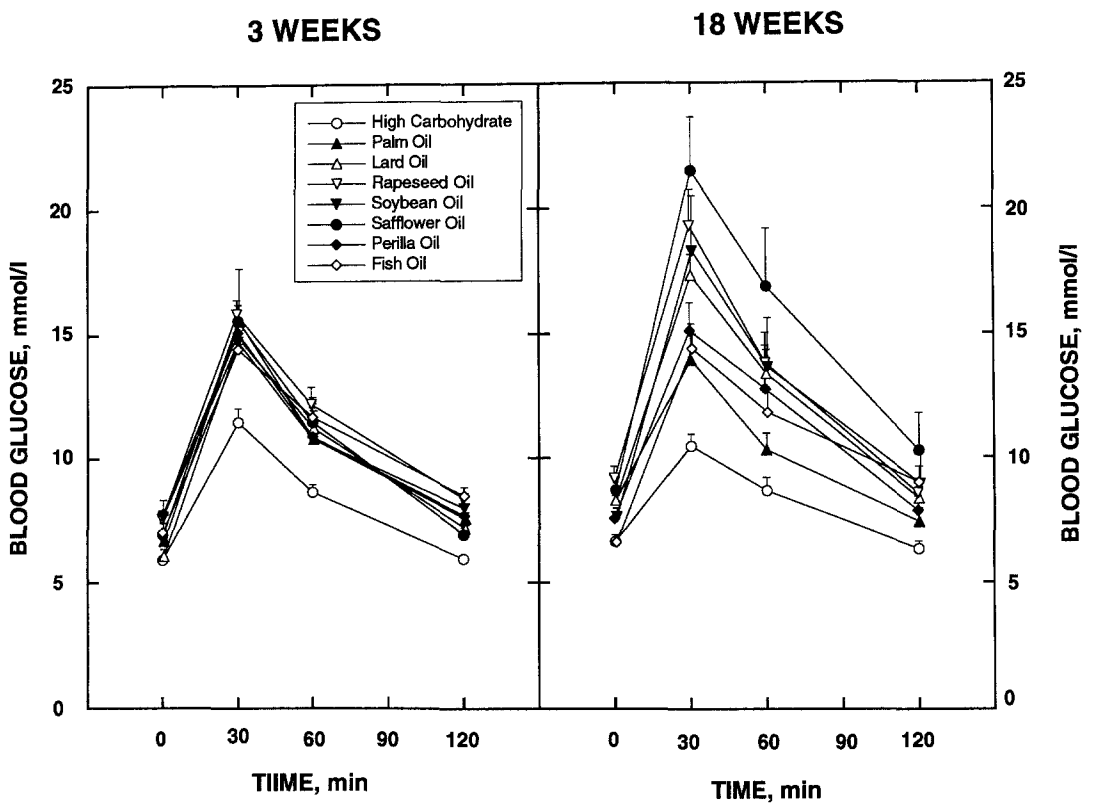

Fig 1. Oral glucose tolerance tests. Each data point represents the mean of 3 to 7 mice at 3 weeks and 4 to 10 mice at 18 weeks. At 3 weeks of feeding: $P < .001$, carbohydrate *v* palm oil, lard oil, rapeseed oil, soybean oil, safflower oil, perilla oil, and fish oil; $P < .05$, lard oil *v* rapeseed oil. At 18 weeks of feeding, $P < .001$, carbohydrate *v* lard oil, rapeseed oil, and safflower oil; $P < .01$, carbohydrate *v* soybean oil, $P < .01$, safflower oil *v* fish oil and palm oil; $P < .05$, carbohydrate *v* perilla oil and fish oil; $P < .05$, safflower oil *v* lard oil and perilla oil.

high-carbohydrate diet 20%, 40%, 60%, and 80% increases of the glucose load resulted in only 12%, −8%, 12%, and 23% increases of blood glucose levels 30 minutes after an oral glucose challenge, which was not significant (n = 3).

In comparison to the high-carbohydrate diet (85 pmol/L), fasting blood insulin significantly increased by threefold with the palm oil diet (245 pmol/L, $P < .001$), but the other values were not significant (Table 3). Fasting triglyceride and cholesterol levels changed reciprocally (Table 3). Thus, in comparison to the high-carbohydrate diet (71 mg/dL), triglyceride levels decreased with lard (52 mg/dL, $P < .05$) and rapeseed oil (41 mg/dL, $P < .001$), soybean oil (42 mg/dL, $P < .01$), safflower oil (35 mg/dL, $P < .001$), perilla oil (42 mg/dL, $P < .001$), and fish oil (36 mg/dL, $P < .001$) diets, whereas total cholesterol levels increased with palm oil (97 mg/dL, $P < .001$) and lard (93 mg/dL, $P < .001$) diets compared with the high-carbohydrate diet (78 mg/dL). These findings are in good agreement with previous reports that most of the dietary vegetable oils were hypotriglyceridemic and dietary animal oils containing saturated fatty acids were hypercholesterolemic.[23,24] It has also been reported that in comparison to a high-fat diet, a high-carbohydrate diet causes hypertriglyceridemia by elevation of very–low-density lipoprotein cholesterol levels.[25]

Because of interactions between the final body weight and fatty acid composition, stepwise multiple regression analysis was used to determine the independent predictors of glycemic control. The sum of glucose levels after glucose challenge (Σglucose) was used as a marker of glycemic control. The actual intake of fatty acids from each group was calculated from the mean food intakes (Table 3) and used in this analysis. The n-3 fatty acid (18:3, 20:5, and 22:6) intake, n-6 (18:2 and 20:4)/n-3 ratio, polyunsaturated to saturated fatty acid ratio, final body weight, WAT weight, and fasting insulin, triglyceride, and cholesterol levels were also included in this regression model. Table 4 shows the partial correlation coefficients relating these variables to Σglucose. (A higher F value indicates a higher contribution to Σglucose.) When all variables were analyzed together, positive relations were found between Σglucose and WAT weight, Σglucose and final body weight, and Σglucose and intake of linoleic acid. Negative relations were found between Σglucose and intake of palmitic acid, and Σglucose and n-3 fatty acids. When WAT weight was chosen as the first independent variable, the correlation with final body weight and with n-3 fatty acid intake disappeared, but the correlation with linoleic and palmitic acid still remained. Interestingly, the n-6/n-3 ratio appeared as the second largest independent variable. When both WAT and n-6/n-3 ratio were chosen as independent variables, the correlation with intake of linoleic or palmitic acid disappeared completely. Two independent variables, WAT weight and n-6/n-3 ratio, were found to explain 42% of Σglucose variations (data not shown).

Table 4. Stepwise Multiple Regression Analysis

Variable	(A) Not Adjusted		(B) Adjusted for WAT		(C) Adjusted for n-6/n-3 and WAT	
	r	F	*r*	F	*r*	F
16:0 (palmitic)	−.37	5.5	−.33	4.0	−.22	1.6
18:0 (stearic)	−.02	0.01	−.03	0.03	.10	0.35
18:1 (oleic)	−.08	0.20	−.14	0.62	.02	0.01
18:2 (linoleic)	.42	7.3	.32	3.8	−.12	0.43
18:3 (linolenic)	−.06	0.11	−.10	0.33	−.01	0.00
n-3	−.36	5.2	−.06	0.12	.18	1.1
n-6/n-3 ratio	.31	3.7	.40	6.5	NA	NA
P/S ratio	.28	2.9	.27	2.6	.05	0.09
Body weight	.46	9.3	−.16	0.91	−.16	0.86
WAT weight	.55	15	NA	NA	NA	NA
Triglyceride	.08	0.22	−.005	0.001	.10	0.31
Cholesterol	.14	0.64	−.02	0.01	.04	0.04
Insulin	.09	0.27	−.11	0.40	−.12	0.43

NOTE. Partial correlation coefficients and F values* are shown (A) before and (B) after adjustment for the effects of WAT weight and (C) after adjustment for the effect of WAT weight and the n-3/n-6 ratio between Σglucose and many variables such as the major fatty acid and n-3 fatty acid intakes, n-6/n-3 and polyunsaturated to saturated fat ratios, final body weight, WAT weight, and fasting triglyceride, cholesterol, and insulin levels.

Abbreviations: P/S, polyunsaturated to saturated fat ratio; NA, not applicable.

*A larger F value indicates a larger contribution to Σglucose.

The contribution of obesity to Σglucose was further investigated by univariate analysis. When individual mice from all groups were plotted, Σglucose was well correlated with body weight (Fig 2A: $r = .67, P < .0001$, n = 47). Also, Σglucose was well correlated with wet WAT weight (data not shown: $r = .67$, $P < .0001$, n = 47). However, there were large variations in body weight increases among individual mice even within specific oil diet–fed groups (Fig 2A). For example, in lard oil–fed mice, the correlation between Σglucose and body weight of individual mice was high (data not shown: $r = .85, P = .008$, n = 7). To examine the effects of various oils and individual differences on obesity-induced hyperglycemia separately, the mean body weight of each group of mice was plotted against Σglucose. When analyzed as a group, there was no significant correlations between body weight and Σglucose (Fig 2B: $r = .60$, $P = .12$, n = 8).

Since the contribution of n-3 fatty acids is minimal after adjustment of WAT weight (Table 4), n-6 intake (mostly linoleic acid) may be responsible for the favorable effects of the n-6/n-3 ratio. Univariate analysis also supported this conclusion. There was a positive correlation between Σglucose and the actual intake amount of linoleic acid (Fig 3A: $r = .83$, $P = .02$, n = 7). However, there was no significant correlation between the mean final body weight and the intake amount of linoleic acid (Fig 3B: $r = .46$, $P = .30$, n = 7). There was no significant correlation between Σglucose and the intake amount of n-3 fatty acid (data not shown: $r = .48$, $P = .27$, n = 7), but there was a significant negative correlation between the mean body weight and the intake amount of n-3 fatty acid (data not shown: $r = -.79$, $P = .04$, n = 7). These data indicate that the increased intake of linoleic acid (or n-6/n-3) and obesity are independent risk factors that lead to abnormal glucose tolerance, and the favorable effects of n-3 fatty acid may be due to the decrease of body weight gain.

The intake of palmitic acid was inversely correlated with Σglucose by multiple regression analysis. The possibility that a higher intake of palmitic acid is better for glycemic control is highly unlikely, because the intake of saturated fatty acid has been reported to induce insulin resistance in humans.[26] Rather, it is simply explained that because of an inverse correlation between the composition of palmitic and linoleic acids in dietary oil subtypes (Table 2), both variables may be chosen in multiple regression analysis. Also, the high n-3/n-6 ratio in the fish oil diet raises a possibility of linoleic acid deficiency.

To ascertain the basis for the loss of glycemic control caused by feeding high-fat diets, levels of GLUT4 protein in gastrocnemius (skeletal muscle) were assessed by Western blot analysis. Low-level overexpression of GLUT4 in transgenic mice is known to prevent high-fat diet–induced hyperglycemia.[27] In comparison to the high-carbohydrate diet, the high-fat diet subtypes resulted in a slight decrease

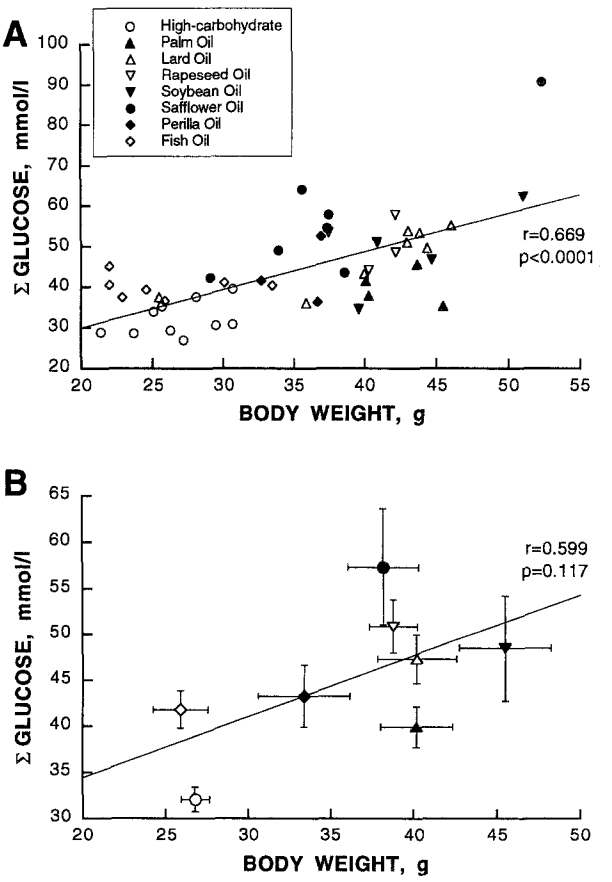

Fig 2. (A) Relationship between the body weight of individual mice and Σglucose at 0, 30, 90, and 120 minutes after oral glucose tolerance tests; $r = .699, P < .0001$, n = 47. (B) Relationship between the mean body weight of each group and Σglucose; $r = .599, P = .117$, n = 8.

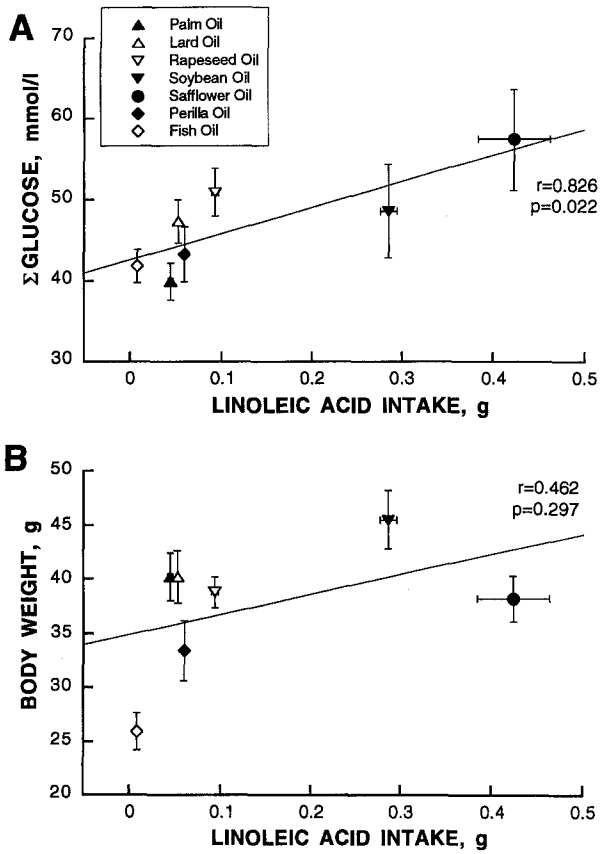

Fig 3. (A) Relationship between intake of linoleic acid and Σglucose at 0, 30, 90, and 120 minutes after oral glucose tolerance tests. The mean linoleic acid intakes (18:2) for each high-fat group were plotted against Σglucose; r = .826, P = .022, n = 7. The actual intake of oleic and linoleic acids was calculated from the amount of daily food intake shown in Table 3. (B) Relationship between linoleic acid intake and final body weight. The mean linoleic acid intakes (18:2) for each high-fat group were plotted against the mean body weight; r = .462, P = .297, n = 7.

of GLUT4 levels, but these were not significant by ANOVA (Fig 4).

DISCUSSION

Most of the high-fat diets in comparison to the high-carbohydrate diet were found to have adverse effects on glucose tolerance and obesity. In human studies, linoleic acid and saturated fat and oleic acid have been linked to the development of insulin resistance[26] and coronary artery disease.[28,29] In this study, both obesity and intake of linoleic acid were found to be independent positive variables for aggravation of glucose tolerance.

Obesity (or WAT weight) was first chosen as an independent variable to determine the glucose tolerance. WAT weight was measured as a marker of visceral obesity. In this strain of mice, Rebuffe-Scrive et al[30] reported that the increase of visceral fat seemed to be a specific characteristic associated with the genetic predisposition for NIDDM. The contribution of visceral fat to abnormal glucose tolerance has been observed in mice and in humans.[31,32]

Using a euglycemic clamp technique, Storlien et al[12] reported that replacement of only 6% of the linoleic acid from safflower oil with n-3 fatty acid from fish oil prevented the development of insulin resistance. In our study, fish and perilla oils that contained a higher amount of n-3 fatty acids showed a less diabetic glucose tolerance curve, but failed to appear as an independent variable. This is because n-3 fatty acid intake has an inverse correlation with obesity (Table 4). It has been reported that in genetically obese *ob/ob* mice, an increase in the n-3 fat content of the diet decreases weight gain despite higher intake.[33] As indicated by Pan and Storlien,[34] the lower weight gain associated with increased tissue levels of n-3 fatty acid might be explained by the "leaky membrane" hypothesis proposed by Else and Hulbert.[35] This hypothesis was further supported by the finding that the increasing amount of membrane n-3 fatty acid resulted in increased proton influx in the rat liver mitochondria, and then resulted in energy consumption.[36] A possible reason for the lack of change in glycemic control with fish oil supplements to humans may be the failure to reduce body weight.[15,17] Indeed, when fish plus safflower oil–fed rats became obese, insulin resistance was manifested in adipocytes.[37]

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 output from the liver.[38] Palm oil–fed mice might have had insulin resistance, but due to an increase of insulin secretion, they showed less diabetic glycemic control. This may lead to a better glycemic control but a body weight increase. It has been reported that high-coconut oil–fed mice show higher fasting plasma insulin levels.[30] These oils from tropical fruits have a higher amount of saturated acids: coconut oil contains mainly 45% to 50% lauric acid (12:0), and palm oil is 45% palmitic acid (16:0). On the other hand, mice fed lard and other vegetable oils did not show hyperinsulinemia, irrespective of insulin resistance. These findings are in good agreement with previous reports that rats fed lard

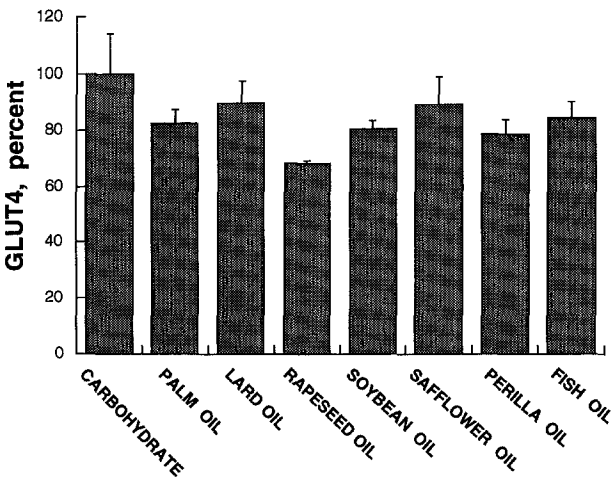

Fig 4. Effect of feeding high-fat oil subtypes on GLUT4 protein in skeletal muscles. Results are the mean ± SE of individual mean values obtained in each of 4 mice; F(7, 24) = 1.6, P = .2.

oil[39] or safflower oil[6,37] showed hypoinsulinemia. The coconut oil- and palm oil–fed mice showed a pathological state similar to that of obese hyperinsulinemic NIDDM patients observed in the United States.[1] The difference in NIDDM types between Americans and Japanese, most of whom are low insulin responders,[40,41] may be due to a difference in intake of dietary oil types.

However, with in vitro studies, fatty acids per se are known to stimulate insulin secretion both in isolated pancreatic islets[42] and in perfused pancreas preparations.[43] Also, Opara et al[44] reported that in isolated perfused murine islets, the effects of fatty acids on insulin release were dependent on the fatty acid subtype, the fatty acid–stimulated insulin secretion was strongest in 5 mmol/L 12:0 but declined with increasing chain length, and insulin secretion was enhanced as the degree of unsaturation of fatty acids increased. The discrepancy for insulin secretion between in vitro studies and our in vivo study may be explained by the fact that with the in vitro studies, the acute effects of lipid or free-fatty acid administration on insulin secretion were examined, whereas in our studies the chronic effects were examined. Indeed, Sako and Grill[45] reported that hyperlipidemia was associated with short-term stimulation but long-term inhibition of glucose-induced insulin secretion.

Although linoleic acid is an independent variable, linoleic acid–induced obesity also contributed to the glucose intolerance. It is clear by stepwise regression analysis that F values for linoleic acid decreased from 7.3 to 3.8 even after consideration of obesity. However, it has not been ruled out that other substances included in these oils but not measured in this study, such as *trans*-fatty acids,[46] have predominant effects on glucose tolerance.

Assuming that higher intakes of most dietary oils result in abnormal glucose tolerance, how are the effects interpreted? Euglycemic clamp studies indicated that rats fed linoleic acid–rich oils showed insulin resistance in skeletal muscles and liver.[6] The mechanisms of high-fat–induced insulin resistance are not clear at present. A decreased GLUT4 intrinsic activity, decreased translocation of GLUT4, or decreased signaling from the insulin receptor to GLUT4-containing vesicles might be involved. However, it has not been ruled out that a small decrease in GLUT4 by high-fat feeding, which was not significant in this study, might be responsible for the changes in glucose uptake observed with the dietary manipulation. Another reason is that most of the vegetable oils are not hyperinsulinemic[6,37,39] (Table 3). Kim et al[47] proposed that decreased of GLUT2 and glucokinase mRNA in pancreatic β cells are responsible for the high-fat–induced decrease of insulin secretion, although they did not describe fat types.

It was found in this study that (1) fasting blood insulin levels varied among fat subtypes: palm oil is hyperinsulinemic, but most of the other vegetable oils are not; (2) a favorable glucose response obtained by fish oil feeding may be mediated by a decrease of body weight; and (3) obesity and a higher intake of linoleic acid are independent risk factors for aggravation of glucose intolerance. However, we cannot rule out that humans might respond differently to dietary fat. Further human studies are necessary to verify this hypothesis.

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