High-Monounsaturated Fat Diet–Induced Obesity and Diabetes in C57BL/6J Mice

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A high–monounsaturated fat diet has been proposed as a palatable alternative to a high-carbohydrate diet in diabetic patients, but it is unknown whether a higher intake of monounsaturated fat induces obesity and diabetes, as usually observed with other types of fat. To answer this question, C57BL/6J mice were divided into three groups: the first group was given a high-carbohydrate diet, and the other two groups were given a high–monounsaturated fat diet (60% of total energy) as olive oil or synthetic triolein for 4 months. It has been previously reported that the C57BL/6J mouse has a genetic predisposition for intraabdominal obesity and non–insulin-dependent diabetes mellitus (NIDDM) by high-polyunsaturated fat (n-6) feeding. Although there were no significant differences in energy intake and fat absorption among these three groups, compared with the high-carbohydrate diet, both high–monounsaturated fat diets produced hyperglycemia, obesity, and triglyceride accumulation in the liver and skeletal muscle. These data indicate that the recently recommended high–monounsaturated fat diet might induce obesity and diabetes.

DIETARY RECOMMENDATIONS for diabetic patients remain controversial. Diets low in saturated fat have been widely recommended to patients with non–insulin-dependent diabetes mellitus (NIDDM).[1] However, debate continues as to whether saturated fat should be replaced by monounsaturated fat or carbohydrate.[2-4] Although there is no proof that the elevated blood triglycerides induced by a high-carbohydrate diet are atherogenic, recent human studies have indicated that a high-carbohydrate/low-fat diet, by stimulating hepatic very–low-density lipoprotein (VLDL)-triglyceride synthesis and secretion,[5] invariably increases fasting and postprandial triglyceride concentrations.[6,7] Compared with a high-carbohydrate diet in NIDDM patients, a high–monounsaturated fat diet decreased plasma triglyceride levels,[8-10] and in rabbits, oleate-rich low-density lipoprotein (LDL) particles were remarkably resistant to oxidative modification, which might lead to atherosclerosis.[11] Thus, a high–monounsaturated fat diet has been proposed as a palatable alternative to a high-carbohydrate diet. However, there is some reservation about using a large amount of high–monounsaturated fatty acid oils in diabetic patients at large because of the possible risk of developing obesity, which may lead to diabetes and atherosclerosis. This question can only be answered by a direct comparison of diets rich in carbohydrates and monounsaturated fatty acids with the primary outcome variables of cardiovascular morbidity and mortality. Such long-term intervention studies have not been undertaken in humans because of economic reasons and practical difficulties.

On the other hand, C57BL/6J mice fed a high-fat diet develop impaired glucose tolerance and intraabdominal obesity, resulting in a condition resembling NIDDM within a relatively short feeding period.[12,13] Using this NIDDM mouse model, we have shown previously that in comparison to the effects of high-carbohydrate feeding in mice, high-fat feeding with n-6 fatty acid–rich oil resulted in hyperglycemia and obesity.[14] In this study, we examined the effects of monounsaturated fat on obesity and diabetes in mice.

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 stabilize the metabolic conditions. The mice were maintained at a constant temperature of 22°C with a fixed artificial light cycle (12-hour light/dark cycle). The mice were allowed free access to either the high-carbohydrate diet or high–monounsaturated fat diets.

Diet

The dietary composition is shown in Table 1. Rapeseed oil was used in these experiments as a source of essential fatty acids both in the control high-carbohydrate diet and the high–monounsaturated fat diets. Fatty acid composition of dietary oils was measured by gas-liquid chromatography and is shown in Table 2. Ingredients for the purified diets were mixed, formed into a dough with water, rolled into pellets, wrapped with Saran Wrap (Asahi Kasei Kogyo, Tokyo, Japan), and stored at −20°C until used, to minimize fatty acid oxidation. 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. Fresh food was provided to the mice biweekly. Casein, sucrose, starch, vitamin mixture, mineral mixture, and cellulose powder were purchased from Oriental Yeast (Tokyo, Japan); DL-methionine from Sigma (St Louis, MO); rapeseed oil from Yonezawa Oil (Saitama, Japan); and olive oil from Miyazawa Yakuhin (Tokyo, Japan).

Triolein Synthesis

Oleic acid (7.08 mol, 2,000 g), glyceride (1.58 mol, 145 g), calcium hydroxide (4.29 g), and activated charcoal (8.58 g) were added to a three-neck round-bottom flask equipped with a Dean-Stark trap bearing a reflux condenser, an efficient mechanical stirrer, and a thermometer. The mixture was heated and stirred vigorously at 240°C for 5 hours under a nitrogen stream. Water generated during the reaction was dried through the trap. After neutralization with phosphoric acid, the mixture

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Table 1. Dietary Composition (%)

Component	High-Carbohydrate	High-Olive Oil	High-Triolein
Rapeseed oil	4.0	5.6	5.6
Supplemented oil	—	26.4	26.4
Casein	23.7	33.1	33.1
Sucrose	10.0	17.6	17.6
α-Starch	50.0	—	—
Vitamin mixture	1.0	1.4	1.4
Mineral mixture	7.0	9.8	9.8
Cellulose powder	4.0	5.6	5.6
DL-Methionine	0.35	0.5	0.5
Energy (kcal/100 g)	343	490	490
Fat energy (% of total energy)	10.7	60.2	60.2

was further heated at 80°C for 30 minutes, and then activated charcoal was removed by filtration. Unreacted oleic acid and monoglycerides and diglycerides were removed by stream distillation from filtered products. The yield of triolein was 1,860 g. The glyceride and fatty acid composition of triolein were analyzed by gas-liquid chromatography. Triglyceride was 97% of total glycerides, and C18:1 was 90% of total fatty acids. Oleic acid was purchased from Tokyo Kasei Kogyo (Tokyo, Japan), and other chemicals from Wako Pure Chemical Industries (Osaka, Japan).

Experimental Procedures

Mice were divided into three groups (n = 5 to 6 per group). The first group was given a high-carbohydrate diet (control) that, on a caloric basis, consisted of 63% carbohydrate, 26% protein, and 11% fat (Table 1). The other two groups were given a high–monounsaturated fat diet as olive oil or triolein, which consisted of 14% carbohydrate, 26% protein, and 60% fat. The mice were fed for 17 weeks. Energy intake, body weight, fasting and feeding blood insulin, triglyceride, cholesterol, and nonesterified fatty acids (NEFA) levels were measured at 16 to 17 weeks of feeding. Also, oral glucose tolerance tests (GTTs) were conducted at 9 and 15 weeks of feeding. The energy intake, fat in collected feces, intestinal absorption rate of fat, and body weight were measured during this experimental period. After killing the mice, the parametrial white adipose tissue (WAT) and liver weight and triglyceride levels in liver and skeletal muscle were measured. Part of the liver was used for fatty acid synthase (FAS), acetyl-coenzyme A (CoA) carboxylase (ACC), and acyl-CoA synthetase (ACS) mRNA assay by Northern blotting.

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

Fatty Acid	Rapeseed Oil	Olive Oil	Triolein
16:0	3.8	14.4	1.3
16:1	0.2	1.5	—
18:0	1.7	2.5	2.7
18:1	59.4	66.2	89.7
18:2n-6	20.2	14.3	4.1
18:3n-3	7.1	0.5	0.4
20:0	0.6	0.4	0.1
20:1	1.7	0.2	0.6
Others	5.3	0	1.1
Ratio of saturated, monounsaturated, and polyunsaturated fatty acid	10:10:4	10:39:9	10:220:11
n-6 to n-3 ratio	2.9	28.6	10.3

NOTE. Values for "others" (fatty acids of rapeseed oil) are as follows: 22:0, 0.3%; 22:1, 1.1%; 24:0, 0.2%; 24:1, 0.3%; and unidentified, 3.4%.

Measurement of Energy Intake

At 12 to 13 weeks of feeding, mice that had been kept in plastic shoebox cages with paper chips (Alpha Dri; Shepherd Specialty Papers, Kalamazoo, MI) were transferred to shoebox cages with wire bottoms. Beneath the wire, newspapers were spread out to collect food spillage. After removing feces on the paper, food spillage was collected and dried in an oven to evaporate water originating from urine. To accommodate the mice to cages with wire bottoms, food intake measurements were started 2 days after transfer to the new cages. Food intake measurement was made every day for 6 days, and then mice were returned to the shoebox 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.

Measurement of Lipid Absorption

For feces collection, at 11 weeks of feeding, to avoid contamination of lipid-containing food into the feces, mice that had been kept in plastic shoebox cages with experimental foods were transferred to new shoebox cages without food. Mice were kept in these new cages for 3 hours, feces excreted during the 3 hours were collected, and then the mice were returned to the old cages. This procedure was repeated twice per day, morning and evening, for 6 days to collect a sufficient amount of feces for one fat measurement. To collect enough feces for lipid measurement at three times, collections were continued for 2 weeks. Lipid measurement in feces was performed by the method described by Saxon.[15] For lipid absorption measurement, the energy intake and amount of feces per day were measured for 6 days simultaneously.

Oral GTT

Nine and 15 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 cutting the tail end before and 30, 60, and 120 minutes after glucose administration. Blood glucose levels were measured using a TIDEX glucose analyzer (Sankyo, Tokyo, Japan).

Triglyceride Measurement in Liver and Skeletal Muscle

Liver, gastrocnemius, and quadriceps tissues (100 mg) were homogenized in 12 mL chloroform-methanol (2:1 vol/vol) by a polytron (Kinematica, Littau/Luzern, Switzerland), and lipid extracts were obtained by the method described by Folch et al.[16] Triglycerides were separated on Sep-Pak silica cartridges (Sep-Pak Plus; Waters, Milford, MA) by the method described by Pan and Storlien.[17] Briefly, about 10 mL lipid extract in hexane was applied to this cartridge and washed twice by 10 mL hexane. Triglyceride was eluted by 30 mL ethyl acetate. Samples were dried by N_2 gas and suspended by 2 mL isopropanol, and thereafter the triglyceride content was measured by enzyme assay kits (determiner LTG; Kyowa Medics, Tokyo, Japan).

Northern Blots

Samples (15 μg per lane) of total RNA isolated by the method of Chirgwin et al[18] were denatured with glyoxal and dimethyl sulfoxide and analyzed by electrophoresis in 1% agarose gels. After transfer to Hybond membranes (Amersham, Buckinghamshire, England) and UV cross-linking, RNA blots were stained with methylene blue to locate 28S and 18S rRNAs and ascertain the amount of loaded RNAs. The

blots were hybridized with rat FAS,[19] ACC,[20] and ACS cDNA probes[21] labeled with ^{32}P-dCTP (ICN, Costa Mesa, CA) by random prime labeling methods.[22] The amount of each mRNA was quantified with an image analyzer (BAS 2000; Fuji Film, Tokyo, Japan).

Other Analyses and Methods

Immunoreactive insulin was determined by an enzyme immunoassay kit using rat insulin as a standard (Morinaga, Yokohama, Japan). Triglyceride and total cholesterol levels were measured by enzyme assays (Kyowa Medics, Tokyo, Japan). NEFA levels were measured by enzyme assay (Wako Chemicals, Osaka, Japan).

Statistical Analysis

Statistical comparisons of the groups were made by one-way ANOVA, and each group was compared with the others by Fisher's PLSD test (Statview 4.0; Abacus Concepts, Berkeley, CA). The glucose tolerance curve of each group was compared by repeated-measures ANOVA (SuperANOVA; Abacus Concepts). Statistical significance is defined as P less than .05. Values are the mean ± SE.

RESULTS

During the 17-week 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 virtually identical (Table 3). On a per-day basis, the total lipid amount in feces increased slightly in both high–olive oil and high-triolein mice compared with carbohydrate-fed mice (Table 3). The calculated lipid absorption rate in the intestine showed that most of the lipids taken orally were absorbed, and no significant inhibition of lipid absorption was observed in each group. Mice fed high–olive oil and high-triolein diets had a 25% increase in body weight compared with mice fed a high-carbohydrate diet (Table 3). Parallel to the body weight increase, both high–monounsaturated fat groups showed an increased wet weight of parametrial WAT, but a liver weight increase was observed only in high-triolein mice (Table 3).

After a 15-week feeding, both high–monounsaturated fat diets resulted in significant increases of blood glucose before and 30 and 60 minutes after oral glucose challenge compared with high-carbohydrate feeding (Table 3 and Fig 1). These differences were also observed after a 9-week feeding (data not shown). Since plasma fasting and feeding insulin levels in each group of mice did not differ significantly (Table 3), the abnormal glucose tolerance observed in the high–monounsaturated fat diet was not due to decreases of insulin secretion, but was instead related to insulin resistance in skeletal muscle and liver. In comparison to the high-carbohydrate diet, blood fasting and feeding triglyceride levels decreased with both monounsaturated fat diets, whereas fasting and feeding total cholesterol levels slightly increased. Fasting and feeding NEFA levels did not differ among these groups (Table 3).

Table 3. Energy and Lipid Intake, Fecal Weight and Lipid, Lipid Absorption Rate, Final Body Weight, WAT Weight, Liver Weight, Σ Glucose of GTT, and Fasting and Feeding Blood Insulin, Triglyceride, Cholesterol, and NEFA Levels

Parameter	High-Carbohydrate	High–Olive Oil	High-Triolein	ANOVA
Energy intake (kcal · mouse^{-1} · d^{-1})	9.0 ± 0.5	8.0 ± 0.3	8.6 ± 0.2	$F(2,15) = 2.15$, $P = .15$
Lipid intake (mg · mouse^{-1} · d^{-1})	105 ± 5	523 ± 17‡	564 ± 16‡	$F(2,15) = 335$, $P < .001$
Fecal weight (g · mouse^{-1} · d^{-1})	1.10 ± 0.08	1.26 ± 0.06	1.36 ± 0.07	$F(2,15) = 3.34$, $P = .06$
Lipid in feces (mg · mouse^{-1} · d^{-1})	8.7 ± 0.6	27.8 ± 1.3‡	19.6 ± 1.0‡	$F(2,15) = 85.2$, $P < .001$
Lipid absorption (%)	92	95	97	
Final body weight (g)	24.6 ± 0.8	30.6 ± 1.6*	30.8 ± 1.6*	$F(2,14) = 5.36$, $P < .05$
WAT weight (g)	0.7 ± 0.2	1.8 ± 0.2†	1.4 ± 0.2*	$F(2,14) = 6.96$, $P < .01$
Liver weight (g)	1.1 ± 0.1	1.1 ± 0.1	1.4 ± 0.1‡	$F(2,14) = 12.5$, $P < .001$
Σ Glucose (mmol/L)	29.0 ± 0.9	38.3 ± 1.8†	44.1 ± 2.7‡	$F(2,14) = 13.2$, $P < .001$
Insulin (pmol/L)				
Fasting	35.4 ± 7.8	49.2 ± 5.2	68.1 ± 13.2	$F(2,14) = 2.88$, $P = .09$
Feeding	237 ± 47	315 ± 49	227 ± 20	$F(2,14) = 1.48$, $P = .26$
Triglyceride (mmol/L)				
Fasting	80.5 ± 12.4	37.8 ± 5.2†	27.1 ± 3.9‡	$F(2,14) = 13.7$, $P < .001$
Feeding	35.6 ± 7.6	20.7 ± 3.1*	15.7 ± 1.7†	$F(2,14) = 5.23$, $P < .05$
Total cholesterol (mmol/L)				
Fasting	75.0 ± 1.3	87.2 ± 3.0*	78.1 ± 4.0	$F(2,14) = 4.05$, $P < .05$
Feeding	74.2 ± 5.6	89.6 ± 2.6*	95.3 ± 3.9†	$F(2,14) = 6.91$, $P < .01$
NEFA (μEq/L)				
Fasting	804 ± 186	585 ± 48	688 ± 74	$F(2,14) = 0.99$, $P = .40$
Feeding	181 ± 20	135 ± 23	217 ± 26	$F(2,14) = 3.12$, $P = .08$

NOTE. Mice were killed at 17 weeks of feeding, and body weight and liver and wet WAT weight were measured. Energy intake and the amount of feces were simultaneously measured for 6 days. Feces used for lipid analysis were collected at different occasions to avoid contamination of lipid in food by transferring mice to new cages. Feces collections were continued for 2 weeks for lipid analysis. Mice fed each diet for 15 weeks were fasted overnight, and then oral GTTs were conducted. Fasting and feeding blood insulin, triglyceride, cholesterol, and NEFA levels were measured at 16 to 17 weeks of feeding. Results are the mean ± SE of individual mean values obtained in each of 5 to 6 mice. High–olive oil and high-triolein diets are compared with high-carbohydrate diet by Fisher's PLSD test. Lipid absorption rate is calculated as (lipid intake − lipid in feces)/lipid intake × 100. Σ glucose is the sum of blood glucose levels at 0, 30, 60, and 120 minutes after oral glucose administration.

*$P < .05$.

†$P < .01$.

‡$P < .001$.

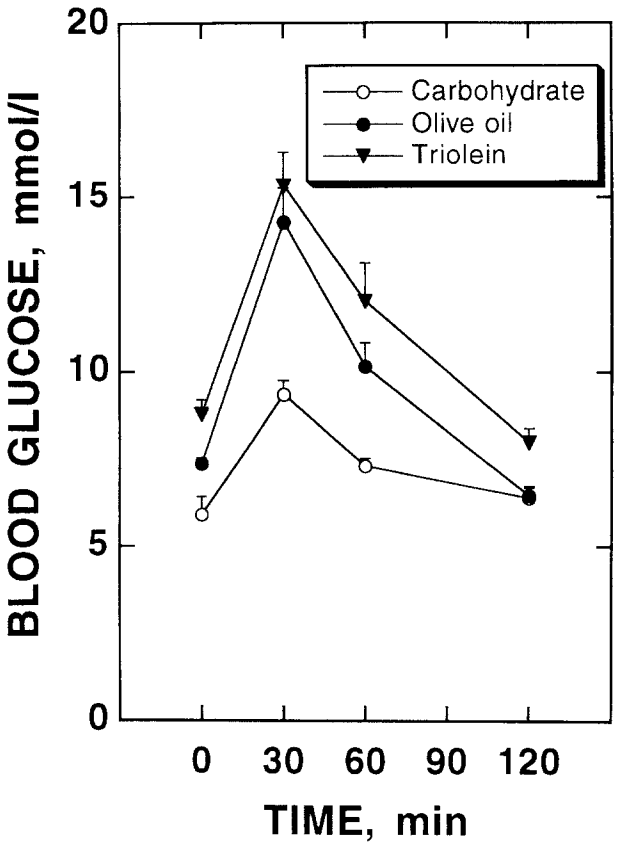

Fig 1. Oral GTT in mice. Mice fed high–olive oil and high-triolein diets for 15 weeks were fasted overnight and then administered D-glucose (1 mg/g body weight) orally by stomach tube. As negative controls, high-carbohydrate–fed mice were included. Blood glucose levels were determined at the times indicated. Each data point represents the mean ± SE of 5 to 6 mice. $P < .0001$, high-carbohydrate *v* other groups by repeated-measures ANOVA.

To investigate the relationship between local accumulation of triglyceride and insulin resistance, tissue triglyceride levels were determined. In the gastrocnemius, some values are significant but others are not. Compared with high-carbohydrate feeding, high–olive oil and high-triolein feeding increased triglyceride levels 1.7-fold and 1.4-fold, respectively, whereas in quadriceps, they increased 1.8-fold and 1.5-fold, respectively. In the liver, high–olive oil and high-triolein feeding increased triglyceride levels 1.4-fold and 2.4-fold, respectively (Table 4).

To elucidate the mechanism of high-triolein diet–induced triglyceride accumulation in the liver, FAS, ACC, and ACS mRNA levels were measured. FAS and ACC play key roles in de novo fatty acid synthesis,[23] while ACS is for triglyceride synthesis in the liver.[21,24] In comparison to the high-carbohydrate diet, the high–olive oil diet slightly decreased and the high-triolein diet increased FAS and ACC mRNA levels (Fig 2). ACS mRNA levels in high–olive oil and high-triolein feeding increased 1.6-fold and 1.5-fold, respectively, but compared with levels in high-carbohydrate mice, they were not significant.

To understand the differences in these two diets, the actual intake of individual fatty acids from each group was estimated by the data for fatty acid composition (Table 2) and data for average lipid intake (Table 3). In comparison to high–olive oil mice, high-triolein mice consumed a 1.4-fold larger amount of monounsaturated fatty acid, but consumption of saturated fatty acids was 68% lower and of polyunsaturated fatty acids 46% lower (Table 5).

DISCUSSION

In the present study, in comparison to a high-carbohydrate diet, ad libitum feeding of two types of monounsaturated fat diets (high–olive oil and high-triolein diets) in C57/BL6J mice resulted in obesity and hyperglycemia. In our previous study to examine the effects of various high-fat dietary oils on obesity and diabetes in C57/BL6J mice, n-6–rich oils such as safflower and soybean oil produced similar levels of obesity and diabetes as monounsaturated fat–rich oils, while n-3–rich oils such as perilla and fish oils showed less obese and diabetic effects.[14] Rapeseed oil, which contains a relatively large amount of oleic acid (59% of total fatty acids), also showed obese and diabetic effects. The present and previous data have clearly shown that even high monounsaturated fat, when given in a large amount, results in obesity and diabetes.

It has been suggested in short-term human studies that under isocaloric conditions, fat and carbohydrate have a similar potency to induce obesity. For example, Leibel et al[25] reported that when 16 human subjects were fed liquid diets with various corn oil content for 2 to 8 weeks in a metabolic ward, even with extremely large differences in the fat to carbohydrate ratio ranging from 0% to 70% of total energy, there were no detectable differences in body weight. Hill et al[26] also reported that when eight adults were given a high-carbohydrate diet (60% of energy from carbohydrate) and a high-fat diet (60% of calories from fat) for 1 week under isocaloric conditions, dietary composition did not affect total energy expenditure, although they did not describe the dietary fat sources and visceral fat level. On the other hand, in agreement with our conclusions, most epidemiological data have suggested that a high intake of dietary fat that includes monounsaturated fat is

Table 4. Triglyceride Levels in Gastrocnemius, Quadriceps, and Liver

Triglyceride (μmol/g)	High-Carbohydrate (n = 5)	High–Olive Oil (n = 6)	High-Triolein (n = 6)	ANOVA
Gastrocnemius	11.1 ± 2.6	18.6 ± 3.8	15.7 ± 3.8	$F(2,14) = 1.08$, $P = .36$
Quadriceps	12.4 ± 2.7	22.3 ± 2.2*	18.5 ± 2.2	$F(2,14) = 4.4$, $P < .05$
Liver	63.2 ± 17.9	91.2 ± 5.1	151.7 ± 21.9†	$F(2,14) = 7.4$, $P < .01$

NOTE. Mice were killed at 17 weeks of feeding, and triglyceride levels in skeletal muscle (gastrocnemius and quadriceps) and liver were measured. Results are the mean ± SE of individual mean values obtained in each of 5 to 6 mice. High–olive oil and high-triolein diets are compared with high-carbohydrate diet by Fisher's PLSD test.

*$P < .05$.

†$P < .01$.

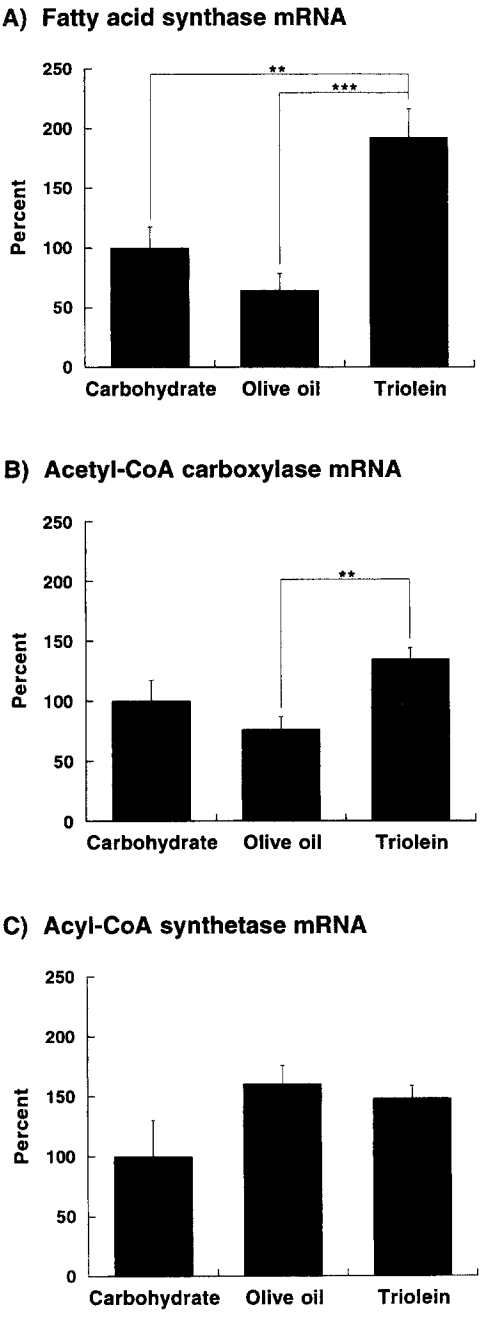

Fig 2. Northern blotting of FAS (A), ACC (B), and ACS (C) mRNA in liver. Mice were fed a high-carbohydrate, high–olive oil, or high-triolein diet for 17 weeks. Total RNA was extracted from the liver under feeding conditions with guanidine thiocyanate and sedimented through a cesium chloride cushion. All samples (15 μg per lane) of total RNA were applied. ^{32}P-labeled rat FAS, ACC, and ACS cDNA probes were used. mRNA levels were quantified by image analyzer and expressed as a relative percent to that from high-carbohydrate–fed mice. Background values were subtracted. Each data point represents the mean ± SE of 5 to 6 mice. **$P < .01$, ***$P < .001$ by ANOVA and Fisher's PLSD test.

associated with increasing adiposity[27] and development of insulin resistance[28,29] irrespective of total energy intake. However, since all of these cross-sectional studies relied on recall methods, the accuracy of measurement of food intake is questionable. The other important aspect of high-fat feeding is its palatability. Duncan et al[30] reported that subjects who were allowed to eat as much of a high-energy diet (high fat and high simple sugar) or low-energy diet (low fat and low simple sugar) as they liked consumed nearly twice as much energy when consuming a high-energy diet compared with a low-energy diet. For similar reasons, Rolls[31] concluded that the best dietary advice for weight maintenance and hunger control is to consume a low-fat/high-carbohydrate diet. Thus, it is likely that small increases in energy intake on a high-fat diet integrated over a prolonged period that is difficult to access by recall methods may lead to obesity. Indeed, in human studies, it has been reported that with a fixed amount of carbohydrate and protein, increased fat intake did not promote its own oxidation, which might lead to obesity.[32,33]

In this mouse experiment, although the total energy intake of each group became nearly identical, high–monounsaturated fat diets resulted in obesity and diabetes. West et al[34] also reported that six strains of mice including C57BL/6J kept in individual cages showed high-fat diet–induced obesity even under isocaloric conditions. Thus, these data suggest that even under isoenergy conditions, high–monounsaturated fat feeding may eventually result in obesity and diabetes. However, because the measurement of food intake was not ideal, as discussed previously,[35] it cannot be ruled out that a small increase of food intake in the high-fat diet may lead to obesity in mice. Also, in this type of experiment, one should pay attention to the possibility of high-fat diet–induced malnutrition due to a shortage of other nutrients. To provide essential fatty acids, a small amount of rapeseed oil used in the high-carbohydrate diet was added to both high-fat diets. Carbohydrate content was also decreased on the high-fat diet. In human studies, Himsworth[36] showed that a low-carbohydrate diet (~8% of total energy) is diabetogenic. The carbohydrate content of the high-fat diet in this mouse study was 14%, about twofold higher than the minimal value in humans. Our previous study indicated that a high–fish oil diet that also contained 14% carbohydrate showed a relatively good glycemic control without a body weight

Table 5. Average Intake of Fatty Acids (mg · mouse^{-1} · d^{-1})

Fatty Acid	High-Carbohydrate	High–Olive Oil	High-Triolein
C16:0	4.0	65.6	9.8
C16:1	0.2	6.7	0.2
C18:0	1.8	12.3	14.3
C18:1	62.1	339.9	476.4
C18:2n-6	21.1	80.2	39.0
C18:3n-3	7.4	8.7	8.9
C20:0	0.6	2.3	1.1
C20:1	1.8	2.4	4.5
Saturated	6.9	80.7	25.6
Monounsaturated	65.6	350.3	482.5
Polyunsaturated	28.6	88.8	47.9
n-6	21.1	80.2	39.0
n-3	7.4	8.7	8.9
n-9	65.4	343.6	482.3

NOTE. Average intake of individual fatty acids for each group of mice is calculated as (fatty acid composition of dietary oils in Table 2) × (lipid intake in mg/mouse/d in Table 3) as follows: for example, C16.0 value for high-carbohydrate–fed mice is calculated as 105 × 0.038 = 4.0.

increase,[14] suggesting that a carbohydrate content of 14% may not be a cause of obesity and diabetes.

Mice fed a high–olive oil diet and high-triolein diet consumed a large amount of monounsaturated fatty acid: 67% and 87% of total fatty acids, respectively (Table 5). Although there are some minor differences in the individual fatty acid intake among each group of mice, a higher intake of monounsaturated fatty acids is a probable cause of obesity and diabetes. In comparison to high–olive oil feeding, a high-triolein diet caused a larger amount of triglyceride accumulation (Table 4) and an increase of FAS and ACC mRNA levels in liver (Fig 2). Since it is known that polyunsaturated fatty acids, but not saturated and monounsaturated fatty acids, decrease FAS and ACC mRNA,[37,38] high-triolein diet–induced increases in FAS and ACC mRNA may be due to a relatively lower content of polyunsaturated fatty acid in a high-triolein diet. Elevated FAS and ACC mRNA levels by high-triolein feeding might lead to further triglyceride accumulation in the liver. However, in high–safflower oil compared with high-carbohydrate feeding, although FAS and ACC mRNA levels decreased, triglyceride accumulation in the liver increased (N. Tsunoda, S. Ikemoto, M. Takahashi, et al, unpublished observation, April 1997). Thus, FAS and ACC mRNA levels may not be a rate-limiting step of high-fat diet–induced triglyceride accumulation in the liver. Increased ACS mRNA levels from both high–monounsaturated fat diets may contribute to triglyceride accumulation in the liver. ACS catalyzes the formation of acyl-CoA from fatty acid, and acyl-CoA is used for triglyceride formation in the liver. ACS mRNA levels were well correlated with triglyceride levels in the liver in high-safflower oil–fed mice.[35] The expression of ACS was markedly increased in rat liver by high-fat (20% soybean oil and 49% sucrose) and high-carbohydrate (69% sucrose) feeding,[21] and in genetically obese Zucker rats.[39] Since fasting and feeding blood NEFA did not increase by both monounsaturated fat diets (Table 3), the increased triglyceride accumulation in the liver may not be due to increased influx of extracellular fatty acids into the liver. The decreases of fasting and feeding blood triglyceride levels observed in both monounsaturated fat diets are in good agreement with data from human studies.[8,10] It is not clear at present whether these decreases are due to inhibition of VLDL secretion in the liver or accelerated triglyceride degradation in the blood.

So, what is the cause of high-monounsaturated fat–induced diabetes? An increase of triglyceride accumulation in the liver might lead to increased glucose output or, as indicated by Storlien et al,[40] accumulation of triglyceride in skeletal muscle might lead to insulin resistance, or it may be simply explained by an increase of intraabdominal fat.

In conclusion, these data suggest that the recently recommended high–monounsaturated fat diet might result in obesity and diabetes. Long-term human intervention studies including visceral fat measurements are required to elucidate whether a high–monounsaturated fatty acid diet is better than a high-carbohydrate diet.

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