Role of Fatty Acid Composition in the Development of Metabolic Disorders in Sucrose-Induced Obese Rats

SATOSHI FUKUCHI, KAZUYUKI HAMAGUCHI, MASATAKA SEIKE, KATSURO HIMENO, TOSHIIE SAKATA, AND HIRONOBU YOSHIMATSU[1]

Department of Internal Medicine I, Faculty of Medicine, Oita University, Hasama, Oita, 879-5593, Japan

Fatty acids have been shown to be involved in the development of insulin resistance associated with obesity. We used sucrose loading in rats to analyze changes in fatty acid composition in the progression of obesity and the related metabolic disorder. Although rats fed a sucrose diet for 4 weeks had body weights similar to those of control animals, their visceral fat pads were significantly larger, and serum triglyceride levels were higher; however, neither plasma glucose nor insulin levels were significantly higher. After 20 weeks of sucrose loading, body weight and visceral and subcutaneous fat pads had increased significantly compared with those in control rats. Moreover, plasma glucose, insulin, and triglyceride levels were significantly higher. An analysis of individual fatty acid components in the blood and peripheral tissues demonstrated phase- and tissue-dependent changes. After 20 weeks of sucrose loading, palmitoleic acid (16:1 n-7) and oleic acid (18:1 n-9), the major components of monounsaturated fatty acid, showed a ubiquitous increase in plasma and all tissues analyzed. In contrast, linoleic acid (18:2 n-6) and arachidonic acid (20:4 n-6), the major components of polyunsaturated fatty acid in the n-6 family, decreased in plasma and all tissues analyzed. After 4 weeks of sucrose loading, these changes in fatty acid composition were observed only in the liver and plasma and not in fat and muscle. This led us to conclude that elevation of plasma glucose and insulin develop at the late phase of sucrose-induced obesity, when changes in fatty acid composition appear in fat and muscle. Furthermore, changes in fatty acid composition in liver seen after 4 weeks of sucrose loading, when increases in neither plasma glucose nor insulin were detected, suggest that liver may be the initial site of fatty acid imbalance and that aberrations in hepatic fatty acid composition may lead to fatty acid imbalances in other tissues. Exp Biol Med 229:486–493, 2004

This study was supported by Grants-in-Aid for Scientific Research 07457225 (T.S.) and 10670066 (H.Y.) from The Japan Society for the Promotion of Science, Japan.

[1]To whom requests for reprints should be addressed at Department of Internal Medicine I, Faculty of Medicine, Oita University, 1-1 Idaigaoka, Hasama, Oita, 879-5593, Japan. E-mail: hiroy@med.oita-u.ac.jp

Received October 8, 2003.
Accepted March 4, 2004.

1535-3702/04/2295-0001$15.00

Key words: sucrose-induced obesity; fatty acids; hyperinsulinemia; hypertriglyceridemia

Insulin resistance associated with obesity has been shown to cause a variety of common disorders, including type 2 diabetes, hyperlipidemia, hypertension, and coronary artery disease. Fatty acids have received considerable attention as one of the factors inducing insulin resistance; changes in fatty acid composition of the blood and muscle have been shown to correlate with insulin resistance in humans (1–3). Similarly, insulin resistance in rats fed a high-fat diet can be modulated by substituting the fatty acid component in the diet (4). Fatty acids play a role in both the cellular and molecular mechanisms of insulin resistance, because they are a determinant of the membrane property that affects insulin sensitivity (5, 6), and they act as a physiological signaling molecule that induces insulin resistance (7, 8). Evidence for these mechanisms is that diet-induced obesity in mice lacking the aP2 gene, which codes for the fatty acid binding protein, neither develop insulin resistance nor express mRNA for tumor necrosis factor–α (TNF-α), a cytokine produced by adipose tissue that has been implicated in obesity-related insulin resistance (9). However, it remains unclear which fatty acid components contribute to the development of insulin resistance. Among the various fatty acids components, ω-3 long chain polyunsaturated fatty acids (PUFA) such as docosahexaenoic acid (DHA; 22:6) in muscle have been shown to be important for efficient insulin action in rats (10). Insulin sensitivity assessed by the glucose clamp method was found to correlate positively with the total percentage of C20-22 PUFA, especially arachidonic acid (20:4), and correlate negatively with monounsaturated fatty acids (MUFA), especially oleinic acid (18:1 n-9) in the phospholipid fraction of human skeletal muscle (4). Another study investigating changes in the fatty acid composition of serum cholesterol ester in patients with type 2 diabetes showed higher proportions of saturated fatty acids (SFA)

and palmitoleic acid (16:1 n-7) as MUFA and a lower proportion of linoleic acid (18:2 n-6) as PUFA (11). Thus, the contribution of individual fatty acid components to insulin resistance appears to differ depending on the species or tissue examined and on the method used to induce insulin resistance. However, with all of these taken together, it can be concluded that MUFA may promote, whereas PUFA may protect against, the progression of insulin resistance.

It has been well established that visceral fat accumulation (also called "visceral fat syndrome") is one of the risk factors that induce obesity-related metabolic dysregulation and hyperinsulinemia (12, 13). Compared with subcutaneous fat tissue, the turnover of lipid metabolism is higher in visceral fat (14). As a result, increased lipogenic activity in visceral fat tissue induces relatively rapid fat deposition in response to excessive energy intake (14). Higher lipolytic activity in this tissue induces the simultaneous hypersecretion of fatty acids into the portal vein, thereby affecting lipid metabolism in the liver (15). As a result of this process, fatty acids contribute to the pathogenesis of visceral fat syndrome (16).

On the other hand, it is well known that skeletal muscle is the principal site of insulin-mediated glucose disposal. Insulin sensitivity has been shown to be associated with fatty acid imbalances in muscle (17). In this regard, it may be hypothesized that visceral fat tissue, liver, and muscle play different roles in the development of insulin resistance, the former two inducing abnormal fatty acid metabolism and the latter as a target site that reflects the fatty acid imbalance in insulin resistance. However, despite numerous studies that have indicated an important role for fatty acids in the development of insulin resistance, the details of which, when, where, and how fatty acids contribute to this disorder remain unclear. Therefore, we aimed to clarify how the fatty acid composition of various tissues change in response to the development of obesity in sucrose-loaded rats.

Material and Methods

Animals. Mature male Wistar King A (WKA) rats weighing 315.9 ± 17.3 g were housed in a room illuminated daily from 0700 to 1900 hr (a 12:12-hr light:dark cycle) and maintained at 21° ± 1°C with a humidity of 55% ± 5%. The animals were allowed free access to standard solid rat chow and tap water, except where described otherwise. All studies were conducted in accordance with Oita University Guidelines, which are based on the National Institutes of Health's *Guide for the Care and Use of Laboratory Animals.*

Sucrose-Induced Obese Rats. Sucrose-induced obesity was produced in the WKA rats by simultaneous exposure to a liquid diet of 34% sucrose and standard rat chow (CE-2; Japan CLEA, Tokyo, Japan) *ad libitum.* The composition of nutrients and fatty acids in CE-2 is shown in Table 1. The body weight was recorded weekly during the study period. Total calorie intake was calculated by measuring the consumption of both sucrose and rat chow at 4 and 20 weeks after the introduction of the obesity-inducing diet. Rats were fasted for 12 hrs and killed by an intraperitoneal (ip) injection of 45 mg/kg sodium pentobarbital anesthesia at 4 and 20 weeks. Plasma and tissues (including liver, muscle, and subcutaneous and visceral fat pads) were then collected. Subcutaneous and visceral fat pads were carefully dissected from each animal according to anatomical landmarks. All visible subcutaneous fat between the lower part of neck and midthigh was considered to be subcutaneous fat, whereas all fat along the mesentery (starting at lesser curvature of the stomach and ending at the sigmoid colon), epididymal fat, perirenal fat, and retroperitoneal fat were considered to be visceral fat. For each rat, the weight of the subcutaneous and visceral fat pads was measured, followed by analysis of the tissue and plasma fatty acid composition as described below.

Extraction of Lipids and Assay of Fatty Acids. The total lipids in each tissue were extracted according to the modified method of Folch *et al.* (18). Approximately 60–90 mg of frozen tissue specimens were homogenized in 2 ml of phosphate-buffered saline (PBS) and a mixture of 6 ml of ice-cold chloroform and methanol (2:1 vol to vol) that contained 0.01% butylated hydroxytoluene as an antioxidant. The total lipids in each 300-µl plasma sample were extracted by a similar method, without homogenization. After centrifugation at 1700 *g* for 5 mins, the lower chloroform layer was collected and subjected to methanolysis in 1.37 *M* HCl-methanol at 100°C for 2 hrs. The methylated lipid extract was then evaporated under a stream of nitrogen. The fatty acid methyl esters were extracted with petroleum ether and analyzed by gas chromatography (Shimadzu GC-17A; Shimadzu, Kyoto, Japan) using a 70% sianoplopyl polisilphenirene–ciroxan capillary column (0.25 mm × 25 m, BPX-70; SGE, Ringwood, Austria). Helium was used as the carrier, and the oven temperature was programmed to maintain 100°C for the first 2 mins and then to increase to 240°C at a rate of 5°C/min and to hold at this temperature for the final 5 mins. The identification and quantification of each fatty acid was made by comparison with an authentic standard mixture (GLC-96; Funakoshi, Tokyo, Japan) using Class 5000 software (Shimadzu, Kyoto, Japan).

Blood Analysis. All the blood samples were centrifuged at 1500 *g* for 15 mins at 5°C and the plasma stored at -20°C until they were analyzed. The plasma glucose concentration was measured using the glucose-oxidase method (Beckman Auto Analyzer, Fullerton, CA), and the plasma insulin concentration was determined by radioimmunoassay using the double antibody/polyethylene glycol (PEG) technique and rat insulin as a standard (Rat Insulin RIA Kit; Linco Research, Inc., St. Charles, MO). Triglyceride concentrations in the plasma samples were determined on an automated analyzer (model RA 1000; Technicon, New York, NY) using commercial enzymatic reagents (GPO-PAP kit 240052; Boehringer-Mannheim,

Table 1. Composition of Nutrients and Fatty Acids in Standard Rat Chow (CE-2)

Nutrient[a]	g/100 g
Moisture	8.7
Crude protein	24.8
Crude fat	4.4
Crude fiber	3.5
Crude ash	7.0
Nitrogen-free extracts (starch)	51.6
Fatty acids	**%**
14:0	0.4
16:0	15.4
16:1 n-7	1.3
18:0	1.8
18:1 n-9	22.7
18:2 n-6	49.4
18:3 n-3	3.4
20:1 n-9	1.0
20:4 n-6	0.2
20:5 n-3	1.8
22:0	0.2
22:1	0.4
22:6 n-3	1.7
Unknown	0.3

[a] Total calories per 100 g of chow are 345.2 kcal, which is calculated as follows: (4 × crude protein) + (9 × crude fat) + (4 × nitrogen-free extracts).

Mannheim, Germany). The triglyceride assay was standardized against a series of dilutions of an elevated control serum (Precilip EL kit 225053; Boehringer-Mannheim), with a normal control serum (Precinorm 781827; Boehringer-Mannheim) included as an internal control in all analyses.

Statistical Analysis. Data were expressed as means ± SD or means ± SE, as indicated in each figure or table. Statistical analyses were carried out using the unpaired Student's *t* test. Weekly body weight data were also analyzed by the unpaired Student's *t* test at each time point of the recording. $P \leq 0.05$ was considered to be statistically significant. All statistical analyses were performed with StatView software (version 5.0; SAS Institute, Cary, NC).

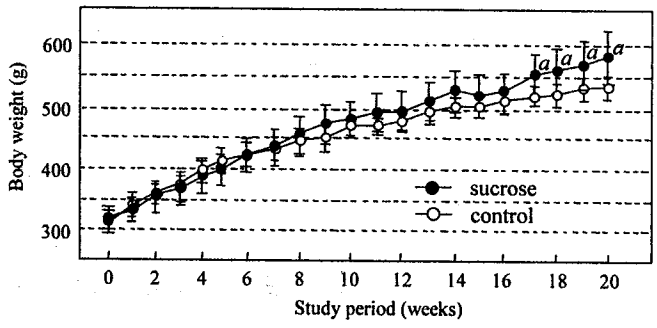

Figure 1. Body weight of control and sucrose-loaded rats during the study period. There were eight animals in each control (open circles) and sucrose (closed circles) group. [a] $P < 0.05$ *vs.* the control value at the same time points.

Results

Effect of Sucrose Loading on Body Weight During the Study Period. Figure 1 shows weekly body weight data from both control and sucrose-loaded rats during the study period. There was no difference in body weight between the two groups until 16 weeks, whereas, after 17 weeks or later of sucrose loading, body weight significantly increased compared with control rats ($P < 0.05$).

Amount and Composition of Dietary Calorie Intake. The composition of dietary calorie intake at 4 and 20 weeks in the sucrose-loaded rats, calculated by consumption of both sucrose and standard rat chow, is indicated in Table 2. Overall calorie intake was significantly increased in sucrose-loaded rats at both 4 and 20 weeks compared with that in control rats. The proportion of caloric increase in sucrose-loaded rats above that in controls at 4 weeks (18.1%) was similar to that at 20 weeks (18.8%). However, the proportion of caloric intake from sucrose above the total caloric intake at 4 weeks (68.9%) became more evident at 20 weeks (81.1%).

Plasma Concentrations of Glucose, Insulin, and Triglycerides. Table 3 shows the plasma concentrations of glucose, insulin, and triglycerides in response to 4 or 20 weeks of sucrose loading. At 4 weeks, the plasma triglyceride concentration was significantly higher in sucrose-loaded rats than in control rats ($P < 0.05$), in contrast to plasma glucose and insulin concentrations, which showed no remarkable changes. However, after 20 weeks of sucrose loading, all three parameters were higher in sucrose-loaded rats than those in control rats ($P < 0.05$ for each).

Visceral and Subcutaneous Fat Deposition in Sucrose-Loaded Rats. Table 4 summarizes body weight and fat pad weight in control and sucrose-loaded rats. The proportion of visceral fat, but not of subcutaneous fat, had increased significantly ($P < 0.05$) at 4 weeks in sucrose-loaded rats. At 20 weeks, the amount of fat tissue in both the visceral and subcutaneous fat pads had increased significantly compared with that in control rats ($P < 0.05$ for each).

Effect of Sucrose Loading on Fatty Acid Composition in the Liver, Plasma, Epididymal Fat, and Muscle. The fatty acid compositions in the liver after 4 and 20 weeks of sucrose loading is summarized in Table 5. The major fatty acid components present in higher proportions relative to total lipid in the liver were 16:0 and 18:0 for SFA; 16:1 n-7 and 18:1 n-9 for MUFA; and 18:2 n-6, 20:4 n-6, and 22:6 n-3 for PUFA. Of the SFAs, 16:0 fatty acid remained constant, whereas 18:0 fatty acid levels were lower after both 4 ($P < 0.05$) and 20 ($P < 0.01$) weeks of sucrose loading. For MUFA, 16:1 n-7 and 18:1 n-9 were increased after 4 ($P < 0.01$ for 16:1 and $P < 0.05$ for 18:1) and 20 ($P < 0.01$ for each) weeks of sucrose loading. In the PUFA component, 18:2 n-6 and 20:4 n-6 were both lower after 4 ($P < 0.01$ for 18:2 n-6 and $P < 0.05$ for 20:4) and 20

Table 2. Amount and Composition of Dietary Caloric Intake in Control and Sucrose-Loaded Rats

	4 weeks		20 weeks	
Nutrient	Control	Sucrose	Control	Sucrose
Crude protein	42.84 ± 3.95	15.73 ± 2.44[a]	49.51 ± 1.90	11.14 ± 3.49[a]
Crude fat	17.10 ± 1.58	6.28 ± 0.98[a]	19.76 ± 0.76	4.45 ± 1.40[a]
Nitrogen-free extracts (starch)	89.13 ± 8.22	32.73 ± 5.10[a]	103.01 ± 3.96	23.18 ± 7.27[a]
Sucrose		121.24 ± 13.94		165.96 ± 0.13
Total calories	149.07 ± 13.75	175.99 ± 6.64[a]	172.29 ± 6.64	204.72 ± 24.70[a]

Data are expressed as kilocalories per day. Total calories was calculated as follows: (4 × crude protein) + (9 × crude fat) + (4 × nitrogen-free extracts). Data are means ± SD, $n = 6$ for each group.
[a] $P < 0.01$ *vs.* control rats at the same time point.

Table 3. Plasma Concentrations of Glucose, Insulin, and Triglycerides in Control and Sucrose-Loaded Rats

	4 weeks		20 weeks	
	Control	Sucrose	Control	Sucrose
Glucose (mmol/l)	5.6 ± 0.4	5.8 ± 0.3	6.2 ± 0.3	6.8 ± 0.6[a]
Insulin (ng/ml)	7.2 ± 3.1	10.3 ± 2.6	13.6 ± 4.5	39.5 ± 17.7[a]
Triglycerides (mmol/l)	1.1 ± 0.2	2.1 ± 0.5[a]	1.4 ± 0.2	2.7 ± 0.6[a]

Data are means ± SD, $n = 6$ for each group.
[a] $P < 0.05$ *vs.* control rats at the same time point.

Table 4. Body Weight and Visceral and Subcutaneous Fat Weight in Control and Sucrose-Loaded Rats

	4 weeks		20 weeks	
	Control	Sucrose	Control	Sucrose
Body weight (g)	385.50 ± 18.86	378.63 ± 23.86	535.86 ± 19.42	583.86 ± 41.43[a]
Visceral fat				
Weight (g)	6.49 ± 0.71	7.73 ± 1.05[a]	10.15 ± 1.24	24.15 ± 6.39[b]
% fat/body weight	1.69 ± 0.18	2.04 ± 0.23[a]	1.89 ± 0.25	4.10 ± 0.88[b]
Subcutaneous fat				
Weight (g)	5.77 ± 0.34	6.31 ± 0.95	6.98 ± 1.02	17.17 ± 6.04[b]
% fat/body weight	1.50 ± 0.11	1.67 ± 0.23	1.31 ± 0.22	2.90 ± 0.85[b]

Data are means ± SD, $n = 6$ for each group.
[a] $P < 0.05$ *vs.* control weight or percentage at the same time point.
[b] $P < 0.01$ *vs.* control weight or percentage at the same time point.

($P < 0.01$ for each) weeks of sucrose loading, whereas 22:6 n-3 remained unchanged during both periods.

Table 6 shows the fatty acid composition in plasma after 4 and 20 weeks of sucrose loading. The major fatty acid components in plasma were 16:0, 18:0, 16:1 n-7, 18:1 n-9, 18:2 n-6, 20:4 n-6, and 22:6 n-3, similar to those measured in the liver. Of the SFAs, 16:0 had increased by both 4 ($P < 0.01$) and 20 ($P < 0.05$) weeks, whereas 18:0 had decreased by both 4 ($P < 0.05$) and 20 ($P < 0.01$) weeks. Sucrose loading caused an increase in the MUFAs 16:1 n-7 and 18:1 n-9 by both 4 ($P < 0.01$ for each) and 20 ($P < 0.05$ for 16:1 n-7 and $P < 0.01$ for 18:1 n-9) weeks but a decrease in PUFAs 18:2 n-6, 20:4 n-6, and 22:6 n-3 by 4 ($P < 0.01$ for each) and 20 ($P < 0.05$ for 18:2 and $P < 0.01$ for the others) weeks.

Table 7 shows the fatty acid composition in epididymal fat tissue after 4 and 20 weeks of sucrose loading. The major components in this tissue were 16:0 and 18:0 for SFA, 16:1 n-7 and 18:1 n-9 for MUFA, and 18:2 n-6 for PUFA. This was similar to the fatty acid composition of both liver and plasma, except that the PUFAs 20:4 n-6 and 22:6 n-3 were present in a relatively lower proportion in fat tissue. In the SFA fractions, 16:0 and 18:0 were unchanged at 4 weeks, but by 20 weeks 16:0 levels had increased ($P < 0.05$), whereas 18:0 levels had decreased ($P < 0.01$). For MUFAs, 16:1 n-7 levels had increased at both 4 and 20 weeks ($P < 0.01$ for each), whereas 18:1 n-9 levels had increased only after 20 weeks of sucrose loading ($P < 0.05$). A similar delayed decrease was observed for the 18:2 n-6 PUFA ($P < 0.05$ at 20 weeks only).

The fatty acid composition in muscle after 4 and 20 weeks of sucrose loading is shown in Table 8. The major

Table 5. Fatty Acid Composition of Liver Total Lipid in Sucrose-Loaded Rats

	4 weeks		20 weeks	
Fatty acid	Control	Sucrose	Control	Sucrose
14:0	0.16 ± 0.02	0.43 ± 0.09	0.12 ± 0.01	0.44 ± 0.05
16:0	21.0 ± 0.1	23.2 ± 0.8	21.5 ± 0.5	22.2 ± 0.8
16:1 n-7	0.7 ± 0.1	4.0 ± 0.4[a]	0.37 ± 0.04	4.2 ± 0.2[a]
18:0	18.9 ± 0.9	14.9 ± 1.2[b]	19.5 ± 0.5	17.2 ± 1.2[a]
18:1 n-9	6.4 ± 0.1	13.2 ± 2.2[b]	5.47 ± 0.01	15.5 ± 0.9[a]
18:1 n-7	4.2 ± 0.3	5.0 ± 0.2[b]	3.5 ± 0.1	7.0 ± 0.6[a]
18:2 n-6	19.4 ± 0.5	14.4 ± 0.6[a]	20.0 ± 0.4	11.1 ± 0.7[a]
18:3 n-3	0.34 ± 0.02	0.20 ± 0.01[a]	0.21 ± 0.01	0.04 ± 0.03[a]
20:0	0.12 ± 0.01	0.05 ± 0.02[b]	0.10 ± 0.01	0.002 ± 0.004[a]
20:1 n-9	0.25 ± 0.02	0.1 ± 0.2[b]	0.24 ± 0.01	0.13 ± 0.01[a]
20:2 n-6	0.88 ± 0.05	0.18 ± 0.01[b]	0.69 ± 0.04	0.11 ± 0.03[a]
20:3 n-6	0.91 ± 0.05	0.82 ± 0.02	0.71 ± 0.03	0.92 ± 0.07[b]
20:4 n-6	17.9 ± 0.9	14.7 ± 1.3[b]	20.6 ± 0.1	12.9 ± 0.6
22:6 n-3	7.7 ± 1.2	7.7 ± 0.7	6.1 ± 0.4	7.5 ± 1.2
24:0	0.02 ± 0.01	0.043 ± 0.004	0.04 ± 0.03	0.02 ± 0.01

Data are means ± SE, $n = 6$ for each group. Fatty acids are expressed as g/100 g fatty acids.
[a] $P < 0.01$ *vs.* the control value of the same fatty acids at the same time point.
[b] $P < 0.05$ *vs.* the control value of the same fatty acids at the same time point.

fatty acid components in this tissue were 16:0, 18:0, 16:1 n-7, 18:1 n-9, 18:2 n-6, 20:4 n-6, and 22:6 n-3, similar to those in plasma and the other tissues, with the exception of fat tissue. For the SFAs, 16:0 did not change after sucrose loading and 18:0 decreased at 4 weeks ($P < 0.05$) but not 20 weeks. For the MUFAs, both 16:1 n-7 and 18:1 n-9 remained unchanged at 4 weeks but had increased by 20 weeks ($P < 0.05$ for each). Of the PUFAs, 18:2 n-6 levels were lower at both 4 ($P < 0.05$) and 20 ($P < 0.01$) weeks, 20:4 n-6 was lower at 20 weeks ($P < 0.05$) but not at 4 weeks, and 22:6 n-3 remained unaltered during the period of sucrose loading.

Discussion

Our results demonstrate that although visceral fat accumulated during the early phase of sucrose loading, body weight and subcutaneous fat deposition remained unchanged during this period. The total caloric intake in the sucrose-loaded rats during this early phase of the study was significantly higher than that of control rats. The excessive energy intake caused by sucrose loading may preferably

Table 6. Fatty Acid Composition of Plasma Total Lipid in Sucrose-Loaded Rats

	4 weeks		20 weeks	
Fatty acid	Control	Sucrose	Control	Sucrose
14:0	0.50 ± 0.02	0.9 ± 0.1	0.29 ± 0.03	1.1 ± 0.1[a]
16:0	20.7 ± 0.4	24.6 ± 0.3[b]	20.2 ± 0.1	23.7 ± 0.7[a]
16:1 n-7	1.3 ± 0.2	6.3 ± 0.6[b]	0.9 ± 0.2	8.7 ± 1.0[a]
18:0	7.01 ± 0.08	6.4 ± 0.2[a]	8.5 ± 0.1	5.8 ± 0.1[b]
18:1 n-9	8.23 ± 0.08	18.5 ± 1.4[b]	6.38 ± 0.07	22.2 ± 1.3[b]
18:1 n-7	3.49 ± 0.08	5.5 ± 0.2[a]	3.9 ± 0.2	8.5 ± 0.5[b]
18:2 n-6	33.1 ± 0.5	19.6 ± 0.9[b]	29.5 ± 0.2	15.3 ± 2.1[a]
18:3 n-3	1.36 ± 0.09	0.73 ± 0.04[b]	0.69 ± 0.06	0.47 ± 0.09
20:0	0.14 ± 0.01	0.12 ± 0.02	0.080 ± 0.004	0.071 ± 0.004
20:1 n-9	0.31 ± 0.01	0.28 ± 0.04	0.23 ± 0.01	0.28 ± 0.01
20:2 n-6	0.49 ± 0.02	0.27 ± 0.01[a]	0.47 ± 0.01	0.14 ± 0.02[a]
20:3 n-6	0.42 ± 0.01	0.52 ± 0.06	0.39 ± 0.02	0.52 ± 0.05[a]
20:4 n-6	11.9 ± 0.4	8.7 ± 0.6[b]	19.4 ± 0.8	6.9 ± 0.3[b]
20:5 n-3	1.9 ± 0.2	1.0 ± 0.1[a]	0.8 ± 0.1	0.78 ± 0.07
22:5 n-3	1.7 ± 0.2	0.86 ± 0.05[a]	1.1 ± 0.2	0.5 ± 0.2[a]
22:6 n-3	5.7 ± 0.1	3.9 ± 0.1[b]	5.4 ± 0.3	3.3 ± 0.3[b]
24:0	0.44 ± 0.03	0.40 ± 0.03	0.44 ± 0.01	0.27 ± 0.01[b]

Data are means ± SE, $n = 6$ for each group. Fatty acids are expressed as g/100 g fatty acids.
[a] $P < 0.05$ *vs.* the control value of the same fatty acids at the same time point.
[b] $P < 0.01$ *vs.* the control value of the same fatty acids at the same time point.

Table 7. Fatty Acid Composition of Epididymal Total Lipid in Sucrose-Loaded Rats

	4 weeks		20 weeks	
Fatty acid	Control	Sucrose	Control	Sucrose
14:0	1.6 ± 0.3	2.0 ± 0.2	0.56 ± 0.04	1.08 ± 0.09[a]
16:0	24.3 ± 2.3	24.9 ± 1.1	18.3 ± 0.2	22.2 ± 1.3[b]
16:1 n-7	3.5 ± 0.8	8.0 ± 1.1[a]	1.1 ± 0.3	8.3 ± 0.3[a]
18:0	5.3 ± 0.9	4.4 ± 0.5	4.3 ± 0.2	2.6 ± 0.2[a]
18:1 n-9	24.3 ± 1.1	25.5 ± 1.0	28.8 ± 0.6	34.8 ± 1.1[b]
18:1 n-7	5.3 ± 0.8	6.8 ± 0.7	4.6 ± 0.3	9.2 ± 0.4[b]
18:2 n-6	29.0 ± 2.3	24.5 ± 1.6	36.3 ± 1.4	20.4 ± 0.6[b]
18:3 n-3	2.9 ± 0.6	1.6 ± 0.3	2.9 ± 1.9	0.69 ± 0.07
20:0	0.3 ± 0.2	0.15 ± 0.04	0.11 ± 0.08	0.05 ± 0.04
20:1 n-9	0.7 ± 0.1	0.6 ± 0.1	1.1 ± 0.2	0.50 ± 0.03[b]
20:2 n-6	0.5 ± 0.1	0.28 ± 0.06	0.4 ± 0.1	0.23 ± 0.05
20:3 n-6	0.18 ± 0.05	0.11 ± 0.04	0.14 ± 0.08	0.07 ± 0.03
20:4 n-6	0.6 ± 0.2	0.39 ± 0.09	0.43 ± 0.04	0.33 ± 0.04
22:6 n-3	1.1 ± 0.5	0.4 ± 0.2	0.6 ± 0.1	0.34 ± 0.08
24:0	0.3 ± 0.1	0.25 ± 0.05	0.34 ± 0.07	0.25 ± 0.02

Data are means ± SE, $n = 6$ for each group. Fatty acids are expressed as g/100 g fatty acids.
[a] $P < 0.01$ *vs.* the control value of the same fatty acids at the same time point.
[b] $P < 0.05$ *vs.* the control value of the same fatty acids at the same time point.

result in the deposition of visceral fat rather than subcutaneous fat. It has been well established that the turnover of lipogenesis and lipolysis is greater in visceral than in subcutaneous fat, probably as a consequence of the greater permeability of fat deposition in visceral tissue (14). In addition, sucrose loading by itself promotes hepatic lipogenesis and the release of very low density lipoprotein (VLDL) into the circulation, which in turn provides fatty acids for adipose tissue to synthesize triglyceride. As a result, sucrose loading is effective in promoting visceral fat deposition. It is well known that visceral fat deposition is a primary factor in the pathogenesis of a variety of obesity-related metabolic disorders, including diabetes, insulin resistance, and hyperlipidemia (13). This was evident in the present study—the sucrose-loaded rats developed hypertriglyceridemia during the early phase when only visceral fat deposition had started to increase. During the late phase, when the accumulation of visceral fat was more pronounced, sucrose loading induced the elevation of plasma glucose and insulin levels.

The liver and skeletal muscle also play a major role in the development of metabolic disorders, through the regulation of lipid metabolism and insulin-induced glucose disposal, respectively. The different time courses of the increases in plasma glucose, insulin, and triglycerides during the course of developing obesity suggest that some

Table 8. Fatty Acid Composition of Muscle Total Lipid in Sucrose-Loaded Rats

	4 weeks		20 weeks	
Fatty acid	Control	Sucrose	Control	Sucrose
14:0	0.45 ± 0.05	0.4 ± 0.2	0.16 ± 0.02	0.4 ± 0.2
16:0	20.0 ± 0.3	21.2 ± 0.9	22.6 ± 0.4	23.6 ± 0.6
16:1 n-7	0.22 ± 0.08	1.0 ± 0.5	0.87 ± 0.07	2.5 ± 0.6[a]
18:0	17.9 ± 0.9	15.1 ± 0.8[a]	15.8 ± 0.4	14.0 ± 1.6
18:1 n-9	7.0 ± 0.6	8.3 ± 1.4	6.8 ± 0.4	14.9 ± 2.8[a]
18:1 n-7	3.7 ± 0.2	4.8 ± 0.2[b]	3.04 ± 0.09	5.5 ± 0.3[a]
18:2 n-6	21.2 ± 0.5	17.7 ± 1.2[a]	22.0 ± 0.3	15.7 ± 0.6[b]
18:3 n-3	0.26 ± 0.04	0.7 ± 0.6	0.20 ± 0.03	0.07 ± 0.04[b]
20:0	0.05 ± 0.05	0.8 ± 0.6	0.04 ± 0.03	0.07 ± 0.02
20:1 n-9	0.17 ± 0.06	0.3 ± 0.2	0.17 ± 0.02	0.06 ± 0.04[a]
20:2 n-6	0.40 ± 0.02	0.4 ± 0.1	0.19 ± 0.01	0.07 ± 0.02[b]
20:3 n-6	1.0 ± 0.7	0.7 ± 0.1	0.30 ± 0.03	0.40 ± 0.04[a]
20:4 n-6	8.9 ± 0.9	9.0 ± 0.5	10.1 ± 0.3	7.6 ± 0.8[a]
22:6 n-3	16.5 ± 1.2	17.0 ± 1.0	17.2 ± 0.5	14.7 ± 1.8
24:0	1.1 ± 0.1	1.2 ± 0.3	0.45 ± 0.05	0.36 ± 0.06

Data are means ± SE, $n = 6$ for each group. Fatty acids are expressed as g/100 g fatty acids.
[a] $P < 0.05$ *vs.* the control value of the same fatty acids at the same time point.
[b] $P < 0.01$ *vs.* the control value of the same fatty acids at the same time point.

time- or tissue-dependent process is necessary to induce these metabolic abnormalities. It is highly probable that fatty acids are involved in this process as signal messengers, given that both the level and composition of fatty acids in the blood are markedly affected by a high-calorie diet or obesity *per se* (10). In addition, it has already been established that some fatty acid composition may be related to insulin resistance (4, 11).

Our results demonstrate that MUFA levels increased and PUFA levels decreased in liver, plasma, the epididymal fat pad, and muscle after 20 weeks of sucrose loading, at which time plasma glucose, insulin, and triglyceride levels had increased in association with an extreme deposition of visceral and subcutaneous fat tissue. These changes in fatty acid composition are consistent with the results of a previous study in humans that showed that insulin sensitivity, as assessed by the glucose clamp method, correlated negatively with MUFA levels and positively with C20-22 PUFA levels in muscle (19). Our detailed analysis of fatty acid composition provides evidence that specific fatty acids that are affected by sucrose loading in rats may be involved in the development of the increase in levels of plasma glucose, insulin, and triglycerides. The magnitude and direction of changes in tissue fatty acid levels in response to sucrose loading showed significant effects on fatty acid composition, specifically palmitoleic acid (16:1 n-7) and oleic acid (18:1 n-9) for MUFA and linoleic acid (18:2 n-6) and arachidonic acid (20:4) for PUFA. In fact, after 20 weeks of sucrose loading, the levels of 16:1 n-7 and 18:1 n-9 fatty acids increased, whereas the levels 18:2 n-6 and 20:4 n-6 fatty acids decreased in muscle, white adipose tissue (WAT), plasma, and liver, with the exception of 20:4 n-6 in WAT. These observations are in accordance with the results of several earlier studies, one of which showed an increase in serum 16:1 n-7 fatty acid levels and a decrease in serum 18:2 n-6 fatty acid levels in patients with type 2 diabetes (20). Another of these studies found that insulin sensitivity in normal human subjects correlated negatively with levels of 18:1 n-9 but not 16:1 n-7 and correlated positively with levels of 20:4 n-6 muscle fatty acids (21). However, in our study, after 4 weeks of sucrose loading, when plasma glucose and insulin levels had increased, these changes in fatty acids were observed only in the liver and plasma, with the exception of 18:2 n-6 in muscle and 16:1 n-7 in WAT. These results indicate that time- and tissue-specific changes in fatty acid composition had occurred in both muscle and fat tissue.

The time course of the development of hyperinsulinemia and fatty acid imbalances in muscle and WAT suggests the importance of these tissues in inducing metabolic disorder in response to abnormal fatty acid metabolism. In contrast, an early imbalance in fatty acid composition was observed in the liver and plasma in the absence of hyperinsulinemia. Given that the plasma triglyceride level also increased during this early phase, even short-term sucrose loading may induce abnormal fatty acid metabolism and rapidly promote hyperlipidemia in the liver and plasma. Although the percentage of dietary fat in the total calorie intake was decreased in sucrose-loaded rats, the composition of the individual fatty acid components in the diet was not different between controls and sucrose-loaded rats. Therefore, it is unlikely that dietary fat composition itself influenced the fatty acid composition in sucrose-loaded rats but rather that the excessive intake of sucrose itself and/or the decreased fat intake affected the endogenous fatty acid production and led to the fatty acid imbalance. In this regard, it is well known that a high-carbohydrate diet accelerates lipogenesis in the liver by affecting hepatic lipogenic enzymes, such as the key enzymes for fatty acid synthesis, acyl coenzyme A (CoA) carboxylase and fatty acid synthase, and an enzyme that is involved in triglyceride synthesis, glycerol 3 phosphate acyltransferase (22). Recently, it has been demonstrated that the sterol regulatory element binding protein–1c (SREBP-1c) is involved in the diet-induced upregulation of these lipogenic enzymes, including sterol CoA desaturase, an enzyme that catalyses the desaturation of SFA (23–25). It has also been reported that PUFA decreases the mRNA expression of SREBP-1c (26). Taken together, during the process in which a high sucrose diet accelerates *de novo* synthesis of fatty acids, it would be expected that the imbalance in the hepatic content of MUFA and PUFA may be accelerated. As a result, the fatty acid imbalances promote lipogenesis and induce VLDL secretion from the liver.

The increase in 16:1 n-7 and 18:1 n-9 fatty acids and the decrease in 18:2 n-6 and 20:4 n-6 fatty acids in the liver and/or plasma that preceded the other changes in our study appear to be important factors in the development of fatty acid imbalances in other target organs. This possibility led us to question how the early disruption of the fatty acid balance in the liver or plasma may influence subsequent abnormalities in glucose metabolism that are associated with the progression of obesity. There is evidence that circulating fatty acids increase hepatic glucose output, probably by increasing gluconeogensis or glycogenolysis (27). This finding and our data suggest that an early disruption of the fatty acid metabolism in the liver and plasma induces hepatic insulin resistance. In response to prolonged sucrose loading, an abnormal fatty acid metabolism develops in the muscle, thereby affecting insulin sensitivity in this tissue. The fatty acids changes that we observed in each tissue are consistent with this process in obesity-related metabolic disorder and hyperinsulinemia.

In summary, our results suggest that time- and tissue-dependent changes in fatty acid composition, especially MUFA and PUFA, may be factors in the development of hyperlipidemia and hyperinsulinemia. Within each fatty acid component, palmitoleic acid (16:1 n-7) and oleic acid (18:1 n-9) as MUFA and linoleic acid (18:2 n-6) and arachidonic acid (20:4 n-6) as PUFA have an important role in determining fatty acid composition. An early disruption in the balance of these fatty acids in the liver may cause a

metabolic derangement that induces the metabolic disorders associated with sucrose-induced obesity.

1. Pelikanova T, Kazdova L, Chvojkova S, Base J. Serum phospholipid fatty acid composition and insulin action in type 2 diabetic patients. Metabolism 50:1472–1478, 2001.
2. Clore JN, Harris PA, Li J, Azzam A, Gill R, Zuelzer W, Rizzo WB, Blackard WG. Changes in phosphatidylcholine fatty acid composition are associated with altered skeletal muscle insulin responsiveness in normal man. Metabolism 49:232–238, 2000.
3. Borkman M, Storlien LH, Pan DA, Jenkins AB, Chisholm DJ, Campbell LV. The relation between insulin sensitivity and the fatty-acid composition of skeletal-muscle phospholipids. N Engl J Med 328:238–244, 1993.
4. Storlien LH, Baur LA, Kriketos AD, Pan DA, Cooney GJ, Jenkins AB, Calvert GD, Campbell LV. Dietary fats and insulin action. Diabetologia 39:621–631, 1996.
5. Ginsberg BH, Jabour J, Spector AA. Effect of alterations in membrane lipid unsaturation on the properties of the insulin receptor of Ehrlich ascites cells. Biochim Biophys Acta 690:157–164, 1982.
6. Grunfeld C, Baird KL, Kahn CR. Maintenance of 3T3-L1 cells in culture media containing saturated fatty acids decreases insulin binding and insulin action. Biochem Biophys Res Commun 103:219–226, 1981.
7. Dresner A, Laurent D, Marcucci M, Griffin ME, Dufour S, Cline GW, Slezak LA, Andersen DK, Hundal RS, Rothman DL, Petersen KF, Shulman GI. Effects of free fatty acids on glucose transport and IRS-1–associated phosphatidylinositol 3-kinase activity. J Clin Invest 103:253–259, 1999.
8. Griffin ME, Marcucci MJ, Cline GW, Bell K, Barucci N, Lee D, Goodyear LJ, Kraegen EW, White MF, Shulman GI. Free fatty acid–induced insulin resistance is associated with activation of protein kinase C theta and alterations in the insulin signaling cascade. Diabetes 48:1270–1274, 1999.
9. Hotamisligil GS, Johnson RS, Distel RJ, Ellis R, Papaioannou VE, Spiegelman BM. Uncoupling of obesity from insulin resistance through a targeted mutation in aP2, the adipocyte fatty acid binding protein. Science 274:1377–1379, 1996.
10. Storlien LH, Hulbert AJ, Else PL. Polyunsaturated fatty acids, membrane function and metabolic diseases such as diabetes and obesity. Curr Opin Clin Nutr Metab Care 1:559–563, 1998.
11. Vessby B, Aro A, Skarfors E, Berglund L, Salminen I, Lithell H. The risk to develop NIDDM is related to the fatty acid composition of the serum cholesterol esters. Diabetes 43:1353–1357, 1994.
12. Matsuzawa Y. Pathophysiology and molecular mechanisms of visceral fat syndrome: the Japanese experience. Diabetes Metab Rev 13:3–13, 1997.
13. Fujioka S, Matsuzawa Y, Tokunaga K, Kawamoto T, Kobatake T, Keno Y, Kotani K, Yoshida S, Tarui S. Improvement of glucose and lipid metabolism associated with selective reduction of intra-abdominal visceral fat in premenopausal women with visceral fat obesity. Int J Obes 15:853–859, 1991.
14. Shimomura I, Takahashi M, Tokunaga K, Keno Y, Nakamura T, Yamashita S, Takemura K, Yamamoto T, Funahashi T, Matsuzawa Y. Rapid enhancement of acyl-CoA synthetase, LPL, and GLUT-4 mRNAs in adipose tissue of VMH rats. Am J Physiol 270:E995–E1002, 1996.
15. Fujioka S, Matsuzawa Y, Tokunaga K, Tarui S. Contribution of intra-abdominal fat accumulation to the impairment of glucose and lipid metabolism in human obesity. Metabolism 36:54–59, 1987.
16. Matsuzawa Y, Shimomura I, Nakamura T, Keno Y, Kotani K, Tokunaga K. Pathophysiology and pathogenesis of visceral fat obesity. Obes Res 3(Suppl 2):187S–194S, 1995.
17. Kim JK, Wi JK, Youn JH. Plasma free fatty acids decrease insulin-stimulated skeletal muscle glucose uptake by suppressing glycolysis in conscious rats. Diabetes 45:446–453, 1996.
18. Folch J, Lees M, Stanley GHS. A simple method for the isolation and purification of total lipids from animal tissues. J Biol Chem 226:497–509, 1957.
19. D'Alessandro ME, Lombardo YB, Chicco A. Effect of dietary fish oil on insulin sensitivity and metabolic fate of glucose in the skeletal muscle of normal rats. Ann Nutr Metab 46:114–120, 2002.
20. Vessby B, Tengblad S, Lithell H. Insulin sensitivity is related to the fatty acid composition of serum lipids and skeletal muscle phospholipids in 70-year-old men. Diabetologia 37:1044–1050, 1994.
21. Manco M, Mingrone G, Greco AV, Capristo E, Gniuli D, De Gaetano A, Gasbarrini G. Insulin resistance directly correlates with increased saturated fatty acids in skeletal muscle triglycerides. Metabolism 49:220–224, 2000.
22. Iritani N. Nutritional and hormonal regulation of lipogenic-enzyme gene expression in rat liver. Eur J Biochem 205:433–442, 1992.
23. Kakuma T, Lee Y, Unger RH. Effects of leptin, troglitazone, and dietary fat on stearoyl CoA desaturase. Biochem Biophys Res Commun 297:1259–1263, 2002.
24. Horton JD, Bashmakov Y, Shimomura I, Shimano H. Regulation of sterol regulatory element binding proteins in livers of fasted and refed mice. Proc Natl Acad Sci U S A 95:5987–5992. 1998.
25. Shimomura I, Shimano H, Korn BS, Bashmakov Y, Horton JD. Nuclear sterol regulatory element-binding proteins activate genes responsible for the entire program of unsaturated fatty acid biosynthesis in transgenic mouse liver. J Biol Chem 273:35299–35306, 1998.
26. Yahagi N, Shimano H, Hasty AH, Amemiya-Kudo M, Okazaki H, Tamura Y, Iizuka Y, Shionoiri F, Ohashi K, Osuga J, Harada K, Gotoda T, Nagai R, Ishibashi S, Yamada N. A crucial role of sterol regulatory element–binding protein–1 in the regulation of lipogenic gene expression by polyunsaturated fatty acids. J Biol Chem 274:35840–35844, 1999.
27. Bergman RN. New concepts in extracellular signaling for insulin action: the single gateway hypothesis. Recent Prog Horm Res 52:359–385, 1997.