# Desaturase Activities in Rat Model of Insulin Resistance Induced by a Sucrose-Rich Diet

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**ABSTRACT:** A sucrose-rich diet, as compared with a similar starch diet, induces a time-dependent typical noninsulin-dependent diabetes syndrome characterized by insulin resistance in rats. Within the first 3 wk, there was glucose intolerance associated with hyperinsulinemia, hypertriglyceridemia, and high plasma FFA. In this study, we examined the effect of the sucrose-rich diet vs. the starch diet during short- (3 wk) and long-term treatment (6 mon) on hepatic Δ9, Δ6, and Δ5 desaturases. These enzymes modulate monounsaturated FA and PUFA biosynthesis, respectively. Sucrose feeding (3 wk) caused an initial hyperinsulinemia that was normalized within 6 mon. In the early period (3 wk), stearoyl-CoA desaturase-1 (SCD-1) mRNA and activity were decreased, whereas Δ6 desaturase mRNA abundance and Δ6 and Δ5 desaturase activities remained unchanged. After 6 mon of sucrose feeding, activities of the Δ9, Δ6, and Δ5 desaturases were each increased. The SCD-1 and Δ6 desaturase mRNA were also correspondingly higher. These increases were consistent with an increase in oleic acid, the 20:4/18:2 ratio, and 22:4n-6 and 22:5n-6 acids in liver and muscle lipids. On the other hand, the percentage of 22:6n-3 acid was decreased. In conclusion, a sucrose-rich diet after 6 mon induces an increase in rat liver SCD-1 and Δ6 desaturase mRNA and enzymatic activities that are opposite to the changes reported in insulin-dependent diabetes mellitus. It appears that neither blood insulin levels nor insulin resistance is a factor affecting the Δ9, Δ6, and Δ5 desaturase changes in mRNA and activity found with the sucrose-rich diet.

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The two major types of diabetes mellitus are type 1, also called insulin-dependent diabetes mellitus (IDDM), which is associated with insulin deficiency, and type 2, also called noninsulin-dependent diabetes mellitus (NIDDM), which is strongly associated with insulin resistance. The latter is the most common form of the disease and has been investigated for many years (1,2). Studies have shown that alterations in carbohydrate and lipid metabolism contribute to the disease (3). Although it is generally considered that there are genetic or polygenetic explanations for type 2 diabetes (4), environmental factors also contribute to the disease process. However, the precise mechanism for these factors is yet to be determined.

Several animal models of type 2 diabetes are generally based on genetic alterations (5). However, we chose to study one based on the induction by fructose, or more specifically by a sucrose-rich diet. Similar to the genetic models, a sucrose-rich diet evokes glucose intolerance associated with hyperinsulinemia, increased plasma FFA, and hypertriglyceridemia (6,7). A three-step metabolic syndrome has been identified (8) depending on the time of administration of the sucrose diet. Step 1: Induction Period (3–5 wk) characterized by hypertriglyceridemia, a moderate increase in plasma FFA, hyperinsulinemia, and impaired glucose tolerance. Step 2: Adaptation Period (5–8 wk) featuring a spontaneous normalization of the aforementioned variables. Step 3: Recurrence Period (after 8 wk) involving moderate hyperglycemia with normoinsulinemia, hypertriglyceridemia, high plasma FFA, and severe glucose intolerance (insulin insensitivity).

A fructose diet also alters Δ9 desaturation activity. Early work (9) in our laboratories showed that after substituting fructose for dextrin in the diet for only 3 d, liver Δ9 desaturation of stearic acid was enhanced in both normal and streptozotocin-diabetic rats. In addition, Waters and Ntambi (10) recently showed that fructose, but not glucose, administration for 24 h to food-deprived diabetic mice increased the hepatic stearoyl-CoA desaturase-1 (SCD-1) mRNA 23-fold in a way similar to insulin administration. They also deduced that normal circulating insulin levels are not required for the fructose induction of the SCD-1 mRNA, and the simultaneous administration of fructose and insulin more than doubles the effect of each factor.

Given this information, we considered it important to extend our knowledge of the effect of a sucrose-rich diet and the resulting insulin resistance on the biosynthesis of PUFA modulated by Δ6 and Δ5 desaturase activities, and to compare these effects with the synthesis of the monounsaturated FA regulated by Δ9 desaturase activity. For this reason, we determined the effect of a sucrose diet on the hepatic activity of Δ6 and Δ5 desaturases, and Δ6 desaturase mRNA transcription. Moreover, the FA composition of different liver and muscle lipids was determined and correlated with changes in

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Abbreviations: IDDM insulin-dependent diabetes mellitus; NIDDM noninsulin-dependent diabetes mellitus; PPAR peroxisome proliferator-activating receptor; SCD-1 stearoyl-CoA desaturase-1.

mRNA and enzymatic activity of hepatic Δ6 desaturase and SCD-1 during the induction (3 wk) and recurrence (6 mon) periods. The results showed that a sucrose-rich diet resulted in an enhancement of Δ9, Δ6, and Δ5 desaturations after 6 mon, which was the reverse of what would be expected of an insulin resistance effect.

## MATERIALS AND METHODS

*Materials*. [1-$^{14}$C]Stearic acid (56 mCi/mmol, 98% radiochemically pure) and [1-$^{14}$C]linoleic acid (55 mCi/mmol, 99% radiochemically pure) were purchased from Amersham Life Science (Amersham, Bucks, United Kingdom). [1-$^{14}$C]-Eicosa-8,11,14-trienoic acid (52 mCi/mmol, 98% radiochemically pure) was provided by New England Nuclear (Boston, MA). Unlabeled FA were provided by Nu-Chek-Prep (Elysian, MN). Cofactors used for enzymatic reactions were obtained from Sigma Chemical (St. Louis, MO). Solvents for HPLC were purchased from Carlo Erba (Milan, Italy).

Rat SCD-1 (Δ9 desaturase) cDNA was a kind gift from Dr. Juris Ozols (Department of Biochemistry, University of Connecticut, Central Health, Farmington, CT), and rat Δ6 desaturase cDNA was a kind gift from Dr. Tsunehiro Aki (Department of Molecular Biotechnology, Hiroshima University, Higashi-Hiroshima, Japan). Restriction enzymes and other DNA nuclei-modifying enzymes were obtained from Promega (Madison, WI), and were used for Northern blot determinations of mRNA (11).

*Animal model and diets.* Male Wistar rats, weighing 180–200 g, were purchased from the National Institute of Pharmacology (Buenos Aires, Argentina), and maintained in an animal room under controlled temperature (23°C) with a fixed 12-h light/dark cycle. Animal care followed international rules for experimentation with animals. They were initially fed a standard laboratory-rat diet (Ralston Purina, St. Louis, MO). After 1 wk, the rats were randomly distributed into two groups. The experimental group received a semisynthetic sucrose-rich diet containing by weight 63% sucrose, 17% vitamin-free casein, 5% corn oil, 10% cellulose, 3.5% salt mixture (AIN-93M-MX; 7), 1% vitamin mixture (AIN-93-VX; 12), 0.2% choline chloride, and 0.3% methionine. The control group received the same semisynthetic diet but with sucrose replaced by starch. The FA composition of both diets (% by weight) was as follows: 12.54% 16:0, 0.20% 16:1, 2.77% 18:0, 32.30% 18:1n-9, 51.52% 18:2n-6, and 0.67% 18:3n-3. The rats had free access to food and water. Diets were isoenergetic, providing ~15.28 kJ/g of food. The weight of each rat was recorded twice each week, and the individual energy intake of five rats in each group was also assessed twice each week. Energy intake in kJ/d, after 3 wk of diet, was as follows: control, 361.21 ± 30.55; sucrose-rich, 372.97 ± 13.62 ($P > 0.05$). Energy intake (kJ/d) after 30 wk of diet was control, 285.4 ± 16.75; sucrose-rich, 353.23 ± 14.8, $P < 0.01$). Rats fed sucrose for 6 mon gained ~15% more weight than controls. Control and experimental rat groups were processed and analyzed after 3 wk or 6 mon.

*Analytical methods.* Blood samples obtained at the times and conditions specified in the text were rapidly centrifuged at 4°C and 200 × *g* and analyzed immediately or stored at −20°C, and examined within the next 3 d. Plasma TG (13), FFA (14), and glucose (15) were determined by spectrophotometric methods. Immunoreactive insulin levels were measured by RIA using the method of Herbert *et al*. (16).

*Liver organelle fractionation.* After the specified times, lots of five rats from each group were killed by decapitation without anesthesia and exsanguinated. The liver and the gastrocnemius muscle of each rat were excised rapidly. The liver was placed in an ice-cold homogenizing solution (1:3 wt/vol) composed of 0.25 M sucrose, 0.15 M KCl, 0.1 mM EDTA, 1.41 mM *N*-acetyl cysteine, 5 mM $MgCl_2$, and 62 mM phosphate buffer (pH 7.4). Microsomes were obtained by differential ultracentrifugation at 100,000 × *g* (Beckman Ultracentrifuge) as described elsewhere (17). Samples were stored at −80°C. Protein concentration was measured according to the procedure of Lowry *et al*. (18).

*Lipid fractionation and analyses.* Lipids were extracted from total liver homogenate, microsomes, and gastrocnemius muscle according to the procedure of Folch *et al*. (19). Total phosphorus was determined by the method of Chen *et al*. (20). Free and esterified cholesterol were separated by TLC on Whatman high-performance TLC plates (Alltech, Deerfield, IL), 20 × 20 cm (Linear-K preabsorbed strip) using hexane/ethyl ether/acetic acid (80:20:1, by vol). Free and esterified cholesterol spots were visualized by the ferric chloride method of Lowry (21). They were quantified by comparison to curves constructed using commercial standards (1–5 μg). After staining, the plates were scanned and densitometric quantitation was performed using ID Image Analyses Software (Kodak, Rochester, NY).

Total phospholipids and TAG were separated by TLC using hexane/ethyl ether/acetic acid (80:20:1 by vol). The FA composition of the lipids was determined by GLC of their methyl esters in a Hewlett-Packard HP 6890 apparatus. They were injected into an Omega Wax 250 (Supelco, Bellefonte, PA) capillary column of 30 m, 0.25 mm i.d. and 0.25 μm film. The temperature was programmed to obtain a linear increase of 3°C/min from 175 to 230°C . The chromatographic peaks were identified by comparison of their retention times with those of authentic standards.

*Δ9, Δ6, and Δ5 desaturation activity determination.* The Δ9, Δ6, and Δ5 desaturations were estimated in hepatic microsomes, using as substrates 50 μM [1-$^{14}$C]stearic acid, 33 μM [1-$^{14}$C]linoleic acid, and 50 μM [1-$^{14}$C]eicosa-8,11,14-trienoic acid, respectively. The acids were incubated with 2.5, 2.0, and 1.95 mg of microsomal protein, respectively, in a final volume of 1.5 mL at 36°C. The reaction mixture consisted of 0.25 M sucrose, 0.15 M KCl, 1.41 mM *N*-acetyl-L-cysteine, 40 mM NaF, 60 μM CoA (sodium salt), 1.3 mM ATP, 0.87 mM NADH, 5 mM $MgCl_2$, and 40 mM potassium phosphate buffer (pH 7.4). After a 1-min preincubation at 36°C, the reaction was started by the addition of microsomal protein, and the mixture was incubated in open tubes for 15 min in a thermoregulated shaking water bath. The desatura-

tion reaction was stopped with 10% (wt/vol) KOH in ethanol, followed by saponification. The extracted FFA were dissolved in methanol/water/acetic acid (85:15:0.2, by vol) and fractionated by RP-HPLC. Separations were performed on an Econosil C18, 10-μm particle size, reversed-phase column (250 × 4.6 mm) (Alltech), coupled to a guard column (10 × 4 mm) filled with pellicular C18. The mobile phase consisted of methanol/water/acetic acid (90:10:0.2, by vol) at a flow rate of 1 mL/min; a Merck-Hitachi L-6200 solvent delivery system (Darmstadt, Germany) was used. The column eluate was monitored by a UV spectrometer at 205 nm for FA identification on the basis of their retention times. The effluent was mixed with Ultima Flo-M scintillation cocktail (Packard Instruments, Downers Grove, IL) at a 1:3 ratio, and the radioactivity was measured by passing the mixture through an on-line Radiomatic Instruments Flo One-β radioactivity detector (Tampa, FL), fitted with a 0.5-mL cell, at a rate of 3 mL/min.

*Measurements of desaturase mRNA.* Total liver RNA of the different rats tested was isolated with a Wizard RNA Isolation System (Promega, Madison, WI) according to the manufacturer's instructions. Total RNA (20 μg) was size fractionated on a 1% formaldehyde gel and then transferred to a Zeta-Probe nylon membrane (Bio-Rad, Richmond, CA). The Δ9 and Δ6 desaturase and β-actin probes were prepared by incorporating [$^{32}$P]dCTP by random prime labeling. Northern blot hybridization analyses were performed as described by Sambrook *et al.* (11). The autoradiographic signals for Δ9 and Δ6 desaturase mRNA were quantified using 1D Image Analysis Software (Kodak) from multiple exposures. They were normalized to mRNA for β-actin, with all mRNA probed on the same gel.

*Statistical analyses.* The results are expressed as means ± SEM or ± SD. Significance was determined by Student's *t*-test (unpaired) or, when appropriate, data were subjected to ANOVA (Instat v. 2.0 Graph Pad Software, San Diego, CA) with diet as the main factor. The Tukey–Kramer multiple comparison test was used. Differences were considered significant at $P < 0.05$.

## RESULTS

*Blood variables.* As reported in other publications (22) and confirmed in this study, a sucrose-rich diet produced significant changes in blood metabolic variables in rats, which evolved with the duration of feeding (Table 1). In samples of blood taken from rats that had been food deprived overnight, it was determined that after 3 wk of feeding a sucrose-rich diet (Induction Period) the glycemia did not change, but the FFA and TG levels increased (Table 1). The insulinemia did not increase. After 6 mon of feeding a sucrose-rich diet (Recurrence Period), the blood samples under food-deprived conditions showed increased glycemia, FFA, and TG levels but normal insulin levels. The blood samples taken from fed rats showed similar increases in FFA and TG variables for rats fed sucrose-rich diets both short term and long term (Table 1). The glycemia was normal for the first 3 wk but increased after 6 mon. In contrast, in rats that were fed, there was a significant increase in the blood insulin level of sucrose-fed rats compared with the controls after 3 wk of treatment, which disappeared after 6 mon (Table 1).

*Effect of a sucrose-rich diet on liver Δ9 desaturation activity and the abundance of SCD-1 mRNA.* The liver microsomal Δ9 desaturation activity for the conversion of labeled stearic acid to oleic acid in control rats and rats fed a sucrose-rich diet for 3 wk and for 6 mon clearly differed (Table 2). After 3 wk of feeding the sucrose-rich diet, the liver Δ9 desaturation activity declined sharply compared with that of the control rats fed starch (Table 2). This response occurred despite the observation that the insulinemia was higher in the sucrose-fed rats. When these results were compared with the relative abundance of SCD-1 mRNA in the livers of rats fed a sucrose-rich diet and control rats, we also found a decrease in the mRNA induced by the sucrose diet (Fig. 1) that correlated rather well with the enzymatic measurements.

However, after 6 mon of sucrose feeding, when the rats were in the Recurrence Period, their Δ9 desaturation activity was more than double the control values (Table 2), despite the similarity of

**TABLE 1**
**Plasma Parameters in Rats Fed a Control or a Sucrose-Rich Diet at Different Times of the Diet[a,b]**

| | 3 wk | | 6 mon | |
|---|---|---|---|---|
| | Control | Sucrose-rich | Control | Sucrose-rich |
| Food-deprived rats | | | | |
| FFA (μequiv/L) | 440.0 ± 28.0 | 578.4 ± 20.0** | 526.0 ± 19.6 | 930.0 ± 29.4*** |
| TG (mM) | 0.33 ± 0.04 | 0.67 ± 0.01*** | 0.54 ± 0.05 | 1.20 ± 0.09*** |
| Glucose (mM) | 5.67 ± 0.2 | 5.54 ± 0.07 | 6.1 ± 0.19 | 6.98 ± 0.16** |
| Insulin (μU/mL) | 8.50 ± 0.44 | 10.34 ± 2.03 | 8.36 ± 1.48 | 7.45 ± 1.34 |
| Fed rats | | | | |
| FFA (μequiv/L) | 257 ± 22 | 487 ± 25*** | 280 ± 20 | 700 ± 40*** |
| TG (mM) | 0.56 ± 0.04 | 1.62 ± 0.07*** | 0.73 ± 0.03 | 2.28 ± 0.14*** |
| Glucose (mM) | 6.20 ± 0.04 | 6.45 ± 0.08 | 6.50 ± 0.06 | 8.20 ± 0.12*** |
| Insulin (μU/mL) | 51.2 ± 4.5 | 80.2 ± 6.1** | 48.1 ± 3.6 | 50.2 ± 4.1 |

[a]Values are means ± SEM, $n = 5$. Asterisks indicate values are different from control ***$P < 0.001$; **$P < 0.01$ evaluated by Student's *t*-test.
[b]Rats were food deprived overnight.

**TABLE 2**
**Δ9, Δ6, and Δ5 Desaturation Activities of Liver Microsomes at 3 wk and 6 mon of Sucrose Feeding[a]**

| | 3 wk | | 6 mon | |
|---|---|---|---|---|
| Desaturase | Control (a) | Sucrose-rich (b) | Control (c) | Sucrose-rich (d) |
| | [nmol product/(min·mg protein)] | | | |
| Δ9 | 0.285 ± 0.040 | 0.072 ± 0.010$^{a***}$ | 0.141 ± 0.039$^{a***}$ | 0.391 ± 0.070$^{b***,c***}$ |
| Δ6 | 0.185 ± 0.030 | 0.177 ± 0.037 | 0.150 ± 0.029 | 0.276 ± 0.056$^{b***,c***}$ |
| Δ5 | 0.111 ± 0.025 | 0.162 ± 0.027 | 0.137 ± 0.018 | 0.213 ± 0.033$^{c**}$ |

[a]Results are means ± SD, $n = 5$. Differences were evaluated using ANOVA. Different roman superscript letters indicate differences between this value and that in the group headed by that letter. ***$P < 0.001$ and **$P < 0.01$.

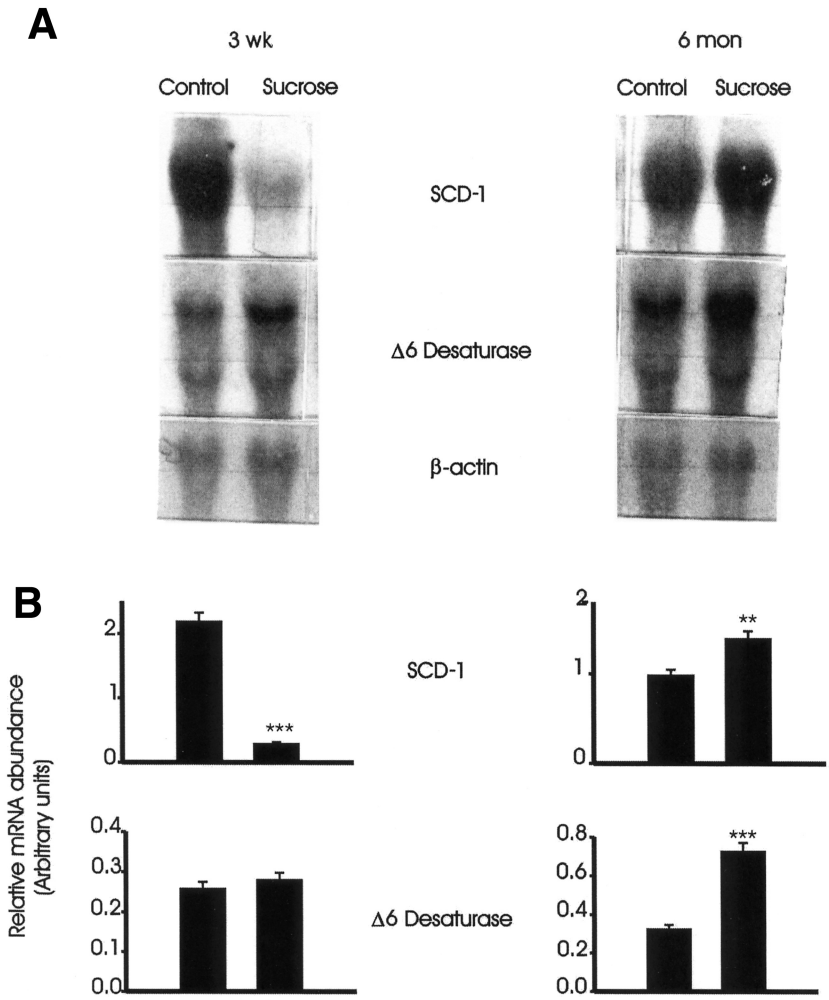


**FIG. 1.** Effect of a sucrose-rich diet on the levels of liver stearoyl-CoA desaturase-1 (SCD-1) and Δ6 desaturase mRNA. (A) Representative autoradiographs of Northern blot analysis of three rats. Total liver mRNA from control and sucrose-fed rats, at the periods indicated, were electrophoresed on a 1% agarose-formaldehyde gel, blotted to nylon membrane, and probed with $^{32}$P random primed cDNA. mRNA levels were compared with β-actin signals. (B) The signals were quantified by ID Image System Software of Northern blots representing the ratio of the intensities of desaturases mRNA to β-actin mRNA. The results are means ± SD, $n = 3$. Asterisks indicate that values are different from control, ***$P < 0.001$; **$P < 0.05$; NS, not significant as evaluated by Student's *t*-test.

blood insulin levels in control and sucrose-fed rats (Table 1). Correspondingly, liver SCD-1 mRNA abundance was also much higher in the sucrose-fed rats than in the controls (Fig. 1).

*Effect of a sucrose-rich diet on liver Δ6 and Δ5 desaturation activity and the abundance of Δ6 desaturase mRNA.* The extent of liver microsomal Δ6 and Δ5 desaturations of labeled

linoleic and eicosatrienoic n-6 acids, respectively, in rats fed a control (starch-rich diet) and a sucrose-rich diet for 3 wk or 6 mon are shown in Table 2. After 3 wk, the Δ6 and Δ5 desaturation activities did not differ between diets. After 6 mon, both the Δ6 and Δ5 desaturation activities were significantly enhanced by the sucrose-rich diet compared with the control diet. The Δ6 and Δ5 desaturation activities of control rats did not change from 3 wk to 6 mon (Table 2).

The measurement of Δ6 desaturase mRNA abundance in rat liver (Fig. 1) showed similar changes to the activities of the enzyme in sucrose-fed rats after 3 wk and 6 mon. Δ6 Desaturase mRNA and enzymatic activity increased in the rats only after 6 mon of sucrose feeding compared with rats fed the control diet for the same time period.

*Effect of diets and time on liver and muscle FA composition of lipids*. Compared with the control starch-rich diet, the sucrose-rich diet did not affect free cholesterol or esterified cholesterol of liver and liver microsomes after 3 wk or 6 mon (Table 3). In addition, the diets did not affect the proportion of phospholipid in the lipids of liver and the corresponding microsomes.

The FA composition of the lipids of rats fed for 3 wk or 6 mon is shown in Table 4 for liver, Table 5 for liver microsomes, Table 6 for liver microsomal phospholipids and TAG, and Table 7 for muscle phospholipids and TAG. In all of the lipids studied, linoleic acid was a very important component due to its high concentration in the diet, whereas oleic acid was a minor component in all of the lipids except for TAG. Arachidonic acid was a major component in all of the lipids except the TAG.

The sucrose-rich diet generally produced some changes in the FA composition in all of the samples. Changes in the level of oleic acid correlated with the Δ9 desaturase activities, showing a significant decrease in the liver microsomal phospholipids of rats fed for 3 wk (Table 6) and a significant increase in the muscle and microsomal liver phospholipids and TAG (Tables 6 and 7) of rats fed the sucrose-rich diet for 6 mon. An enhancement of the 20:4/18:2 ratio and the n-6 PUFA, 22:4n-6, and 22:5n-6 in total liver lipids (Table 4) correlated with the increase in Δ6 and Δ5 desaturase activity in rats after 6 mon of sucrose feeding. Similar results were observed in liver microsomes (Table 5), and in phospholipids of liver microsomes (Table 6) and muscle (Table 7). The sucrose-rich diet increased the n-6 PUFA/18:2n-6 ratio after 6 mon, whereas the same diet simultaneously and antagonistically decreased the proportion of DHA (22:6n-3) of the n-3 family (Tables 4–7) at both 3 wk and 6 mon.

**TABLE 3**
**Effect of the Diets on the Free and Esterified Cholesterol and Phospholipids in Total Liver and Liver Microsomes[a]**

| | 3 wk | | 6 mon | |
|---|---|---|---|---|
| | Control | Sucrose-rich | Control | Sucrose-rich |
| | (% of total lipid weight) | | | |
| Free cholesterol | 4.01 ± 0.20 | 3.71 ± 0.18 | 2.98 ± 0.43 | 3.31 ± 0.46 |
| Cholesterol esters | 2.23 ± 0.53 | 2.08 ± 0.38 | 2.34 ± 0.60 | 1.41 ± 0.53 |
| Phospholipids | 59.31 ± 2.22 | 58.79 ± 1.11 | 60.86 ± 4.52 | 66.49 ± 8.46 |
| Liver microsomes | | | | |
| Free cholesterol | 2.93 ± 0.35 | 3.48 ± 1.08 | 3.06 ± 0.21 | 2.92 ± 0.22 |
| Cholesterol esters | 1.43 ± 0.51 | 0.91 ± 0.12 | 1.06 ± 0.30 | 0.86 ± 0.25 |
| Phospholipids | 84.59 ± 5.08 | 73.96 ± 7.66 | 74.78 ± 5.48 | 65.77 ± 2.91 |

[a]Results are means ± SD, $n = 4$. Groups did not differ when analyzed by Student's *t*-test.

**TABLE 4**
**FA Composition of Total Liver[a,b]**

| | 3 wk | | 6 mon | |
|---|---|---|---|---|
| FA | Control (a) | Sucrose-rich (b) | Control (c) | Sucrose-rich (d) |
| | (g/100 g) | | | |
| 16:0 | 22.95 ± 3.08 | 21.75 ± 2.08 | 22.32 ± 1.14 | 21.04 ± 0.67 |
| 16:1 | 0.96 ± 0.20 | 1.41 ± 0.31 | 1.89 ± 0.30 | 1.69 ± 0.19 |
| 18:0 | 21.00 ± 2.88 | 20.36 ± 0.96 | 14.10 ± 1.39 | 15.44 ± 0.41 |
| 18:1n-9 | 6.31 ± 0.58 | 8.08 ± 1.00 | 10.74 ± 2.07 | 12.03 ± 0.55 |
| 18:2n-6 | 18.87 ± 2.14 | 14.65 ± 0.36 | 22.65 ± 1.35 | 16.68 ± 0.94$^{c***}$ |
| 20:4n-6 | 20.77 ± 2.50 | 22.87 ± 1.95 | 18.29 ± 1.41 | 20.52 ± 1.00 |
| 22:4n-6 | 0.42 ± 0.24 | 0.97 ± 0.16$^{a**}$ | 0.65 ± 0.08 | 1.10 ± 0.19$^{c*}$ |
| 22:5n-6 | 0.37 ± 0.14 | 2.55 ± 0.52$^{a***}$ | 0.94 ± 0.17 | 3.26 ± 0.72$^{c***}$ |
| 22:6n-3 | 6.10 ± 0.77 | 3.69 ± 0.16$^{a***}$ | 4.10 ± 0.64 | 2.96 ± 0.44 |
| 20:4/18:2 | 1.23 | 1.56 | 0.81 | 1.23 |

[a]Results are means ± SD, $n = 4$. Differences were analyzed by ANOVA. Different roman superscript letters indicate differences between this value and that in the group headed by that letter. $^{***}P < 0.001$, $^{**}P < 0.01$, $^{*}P < 0.05$.
[b]Only the main FA are tabulated. Other FA make 100%.

**TABLE 5**
**FA Composition of Liver Microsomes[a,b]**

| | 3 wk | | 6 mon | |
|---|---|---|---|---|
| FA | Control (a) | Sucrose-rich (b) | Control (c) | Sucrose-rich (d) |
| | | (g/100 g) | | |
| 16:0 | 19.95 ± 0.35 | 19.32 ± 0.76 | 21.37 ± 1.21 | 20.83 ± 2.04 |
| 16:1 | 0.90 ± 0.44 | 0.70 ± 0.13 | 0.80 ± 0.55 | 0.98 ± 0.22 |
| 18:0 | 21.39 ± 1.23 | 23.50 ± 0.47 | 18.18 ± 0.82 | 19.18 ± 1.77 |
| 18:1n-9 | 4.77 ± 0.35 | 4.73 ± 0.16 | 6.20 ± 1.46 | 7.13 ± 0.23 |
| 18:2n-6 | 13.84 ± 0.73 | 10.39 ± 0.48$^{a**}$ | 14.57 ± 1.64 | 11.41 ± 0.93$^{c**}$ |
| 20:4n-6 | 26.44 ± 0.45 | 29.47 ± 0.40 | 26.83 ± 1.97 | 27.49 ± 2.65 |
| 22:4n-6 | 0.82 ± 0.27 | 1.16 ± 0.22 | 0.59 ± 0.09 | 1.10 ± 0.22$^{c*}$ |
| 22:5n-6 | 0.52 ± 0.49 | 2.86 ± 0.60$^{a**}$ | 0.79 ± 0.16 | 3.71 ± 0.98$^{c***}$ |
| 22:6n-3 | 7.11 ± 0.69 | 4.80 ± 0.34$^{a**}$ | 6.21 ± 0.97 | 3.38 ± 0.32$^{c***}$ |
| 20:4/18:2 | 1.91 | 2.84 | 1.84 | 2.41 |

[a]Results are means ± SD, $n = 4$. Differences were analyzed by ANOVA. Different roman superscript letters indicate differences between this value and that in the group headed by that letter. $^{***}P < 0.001$, $^{**}P < 0.01$, $^{*}P < 0.05$.
[b]Only the main FA are tabulated. Other FA make 100%.

**TABLE 6**
**FA Composition of Hepatic Microsomal Phospholipids and TAG[a,b]**

| | 3 wk | | 6 mon | |
|---|---|---|---|---|
| FA | Control (a) | Sucrose-rich (b) | Control (c) | Sucrose-rich (d) |
| | | Phospholipids (g/100 g) | | |
| 16:0 | 22.95 ± 3.08 | 21.75 ± 2.08 | 22.32 ± 1.14 | 21.04 ± 0.67 |
| 16:0 | 20:17 ± 0.70 | 21.83 ± 3.93 | 22.36 ± 0.41 | 20.39 ± 1.19 |
| 16:1 | 0.56 ± 0.06 | 0.54 ± 0.05 | 0.64 ± 0.05 | 0.66 ± 0.15 |
| 18:0 | 23.42 ± 0.81 | 25.74 ± 1.06 | 21.67 ± 1.39 | 20.79 ± 0.40 |
| 18:1n-9 | 3.81 ± 0.28 | 2.65 ± 0.06$^{a***}$ | 3.24 ± 0.16 | 4.42 ± 0.27$^{c***}$ |
| 18:2n-6 | 12.60 ± 0.58 | 7.74 ± 0.53$^{a***}$ | 11.33 ± 0.64 | 9.25 ± 0.80$^{c*}$ |
| 20:4n-6 | 27.66 ± 0.69 | 29.90 ± 1.91 | 20.14 ± 1.19 | 30.79 ± 0.59$^{c***}$ |
| 22:4n-6 | 0.25 ± 0.03 | 0.95 ± 0.11$^{a***}$ | 0.50 ± 0.06 | 1.13 ± 0.08$^{c***}$ |
| 22:5n-6 | 0.17 ± 0.02 | 2.88 ± 0.78$^{a*}$ | 0.83 ± 0.28 | 4.11 ± 1.37$^{c**}$ |
| 22:6n-3 | 7.05 ± 0.53 | 4.79 ± 0.43$^{a*}$ | 6.16 ± 0.92 | 3.44 ± 0.55$^{c**}$ |
| 20:4/18:2 | 2.19 | 3.88 | 2.57 | 3.33 |
| | | TAG (g/100 g) | | |
| 16:0 | 31.34 ± 2.44 | 31.89 ± 4.49 | 23.88 ± 1.56 | 25.42 ± 0.61 |
| 16:1 | 2.41 ± 0.49 | 2.90 ± 0.19 | 2.75 ± 0.32 | 2.96 ± 0.22 |
| 18:0 | 10.41 ± 2.25 | 7.54 ± 0.99 | 5.16 ± 0.72 | 5.80 ± 0.81 |
| 18:1n-9 | 16.88 ± 1.55 | 18.47 ± 0.05 | 20.18 ± 1.73 | 26.61 ± 0.82$^{c***}$ |
| 18:2n-6 | 27.86 ± 1.45 | 27.46 ± 4.53 | 38.90 ± 0.18 | 30.41 ± 0.58$^{c*}$ |
| 20:4n-6 | 5.55 ± 0.95 | 7.35 ± 0.73$^{a*}$ | 6.86 ± 0.07 | 5.43 ± 0.32 |
| 22:6n-3 | 2.82 ± 0.24 | 0.55 ± 0.09$^{a**}$ | Trace | Trace |
| 20:4/18:2 | 0.19 | 0.27 | 0.17 | 0.18 |

[a]Results are means ± SD, $n = 4$. Differences were analyzed by ANOVA. Different roman superscript letters indicate differences between this value and that in the group headed by that letter. $^{***}P < 0.001$, $^{**}P < 0.01$, $^{*}P < 0.05$.
[b]Only the main FA are tabulated. Other FA make 100%.

## DISCUSSION

*Blood parameters and insulin resistance*. The data clearly show that during the Induction Period (3 wk), a sucrose-rich diet produces the typical plasmatic changes previously described (22), including increases in FFA and TG as well as hyperinsulinemia with normoglycemia. It is interesting to remark that this increase in plasma TG was shown (6) to be produced together with correlative increases in liver and heart TG. In addition, the group led by Lombardo (22) also demonstrated that during this period, the sucrose-rich diet moderately increased basal lipolysis and decreased the antilipolytic action of insulin in the adipocytes. Moreover, Lombardo *et al*. (7) showed that pancreas samples obtained from rats fed the sucrose-rich diet for 3 wk released three- to sixfold more insulin than those of the controls in the absence or presence of physiologic concentrations of glucose. Despite the changes in insulin secretion, a drop in glucose tolerance was shown, suggesting that a state of insulin resistance had developed. After 6 mon in the Recurrence Period, rats fed the sucrose-

**TABLE 7**
**FA Composition of Muscle Phospholipids and TAG in Rats Fed a Sucrose-Rich Diet for 6 mon[a,b]**

| | Phospholipids | | TAG | |
|---|---|---|---|---|
| FA | Control | Sucrose-rich | Control | Sucrose-rich |
| | (g/100 g) | | | |
| 16:0 | 23.82 ± 0.85 | 23.63 ± 1.08 | 24.84 ± 1.01 | 23.49 ± 2.62 |
| 16:1 | 0.91 ± 0.14 | 0.74 ± 0.22 | 5.34 ± 0.91 | 4.49 ± 0.40 |
| 18:0 | 13.72 ± 0.16 | 13.65 ± 0.72 | 9.14 ± 3.49 | 5.12 ± 1.44 |
| 18:1n-9 | 4.65 ± 0.27 | 5.84 ± 0.54** | 26.02 ± 0.68 | 33.19 ± 2.14** |
| 18:2n-6 | 17.55 ± 0.91 | 15.26 ± 1.24** | 30.39 ± 1.54 | 28.50 ± 2.64 |
| 20:4n-6 | 20.22 ± 0.71 | 22.44 ± 1.04** | 0.99 ± 0.10 | 1.10 ± 0.19 |
| 22:4n-6 | 0.74 ± 0.05 | 1.28 ± 0.14*** | ND | ND |
| 22:5n-6 | 0.87 ± 0.13 | 4.47 ± 1.15*** | ND | ND |
| 22:6n-3 | 10.84 ± 1.26 | 6.90 ± 0.53*** | ND | ND |
| 20:4/18:2 | 1.15 | 1.47 | 0.03 | 0.04 |

[a]Results are means ± SD, $n = 5$. Asterisks indicate difference from controls, ***$P < 0.001$, **$P < 0.01$ (Student's *t*-test).
[b]Only the main FA are tabulated. Other FA make 100%; ND, not detected.

rich diet progressed to normoinsulinemia compared with controls; however, increased glycemia was now present, and plasma TG and FFA remained elevated.

Similar alterations in plasma variables were shown by Soria et *al*. (22) to be evident as early as 15 wk after treatment. These data suggest a minor response of the pancreas to the glycemic stimulus. To support this suggestion, Lombardo *et al*. (23) found that a prolonged period (30 wk) of sucrose-rich diet consumption significantly increased both islet number and β-cell area in the pancreas. However, this was not accompanied by an increase in immunoreactive insulin. Therefore, it was postulated that the newly emerged β-cells had some sort of derangement due to the increased insulin demand resulting from insulin resistance induced by the long-term feeding of the sucrose-rich diet. Tobey *et al*. (6) proposed in an early work that the insulin resistance resulting from chronic fructose feeding was due to the diminished ability of insulin to suppress hepatic glucose output, and not to a decrease in insulin-stimulated glucose uptake in muscle.

However, it is important to compare this insulin-resistance syndrome evoked by the sucrose-rich diet in rats with modern results and interpretations of the human insulin resistance found in NIDDM patients. In this respect, Shulman (2) found that under steady-state plasma concentrations of insulin and glucose, muscle glycogen synthesis was ~50% lower in diabetic subjects. Because muscle glycogen synthesis accounted for most of the whole-body glucose uptake, he considered it to play a major role in causing insulin resistance in NIDDM patients. Considering the possible rate-controlling steps in this process, including glycogen synthase, hexokinase II, and glucose transport, the Shulman laboratory (2) proved that glucose transport was the most effective. Moreover, FFA are associated with insulin resistance, and their increase causes a reduction of ~50% in the insulin-stimulated rates of muscle glycogen synthesis and whole-body glucose oxidation, consequently challenging some aspects of the old model of Randle *et al.* (24). Therefore, these analyses could help to explain the relevance and importance of lipid metabolism in muscle, liver, and adipose tissue.

In addition, peroxisome proliferator-activating receptors (PPAR) are involved in insulin resistance. Thiazolidinediones, which are high-affinity ligands of the nuclear receptor PPAR-γ, enhance target tissue sensitivity to insulin *in vivo* (25) and induce other antidiabetic effects. In fructose-fed rats, it was also shown that oral administration of troglitazone, a thiazolidinedione derivative, normalizes plasma TG and FFA concentration and glucose homeostasis, and completely prevents insulin resistance in either the 3-wk (26) or 6-mon long (8) experiments, without detectable changes in plasma insulin levels. PPAR-γ plays a specific role in fat cells and in other tissues, but not in liver. It has been suggested that PPAR-γ downregulates resistin, an adipocyte-secreted signaling molecule that induces insulin resistance, which has been suggested to increase in NIDDM (27). Although this conclusion has been refuted by some investigators (28–30), Stumvoll and Häring (31) indicated that the Pro12 Ala polymorphism in PPAR-γ2 represents the first genetic variant with a notable effect on the risk of common type 2 diabetes, modulating the production and release of adipose-derived factors, i.e., insulin-desensitizing FFA, tumor necrosis factor-α, resistin, and the insulin-sensitizing hormone adiponectin. Therefore, these aspects of human type 2 diabetes mellitus and the dietary fructose effect are very similar, consequently suggesting similar mechanisms of development.

*Effect on Δ9 desaturase and monounsaturated FA biosynthesis*. The presence of high levels of fructose in a sucrose-rich diet compared with a similar diet containing only starch altered liver Δ9 desaturase (SCD-1) activity and its mRNA abundance. However, the changes were quite different depending on the duration of the sucrose-rich diet feeding.

After 3 wk, comparative decreases were found in both the Δ9 desaturation activity and mRNA in spite of the significant increase found in the blood insulin level (Table 1). It has been known for a long time (32) that insulin injection activates the

depressed Δ9 desaturation activity found in livers of experimental insulin-dependent diabetic rats by a specific protein synthesis enhancement. However, from the present results, it may be deduced that the increased blood insulin levels resulting from administration of a sucrose-rich diet for 3 wk had no activating effect on the transcription of SCD-1 mRNA and its enzymatic activity, and that insulin was incapable of modulating this enzyme.

In addition, the FA compositions of the liver and muscle lipids show that the sucrose-rich diet evoked after 3 wk a significant, correlative decrease of oleic acid in liver microsomal phospholipids, but no differences in oleic or palmitoleic acids were found in other lipids. Therefore, the changes in these FA in this short period were limited.

A different scenario emerges in the biosynthesis of monounsaturated FA after 6 mon of sucrose feeding. Compared with control rats, a significant increase in the liver SCD-1 mRNA that correlated with a corresponding increase in the enzymatic activity was observed. In this case, the increased activity of Δ9 desaturation of stearic to oleic acid was also correlated with significant increases in oleic acid in the phospholipids and TAG of muscle and in total liver microsomes, and their phospholipid and TAG fractions. In these rats, the blood insulin level had no direct effect on the increased transcription of the SCD-1 mRNA and enzymatic activity because blood insulin levels were normal (Table 1). Moreover, an insulin resistance mechanism may also be rejected because it would have exactly the opposite effect to what was observed.

The SCD-1 isoform was tested in our experiments and this isoform is the only one present in rat liver. It is also present in adipocytes together with the SCD-2 isoform, and is the one depressed there by thiazolidinedione-specific ligands of PPAR-γ (33), demonstrating its importance in enzyme modulation. Consequently, the change in SCD-1 activity in liver of sucrose-fed rats cannot be ascribed to a direct effect of PPAR-γ; however, an indirect effect may be possible. This would be in accordance with the beneficial effects shown in the normalization of plasma TG, FFA, glucose, and insulin levels, and the elimination of insulin resistance by treatment of rats fed the sucrose-rich diet with troglitazone (8). However, a recently published work (34) showed that of all the thiazolidinediones tested, only troglitazone is able to induce PPAR-γ expression in rat liver, opening questions about PPAR-γ organ specificity.

*Effect on Δ6 and Δ5 desaturases and PUFA biosynthesis.* The effect of the sucrose-rich diet vs. a starch-rich diet on the Δ6 and Δ5 desaturation activities of liver microsomes in comparison with the blood insulin level showed that the 3-wk period of sucrose administration did not change their activity in spite of the hyperinsulinemia. After 6 mon, both enzymes were significantly activated in spite of invariance in insulinemia. Therefore, an insulin resistance mechanism affecting this enhancing activation must also be rejected.

We could measure the effect of sucrose on the mRNA transcription of only the Δ6 desaturase; correlative to the enzymatic activity, the abundance of Δ6 desaturase mRNA was not altered during the first 3 wk of sucrose treatment. After 6 mon, the increased activity of the Δ6 desaturation could be ascribed to a relevant enhancement of its mRNA transcription (Fig. 1).

The sucrose activation of the Δ6 and Δ5 desaturases after 6 mon was expressed in the FA composition of liver and muscle lipids in which the 20:4n-6/18:2n-6 ratio increased in each tissue examined. However, as indicated, the proportion of DHA (22:6n-3) was decreased at all times in all of the lipids in spite of the increase in the Δ6 and Δ5 desaturation activities.

The activity of these enzymes is a very important modulating factor in the biosynthesis of 20-carbon polyunsaturated EFA of both the n-6 and n-3 families, but this modulation does not always work in controlling the level of the n-3 acid DHA (22:6n-3) (35). This difference may be ascribed to the additional microsomal steps of chain elongations from 20:5n-3 to 22:5n-3 and 24:5n-3 and further Δ6 desaturation to 24:6n-3 acid, and a peroxisomal step of β-oxidation of the 24:6n-3 to 22:6n-3 acid, which are necessary after the sequence of desaturations and elongations of 18:3n-3 to the 20-carbon PUFA controlled by the Δ6 and Δ5 desaturases.

However, the specific depression of n-3 DHA is difficult to attribute to the above-mentioned peroxisomal steps because the same steps are required for the biosynthesis of docosapentaenoic acid (22:5n-6) of the n-6 family, which is significantly increased in all of the lipids. Therefore, we hypothesize that the effect is produced at a later step of oxidation, which would selectively differentiate the n-3 from the n-6 22-carbon PUFA. Nevertheless, both the starch-rich and sucrose-rich diets were n-3 FA deficient (0.03 wt% 18:3n-3 in total food). Consequently, the data indicate that a sucrose-rich diet exacerbates this deficiency. The present results suggest that the antagonistic behavior of the NIDDM syndrome is induced by the sucrose-rich diet compared with the experimental streptozotocin diabetes (IDDM) syndrome in which the diabetes status, while decreasing the 20:4n-6/18:2n-6 ratio through a depression of Δ6 (36) and Δ5 desaturases, increases the proportion of DHA (22:6n-3) in liver lipids as shown in our laboratory (35) and confirmed by others (37). The decrease in DHA may be important from many points of view because it has been shown (38) that replacement of corn oil by cod liver oil (rich in 22:6n-3) in the sucrose-rich diet provokes a reversal of the abnormal hypertriglyceridemia, high plasmatic FFA levels, and diminished peripheral insulin sensitivity. Moreover, Storlien *et al.* (39) suggested that in muscles, n-3 PUFA counteract insulin resistance, whereas saturated acids esterified in the muscle membrane phospholipids would enhance it, and a high n-6/n-3 ratio would be deleterious.

However, in spite of the similarity of the Δ9, Δ6, and Δ5 desaturase modulation of the activity and independence from insulin and insulin resistance found in sucrose-fed rats, different mechanisms modulate the Δ9 desaturases and the Δ6 and Δ5 desaturases, and although hormones such as glucocorticoids, mineralocorticoids, testosterone, and estradiol activate Δ9 desaturase, they deactivate Δ6 and Δ5 (37–40).

In the case of the increased expression of Δ6 and Δ5 desaturase, the contribution of PPAR-α, a nuclear receptor very important in liver and specifically activated by fibrates, and not by thiazolidinediones, has been proved (41). Therefore, it is possible that PPAR-α might be involved in some way in the sucrose modulation of these enzymes. This hypothesis gains special importance when we consider the latest findings of Nagai *et al*. (42), showing that a fructose-rich diet modifies the hepatic PPAR-α mRNA content and activity. In addition, the fructose-rich diet also increases the gene expression of sterol regulatory element binding protein-1 which, as recently shown (41), raises Δ6 and Δ5 desaturase mRNA expression. Therefore, those effects are coincident with the effects found in the present experiment with sucrose, and suggest another possible mechanism of enzyme activation.

It is important to remark that the basic underlying mechanism that brings about the effects shown in our experiments resides in the metabolic differences and interactions existing between fructose (sucrose) and glucose (starch). Moreover, because the metabolic effects induced by the sucrose-rich diet are very similar to those found in human NIDDM, it is an excellent and appropriate tool for studying and disclosing the developmental steps of the disease.

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