Hepatic Δ9, Δ6, and Δ5 Desaturations in Non-Insulin-Dependent Diabetes Mellitus eSS Rats

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ABSTRACT: Both diabetes mellitus type 1 and diabetes mellitus type 2 are widespread diseases that alter carbohydrate and lipid metabolism. e Stilmann-Salgado (eSS) rats are experimental animals that spontaneously evolve to a state similar to that of young people affected by non-insulin-dependent diabetes mellitus (NIDDM; type 2). Using 6-mon-old eSS rats that, according to the literature [Martinez, S.M., Tarrés, M.C., Montenegro, S., Milo, R., Picena, J.C., Figueroa, N., and Rabasa, S.R. (1988) Spontaneous Diabetes in eSS Rats, *Acta Diabetol. Lat. 25*, 303–313], had already developed insulin resistance, we investigated the changes evoked on Δ9, Δ6, and Δ5 liver desaturases. The abundance of mRNA and enzymatic activities were measured, as well as the FA composition of liver microsomal lipids. Compared to control rats, the mRNA content and activity of SCD-1 (stearoyl CoA-desaturase, isoform of the Δ9 desaturase) were significantly higher, whereas the mRNA and activities of Δ6 and Δ5 desaturases were not significantly modified. Correspondingly, the proportion of 18:1n-9 and the ratios of 18:1n-9/18:0 and 16:1/16:0 in lipids were significantly increased, whereas the proportion of 20:4n-6 was unaltered. These effects were found while glycemia was constant or increased. The results are completely opposite those described in insulin-dependent diabetes mellitus (type 1), in which a depression of all the desaturases is found. They suggest that in eSS rats, the activities of the desaturases were not modified by an insulin-resistance effect. Moreover, we suggest that the enhancement of SCD-1 activity might be considered as another typical sign of the NIDDM syndrome, because it has also been found in other animal models of NIDDM, for example, the ones evoked by the sucrose-rich diet and in the Zucker rat.

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The two main types of diabetes are insulin-dependent diabetes mellitus (IDDM), characterized principally by its dependence on insulin secretion, and non-insulin-dependent diabetes mellitus (NIDDM), recognized fundamentally by its insulin resistance. Of the two diseases, the latter is the more common form of diabetes, and its prevalence is increasing at present. NIDDM is considered to have a polygenetic origin associated with environmental effects. Several animal models have been used to study NIDDM experimentally. Most of them are based on genetic modifications (1)—e.g., the ones called the Agouti mouse, tubby mouse, fat mouse, Zucker rat, and eSS (e Stilmann-Salgado) rat (2)—and some others are produced by dietary manipulations, e.g., the administration of a sucrose-rich diet (3).

NIDDM alters carbohydrate and lipid metabolism, and the alterations progress with aging. Plasmatic changes are generally represented by hypertriglyceridemia, an increase in FFA, increased or normoinsulinemia, and increased or normoglycemia, accompanied by glucose intolerance.

However, the effect of NIDDM on the FA desaturases and PUFA enzymatic biosynthesis has been investigated less; only in the last decade were the first studies initiated (4). In contrast, research into both monounsaturated FA and PUFA enzymatic biosynthesis in experimental IDDM started as early as the 1960s. In 1964 (5) insulin-dependent Δ9 desaturase modulation of monounsaturated FA biosynthesis was demonstrated, and in 1966 (6) a similar modulation of Δ6 desaturase activity and PUFA biosynthesis was shown.

In the present experiments we used the rat model called the eSS strain, obtained in Rosario, Argentina, by genetic manipulations (2,7). The generation of eSS rats was described in detail by Martínez *et al.* (2). These rats spontaneously progress to a diabetic state that resembles the NIDDM of young people (2,7), but the clinical repercussions are moderate. They specifically show diabetic blood glucose levels and low glucose tolerance as early as the second month of age, followed by hypertriglyceridemia. Obesity is not regularly associated with this model. Insulinemia is increased early on (7), but the production of insulin decreases with aging (2). Whereas the pancreatic islets are normal in 1-mon-old eSS rats, 6-mon-old eSS animals show disruption of the islet architecture, and the volume density of endocrine tissue and the percentage of β-cells are diminished (8).

That Δ9 desaturase modulates the biosynthesis of monounsaturated FA is well known, and by altering the ratio of 18:1n-9/18:0 FA in membrane phospholipids, it may modify cell membrane structure and fluidity. This enzyme has not been studied until now in eSS rats. On the other hand, it has been fully proved that the Δ6 and Δ5 desaturases are key enzymes in the biosynthesis and regulation of PUFA of the n-6 and n-3 families, which are relevant in mammalian physiology.

In consequence, we decided to investigate the changes

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Abbreviations: ABCA-1, ATP binding cassette transporter A-1; eSS rats, e Stilmann-Salgado rats; IDDM, insulin-dependent diabetes mellitus; NIDDM, non-insulin-dependent diabetes mellitus; PtdCho, phosphatidylcholine; SCD-1, stearoyl-CoA desaturase-1.

evoked in eSS rats on the gene expression and catalytic activity of rat liver Δ9, Δ6, and Δ5 desaturases, as well as the corresponding FA compositions of liver microsomal lipids. The results obtained were compared to data previously published using (i) the same type of rats (4,9); (ii) sucrose-fed rats (10), which also show a time-dependent NIDDM syndrome with an increase in all the desaturase activities; and (iii) streptozotocin-treated rats (11,12), which show an IDDM syndrome with a decay in all the desaturase activities.

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 (Buckinghamshire, 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 Co. (St. Louis, MO). Analytical grade solvents were purchased from Carlo Erba (Milan, Italy).

Rat cDNA of SCD-1 (stearoyl-CoA desaturase-1, isoform of the Δ9 desaturase), Δ6 desaturase, and Δ5 desaturase were kind gifts from Dr. Juris Ozols (Department of Biochemistry, University of Connecticut, Central Health, Farmington, CT), Dr. Tsunehiro Aki (Department of Molecular Biotechnology, Hiroshima University, Higashi-Hiroshima, Japan), and Drs. Reza Zolfaghari and A. Catharine Ross (Department of Nutritional Sciences, The Pennsylvania State University, University Park, PA), respectively. Restriction enzymes and other molecular biology reagents were obtained from Promega (Madison, WI). They were used for further Northern blot determinations of mRNA (13). TLC plates of silica gel 60 were from Merck (Darmstadt, Germany).

Animals. The study was performed under the international rules for animal care. Six-month-old male eSS rats were provided by the University of Rosario, School of Medicine (Rosario, Argentina). eSS and control rats were fed on a complete commercial diet (Cargill, Buenos Aires, Argentina) and water *ad libitum*. The percent FA composition (wt%) of the food was 22.5% 16:0, 1.3% 16:1, 13.7% 18:0, 25.9% 18:1n-9, 2.5% 18:1n-7, 30.7% 18:2n-6, and 3.4% 18:3n-3.

Blood samples. Blood samples, obtained by cardiac puncture, were centrifuged rapidly at 4°C and plasma was immediately stored at −20°C until further analysis. Blood glucose and TG levels were measured by commercial enzymatic methods (Wiener Lab. Test, Rosario, Argentina).

Liver subcellular fractionation. Animals were killed by decapitation without anesthesia and exsanguinated. The livers were excised rapidly and 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 (14). The microsomal fractions were stored at −80°C. Protein concentration was measured according to the procedure of Lowry *et al.* (15).

Lipid analysis. Lipids were extracted from microsomes according to the procedure of Folch *et al.* (16). Phosphatidylcholine (PtdCho) was separated from other microsomal lipids by TLC using chloroform/methanol/acetic acid/water (50:37.5:3.5:2, by vol).

FA compositions from both total lipids and PtdCho were determined by GLC of their methyl esters in a Hewlett-Packard HP 6890 apparatus. They were injected into an OMEGAWAX 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 determinations. Δ9, Δ6, and Δ5 desaturations were measured in hepatic microsomes using as substrates 50 μM [1-^{14}C]stearic acid, 50 μM [1-^{14}C]-linoleic acid, and 30 μM [1-^{14}C]eicosa-8,11,14-trienoic acid, respectively. Substrates were incubated with 2.5 mg of microsomal protein, in a final volume of 1.5 mL at 36°C. The reaction 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 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 desaturation 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 C_{18}, 10 μm particle size, reversed-phase column (250 × 4.6 mm) (Alltech Associates, Inc., Deerfield, IL), coupled to a guard column (10 × 4 mm) filled with pellicular C_{18}. The mobile phase consisted of methanol/water/acetic acid (90:10:0.2, by vol) at a flow rate of 1 mL/min, and 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 detector fitted with a 0.5-mL cell at a rate of 3 mL/min.

Measurements of mRNA desaturases. Total liver RNA of different animals tested was isolated with Wizard RNA Isolation System (Promega) according to the manufacturer's instructions. Twenty micrograms of total RNA was size-fractionated on a 1% agarose-formaldehyde gel and then transferred to a Zeta-Probe nylon membrane (Bio-Rad, Richmond, CA). The SCD-1, Δ6 and Δ5 desaturases, 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.* (13). The autoradiographic signals for SCD-1, Δ6 desaturase, and Δ5 desaturase mRNA were quantified using 1D Image Analysis Software (Kodak, Rochester, NY) from multiple exposures. They were normalized to mRNA for β-actin, with all the mRNA probed on the

same gel. Northern blot analyses were performed using an unpaired *t*-test.

Statistical analyses. Results are expressed as means ± SD. Statistical significance was determined by Student's *t*-test. $P < 0.05$ was accepted as statistically significant.

RESULTS

Experiment 1. In a first experiment, the fasting glucose concentration of 6-mon-old male eSS rats revealed a glycemic profile similar to but statistically higher than ($P < 0.05$) that of control rats (controls 1.19 ± 0.08 g/L; eSS rats 1.36 ± 0.05 g/L), equivalent to that found by Martínez *et al.* (2) in eSS rats of that age. At age 6 mon this type of rat has already developed mild NIDDM with an abnormal glucose tolerance test, as described by Martínez *et al.* (2,9).

(i) FA desaturation activity. Δ9 desaturation activity, which may be considered to measure the eSS-1 isoform, and Δ6 desaturation activity of liver microsomes of the eSS rats compared to controls are displayed in Table 1. Results showed a 6.7-fold increase in Δ9 desaturase activity, as measured by conversion of labeled stearic acid to oleic acid, but no statistically significant change in the Δ6 desaturation of linoleic acid was observed.

(ii) mRNA of SCD-1 and Δ6 and Δ5 desaturases. Changes in the abundance of mRNA of the SCD-1 isoform of Δ9 desaturase and Δ6 desaturase in eSS rat livers compared to controls are shown in Figure 1. In this assay the comparative increase in the SCD-1 mRNA found in eSS rats correlates quite well with the enhancement of liver Δ9 desaturase activity, as shown in Table 1. However, the Δ6 desaturase mRNA was higher in eSS rats (Fig. 1), whereas the enzymatic activity of this desaturase (Table 1) was constant. Therefore, it did not increase correlatively as expected. The Δ5 desaturase mRNA was not modified.

(iii) FA composition of liver microsomes. From the preceding results, it might be expected that the proportions of palmitoleic (16:1) and oleic (18:1n-9) acids and the ratios of 16:1/16:0 and 18:1n-9/18:0 in the microsomal liver lipids of eSS rats would be enhanced owing to the increase in Δ9 desaturase. These results are shown in Table 2, which gives FA compositions of liver microsomes. The FA 16:1 and 18:1n-9 increased by 1.6- and 0.6-fold, respectively, in the total lipid fraction of eSS diabetic rats compared to controls. Additionally, the ratios of 16:1/16:0 and 18:1n-9/18:0 were 1.4- and 0.7-fold higher, respectively, in the diabetic rats compared to normal control rats.

On the other hand, the absence of a statistically significant change in arachidonic acid (20:4n-6) (Table 2) correlates with the unchanged Δ6 and Δ5 desaturase activities. Notwithstanding, a small increase in the minor FA 20:3n-6 was found in eSS rats, and this enhancement correlated with a decrease in 18:2n-6, which might suggest an activation of Δ6 desaturation. A significant increase (38%) in DHA (22:6n-3) of the n-3 family is shown in Table 2, indicating a different type of effect compared to the 20-carbon PUFA generally found in diabetic rats (11,12).

Experiment 2. In this experiment, a new lot of 6-mon-old eSS rats was studied to check the previous results. This lot showed a glycemia that was not statistically significant compared to control animals (controls 1.25 ± 0.09 g/L, eSS rats

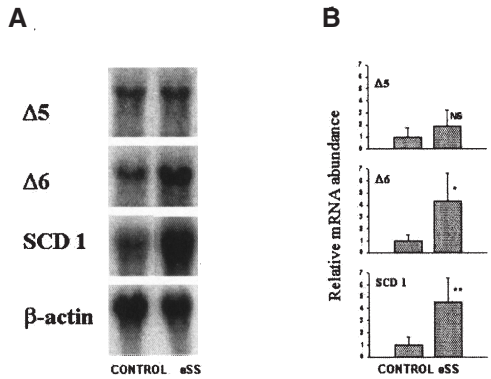

FIG. 1. mRNA levels of liver stearoyl-CoA desaturase-1 (SCD-1) and Δ6 and Δ5 desaturases in Experiment 1. (A) Representative autoradiographs of a Northern blot analysis. Total liver mRNA were electrophoresed on a 1% agarose formaldehyde gel, blotted to nylon membrane and probed with ^{32}P random-primed cDNA. mRNA levels were compared to β-actin samples. (B) The signals of Northern blots representing the ratio of the intensities of desaturase mRNA to β-actin mRNA were quantified by 1D Image Analysis Software (Kodak, Rochester, NY) and normalized. Results are the mean ± SD, $n = 3$. Statistical significance was analyzed by Student's *t*-test. $^{**}P < 0.01$, $^{*}P < 0.05$, NS, not significant. eSS, e Stilman-Salgado rats.

TABLE 1
Δ9 and Δ6 Desaturation Activities of Liver Microsomes in Experiment 1[a]

Desaturases	Control	eSS
Δ9	0.013 ± 0.001	0.100 ± 0.015***
Δ6	0.174 ± 0.067	0.265 ± 0.035

[a][1-^{14}C]Stearic acid and [1-^{14}C]linoleic acid, respectively, were used as substrates. Results, expressed as nmol product/min·mg protein, are the mean ± SD, $n = 3$. They were evaluated by Student's *t*-test. $^{***}P < 0.001$. eSS, e Stilmann-Salgado rats.

TABLE 2
FA Composition (g/100 g) of Liver Microsomes in Experiment 1[a]

FA	Control	eSS
16:0	17.80 ± 0.23	18.76 ± 1.12
16:1	0.26 ± 0.14	0.67 ± 0.04*
18:0	24.95 ± 1.12	22.68 ± 0.18
18:1n-9	4.30 ± 0.54	6.78 ± 0.72**
18:1n-7	1.78 ± 0.51	2.39 ± 0.07
18:2n-6	13.87 ± 1.15	10.28 ± 0.09*
20:3n-6	0.32 ± 0.03	0.61 ± 0.01***
20:4n-6	28.75 ± 1.04	27.42 ± 1.07
22:4n-6	0.38 ± 0.05	0.35 ± 0.02
22:5n-6	0.14 ± 0.04	0.20 ± 0.05
22:5n-3	0.95 ± 0.11	0.85 ± 0.05
22:6n-3	6.50 ± 0.41	9.01 ± 0.50**
16:1/16:0	0.015	0.036
18:1n-9/18:0	0.172	0.299
20:4n-6/18:2n-6	2.073	2.667

[a]Only the principal FA were considered. Data are the mean ± SD, $n = 3$. $^{***}P < 0.001$; $^{**}P < 0.01$; $^{*}P < 0.05$, evaluated by Student's *t*-test. For abbreviation see Table 1.

TABLE 3
Δ6 and Δ5 Desaturation Activities of Liver Microsomes in Experiment 2[a]

Desaturases	Control	eSS
Δ6	0.174 ± 0.036	0.181 ± 0.026
Δ5	0.147 ± 0.039	0.096 ± 0.020

[a][1-^{14}C]Linoleic acid and [1-^{14}C]eicosatrienoic n-6 acid, respectively, were used as substrates. Results, expressed as nmol product/min·mg protein, are the mean ± SD, $n = 4$. Differences were not significant when evaluated by Student's *t*-test. For abbreviation see Table 1.

1.34 ± 0.07 g/L). Gómez Dumm *et al.* (9) found similar results for eSS rats of the same age.

The triglyceridemia measured in these eSS rats (four animals) indicated a statistically significant ($P < 0.005$) increase (eSS rat TG 2.14 ± 0.61 g/L vs. control rat TG 0.49 ± 0.10 g/L), typical of the NIDDM syndrome.

(i) FA desaturation activity and abundance of mRNA. Δ6 and Δ5 desaturations of [1-^{14}C]linoleic and [1-^{14}C]eicosa-8,11,14-trienoic acids, respectively, found in the eSS rat liver are shown in Table 3. The activity of both enzymes was not modified significantly in eSS rats compared to the controls. The results for Δ6 desaturase reproduced those found in the first experiment (Table 1). They correlated well with the absence of alteration in the level of mRNA of both Δ6 and Δ5 desaturases in the liver (Fig. 2), indicating the absence of a change in their expression.

In the second experiment (Fig. 2), the eSS animals again showed a significant, approximately fourfold enhancement of liver SCD-1 mRNA.

(ii) FA composition of liver microsomal lipids. The FA compositions of liver microsomal total lipids and PtdCho of eSS rats are listed in Table 4. They showed, first, that oleic acid (18:1n-9) and the ratio of 18:1n-9/18:0 were increased in both types of lipids when compared to control rats (*cf.* Table 2). Palmitoleic acid (16:1n-7) was also increased in PtdChol and the ratio of 16:1/16:0 was increased in both lipids. Moreover, microsomal PtdCho also showed a statistical increase of 18:1n-7, which would be the elongated product of 16:1n-7. Therefore, the changes in 18:1n-9, 16:1, and 18:1n-7 were in accordance with an increase in Δ9 desaturation activity.

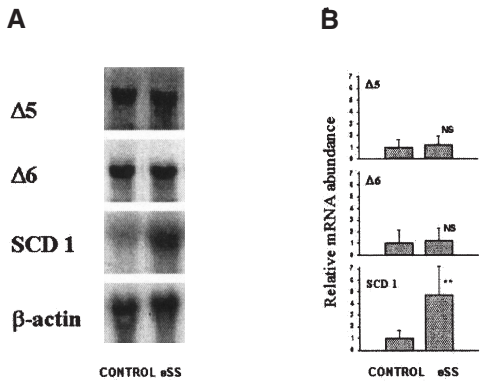

FIG. 2. mRNA levels of liver SCD-1 and Δ6 and Δ5 desaturases in Experiment 2. (A) Representative autoradiographs of a Northern blot analysis; (B) quantified signals. Procedures are as described in Figure 1. Results are the mean ± SD, $n = 3$. Statistical significance was analyzed by Student's *t*-test $^{**}P < 0.01$, NS, not significant; for other abbreviations see Figure 1.

TABLE 4
FA Composition (g/100 g) of Liver Microsomal Lipids of Experiment 2[a]

	Total lipids	
FA	Control	eSS
16:0	20.79 ± 4.04	19.95 ± 1.14
16:1	0.93 ± 0.64	1.03 ± 0.24
18:0	25.69 ± 0.42	24.17 ± 0.16***
18:1n-9	4.69 ± 0.81	6.32 ± 0.22**
18:1n-7	1.95 ± 0.65	2.24 ± 0.11
18:2n-6	13.44 ± 1.33	14.16 ± 0.98
20:3n-6	0.49 ± 0.06	0.96 ± 0.14***
20:4n-6	25.04 ± 3.56	25.16 ± 1.24
22:4n-6	0.31 ± 0.12	0.27 ± 0.02
22:5n-6	0.02 ± 0.05	0.14 ± 0.02**
22:5n-3	0.71 ± 0.14	0.74 ± 0.07
22:6n-3	5.94 ± 1.44	4.86 ± 0.37
16:1/16:0	0.045	0.052
18:1n-9/18:0	0.183	0.261
20:4n-6/18:2n-6	1.863	1.777
	Phosphatidylcholine of microsomal lipids	
FA	Control	eSS
16:0	21.57 ± 1.17	21.83 ± 0.17
16:1	0.79 ± 0.23	1.26 ± 0.09**
18:0	27.26 ± 0.63	25.73 ± 0.35**
18:1n-9	3.83 ± 0.19	5.08 ± 0.36***
18:1n-7	1.68 ± 0.01	2.24 ± 0.17***
18:2n-6	13.82 ± 1.10	13.71 ± 1.24
20:3n-6	0.63 ± 0.08	0.90 ± 0.16*
20:4n-6	25.58 ± 1.98	25.51 ± 1.39
22:4n-6	0.20 ± 0.14	0.11 ± 0.13
22:5n-6	0.01 ± 0.03	0.09 ± 0.02**
22:5n-3	0.46 ± 0.09	0.41 ± 0.07
22:6n-3	4.16 ± 0.55	3.13 ± 0.52*
16:1/16:0	0.036	0.058
18:1n-9/18:0	0.140	0.197
20:4n-6/18:2n-6	1.851	1.861

[a]Only the principal FA were considered. Data are the mean ± SD, $n = 4$. $^{***}P < 0.001$; $^{**}P < 0.01$; $^{*}P < 0.05$, evaluated by Student's *t*-test. For abbreviation see Table 1.

The lack of change in the amounts of arachidonic acid (20:4n-6) and the invariant 20:4n-6/18:2n-6 ratio shown in microsomal lipids and PtdCho (Table 4) were also in accordance with the unaltered Δ6 and Δ5 desaturations and mRNA levels of both enzymes, as illustrated in Table 3 and Figure 2. However a small increase was found in the minor acids 20:3n-6 and 22:5n-6.

Unlike the microsomal FA composition shown in Table 2, the amount of the n-3 FA DHA (22:6n-3) remained almost unchanged in the second experiment.

DISCUSSION

Previous studies (2,7) have shown that eSS rats develop fasting hyperglycemia, abnormal glucose tolerance, and hyperinsulinemia from a rather early age. The percentage of male eSS animals showing hyperglycemia increased from 16% at 1 mon to 64.7% at 2.5 mon and to 90.5% at 10 mon. Marked glucose intolerance was found at 4, 6–8, 10–13, and 15–18 mon of age, together with slowly declining hyperinsulinemia measured at the same ages, which was no longer significant at 15–18 mon.

In addition, a sequential morphological study of pancreatic islets (8) did not reveal any significant changes in 1-mon-old eSS rats. But at 6 mon they showed a disruption of the islet architecture and fibrosis in the stroma, suggesting a hyperplasia of β-cell mass. At 18 mon eSS rats also showed conspicuous islet lesions, which might indicate that the capacity for β-cell replication was much lower or exhausted. All these results led to the conclusion that eSS rats developed insulin resistance rather early, as evidenced by their reduced capacity to normalize glucose homeostasis. This insulin ineffectiveness was partially compensated for early on by an increase in insulin secretion. However, in older rats insulin secretion was also diminished.

In the present study we chose 6-mon-old eSS rats because previously published data (8) indicated they would be in a rather early period of NIDDM, but with a net NIDDM syndrome. In 5-mon-old rats, Gómez Dumm *et al.* (9) also found another typical sign of NIDDM syndrome in the eSS rats, i.e., an increase in triglyceridemia, but they also found normoglycemia.

The eSS rats used in the present experiments were raised in the same laboratory where this line of animals was first bred and that provided the rats for earlier investigations (2,7–9). Similar to the results of Martínez *et al.* (2) and Gómez Dumm *et al.* (9), our results obtained using eSS rats showed hypertriglyceridemia and normo- or hyperglycemia compared to control rats. These data and others reported by the authors mentioned in previous works (2,9) indicated that 6-mon-old eSS rats showed insulin resistance in the regulation of glucose homeostasis.

In experimental IDDM, it was shown long ago (5,6), and since then fully and repeatedly confirmed (12,17,18), that insulin promotes the recovery of SCD-1, Δ6 and Δ5 desaturase mRNA, and enzymatic activities depressed in IDDM. However, in the 6-mon-old eSS rats described in previous publications (2,7) that were considered to have developed insulin resistance, we found a very significant increase in both mRNA and the enzymatic activity of the liver SCD-1 isoform of Δ9 desaturase. Moreover, liver Δ6 and Δ5 desaturase mRNA and enzymatic activities, instead of being decreased as would be expected, were unmodified except for one case in which the Δ6 desaturase mRNA was increased (Fig. 1).

It is important to remark that the enhancement of mRNA and the activity of liver SCD-1 shown in this study were correlated with increases in oleic acid (18:1n-9), in the ratios of 18:1n-9/18:0 and 16:1/16:0 in liver microsomal lipids (Tables 2 and 4), and in microsomal PtdCho (Table 4). In this phospholipid an increase in 18:1n-7 was also shown. Since Brenner (11,12) and others (19) have repeatedly shown that the changes in unsaturated FA composition of liver microsomal phospholipids and, more precisely, of PtdCho are good sensors of changes in FA desaturation, the data in Tables 2 and 4 would confirm the increase in activity of Δ9 desaturase (SCD-1 isoform). Also, they indicate the increase in activity of this enzyme and a relevant effect on the lipid composition and the structural and biophysical properties of liver membranes, as well as other possible and outstanding effects such as the enhancement of TG biosynthesis or an increase in plasma TG, as shown by Ntambi's group (20). In this respect, according to Listenberger *et al.* (21), the increase in oleic acid (18:1n-9) at the cellular level that leads to the accumulation of TG rescues palmitate-induced apoptosis by channeling palmitate into TG pools and away from pathways leading to apoptosis. Moreover, an increase in the ratios of monounsaturated/saturated FA in the membrane phospholipids of eSS rats, as mentioned above, would favor an increase in the proportion of disordered liquid crystalline domains of the bilayers at the expense of ordered membrane regions, evoking an enhancement of fluidity and permeability (22). In that respect, Sun *et al.* (22) demonstrated that whereas overexpression of SCD-1 and SCD-2 activity increased passive cholesterol efflux in cells, it inhibited ATP-binding cassette transporter A-1 (ABCA-1) transporter-mediated cholesterol efflux.

The results of this study for NIDDM rats are quite opposite those detected in IDDM experimental animals (18,23), in which the ratios of monounsaturated/saturated FA are depressed by the effect of decrease in Δ9 desaturation of the saturated FA, producing a decline in the disordered liquid crystalline domains of membranes. Similarly, increases in arachidonic acid and the ratio of 20:4n-6/18:2n-6 were not found in the same microsomal lipids (Table 4), which is in good accordance with the unchanged Δ6 and Δ5 desaturase activities (Table 3) and the corresponding mRNA (Fig. 2).

In consequence, all these data regarding Δ9, Δ6, and Δ5 desaturases cannot be ascribed to and are apparently opposite to an insulin-resistance effect.

However, research done in 1993 by Gómez Dumm *et al.* (4) investigating the activities of the Δ6 and Δ5 desaturases in 10-mon-old eSS rats found that the animals were slightly hyperglycemic, that they showed a diabetic glucose-tolerance profile, and that the hepatic Δ6 and Δ5 *in vitro* desaturations of linoleic and eicosa-8,11,14-trienoic acids, respectively, were decreased. Therefore, these results might be attributed to an insulin-resistance effect. On the other hand, we found (10) that a sucrose-rich diet provoked the NIDDM syndrome in normal rats after 6 mon as characterized by hyperglycemia, hypertriglyceridemia, and high plasma FFA but normoinsulinemia. In these animals we also found increased mRNA of the hepatic SCD-1 and Δ6 desaturase, which correlated with increased activities of both desaturases. In addition, Δ5 desaturation activity was also enhanced. In those animals, which also showed the NIDDM syndrome, the independence of desaturase activities from an insulin-resistance effect was found to be similar to the eSS rats in our present investigation. Waters and Ntambi (18) also found an increase in SCD-1 gene expression independent of insulinemia in streptozotocin diabetic mice when using a fructose diet.

Therefore, in comparing all these results, we may conclude that eSS rats exhibit an increase in liver SCD-1 gene expression and enzymatic activity along with the development of the NIDDM syndrome. Moreover, Δ9, Δ6, and Δ5 desaturase activities are not altered by an insulin-resistance effect, and a direct effect of insulinemia is doubtful. They would show that in eSS rats and in animals receiving a sucrose-rich diet (10), liver insulin resistance is selective. It would impair the suppression of gluconeogenesis and glycemic homeostasis without an impairment of Δ9 desaturation of FA.

In addition, our present results with the eSS rats, with Zucker rats (24,25), and with the effect of a sucrose-rich diet (10),

together with the results of Ntambi's group (20), lead us to suggest that an increase in Δ9 desaturase activity may be added as a typical and very important signal to the other previously known alterations of carbohydrate and lipid metabolism found in the NIDDM syndrome. This increase in Δ9 desaturase may even be a means by which cells react in an attempt to protect themselves from apoptosis and other deleterious effects evoked by FFA.

In consequence, we reached the interesting conclusion that, in our experimental group of eSS rats and in rats fed a sucrose-rich diet (10), both showing the NIDDM syndrome, other factors different from insulin level may have been altered and are modulating the activity of the two groups of desaturases, the Δ9 and the Δ6 and Δ5. With respect to this conclusion, we have to consider that many biological factors such as peroxisome proliferative-activated receptor-α and -γ, sterol regulatory element-binding proteins, leptin, and ABCA-1 transporters are known to be involved in modulation of FA desaturation; moreover, they are apparently related to insulin effects and therefore may be involved in the effects described in eSS rats.

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