Long-Term Administration of a Sucrose-Rich Diet to Normal Rats: Relationship Between Metabolic and Hormonal Profiles and Morphological Changes in the Endocrine Pancreas

Y.B. Lombardo, S. Drago, A. Chicco, P. Fainstein-Day, R. Gutman, J.J. Gagliardino, and C.L. Gomez Dumm

The aim of the present investigation was to study normal rats fed a sucrose-rich diet (SRD) for a prolonged period (up to 30 weeks) (1) to obtain additional data on the hormonal and metabolic patterns induced by this treatment and (2) to provide information on changes taking place in the pancreatic islet cell populations. We found that long-term feeding with a SRD resulted in a steady state of hypertriglyceridemia and hyperglycemia in which insulin levels remained unchanged and unable to compensate for the increased demands of the developing metabolic changes. The endocrine pancreas showed a significant increase of both islet number and B-cell area, as well as changes in the profile of islet cell distribution. However, these changes were not accompanied by an increase in the pancreatic content of immunoreactive insulin (IRI). It may therefore be postulated that the newly emerged B-cell mass has some sort of derangement with the increased insulin demand resulting from insulin resistance induced by the long-term SRD feeding. Thus, feeding a SRD to normal rats may prove to be an attractive animal model for studying the role of environmental nutritional factors in the unsettled issue of the relationship between insulin resistance and relative insulin deficiency. The model might provide key information for understanding the pathophysiology of human diseases such as type II diabetes, dyslipidemia, and a number of entities included in so-called syndrome X.

NORMAL RATS fed a sucrose- or fructose-rich diet develop hypertriglyceridemia, hyperinsulinemia, impaired glucose tolerance, and decreased insulin action (insulin resistance) in several target tissues, as reported by many investigators, including ourselves.[1-7] It has also been shown that insulin resistance is associated with an oversupply of lipids, either through increased circulating free fatty acids (FFA) and triacylglycerol or by increased triacylglycerol storage in insulin-responsive tissues.[8-9]

In most of the studies mentioned above, the sucrose-rich diet (SRD) was administered for a short period (3 to 5 weeks), usually referred to as an induction period. However, we have shown that the metabolic and hormonal profile obtained in rats fed for up to 15 weeks highly depends on both the length of time and the amount of sucrose administered.[10-12]

There are limited data available at present on the effects of administering a SRD or fructose-rich diet for longer than 4 months on glucose metabolism, insulin secretion, and insulin sensitivity. Nearly two decades ago, Cohen et al[13-14] reported decreased glucose tolerance, increased plasma triglyceride and cholesterol levels, and insulin resistance in genetically selected rats fed on high fructose or sucrose (72% wt/wt) for 8 months. Boot-Handford and Heath[15] showed increased kidney weight and glomerulosclerosis in normal male Wistar rats after feeding a SRD (68% wt/wt) for 6 months. Administration of a moderately rich (15% to 20% wt/wt) fructose diet for 9 months to Wistar rats resulted in an increase of hepatic and epididymal fat pad lipogenic enzyme activities, whereas plasma triglycerides and cholesterol levels remained unchanged.[16] Moreover, impaired glucose tolerance and increased insulin response to a glucose load were observed in rats after 3 to 9 months on the same diet.[16-17] Finally, a SRD increases the severity of glucose intolerance and glucosuria in rats with streptozotocin-induced diabetes.[18]

Since we have previously shown that plasma glucose and insulin levels in hypertriglyceridemic rats fed a SRD for 15 weeks evolve from normoglycemia with hyperinsulinemia (induction period, 3 to 5 weeks on SRD) to hyperglycemia with normoinsulinemia (recurrence period, 10 to 15 weeks on SRD), the aim of the present investigation was to study normal rats fed a SRD for a prolonged period (up to 30 weeks) (1) to obtain additional data on the hormonal and metabolic patterns induced by this treatment and (2) to provide information on changes taking place in the pancreatic islet cell populations.

MATERIALS AND METHODS

Animals and Diets

Male Wistar rats weighing 180 to 200 g (National Institute of Pharmacology, Buenos Aires, Argentina) were maintained in a temperature-controlled room (23°C) with a fixed 12-hour light/dark cycle. They were initially fed standard rat laboratory chow (Ralston Purina, St Louis, MO) to standardize the nutritional status, and given free access to water. After 1 week, the rats were randomly divided into two groups: the experimental group received a semisynthetic SRD (63% wt/wt), and rats on the control diet (CD) received the same semisynthetic diet but with sucrose replaced by starch (63% wt/wt). The preparation, nutritional components, and handling of the SRD and CD have been previously reported in detail.[12] Both diets provided approximately 15.28 KJ/g chow. The animals had free access to food and water and were maintained on the respective diets for a period of 30 weeks.

The weight of each animal was recorded twice per week during the experimental period. In a separate experiment, the individual caloric intake and weight gain of at least 10 animals in each group

From the Department of Biochemistry, University of Litoral, Santa Fe; Department of Med Expt, Hospital Italiano, Buenos Aires; and Centro de Endocrinologia Experimental y Aplicada, University of La Plata, Conicet, La Plata, Argentina.

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Address reprint requests to Y.B. Lombardo, PhD, Department of Biochemistry, University of Litoral, Ciudad Universitaria, Paraje "El Pozo," C.C. 530 (3000) Santa Fe, Argentina.

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were assessed twice per week. Details of the methodology used have been described elsewhere.[12]

On the day of the experiment, food was removed at 9:00 AM unless otherwise indicated, and experiments were performed between 9:00 AM and noon. The experimental protocol was approved by the Human and Animal Research Committee of the School of Biochemistry, Universidad del Litoral, Argentina.

Analytical Methods

Rats were anesthetized with an intraperitoneal injection of pentobarbital (60 mg/kg body weight); blood samples were drawn from the jugular vein and immediately centrifuged at 4°C. The serum samples obtained were assayed either immediately or within the next 3 days after having been stored at −20°C. Serum triacylglycerol,[19] FFA,[20] and plasma glucose[21] were determined by spectrophotometric methods. Immunoreactive insulin (IRI) levels were measured by radioimmunoassay (RIA) using the method of Herbert et al.[22]

Intravenous Glucose Tolerance Test

Animals fasted for 16 to 18 hours received an intravenous (IV) injection of 25% glucose (1 mL/100 g body weight) and were kept in a 28°C environment throughout the blood-sampling period. Details of the methodology used have been reported previously.[10]

Pancreatic Insulin Content

Pancreata from anesthetized rats were immediately removed and minced into small pieces (2 to 3 mm) in 27 mL acid-ethanol at 4°C. Insulin was extracted from the pancreas according to the procedure described by Davoren.[23] The resulting precipitate of crude insulin was separated by centrifugation at room temperature for 20 minutes at 10,000 × *g*, resuspended in a small volume (~0.3 mL) of 0.01N HCl, diluted properly, and measured by RIA.[22] Insulin content was expressed as milliunits per milligram wet tissue.

Islet Cell Population Studies

After removal of the whole pancreas, the fat tissue was carefully dissected away and the wet weight of the total gland recorded. Samples of the tail of the pancreas were then fixed in Bouin's fluid and embedded in paraffin, and serial sections thinner than 5 μm were obtained from different levels of the block. Each section from a given series was mounted on separate slides to stain adjacent sections for immunocytochemical identification of (1) insulin-secreting cells (B cells) and (2) glucagon-, somatostatin-, and pancreatic polypeptide–secreting cells (non-B cells). For this purpose, specimens were incubated with appropriate dilutions of (1) our own guinea pig antiinsulin serum and (2) a mixture of the other three rabbit antisera: antiglucagon, anti–pancreatic polypeptide (both kindly provided by Dr Lise Heding, Novo Nordisk, Copenhagen, Denmark), and antisomatostatin (a gift from Dr Suad Efendic, Department of Endocrinology, Karolinska Institute). Controls for serologic specificity were made by preincubating a given antiserum with an excess of the corresponding hormone for 24 hours at 4°C.[24] Sections were counterstained with hematoxylin. Morphometrical analysis was made by the point-counting method[25] using an 8- × 8-mm grid (256 squares and 289 intersections) mounted on the eyepiece of the microscope. To obtain the values of different parameters for B and non-B cells in the same section, we first processed the tissue for detection of B cells by using antiinsulin serum, and then made the morphometrical measurements. In a second step, and on the top of the same section, we added the immunostain for detecting non-B cells and made the measurement again. Values for non-B cells resulted from the difference between both evaluations. Using this procedure, 11,639 and 20,314 insular cells were surveyed for the CD and SRD groups, respectively.

With the data recorded, we calculated a unit area of total pancreas (15,872 intersections of the grid = 10 μm) excluding connective tissue. We were then able to obtain the area occupied by endocrine pancreas, exocrine pancreas, total pancreas, B cells, and non-B cells, and several ratios and relationships as described in the results. We also estimated the number of islets per unit area. In addition, the ratio between islet cell area and the number of islet cells (B- and non-B) were calculated to obtain cell sizes.

Every islet or small group of endocrine cells was recorded in each section, thus obtaining the number and area of both B and non-B cells. We were therefore able to make histograms on the composition and frequency of endocrine cells associated in groups. The data obtained approached a log-normal distribution; consequently, the islet areas were log-transformed before the histograms were drawn.

Statistical Analysis

The results were expressed as the mean ± SEM. Statistical significance was determined by Student's *t* test[26] or, when appropriate, the data were subjected to a two-way ANOVA (BMDP 1985; University of California, Los Angeles, CA) with diet and time as the main effects. When significance was found, Scheffe's post hoc comparisons were made; *P* less than .05 was accepted as statistically significant.

RESULTS

Food Consumption and Body Weight Gain

Comparable weight gain and food intake (expressed as caloric intake in kilojoules per day) were recorded in SRD and CD rats during the first 15 weeks of observation. However, a significant increase in weight gain (20%) and food intake was observed in rats fed a SRD during the second half of the experimental period (Table 1).

IRI Concentrations

Before starting the SRD, the plasma concentration of the following parameters (mean ± SEM) was recorded: triacylglycerol, 0.43 ± 0.05 mmol/L; FFA, 248 ± 25 μmol/L;

Table 1. Effects of SRD on Food Intake and Weight Gain in Rats Fed for 15 or 30 Weeks

Diet	Time on Diet (wk)	Weight (g)	Weight Gain ($g \cdot d^{-1}$)	Caloric Intake ($kJ \cdot d^{-1}$)
CD	15	370.0 ± 10.1[a]	2.03 ± 0.11[a]	298.0 ± 25.6[a]
SRD	15	387.0 ± 7.3[a]	2.18 ± 0.07[a]	300.0 ± 11.8[a]
CD	30	470.0 ± 13.1[b]	1.13 ± 0.17[b]	295.0 ± 22.0[a]
SRD	30	556.0 ± 8.3[b]	1.87 ± 0.11[b]	404.0 ± 18.1[b]
2 × 2 ANOVA				
Diet		S	S	S
Time		S	S	S
Diet × time		S	S	S
Residual mean square		526.4	0.152	2,344.66

NOTE. Values are the mean ± SEM (8 animals were included in each group). Values in each column that do not share the same superscript were significantly ($P < .05$) different when 1 mean at a time was compared by Scheffe's test.

Abbreviation: S, significant effect ($P < .05$).

Table 2. Plasma Metabolites and Insulin Concentrations in Rats Fed a SRD or CD

Diet	Time on Diet (wk)	Triacylglycerol (mmol/L)	FFA (μmol/L)	Glucose (mmol/L)	Insulin (μU · mL^{-1})
CD	15	0.51 ± 0.05[a]	286 ± 28[a]	6.63 ± 0.10[a]	42.2 ± 2.2[a]
SRD	15	1.50 ± 0.16[b]	602 ± 22[b]	7.90 ± 0.09[b]	40.6 ± 5.2[a]
CD	30	0.47 ± 0.04[a]	269 ± 24[a]	6.62 ± 0.17[a]	50.8 ± 5.8[a]
SRD	30	1.57 ± 0.18[b]	638 ± 40 [b]	8.11 ± 0.14[b]	55.0 ± 4.9[a]
2 × 2 ANOVA					
Diet		S	S	S	NS
Time		NS	NS	NS	NS
Diet × time		NS	NS	NS	NS
Residual mean square		0.16	6,438	0.15	134

NOTE. Values are the mean ± SEM (8 animals were included in each group). Values in each column that do not share the same superscript were significantly ($P < .05$) different when 1 mean at a time was compared by Scheffe's test.

Abbreviation: S, significant effect ($P < .05$); NS, nonsignificant.

glucose, 6.1 ± 0.15 mmol/L; and IRI, 45.0 ± 8.0 μU/mL. According to previous reports from our laboratory,[10] plasma triacylglycerol, FFA, and glucose concentrations were significantly higher in rats fed a SRD for 15 weeks compared with age-matched controls fed the CD. However, comparable plasma insulin levels were recorded in both groups at this time. Similar changes, although at a slightly higher range, were observed when the intake of the SRD was prolonged for up to 30 weeks (Table 2). Moreover, values obtained for all the above-mentioned parameters at 18, 21, 24, and 27 weeks were similar to those observed at 15 or 30 weeks (data not shown).

IV Glucose Tolerance Test, Insulin Release In Vivo, and Pancreatic Insulin Content

After 15 weeks on the SRD, rats developed severe glucose intolerance despite the release of an amount of insulin in response to IV glucose comparable to that of normal age-matched rats on the CD. A more pronounced deterioration of glucose tolerance was observed when the SRD was maintained for 30 weeks. Although not significantly different, the insulin response to the IV glucose challenge in SRD rats was smaller than that observed at 15 weeks. Pancreatic IRI content was comparable in both groups at either 15 or 30 weeks of treatment (Table 3).

The general histologic aspect of both exocrine and endocrine pancreas, the architecture of the islets, and the topographic distribution of the different endocrine cell types were all normal and comparable in both CD and SRD rats (Fig 1).

However, morphometrical studies revealed clear-cut differences between the two groups (Table 4). The number of pancreatic islets per unit area and the endocrine area (B plus non-B cells) were significantly increased in SRD rats. In contrast, the percentage of B and non-B cells in the endocrine pancreas did not vary. However, significant differences were observed when the area occupied by endocrine cells in relation to the area of the total pancreas was considered. Thus, the B-cell area was considerably increased in SRD rats ($P < .001$). The non–B-cell area was also larger in this group, although to a lesser extent ($P < .05$).

The ratio between the islet cell area and the number of islet cells, which expresses the cell size (mean ± SEM in μm^2: B cells, 121.48 ± 9.73 for CD and 118.35 ± 6.46 for SRD; non-B cells, 97.55 ± 6.24 for CD and 95.59 ± 7.05 for SRD), did not show any significant differences (ANOVA) between the two groups studied. These data would indicate that the increase in B-cell and non–B-cell areas described above might result from an increase in the number of cells (hyperplasia) rather than from an increase in the size (hypertrophy).

Histograms (Fig 2A and B) representing the frequency of the endocrine cell groups according to the area occupied (B

Table 3. Plasma Glucose Fractional Coefficient Disappearance Rate (Kg), Plasma Insulin (IRI) Response After IV Glucose, and Pancreatic Insulin Content in Rats Fed a SRD or CD

Diet	Time on Diet (wk)	Kg (×10^{-2})	Plasma IRI AUC (μU · min^{-1} · mL^{-1})	Pancreatic Insulin (mU · pancreas^{-1})
CD	15	2.21 ± 0.17[a]	854 ± 70[a]	2.24 ± 0.12[a]
SRD	15	0.98 ± 0.08[b]	838 ± 62[a]	2.19 ± 0.37[a]
CD	30	2.62 ± 0.14[a]	782 ± 86[a]	2.06 ± 0.21[a]
SRD	30	0.65 ± 0.08[c]	636 ± 87 [a]	2.05 ± 0.40[a]
2 × 2 ANOVA				
Diet		S	NS	NS
Time		NS	NS	NS
Diet × time		S	NS	NS
Residual mean square		0.16	35,745	0.44

NOTE. Values are the mean ± SEM (8 animals were included in each group). Values in each column that do not share the same superscript are significantly ($P < .05$) different when 1 mean at a time was compared by Scheffe's test.

Abbreviation: AUC, area under the curve between 0 and 30 minutes, using the trapezoidal rule; S, significant effect ($P < .05$); NS, nonsignificant.

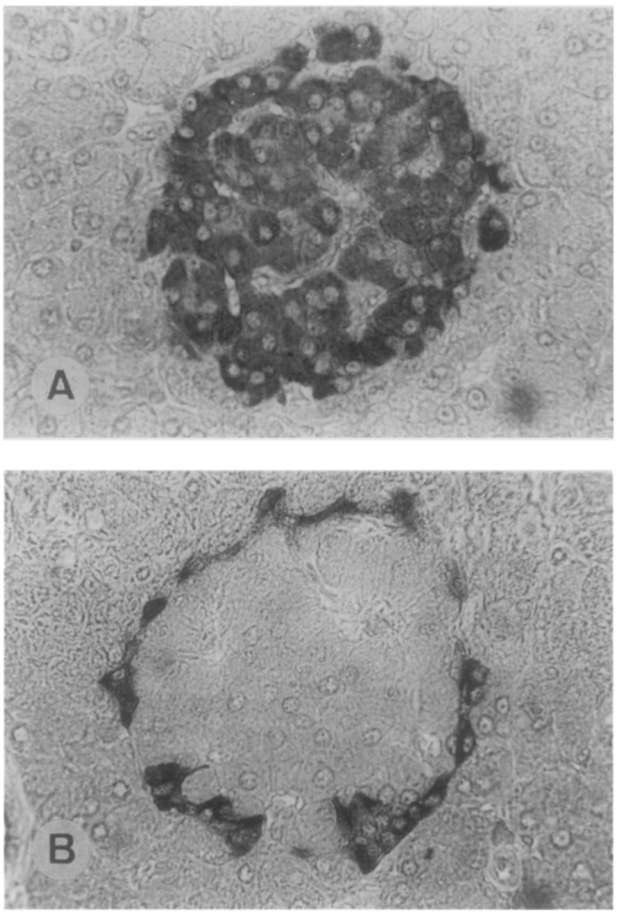

Fig 1. Pancreatic islets from a SRD rat after immunocytochemical identification of (A) insulin (B cells) and (B) glucagon + somatostatin + pancreatic polypeptide (non-B cells). Sections were counterstained with hematoxylin (original magnification ×40).

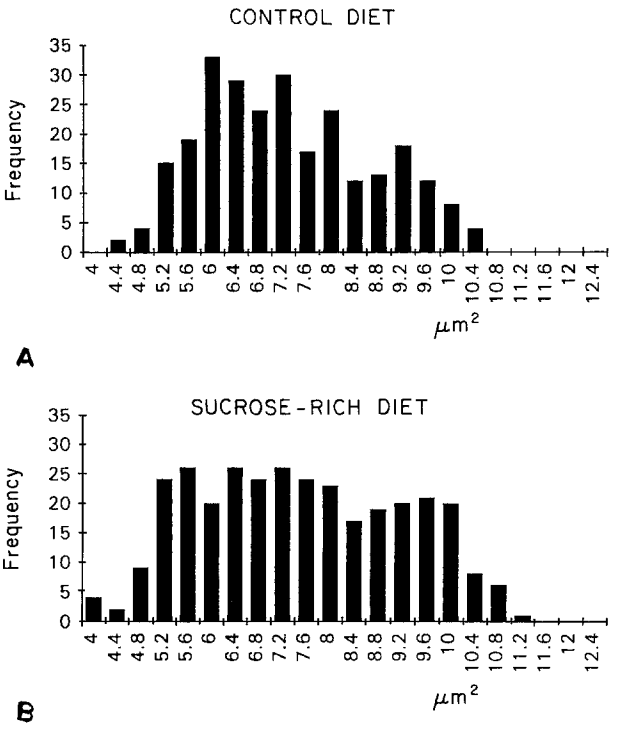

Fig 2. Distribution-frequency of the log-transformed area of endocrine cell groups (B and non-B) found in CD (A) and SRD (B) rats. Note that in the latter, the bars start at lower values (3.6, *v* 4.4 in CD rats) and the frequency of small areas is larger than in CD rats.

and non-B) in CD and SRD rats showed that in SRD rats, smaller cell groups appeared more frequently than larger cell groups. Although it should be interpreted with caution, this phenomenon might represent the appearance of new endocrine cell groups in the SRD group.

DISCUSSION

We have previously shown that sustained administration of a SRD to normal rats for 15 weeks resulted in a three-step metabolic syndrome: (1) induction (3 to 5 weeks on SRD), characterized by the presence of hypertriglyceridemia, hyperinsulinemia, and impaired glucose tolerance; (2) adaptation (5 to 8 weeks), in which spontaneous normalization of the above-mentioned parameters occurred; and (3) recurrence (10 to 15 weeks), where increased plasma glucose, hypertriglyceridemia, and severe glucose intolerance in the face of a normal insulin response to an IV glucose challenge were observed.[10]

Our present data showed that long-term consumption of such a diet for longer periods resulted in a steady state of hypertriglyceridemia and hyperglycemia in which insulin levels remained unchanged and unable to compensate for the increased demands of the developing metabolic changes. These observations strongly suggest the existence of a state of insulin resistance, together with a relative deficit of insulin secretion, which leads to a mild diabetic syndrome that closely resembles the main metabolic and hormonal traits of human non–insulin-dependent diabetes mellitus. The long-term (30 weeks) sucrose feeding induced a relative increase in caloric intake and a significant gain in body weight, which could have also contributed to the decreased insulin sensitivity observed.

Blakely et al[16] also reported hyperglycemia accompanied by hyperinsulinemia, both basally and after an oral glucose

Table 4. Morphometrical Analysis of Pancreases From Rats Fed a SRD or CD for 30 Weeks

Diet	Endocrine Area/Total Pancreatic Area (×10²)	B-Cell Area/ Endocrine Area (×10²)	Non–B-Cell Area/ Endocrine Area (×10²)	B-Cell Area/ Total Pancreatic Area (×10²)	Non–B-Cell Area/ Total Pancreatic Area (×10²)	No. of Islets per 10^6 μm²
CD	0.4725 ± 0.064[a]	81.56 ± 1.3[a]	18.44 ± 1.28[a]	0.3861 ± 0.194[a]	0.0863 ± 0.0136[a]	2.01 ± 0.2[a]
SRD	1.1449 ± 0.223[b]	82.33 ± 2.5[a]	17.72 ± 2.53[a]	0.9518 ± 0.121[b]	0.1930 ± 0.0347[b]	3.29 ± 0.1[b]

NOTE. Values are the mean ± SEM of 4 rat pancreases included in each group. Values in each column that do not share the same superscript were significantly different ($P < .05$) when 1 mean at a time was compared by ANOVA.

challenge, in normal Wistar rats fed a moderate amount of fructose (15% to 20%) for 7 months. However, they recorded no differences either in body weight or in relative food intake as compared with age-matched controls. This discrepancy could be explained by differences in the age of the animals at the beginning of the experiment (weanlings *v* young adults) in addition to smaller amounts of fructose in the diet.

Steady-state hypertriglyceridemia and elevated FFA observed in rats fed the SRD long-term would perhaps be the main condition that leads to the appearance of permanent hyperglycemia. Thus, impaired insulin action was observed in skeletal muscle of rats fed either a short-term SRD or a high-fat diet, resulting in high circulating or intramuscular triglyceride levels.[8,27] Furthermore, the induction of lipid oversupply by feeding a high-fat diet was shown to be accompanied by insulin resistance in the liver and in several other peripheral insulin target tissues.[9] A number of reports[28-30] have demonstrated that FFA also exert an important modulatory effect on insulin action; thus, a chronic elevation of plasma FFA rapidly leads to the development of insulin resistance in skeletal muscle and the whole body by promoting an excessive accumulation of glycogen and lipids in skeletal muscle.[31] Further evidence that changes in FFA metabolism affect hepatic carbohydrate metabolism arises from the demonstration that compounds that reduce the activity of the carnitine palmitoyl transferase system, thereby interfering with long-chain fatty acid oxidation, can decrease plasma glucose levels in normal and diabetic rodents.[32-34]

It may be hypothesized that a sustained increased demand for insulin secretion consecutive to the lower insulin sensitivity is an unbearable burden for the B cell. In the early stage of SRD feeding (induction period), B-cell activity can efficiently compensate for an increased demand and thus maintain fasting serum glucose within the normal range.[4] However, in the long-term the B cell appears to fail to maintain such hyperactivity. The fact that insulin levels of SRD rats in the recurrence period remained within the same range as those recorded in CD animals despite the high serum glucose levels would indicate a B-cell deficit in coping with the increased demand for insulin.

No morphological signs of B-cell reactivity were recorded during the early steps of SRD feeding, despite the fact that a highly increased insulin response was a hallmark of the induction period (data not shown). In contrast, a clear islet reactivity was evident in the latter step of SRD feeding, where the endocrine pancreas showed a significant increase of both islet number and B-cell area, as well as changes in the profile of islet size distribution. The lack of significant changes in endocrine cell size, together with the increase in the number of islets per area and the more frequent appearance of smaller cell groups in SRD rats, suggests that the increase in the B and non-B cell mass is the consequence of hyperplasia rather than of a hypertrophy of these cells. Therefore, it is possible that the sustained administration of sucrose may induce mitosis in adult pancreatic endocrine cells, as glucose does in fetal[35] and adult[36] pancreatic islet cells. Further studies using direct indicators of cell division are required to confirm this assumption.

The morphological incremental changes were not accompanied by an increase in the pancreatic content of IRI. It may therefore be suggested that the newly emerged B-cell mass has some sort of derangement that renders it unable to functionally cope with the increased insulin demand resulting from the state of insulin resistance induced by long-term SRD feeding. On the other hand, it is known that the extracellular matrix of actively proliferating epithelial cells exerts profound influences on gene expression via transmembrane protein components.[37,38] Therefore, perhaps the induced proliferation of endocrine cells in the islets of SRD rats decreases the transcription of the insulin gene, downregulating its expression and thus leading to impaired insulin production and release.

Although similar hormonal and islet morphological changes were also reported in genetic models of type II diabetes,[39-43] it is worth pointing out that the present data were obtained by dietary manipulation of otherwise normal Wistar rats. Thus, feeding a SRD to normal rats may prove an attractive animal model for studying the role of environmental nutritional factors in the unsettled issue of the relationship between insulin resistance and relative insulin deficiency. The model might provide key information for understanding the pathophysiology of human diseases such as type II diabetes, dyslipidemia, and a number of entities included in so-called syndrome X.

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