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# The reduction of dietary sucrose improves dyslipidemia, adiposity, and insulin secretion in an insulin-resistant rat model

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**Abstract**

**Objective:** The purpose of the present work was to investigate whether changes in the type of carbohydrate in the diet are able to improve and/or reverse hyperlipemia, impaired glucose homeostasis, and insulin secretion from β-cells induced in rats by chronically feeding a high sucrose intake.

**Methods:** For 30 wk male Wistar rats received a sucrose-rich diet (63% w/w) or a control diet in which sucrose was replaced by starch. After this period, the sucrose-fed animals were randomly divided into two groups: the first group continued with this diet up to 42 wk and the other received the same diet but with a 20% reduction in the amount of sucrose and the rest of the carbohydrate being replaced by starch. Rats were fed with this diet for the next 12 wk.

**Results:** The reduction of the amount of sucrose in the diet showed a substantial improvement ($P < 0.05$) of dyslipidemia associated with an amelioration of "in vivo" very low-density lipoprotein-triacylglycerol secretion and triacylglycerol removal rate from the circulation. Glucose homeostasis and glucose-induced insulin release from β-cells were improved ($P < 0.05$), although these values did not reach those observed in rats fed a control diet. Visceral adiposity was also significantly reduced ($P < 0.05$).

**Conclusion:** These data are consistent with the suggestion that the composition of the diet could contribute to improvements in dyslipidemia, insulin resistance, and adiposity by direct effects on the lipid metabolism and insulin action and indirectly through the reduction of visceral fat mass and distribution. 

*Keywords:* Sucrose-rich diet; Dyslipidemia; Insulin resistance; Refined sugars; Visceral obesity

## Introduction

The prevalence of the metabolic syndrome—a constellation of disturbed carbohydrate and insulin metabolism, together with central obesity, dyslipidemia, and hypertension—has emerged in recent decades as an epidemic not only in the industrialized but also in the developing world [1]. Many causes have been proposed to account for this problem including changes over time in dietary patterns and lifestyle (e.g., physical activity). In this regard, the daily intake of refined sugars (such as sucrose and fructose), which has been steadily increasing during the past 20 y mainly in children, seems to play an important role in the development of obesity [2]. Although there is little evidence that moderate amounts of refined sugars have detrimental effects on carbohydrate and lipid metabolisms, large doses have been associated with numerous metabolic abnormalities in humans and animals [3,4].

Our laboratory and others [5–10] have reported that rats chronically fed (15–40 wk) with diets rich in sucrose develop dyslipidemia (increase in plasma triacylglycerol and free fatty acids levels), moderate overweight, visceral adiposity, and reduced whole-body insulin sensitivity. Islets isolated from these rats showed a secretion pattern of the

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hormone characterized by the absence of the first peak and the enhancement of the second phase of insulin released in response to glucose. All these metabolic effects induced by feeding a long-term sucrose-rich diet (SRD) closely resemble those found in humans with overweight and/or type 2 diabetes.

Nutritional (e.g., replacement of the usual oil by fish oil in the rats' diet) [11] or pharmacologic (e.g., diazoxide, prazosin) [12,13] interventions similar to an intake of an SRD for a short period (2–4 wk) were able to prevent the development of hypertriglyceridemia and improve the insulin effect on glucose utilization. However, relatively few reports have focused on the effectiveness of dietary manipulation in reversing the metabolic abnormalities previously described induced by the chronic administration of the SRD. A recent study from our laboratory has shown that dyslipidemia and insulin resistance after long-term feeding of normal rats on a SRD could be completely reversed by shifting the source of fat in the diet (corn oil replaced by cod liver oil) [14]. Cohen and Teitelbaum [9] showed that the impaired glucose tolerance induced in rats fed a SRD (67% w/w) for 3 mo could be reversed when the source of carbohydrate in the diet (sucrose) was replaced by starch for 1 mo. Moreover, when the rats were again fed a SRD, the impaired glucose tolerance reappeared within just 1 wk. Further, in rats fed diets containing 10% fructose for up to 14 wk, an increase in the secretion rate of very low-density lipoprotein (VLDL-triacylglycerol) and plasma triacylglycerols can be observed [15].

Because it is practically impossible and even undesirable to abolish fructose and/or sucrose from the diet, the present study further investigated the effect of reducing the amount of dietary sucrose (63% to 20%) on the altered lipid and glucose homeostasis present in rats chronically fed a SRD. In these animals, a well-established dyslipidemia and insulin resistance was observed before the amount of refined sugar was isocalorically replaced by corn starch for 12 wk. We studied whether changes in this macronutrient could improve and/or reverse hyperlipemia, impaired glucose homeostasis, and insulin secretion from $\beta$-cells induced by a high sucrose intake.

## Materials and methods

Male Wistar rats weighing 180–190 g and purchased from the National Institute of Pharmacology (Buenos Aires, Argentina) were maintained under controlled temperature (22 ± 1°C), humidity, and air flow conditions, with a fixed 12-h light/dark cycle (light on 0700 to 1900 h). They were initially fed a standard laboratory chow (Ralston Purina, St. Louis, MO, USA) containing by weight (grams per 100 g) 63 starch (corn, sorghum, wheat oats, and barley), 22.5 protein, 3.5 fat, 6.0 fiber, and 4 vitamin mixture and salt mixture. After 1 wk of acclimatation, the rats were randomly divided into two groups. The experimental group ($n$ = 72) received a semisynthetic SRD (63 g/100 g), whereas the control group (n = 24) received the same semisynthetic diet except that sucrose was replaced by corn starch (63 g/100 g; high starch diet [CD]). The experimental group received the SRD for 30 wk. At this time rats were randomly subdivided into three subgroups. The first group was immediately sacrificed for each procedure as described previously. The rats in the second subgroup continued with the SRD-63% up to 42 wk of the feeding (Table 1). The third subgroup (SRD-20%) received the same semisynthetic SRD in which the amount of sucrose had been reduced to 20%. In this group, the remaining carbohydrate was replaced by corn starch (43%) from week 30 to 42. The control group received the CD throughout the experimental period. Details of the composition of the diets are presented in Table 1. The preparation and handling of the diets have been previously reported in detail [6,7]. All diets provided approximately 16.04 kJ/g of chow and animals had free access to food and water. The weight of each animal was recorded twice a week during the experimental period. In a separate experiment, individual caloric intake and weight gain of at least eight animals in each group were assessed twice a week. On the day of the experiment, food was removed at 0700 h (end of dark period) unless otherwise indicated, and the experiments were performed between 0800 and 1200 h. At least six rats from the three dietary groups were used in each procedure. Rats were anesthetized with intraperitoneal pentobarbital sodium (60 mg/kg of body weight). Blood samples were obtained from the jugular vein and were rapidly centrifuged and plasma was immediately assayed or stored at −20°C. The liver, skeletal muscle (gastrocnemius), epididymal tissue, and retroperitoneal adipose tissue were totally removed, weighed, and immediately frozen and stored at the temperature of liquid nitrogen. The experimental protocol was approved by the

Table 1
Composition of experimental diets (grams per 100 g)*

| Components | CD | SRD-63% | SRD-20% |
|---|---|---|---|
| Corn starch | 63 | — | 43 |
| Sucrose | — | 63 | 20 |
| Casein-free vitamin | 17 | 17 | 17 |
| Corn oil | 7 | 7 | 7 |
| Vitamin mix† | 1 | 1 | 1 |
| Cellulose | 8 | 8 | 8 |
| Salt mix‡ | 3.5 | 3.5 | 3.5 |
| Choline bitartrate | 0.2 | 0.2 | 0.2 |
| DL-methionine | 0.3 | 0.3 | 0.3 |

CD, control diet; SRD-20%, sucrose-rich diet 20% w/w; SRD-63%, sucrose-rich diet 63% w/w

* The CD group consumed the CD for 42 wk, the SRD-63% group consumed the SRD-63% for 42 wk, and the SRD-20% group consumed the SRD-63% for 30 wk and the SRD containing 20% of sucrose plus 43% corn starch for the last 12 wk of the experiment.

† Vitamin mix (AIN-93M-VX).

‡ Salt mix (AIN-93M-MX).

human and animal research committee of the School of Biochemistry, University of Litoral (Santa Fe, Argentina).

*Triacylglycerol secretion rate*

The triacylglycerol secretion rate (TGSR) was evaluated in fasting rats (16–18 h) by blocking the removal of plasma triacylglycerol with Triton WR 1339 (600 mg/kg of body weight) dissolved in 0.9% NaCl. The TGSR was calculated from the linear increase of triacylglycerol versus time according to the procedure described by Wang and Hegsted [16] and previously used by our research group [14].

*Intravenous fat tolerance test*

The intravenous fat tolerance test was performed in rats fasted for 16–18 h by intravenous injection of Intralipid (Kabivitrium, Inc., Alameda, CA, USA) (0.1 mL of 10% Intralipid/100 g of body weight), a soybean oil fat emulsion that was found to be a useful tracer for the study of the fractional turnover rate of circulating triacylglycerol [14]. The disappearance of Intralipid in plasma was measured by nephelometry [17]. The values were plotted on a semilogarithmic scale versus time. The first-order rate constant ($K_2$) of elimination of fat emulsion from the blood stream (fractional removal rate) was calculated by the least squares methods as described elsewhere [14].

*Perifusion of isolated islets*

Rats were decapitated and the islets were isolated by collagenase digestion and collected under the stereoscopic microscope as previously described [6,14,18]. After the islets were washed twice with Krebs-Ringer bicarbonate buffer (KRB), groups of 30–40 islets isolated from each rat were loaded in a 13-mm chamber containing a 5-$\mu$m nylon membrane filter. Islets were perifused with KRB containing 3 mmol/L of glucose, 250 mg/L of essentially fatty acid–free bovine serum albumin, and 40 mg/L of dextran 70, pH 7.4 at 37°C (constantly gassed with 95% $O_2$/5% $CO_2$) at a flow rate of 0.9–1.2 mL/min. After a prewash period of 30 min, two basal samples were obtained. Then the KRB containing a high glucose concentration (16.5 mmol/L) was used until the end of the perifusion period (40 min). Aliquots from the effluent were collected at 1-min intervals until minute 40 and were stored at −20°C until insulin assays. Complete details of the methodology used have been previously described [6,14,18].

*Carcass composition*

Rats from each dietary group were anesthetized as previously described. Anesthetized rats were shaved, and the visceral organs were removed. Carcasses were weighed, placed in plastic bags, and frozen at −20°C. Each frozen carcass was ground to a homogeneous mixture with a mill cooled with liquid nitrogen, and each ground carcass was stored at −20°C. Carcass water was determined by drying ~10-g sample in a drying oven (75–80°C) for 24 h [19]. The weight differences before and after drying were used for its calculation. The dehydrated carcass sample was subsequently used for the determination of ether-extractable fat. Nitrogen determination on 2 g of non-dehydrated ground carcass was used to estimate protein content. Ash content was estimated by combustion of ~1 g of dehydrated ground carcass in a muffle furnace [19]. All measurements were done in duplicate.

*Analytical methods*

Plasma triacylglycerols, free fatty acids, and glucose levels were determined by spectrophotometric methods as previously described [6,14,18]. The immunoreactive insulin was measured by the method proposed by Herbert et al. [20]. The insulin assays were calibrated against rat insulin standard (Novo Nordisk, Copenhagen, Denmark). The triacylglycerol content was determined in homogenates of the frozen liver and muscle (gastrocnemius) powder by the method of Laurell [21]. The adipose lipoprotein lipase (LPL) activity in epididymal fat tissue was measured as previously described [22,23] and was expressed as picomoles of substrate transformed per second (picokatal) per gram of fresh tissue.

*Statistical analysis*

Sample sizes were calculated on the basis of measurements previously made in our laboratory with rats fed a CD or a SRD [6,14,22–24] considering 80% power. Results were expressed as mean ± SEM. Statistical comparisons were done transversely between different dietary groups at each time of the study (30 and 42 wk). Statistical significance between groups was determined by one-way analysis of variance, with one factor (diet) followed by the inspection of all differences between pairs of means by the Newman-Keuls test [25]. Differences with $P$ values $<0.05$ were considered statistically significant. In all cases the interclass correlation coefficients were $\geq 0.73$.

## Results

*Body weight and energy intake*

Body weight and energy intake were carefully monitored in all groups of rats throughout the experimental period. By the end of the 30-wk period, the body weight and energy intake of the SRD-63% group were significantly higher than those observed in rats fed a CD (Table 2). These differences were still present when the SRD-63% was extended up to 42 wk. In contrast, the body weight gain and energy intake were lower in the group of animals in which the amount of

Table 2
Body weight and energy intake in rats fed CD, SRD-63%, or SRD-20%*

| Diet | Body weight (g) | | | Energy intake (kJ/d) | |
|---|---|---|---|---|---|
| | Initial | 30 wk | 42 wk | Initial to 30 wk | 30–42 wk |
| CD | 190.2 ± 9.9[a] | 409.1 ± 18.0[b] | 443.5 ± 18.4[b] | 254.1 ± 11.9[b] | 243.2 ± 11.3[b] |
| SRD-63% | 194.0 ± 5.5[a] | 462.5 ± 10.2[a] | 509.8 ± 14.1[a] | 320.2 ± 12.7[a] | 328.4 ± 13.6[a] |
| SRD-20%† | | | 490.9 ± 11.7[ab] | | 242.8 ± 7.8[b] |

CD, control diet; SRD-20%, sucrose-rich diet 20% w/w; SRD-63%, sucrose-rich diet 63% w/w

* Values are expressed as mean ± SEM. Eight animals were included in each experimental group. Values in a column without a common letter differ at $P < 0.05$.

† SRD-20% group consumed the SRD-63% for 30 wk and the SRD-20% for the last 12 wk of the experiment.

sucrose in the diet was decreased to 20% for 12 wk compared with the SRD-63%–fed rats.

### *Carcass composition, fat pad weight, and LPL activity in epididymal fat tissue*

Rats fed the SRD-63% for 30 or 42 wk showed an increase of carcass weight and carcass fat content, whereas carcass water content was decreased compared with rats fed a CD for 42 wk. When the percentage of the sucrose in the diet was reduced to 20% from weeks 30 to 42, a significant ($P < 0.05$) reduction of carcass fat content was observed, although the lipid content was still above the values recorded in age-matched controls fed a CD. This was accompanied by a moderate reduction of carcass weight and an increase of water content. The amounts of protein and ash were similar in all diet groups (Table 3).

Epididymal and retroperitoneal fat pad weights were significantly increased ($P < 0.05$) in rats fed a SRD-63% at 30 or 42 wk. A significant reduction of the two fat pad weights was observed in rats in which the amount of sucrose in the diet had been reduced (SRD-20%). The increase of LPL activity in epididymal fat pad observed in SRD-63%–fed animals decreased with the reduction of the percentage of sucrose in the diet during the last 12 wk (Table 3).

### *Plasma and liver triacylglycerol content, TGSR, and fractional removal rate of fat emulsion*

An increase of plasma triacylglycerol levels and triacylglycerol content in the liver developed in rats fed an SRD-63% for 30 or 42 wk (Fig. 1A,B). This was accompanied by an increase of "in vivo" TGSR (Fig. 1C) and a decreased fractional removal rate ($K_2$ percentage per minute) of the intravenously injected fat emulsion (Intralipid) when compared with age-matched control rats fed a CD (Fig. 1D). When the amount of sucrose in the diet was reduced to 20% (SRD-20%) for 12 wk, a complete normalization of plasma triacylglycerol level, TGSR, and fractional removal rate of fat emulsion was observed (Fig. 1A,C,D). Liver triacylglycerol content and

Table 3
Carcass composition, fat pad weight, and epididymal LPL activity in rats fed CD, SRD-63%, and SRD-20%*

| Diets | CD | SRD-63% | SRD-63% | SRD-20% |
|---|---|---|---|---|
| Time on diets (wk) | 42 | 30 | 42 | 42 |
| Carcass | | | | |
| Carcass weight (g) | 340.0 ± 6.5[b] | 373.0 ± 8.9[a] | 411.1 ± 20.0[a] | 374.0 ± 11.0[a] |
| Protein (% wet weight) | 20.7 ± 0.5 | 19.7 ± 0.5 | 19.2 ± 0.6 | 19.9 ± 0.6 |
| Fat (% wet weight) | 14.3 ± 0.9[c] | 25.0 ± 0.8[a] | 25.5 ± 1.0[a] | 18.9 ± 1.5[b] |
| Water (% wet weight) | 59.3 ± 1.5[a] | 51.3 ± 1.4[b] | 52.8 ± 1.2[b] | 57.3 ± 1.2[a] |
| Ash (% wet weight) | 4.0 ± 0.3 | 3.9 ± 0.4 | 3.7 ± 0.3 | 3.8 ± 0.2 |
| Retroperitoneal fat | | | | |
| Total weight (g) | 6.20 ± 0.68[c] | 12.59 ± 0.70[a] | 13.88 ± 0.91[a] | 10.20 ± 0.59[b] |
| Relative weight (g/100 g body weight) | 1.32 ± 0.14[c] | 2.72 ± 0.11[a] | 2.74 ± 0.13[a] | 2.21 ± 0.15[b] |
| Epididymal fat | | | | |
| Total weight (g) | 7.12 ± 0.58[c] | 12.75 ± 0.82[a] | 14.08 ± 0.79[a] | 10.39 ± 0.36[b] |
| Relative weight (g/100 g body weight) | 1.63 ± 0.11[c] | 2.75 ± 0.13[a] | 2.80 ± 0.11[a] | 2.28 ± 0.10[b] |
| LPL activity | | | | |
| pKatal/g wet tissue | 1886 ± 273[b] | 3985 ± 294[a] | 4007 ± 306[a] | 2239 ± 297[b] |
| pKatal/total weight | 14 450 ± 1200[c] | 55 158 ± 4216[a] | 55 158 ± 4216[a] | 24 032 ± 3073[b] |

CD, control diet; LPL, lipoprotein lipase; pKatal, picoKatal: pico mol/seg; SRD-20%, sucrose-rich diet 20% w/w; SRD-63%, sucrose-rich diet 63% w/w

* Values are expressed as mean ± SEM. Six animals were included in each experimental group. Values in each line that do not share the same superscript letter are significantly different ($P < 0.05$) when one variable at a time was compared by Newman-Keul test.

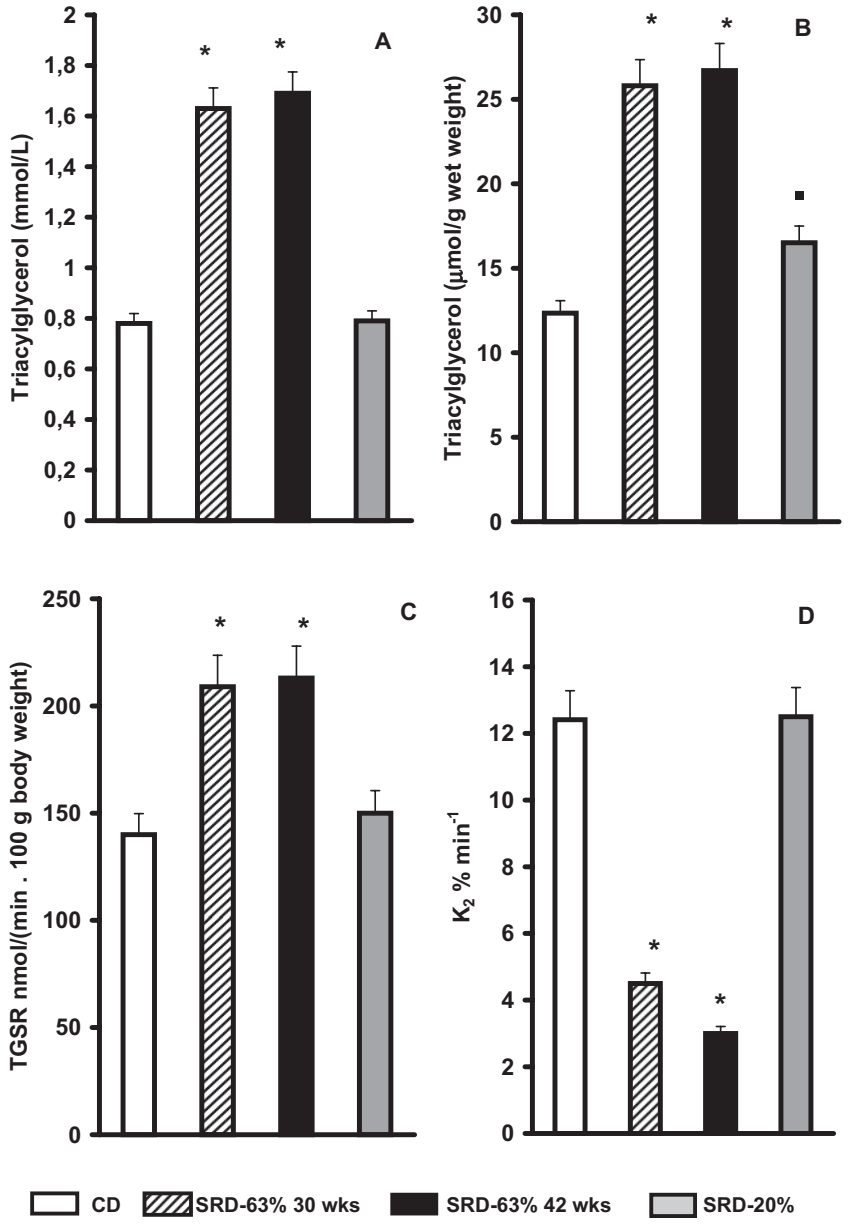


Fig. 1. Plasma triacylglycerol (A), liver triacylglycerol content (B), TGSR (C), and $K_2$ of a fat emulsion, Intralipid, (D) in rats fed a control diet (white bars), sucrose 63% w/w for 30 wk (dark gray bars) or 42 wk (black bars), or sucrose 20% w/w (light gray bars) diet. Values are expressed as mean ± SEM. Six animals were included in each experimental group. $^{*}P < 0.01$, sucrose 63% versus control diet and sucrose 20%; $^{\blacksquare}P < 0.01$ sucrose 20% w/w versus control diet. $K_2$, fractional removal rate; TGSR, triacylglycerol secretion rate.

TGSR significantly decreased ($P < 0.05$), although values were still above those recorded in the CD group. Moreover, the expanded triacylglycerol pool size present in the SRD-63% group was completely normalized after the reduction of sucrose in the diet during the last 12 wk of the experimental period. Values (mean ± SEM, $n = 6$, nanomoles per 100 g of body weight) were 1331 ± 120 in the CD group, 3608 ± 21 in the SRD-63% group for 30 wk, 3630 ± 180 in the SRD-63% for 42 wk, and 1571 ± 100 in the SRD-20% group ($P < 0.05$, SRD-63% 30 or 42 wk versus CD and SRD-20%).

However, the increase of muscle triacylglycerol contents observed in the SRD-63%–fed rats did not improve when the amount of sucrose in the diet was reduced to 20%. Data (mean ± SEM, $n = 6$, micromoles per grams of wet weight) were 3.03 ± 0.42 in the CD group, 7.21 ± 0.28 in the SRD-63% for 30 wk, 7.82 ± 0.30 in the SRD-63% for 42 wk, and 6.61 ± 0.50 for the SRD-20% group ($P < 0.05$, SRD-63% 30 or 42 wk and SRD-20% versus CD).

In contrast, a reciprocal relation between fasting plasma triacylglycerol levels and the fractional removal rate of the intravenously injected fat emulsion was observed (Fig. 2).

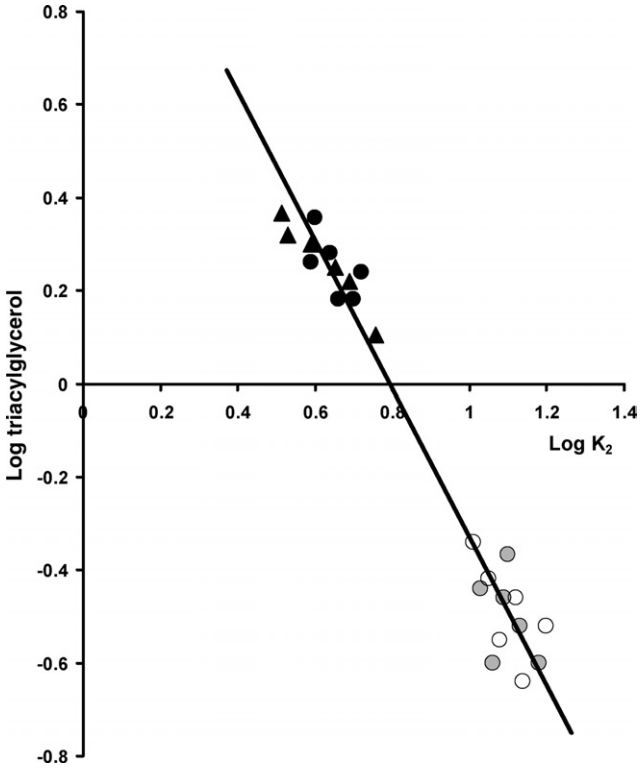


Fig. 2. Correlation between fasting plasma triacylglycerol and $K_2$ (fractional removal rate of the injected fat emulsion, Intralipid; $n = 18$, $r = -0.964$, $P < 0.05$) in rats fed a control diet (weeks 1 to 42; white circles), sucrose 63% w/w for 30 wk (triangles) or 42 wk (black circles) or sucrose 20% w/w (gray circles). $K_2$, fractional removal rate.

*Plasma glucose, free fatty acids, and insulin levels*

Figure 3 depicts a high correlation between plasma glucose and free fatty acid levels in the three dietary groups of rats. As previously reported by our laboratory [5–8] and confirmed by the present findings, the plasma glucose and free fatty acids levels were significantly higher ($P < 0.05$) in the SRD-63%–fed rats compared with controls fed a CD. The present results show that the reduction of the percentage of sucrose in the diet (SRD-20%) from week 30 to 42 significantly improved plasma glucose and free fatty acid levels. However, the concentration of these metabolites was still higher than that recorded in the CD-fed animals. Moreover, no statistical difference in plasma insulin levels was detected at the end of the experimental period across the three dietary groups. Values (mean ± SEM, $n = 6$, microunits per milliliter) were 44.7 ± 5.4 in the CD group, 48.6 ± 2.9 in the SRD-63% for 30 wk, 49.1 ± 5.1 in the SRD-63% for 42 wk, and 46.5 ± 3.6 in the SRD-20% group.

*Perifusion of isolated islets*

The classic biphasic pattern of glucose (16.5 mmol/L) stimulated insulin secretion observed in rats fed a CD diet was completely altered in rats fed a SRD-63% for 42 wk. Similar values were observed at 30 wk on a SRD (data not shown). Perifused islets from SRD-63%–fed rats showed an absence of the first peak and an increase of the second-phase hormone secretion compared with the CD-fed rats (Fig. 4). In contrast, when the percentage of sucrose was reduced (from 63% to 20%) from week 30 to 42, the glucose-induced insulin secretion from perifused islets showed a clear presence of the first peak, although this was lower than that observed in the CD-fed rats. Although the second phase of hormone secretion was significantly reduced compared with those observed in rats fed a SRD-63%, it did not achieve the values observed in the CD groups. Thus, the decrease of the amount of sucrose in the diet improved glucose-induced insulin release from the isolated $\beta$-cells.

## Discussion

Substantial changes in the macronutrient composition of the diet (e.g., refined sugar, saturated fat, etc.) and their interaction with other environmental factors such as lack of physical activity—besides genetic factors—contribute to increase the prevalence of disturbed carbohydrate and lipid metabolisms in addition to obesity, dyslipidemia, and hypertension.

In this study and in agreement with previous work [26], it has been found that the long-term feeding of rats on a high sucrose diet induces dyslipidemia, glucose intolerance, and

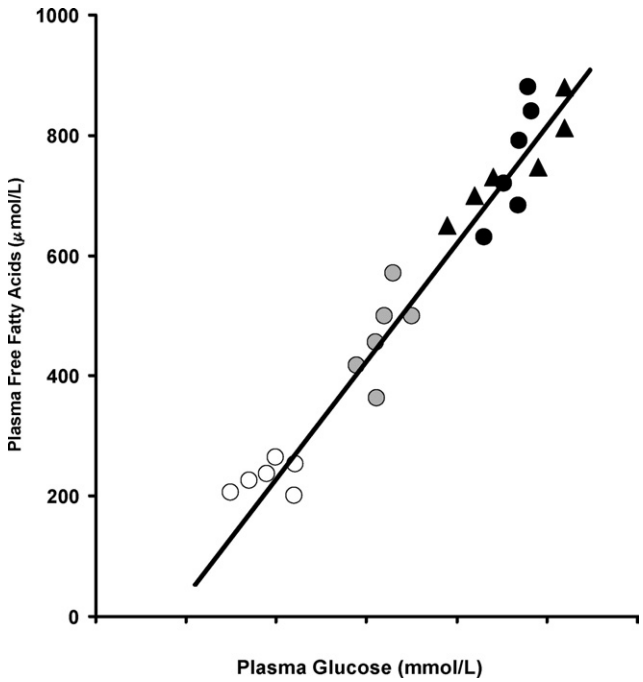


Fig. 3. Correlation between plasma free fatty acids and plasma glucose ($n = 18$, $r = 0.941$, $P < 0.05$) in rats fed a control diet (white circles), sucrose 63% w/w for 30 wk (triangles) or 42 wk (black circles), or sucrose 20% w/w (gray circles).

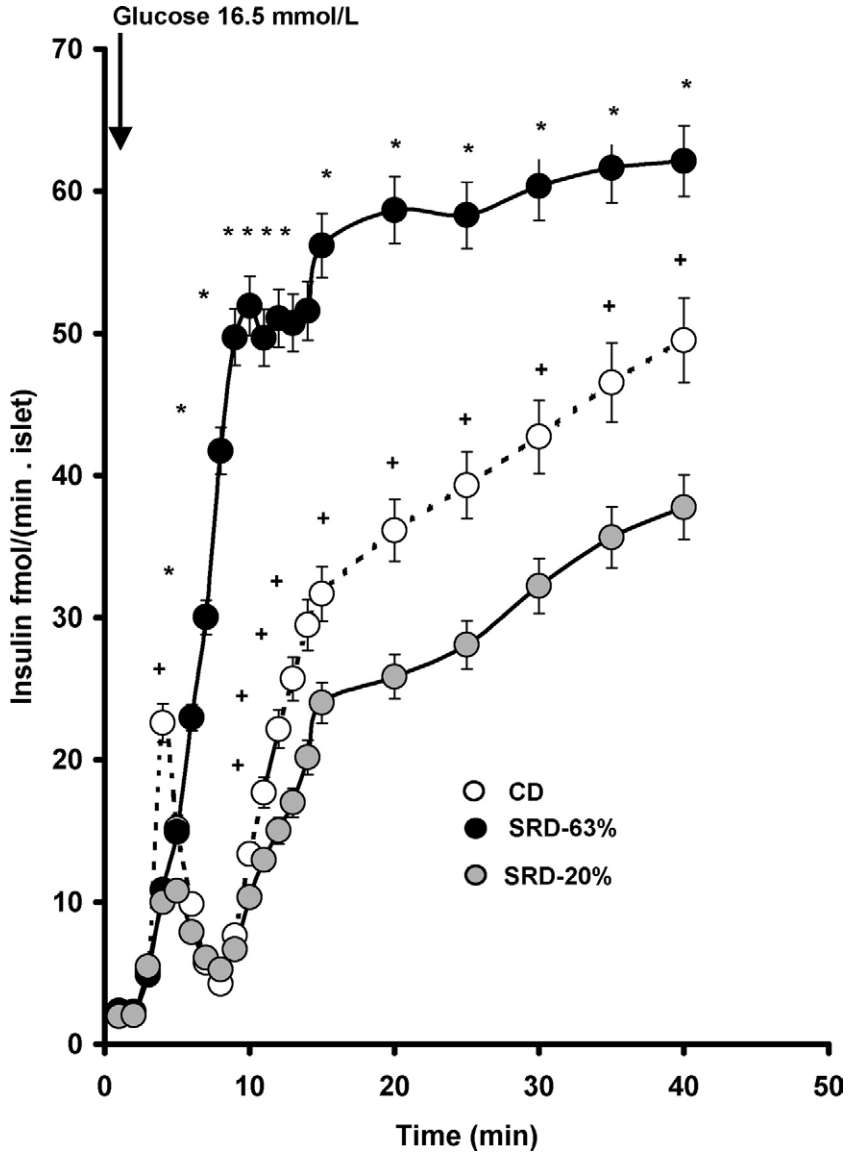


Fig. 4. Insulin secretion in perifused pancreatic islets from rats fed the CD, SRD-63%, or SRD-20%. Glucose (16.5 mmol/L) was added to the perfusion buffer from minutes 3 to 40. Values are expressed as mean ± SEM. Six animals were included in each experiment; $^{*}P < 0.05$, SRD-63% versus CD and SRD-20% at each time point; $^{+}P < 0.05$, SRD-20% versus CD at each time point. CD, control diet; SRD-20%, sucrose-rich diet, 20% w/w; SRD-63%, sucrose-rich diet, 63% w/w.

adiposity. The new findings reported here show that the well-established dyslipidemia (increased plasma triacylglycerol and free fatty acid levels) and the impaired glucose homeostasis after a long-term feeding (42 wk) of a SRD could be substantially improved by partially substituting the amount of sucrose in the diet (63% to 20% w/w) by starch during the last 12 wk of the experimental period. These changes that were accompanied by a significant amelioration of the altered glucose-induced insulin released from isolated $\beta$-cells and the adiposity took place despite the absence of changes in plasma circulating insulin levels. Moreover, the improvement of these abnormalities were the result of reducing the amount of sucrose in the diet and could not be attributed to aging per se because in age-matched control rats fed a CD, none of the parameters analyzed appreciably changed during the course of the study with the only exception of body weight.

There is considerable evidence supporting the ability of the high sucrose diet to upregulate the lipogenesis pathway leading to increased triacylglycerol production [1]. Nagai et al. [27] showed that, after feeding rats for 8 wk with a diet high in fructose (a component of sucrose), the mRNA content of peroxisome proliferators-activated receptor (PPAR$\alpha$), protein, and its activity and the protein expression of fatty acid oxidation enzymes were reduced. In contrast, the gene expression of sterol regulatory element-binding protein (SREBP-1) and the lipogenic enzymes in the liver were increased by high fructose feeding, suggesting

that fructose or its metabolites can regulate lipid oxidation and synthesis leading to an increase of plasma triacylglycerol [28].

Plasma triacylglycerol levels are the result of the VLDL-Tg secretion rate from the liver and the triacylglycerol removal rate from the circulation. In the present study the hypertriglyceridemia observed in the SRD-63%–fed rats could be the consequence of a combination of both processes: an increase of VLDL-Tg secretion and a defective removal of triacylglycerol from the circulation (low fractional removal rate). Moreover, in recent studies [21,22] in these rats, we demonstrated an increase of basal and stimulated lipolysis from isolated adipocytes of epididymal fat pad. Thus, the increased availability of plasma fatty acids, as shown in the present work, may also contribute to hepatic triacylglycerol formation and subsequent VLDL-Tg secretion. Rats fed during the last 12 wk of the experimental period on a diet in which sucrose was partially replaced by starch showed that the reduction of this macronutrient plays a key role in the changes observed in triacylglycerol levels. The VLDL-Tg secretion was completely normalized, possibly through a mechanism that included opposite changes in SREBP-1 and PPAR$\alpha$ expression leading to a significant decrease of hepatic "de novo" lipogenesis and liver Tg contents. Further, plasma triacylglycerol levels and clearance reached values similar to those observed in rats fed a CD. Lithell [29] showed that the fractional removal rate (clearance) of triacylglycerol is positively correlated with plasma triacylglycerol levels and skeletal muscle LPL. In contrast, plasma free fatty acid levels were still above normal. The lack of complete normalization of this metabolite might contribute to the increase of muscle triacylglycerol content observed in this group of rats.

The increased visceral adiposity and the moderate increase of body weight in the SRD-63%–fed rats improved after the percentage of sucrose in the diet was reduced. The decrease of energy intake observed in rats fed a SRD-20%, which was similar to the animals fed a CD, could decrease the deposition of fat visceral adipose tissue. In addition, the normalization of plasma triacylglycerol levels and adipose tissue LPL activity observed in these rats could also contribute to the reduction of fat pad weight. In addition, adiposity associated to high fructose intake may result from the effect of fructose on hormones related to satiety (e.g., insulin, leptin, ghrelin). These hormones play a key role through the central nervous system in the long-term regulation of the energy balance [30]. It has been proposed that glucose but not fructose provides satiety signals to the brain, because the latter is not transported into this tissue [31]. Plasma insulin concentration did not change in the SRD-20%–fed rats and we are unaware of any data concerning plasma leptin and ghrelin levels. However, it is possible that this mechanism might also contribute to the decrease of energy intake and adiposity observed in these rats.

It has been demonstrated that a high sucrose diet impairs hepatic and whole-body peripheral insulin actions in rats [32]. We recently reported that peripheral insulin resistance induced by the long-term feeding on a SRD was closely associated with an increase of plasma triacylglycerol and free fatty acid levels and muscle lipid storage (e.g., triacylglycerol, long-chain acyl coenzyme A). Moreover, in these animals the non-oxidative and oxidative pathways of glucose were impaired [6]. Further evidence that changes in free fatty acid metabolism affect hepatic and whole-body carbohydrate metabolism arises from the demonstration that compounds decreasing plasma free fatty acid levels (acipimox) increase insulin sensitivity in the liver and peripheral tissues [33]. Rats fed SRD-20% showed a moderate decrease of plasma glucose, although still above the normal values, that positively correlates with free fatty acid levels. In this regard, we previously reported [34] that, in SRD-63%–fed rats when sucrose is avoided in the diet, it takes 14 wk for plasma free fatty acids and glucose levels to become completely normalized. This suggests that the percentage of refined sugar and the period that the diet is administered play a role in the improvement or normalization of glucose homeostasis and plasma lipid levels.

We recently demonstrated that the sustained increase of plasma glucose and free fatty acid levels in the SRD-63%–fed rats impairs glucose stimulated insulin secretion "in vivo" and "in vitro" and has a deleterious effect on $\beta$-cell function, possibly through glucolipotoxicity [26,35]. Increased triacylglycerol storage within the $\beta$-cell and diminished glucose oxidation, a signal for insulin secretion and synthesis, were observed in these rats. Moreover, the addition of fish oil in the SRD diet, which normalizes dyslipidemia and glucose levels, was able to return to normalize the altered insulin secretion pattern under the stimulus of glucose. Interestingly, the present study shows that the reduction of the percentage of sucrose in the diet significantly improves the first phase of glucose-stimulated insulin secretion from perifused islets of rats fed SRD-20%. However, the insulin secretion in the second phase was still lower than that in the control group. The latter was an unexpected finding. Although we did not investigate the mechanisms within the $\beta$-cell that could explain this result, the total or partial normalization of plasma lipid levels observed in the SRD-20%–fed rats suggests that the decreased availability of these metabolites in the circulation could contribute to improve $\beta$-cell function and glucose homeostasis.

## Conclusion

In brief, these data are consistent with the suggestion that the composition of the diet (decreased percentage of sucrose) could contribute to improve dyslipidemia, insulin resistance, and visceral adiposity in this experimental animal model. The probable mechanisms include: 1) direct effects on the lipid metabolism and insulin action and 2) indirect effects through the reduction of visceral fat mass and distribution. Our results cannot be directly extrapolated

into human medicine. However, the increased consumption of sucrose and fructose in the world and its possible contribution to the development of obesity and the metabolic syndrome suggest that the amount of this macronutrient could play an important role in the management of these metabolic abnormalities.

## References


[1] Basciano H, Federico L, Adeli K. Review: fructose, insulin resistance, and metabolic dyslipidemia. Nutr Metab 2005;2:1–14.

[2] Bray GA, Joy Nielsen S, Popkin BM. Consumption of high-fructose corn syrup in beverages may play a role in the epidemic obesity. Am J Clin Nutr 2004;79:537–43.

[3] Hallfrish J. Metabolic effects of dietary fructose. FASEB J 1990;4: 2652–60.

[4] Henry RR, Crapo P, Thornburn AW. Current issue in fructose metabolism. Annu Rev Nutr 1991;11:21–39.

[5] Gutman R, Basílico MZ, Bernal C, Chicco A, Lombardo YB. Long-term hypertriglyceridemia and glucose intolerance in rats fed chronically an isocaloric sucrose rich diet. Metabolism 1987;36:1013–20.

[6] Chicco A, D'Alessandro ME, Karabatas L, Pastorale C, Basabe JC, Lombardo YB. Muscle lipid metabolism and insulin secretion are altered in insulin-resistant rats fed high sucrose diet. J Nutr 2003; 133:127–33.

[7] Lombardo YB, Drago S, Chicco A, Fainstein-Day P, Gutman R, Gagliardino JJ, et al. Long term administration to a sucrose-rich diet to normal rats: relationship between metabolic and hormonal profiles and morphological changes in the endocrine pancreas. Metabolism 1996;45:1527–32.

[8] Del Zotto A, Gomez-Dumm CXL, Drago S, Fortino A, Luna GC, Gagliardino JJ. Mechanism involved in the $\beta$ cell mass increase induced by chronic sucrose feeding to normal rats. J Endocrinol 2002;174:225–31.

[9] Cohen AM, Teitelbaum A. Effect of dietary sucrose and starch on oral glucose tolerance and insulin-like activity. Am J Physiol 1964; 206:105–8.

[10] Pagliassotti MJ, Prach PA. Quantity of sucrose alters the tissue pattern and time course of insulin resistance in young rats. Am J Physiol 1995;269:R641–6.

[11] Klimes I, Sebokova E, Vrana A, Kazdova C. Raised dietary intake of n-3 polyunsaturated fatty acids in high sucrose-induced insulin resistance. Animal studies. In: Klimes I, Howard BV, Storlien LH, Sbokova E, editors. Dietary lipids and insulin action. Volume 683. Second International Smolenice Insulin Symposium. New York: Annals of the New York Academy of Sciences; 1993, p. 69–81.

[12] Gutman R, Basílico MZ, Mochiutti N, Chicco A, Lombardo YB. Diazoxide prevents the development of hormonal and metabolic abnormalities present in rats fed a sucrose-rich diet. Horm Metab Res 1985;17:491–4.

[13] Belohsen R, Deshais Y. Involvement of insulinemia in the postprandial hypotriacylglycerolemia induced prazosin in the rat. Metabolism 1993;42:1301–9.

[14] Lombardo YB, Chicco A, D'Alessandro ME, Martinelli M, Soria A, Gutman R. Dietary fish oil normalizes dyslipidemia and glucose intolerance with unchanged insulin levels in rats fed a high sucrose diet. Biochem Biophys Acta 1996;1299:175–82.

[15] Schonfeld G, Pfleger B. Utilization of exogenous free fatty acids for the production of very low density lipoprotein triglyceride by livers of carbohydrate-fed rats. J Lipid Res 1971;12:614–21.

[16] Wang CF, Hegsted DM. Normal blood volume, plasma volume and thiocyanate space in rats and their relation to body weight. Am J Physiol 1949;156:218–27.

[17] Rösner S, Studies on an intravenous fat tolerance test. Methodological, experimental and clinical experiences with Intralipid. Acta Med Scand 1974;564(suppl):1–24.

[18] Chicco A, Basabe JC, Karabatas L, Ferraris N, Fortino A, Lombardo Y. Troglitazone (CS-045) normalizes hypertriglyceridemia and restores the altered patterns of glucose-stimulated insulin secretion in dyslipidemic rats. Metabolism 2000;49:1346–51.

[19] Cunniff P, editor. Official method of analysis of Association of Official Agricultural Chemists. 16th ed. Gaithersburg, Maryland, USA: AOAC International; 1999.

[20] Herbert V, Lau KS, Gottlieb CH, Bleicher S. Coated charcoal immunoassay of insulin. J Clin Endocrinol Metab 1965;25:1375–84.

[21] Laurell S. A method for the routine determination of plasma triglyceride. Scan J Clin Lab Invest 1966;18:668–72.

[22] Soria A, D'Alessandro ME, Lombardo YB. Duration of feeding on a sucrose-rich diet determines metabolic and morphological changes in rat adipocytes. J Appl Physiol 2001;91:2109–16.

[23] Soria A, Chicco A, D'Alessandro ME, Rossi A, Lombardo YB. Dietary fish oil reverse epididymal tissue adiposity, cell hypertrophy and insulin resistance in dyslipidemic sucrose fed rat model. J Nutr Biochem 2002;13:209–18.

[24] D'Alessandro ME, Chicco A, Lombardo YB. A long term sucrose-rich diet increases diacylglycerol content and membrane nPKC$\theta$ expression and alters glucose metabolism in skeletal muscle of rats. Nutr Res 2006;26:209–18.

[25] Snedecor GWP, Cochran WG. Statistical methods. Ames: Iowa State University Press; 1967.

[26] Lombardo YB, Chicco A. Effects of dietary polyunsaturated n-3 fatty acids on dyslipidemia and insulin resistance in rodents and humans. A review. J Nutr Biochem 2006;17:1–13.

[27] Nagai Y, Nishio Y, Nakamura T, Maegawa H, Kikkawa R, Kashiwagi A. Amelioration of high fructose induced metabolic derangements by activation of PPAR$\alpha$. Am J Physiol Endocrinol Metab 2002;282:E1180–90.

[28] Kelley GL, Allan G, Azhar S. High dietary fructose induces a hepatic stress response resulting in cholesterol and lipid dysregulation. Endocrinology 2004;145:548–55.

[29] Lithell H. Lipoprotein lipase activity in human skeletal muscle and adipose tissue. Acta Univ Uppsala 1976;272, 1–19.

[30] Porte D Jr, Baskin DG, Schwartz MW. Leptin and insulin action in the central nervous system. Nutr Rev 2002;60:S20–9.

[31] Miller CC, Martin RJ, Whitney ML, Edwards GL. Intracerebroventricular injection of fructose stimulates feeding in rats. Nutr Neurosci 2002;5:359–62.

[32] Bergman RN, Finegood DT, Ader M. Assessment of insulin sensitivity in vivo. Endocrinol Rev 1985;6:45–86.

[33] Lee KJ, Park KS, Park SW. Effect of decreasing plasma free fatty acid by acipimox on hepatic glucose metabolism in normal rats. Metabolism 1996;45:1408–15.

[34] Chicco A, Bernal C, Soria A, Giangrossi G, Lombardo YB. Dietary effects of partial or total substitution of sucrose for starch on glucose and lipid metabolism in dyslipidemic rats. Nutr Res 1999;19:281–93.

[35] Pighin D, Karabatas L, Rossi A, Chicco A, Basabe JC, Lombardo YB. Fish oil affects pancreatic fat storage, pyruvate dehydrogenase complex activity and insulin secretion in rats fed a sucrose-rich diet. J Nutr 2003;133:4095–101.