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Maternal sucrose-rich diet and fetal programming: changes in hepatic lipogenic and oxidative enzymes and glucose homeostasis in adult offspring

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Nutritional insults during pregnancy and lactation (P + L) are often associated with offspring health risks. We investigated the effect of maternal exposure to a sucrose-rich diet (SRD) during P + L on glucose and lipid metabolism of adult offspring regardless of post-weaning diet. Dams were fed an SRD or a control diet (CD) during P + L. After weaning, male offspring from SRD and CD dams were divided into two groups and fed a CD or SRD until 150 days old forming CD–CD, CD–SRD, SRD–SRD and SRD–CD groups. Offspring where SRD was fed at any period of life showed: (1) increased adipose tissue weight without changes in the final body weight; (2) dyslipidemia as a result of increased very low density lipoprotein triglyceride secretion rate and decreased triglyceride clearance; (3) hepatic steatosis associated with increased activity of key enzymes involved in liver *de novo* lipogenesis and significant decrease of the activity of mitochondrial fatty acid oxidation enzyme. These results were more pronounced in CD–SRD and SRD–SRD groups. (4) Hyperglycemia without changes in insulin levels, plus a deterioration of intravenous glucose tolerance and intraperitoneal insulin tolerance test. We hypothesized that SRD during P + L could be associated with a programming effect on glucose homeostasis and hepatic lipid metabolism that predispose offspring to develop later-life insulin resistance and metabolic disorders, regardless of post-natal diet.

Introduction

Increasing evidence in humans and experimental animal models suggests that perturbations of the developmental *milieu* during the pre-natal period or early stages of the post-natal life constitute health risks for progeny, leading to an enhanced susceptibility to diseases later in life.[1,2] Since Barker's "fetal origins hypothesis" was first introduced, accumulated experimental data have demonstrated that malnutrition – considered as poor nutrition, low or high protein diet or high fat diet – during pregnancy and/or early post-natal life may lead to the development of dyslipidemia, insulin resistance (IR), endothelial dysfunction and hypertension, in the adult life of offspring.[3–5] These abnormalities are included in the metabolic syndrome, whose incidence is rapidly increasing worldwide. Although the relative and absolute terms of dietary fat intake, mainly as saturated fat, have declined in developed countries,[6] the consumption of simple sugars such as high fructose corn syrup has registered a four- to five-fold increase in the past few decades.[7]

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Diets rich in fructose/sucrose, in adult humans and experimental animals, are associated with increased risk of dyslipidemia, IR, hypertension, obesity and other metabolic alterations.[8] Although different experimental studies have focused in the incidence of these carbohydrate intakes during gestation on different parameters of maternal and fetal metabolism,[9–11] there is not enough information about the later-life impact of early exposure to an excess of fructose/sucrose *via* fetal metabolic programming. Moreover, there are also differences in the age of progeny in whom the studies were performed. Rawana *et al.*[12] reported hyperinsulinemia in offspring at weaning from dams fed low fructose (10% in tap water) during pregnancy and lactation. In other studies 20% or 60% fructose levels were also present in the progeny in post-natal life or after weaning.[13,14]

The type of carbohydrate fed during the early post-weaning period can also have important effects on the metabolism of adult animals.[15] Samuelsson *et al.*[16] demonstrated that a sugar-rich diet *in utero* and during the suckling period led to hyperinsulinemia, increased adiposity and impaired glucose tolerance in the female offspring weaned on a control diet at month 3 of age. Moreover, D'Alessandro *et al.*[17] recently reported altered lipid metabolism and glucose homeostasis in 100 day old offspring (young adults) from dams fed a sucrose-rich diet during pregnancy and lactation regardless of offspring weaning diet.

In view of the evidence described above, and considering that the incidence and prevalence of metabolic diseases depend not only on the amount and type of carbohydrate but also on the time of intake, the present work expands D'Alessandro's work to consider 150 day old offspring. We investigated whether a high sucrose feeding during pregnancy and lactation with or without exposure to a high sucrose diet in post-natal life modified or impaired the altered pattern of lipid metabolism and glucose homeostasis described in young adults. For this purpose we analyzed: (1) liver triglyceride content and the activities of the enzymes involved in *de novo* lipogenesis and fatty acid oxidation; (2) plasma metabolites, triglyceride secretion rate and intravenous fat tolerance test; (3) glucose utilization in response to an i.v. glucose challenge and whole body insulin action using an insulin tolerance test. Changes in body weight and energy intake were also determined.

Methods

Animal models and diets

Female Wistar rats (200–230 g) purchased from the National Institute of Pharmacology (Buenos Aires, Argentina) were housed in a colony room with a 12 h light–dark cycle and constant temperature (22 °C) and humidity. The experimental protocol was approved by the Human and Animal Research Committee of the School of Biochemistry, University of Litoral, Santa Fe, Argentina, and adequate measures were taken to minimize the pain or discomfort of the rats. Oestrous cycle by vaginal smears was conducted collecting material with the aid of a dropper containing 0.9% saline solution. The vaginal epithelium of the females was rinsed with this solution and the secretion thus collected was immediately analyzed by optical microscopy to determine the current phase of the oestrous cycle. A female in the proestrus phase that exhibited signals of sexual receptivity was mated with a male of the same strain. The following morning, females were considered possibly pregnant if the spermatozoa were found in the vaginal canal. This day was considered as day 1 of gestation. Rats were fed a standard chow before and during mating. Pregnant rats were transferred to individual cages. Through gestation and lactation rats were fed either a purified sucrose-rich diet (SRD; $n = 16$) containing (% of energy) carbohydrate: sucrose 65; protein: casein free vitamin 19; fat: corn oil 16 or a control diet (CD, $n = 16$) constituted by the same purified diet in which sucrose was replaced by corn starch. Table 1 shows further details about the dietary constituents. The preparation and handling of the diets have been reported elsewhere[18] according to the final report of the American Institute of Nutrition.[19] The diets were isoenergetic (16.3 kJ g^{-1} of food) and they were prepared weekly.

At birth, pups were weighed and litter size reduced to eight pups per dam, with an equal number of male and female pups whenever possible. The pups were kept with their own mother until weaning. At this time (21 days post-partum), the male offspring of CD- and SRD-fed dams were weighed and assigned to either a CD or an SRD diet until 150 days of age. The present study was conducted in male offspring only to avoid the effects of different sexual hormones on the lipid metabolism. Offspring born to SRD dams fed a CD or SRD diet after weaning formed the SRD–CD and SRD–SRD groups, respectively. Similarly, offspring born to CD dams fed a CD or SRD diet after weaning formed the CD–CD or CD–SRD groups, respectively. Throughout the experimental period, dams and offspring had free access to food and water and were kept under controlled room conditions, as described above. Food intake and the body weights of offspring were monitored weekly. Briefly, the offspring were individually housed and body weight was measured weekly at 0900 h in all animals starting at post-weaning until the end of the experimental period (150 days of life) using a balance (Ohaus 730-00 veterinary balance, Parsippany, NY, USA). To measure food intake, food was weighed (Ohaus, Adventure Lindavista, Mexico, D.F. Mexico) and then placed into the food container of the cage and the food remaining 24 h later was weighed again. The difference corrected for the food spillage represented the daily food intake.

At the end of the experimental period, food was removed at 0700 h and, unless otherwise indicated, experiments were performed between 0700 and 0900 h. At least six rats from each dietary group were used in each experiment. Rats were anesthetized with sodium pentobarbital (60 mg kg^{-1} i.p.). In one group of animals, *in vivo* experiments were conducted as described below. In another group, blood samples were obtained from the jugular vein, collected in tubes containing EDTA as anticoagulant and centrifuged at 2700*g* for 10 min at room temperature; the plasma obtained was either immediately assayed or stored at −20 °C until use. In addition to blood sampling, the liver was removed from the second group of rats, weighed, frozen and stored at −80 °C until use. Additionally, the epididymal and retroperitoneal adipose tissues were removed and weighed.

Table 1 Composition of the experimental diets[a]

	Control diet (CD)		Sucrose-rich diet (SRD)	
Diet ingredients	(g per 100 g)	(% Energy)	(g per 100 g)	(% Energy)
Corn starch	62.5	65	—	—
Sucrose	—	—	62.5	65
Casein free vitamin	18	19	18	19
Corn oil	7	16	7	16
Vitamin mix[b]	1		1	
Cellulose	7.5		7.5	
Salt mix[c]	3.5		3.5	
Choline bitartrate	0.2		0.2	
DL-Methionine	0.3		0.3	

[a] Diets are based on the AIN-93 diet. [b] Vitamin mix is based on vitamin mix AIN-93M (in g per kg of diet): niacin 3.00; calcium pantothenate, 1.60; pyridoxine HCl, 0.70; thiamin HCl, 0.60; riboflavin, 0.60; folic acid, 0.20; D-biotin, 0.02; vitamin B-12, 2500 units; vitamin E (500 IU per g), 15.00; vitamin A (500 000 IU per g) 0.80; vitamin D_3 (400 000 IU), 0.25; vitamin K, 0.075. [c] Salt mix is based on salt mix AIN-93M (in g per kg of diet): calcium carbonate, 37.0; potassium phosphate (monobasic) 250.0; sodium chloride, 74.0; potassium sulfate, 46.6; potassium citrate, tri-potassium (monohydrate) 28.0; magnesium oxide, 34.0; ferric citrate, 6.06; zinc carbonate, 1.65; manganese carbonate, 0.63; cupric carbonate, 0.30; potassium iodate, 0.01; sodium selenate, 0.01025; ammonium paramolybdate, 0.00795; chromium potassium sulfate, 0.2174.

Analytical methods

Commercially available analytical kits were employed to determine plasma glucose and triglyceride (Tg) concentration (Wiener lab., Rosario, Santa Fe, Argentina). Plasma free fatty acids (FFA) were determined using an acyl-CoA oxidase based colorimetric kit (Wako NEFA-C, Wako Chemicals, Neuss, Germany). Immunoreactive insulin was measured using the method of Herbert *et al.*[20] The immunoreactive insulin assays were calibrated against rat insulin standard (Novo Nordisk, Copenhagen, Denmark). The liver triglyceride content was determined by the method described by Laurell.[21] Briefly, liver lipids were extracted using isopropyl ether : ethanol (95 : 5 v : v), phospholipids were removed by adsorption on silicic acid and glyceride esters were saponified to glycerol. Glycerol was determined by oxidation with peryodate, using a colorimetric measurement of the product from the reaction of formaldehyde and chromotropic acid in sulphuric acid. An UV-Visible Hitachi U1500 (Hitachi High Technology, Japan) was used for all spectrophotometric assays.

Very-low density lipoprotein-Tg (VLDL-Tg) secretion rate

The VLDL-Tg secretion rate was evaluated in fasting rats (16–18 h) by blocking the removal of plasma Tg with Triton WR 1339 (600 mg kg^{-1}) dissolved in 0.9% NaCl. The VLDL-Tg secretion rate was calculated from the linear increase of Tg *versus* time, according to the procedure described by Lombardo *et al.*[22]

Intravenous fat tolerance test

The intravenous fat tolerance test was performed in rats fasted for 16–18 h by injecting i.v. Intralipid® (Sigma-Aldrich, St Louis, MO, USA, 10% at 0.1 mL per 100 g body weight), a soybean oil fat emulsion.[22] The first order rate constant (K_2) of elimination of fat emulsion from the bloodstream (fractional removal rate) (Turbidimeter Micro 100IR Topac Instruments, Cohasset, MA, USA) was calculated by the least squares method.[22]

Glucose tolerance test

An intravenous glucose tolerance test (IVGTT) was performed on anesthetized rats fasted for 16–18 h after the administration of the glucose solution (500 mg glucose per kg body weight). The IVGTT methodology has been described in detail elsewhere.[17,23] A constant for blood glucose removal (K_g) during the glucose tolerance test and the area under the curve (AUC) during the IVGTT was calculated as previously described.[22]

Insulin tolerance test

Whole-body insulin action was determined by an insulin tolerance test (ITT) in anesthetized rats after a 5 h fast, as previously described.[17] A blood sample was obtained from the jugular vein (time 0), and then insulin (0.75 U per kg, Humulin; Lilly, Indianapolis, IN, USA) was injected i.p. Blood samples were subsequently collected 10, 20, 30, 40, 50 and 60 min after injection and the blood glucose concentration was determined using a glucose meter (Accu-Check Performa; Roche, Mannheim, Germany). A constant for blood glucose removal (K_{ITT}) during the ITT and the AUC during the ITT was calculated as described for the IVGTT.

Liver enzymatic activity assays

Acetyl-CoA carboxylase (ACC) and glucose-6-phosphate dehydrogenase (G-6-PDH). Liver tissue samples were homogenized (motor-driven Teflon glass homogenizer, Thomas Scientific Swedesboro, NJ, USA) in an ice-cold buffer [9 mmol L^{-1} KH_2PO_4, 85 mmol L^{-1} K_2HPO_4, 1 mmol L^{-1} DTT, and 70 mmol L^{-1} $KHCO_3$, (pH 7)] and centrifuged at 100 000*g* for 1 h at 4 °C (Beckman Coulter, LE80, Palo Alto, CA, USA). The cytosolic fractions were used for the assay of enzyme activities. ACC activity was measured using an NADH-linked assay, with the slight modifications described by Zimmermann.[24] G-6-PDH was investigated following the increase of NADPH absorption at 340 nm according to Cohen *et al.*[25] as previously described.[26]

Fatty acid synthase (FAS). Liver tissue samples were homogenized as described above in an ice-cold buffer [0.25 mmol L^{-1} sucrose, 1 mmol L^{-1} dithiothreitol and 1 mmol L^{-1} ethylenediaminetetraacetic acid (EDTA) (pH 7.4)] and centrifuged at 100 000*g* for 1 h at 4 °C. FAS was immediately assayed in cytosolic fraction in duplicate by measuring malonyl CoA-dependent oxidation of nicotinamide adenine dinucleotide phosphate (NADPH) at 37 °C.[27]

Malic enzyme (ME). Liver tissue samples were homogenized as described for ACC in ice-cold 0.25 mmol L^{-1} sucrose solution and then centrifuged for 10 min at 40 000*g* at 0–3 °C. The resulting aqueous supernatant fractions were used for the assay of ME activity by measuring the rate of NADPH formation by spectrophotometric measurement at 340 nm and 37 °C.[28]

Carnitine-palmitoyl transferase I (CPT I). CPT I activity was analyzed as described by Karlic *et al.*[29] Briefly, pieces of frozen liver were homogenized in an ice-cold buffer (0.25 mol L^{-1} sucrose, 1 mmol L^{-1} EDTA, 0.1% ethanol, 1 mg L^{-1} antipain, 2 mg L^{-1} aprotinin, 1 mg L^{-1} leupeptin, 0.7 mg L^{-1} pepstatin, 0.2 mol L^{-1} phenyl methyl-sulfonil fluoride) using an Ultra-Turrax Type X120 (Ingenieurbüro CATM Zipperer GmbH, Germany) at maximum speed for 30 s and centrifuged at 300*g* at 4 °C for 10 min. The precleared supernatant was centrifuged at 12 000*g* at 4 °C for a further 5 min. CPT I activity was assayed in these supernatants spectrophotometrically by following the release of CoA-SH from palmitoyl-CoA using the thiol reagent 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB).

Statistical analysis

Sample sizes were calculated on the basis of measurements previously made with rats fed either a CD or an SRD,[8,10,17,22,23,30] considering 80% power. Results are expressed as mean ± SEM. Statistical analyses were performed with SPSS version 17.0 (SPSS, Chicago, IL, USA). The statistical significance of differences was determined by Student's *t*-test or, when appropriate, the data were subjected to a two-way analysis of variance

(ANOVA), with pregnancy and lactation and post-weaning diet as main effects. For the IVGTT and ITT, the results were also analyzed by ANOVA at each time point. If there was a significant interaction between variables, then a one-way ANOVA, followed by Tukey's *post hoc* test, was used to determine specific statistical differences, which were considered significant at $p < 0.05$.[31]

Results

Body weight, energy intake, liver and adipose tissue weight

Similar to previous results,[17] a significant reduction in birth weight was observed in offspring from SRD-fed dams compared with the offspring from CD-fed dams. Moreover, sucrose intake during pregnancy and lactation significantly increased the body weight of pups at weaning as well as the weight gain from birth until weaning (Table 2). Similar results were obtained when only 8 pups were considered in each group (data not shown). After weaning and throughout the experimental period, both energy intake and body weight were carefully monitored in pups weaned on a CD or SRD. Table 3 shows comparable body weight and energy intake recorded in each experimental group until 150 days of age on their respective diets. Although no changes were observed in liver weight between the different dietary groups, an increased visceral adiposity (epididymal and retroperitoneal weight, g per 100 g body weight) was recorded when SRD was present at any stage of life compared with offspring weaned on a CD from CD-fed dams. It is important to note that retroperitoneal tissue weight expressed as % of total body weight was also significantly different in the SRD–CD group compared with levels in offspring fed the SRD after weaning.

Table 2 Birth and weaning weights in offspring from dams fed a control (CD) or a sucrose-rich diet (SRD)[a]

Group	Birth weight (g)	Weaning weight (g)	ΔBody weight (1–21 days, g)
CD (50)	5.82 ± 0.049	34.20 ± 0.50	27.92 ± 0.52
SRD (50)	5.66 ± 0.046†	36.98 ± 0.50†	31.54 ± 1.51†

[a] Values are expressed as mean ± SEM; ( ) number of offspring per group. † $p < 0.05$ SRD *vs.* CD.

Plasma glucose and insulin levels, glucose tolerance and insulin sensitivity

Table 4 shows glucose homeostasis in offspring from the different dietary groups at 150 days of age. When sucrose was present only at an early stage of life (pregnancy and lactation) or in both early stage and post-weaning, offspring plasma glucose levels were significantly higher compared with those from offspring from CD-dams weaned on the same diet. There were no significant differences in plasma insulin levels in any rat group.

To assess glucose handling *in vivo*, glucose was administered as a single i.v. bolus. The glucose disappearance rates (K_g values) decreased significantly ($p < 0.05$) when sucrose was present in the dams' diet and/or in the offspring after weaning compared with the CD–CD group. However, incremental glucose values integrated over a 60 min period after glucose injection (ΔG 0–60) were similar in all groups (Table 4).

Insulin sensitivity was determined by ITT. The K_{ITT} in the offspring decreased significantly ($p < 0.05$) when sucrose was present during pregnancy and lactation and/or after weaning. However, the AUC integrated during ITT was similar in all groups of rats (Table 4).

Since FFA levels are closely related to insulin sensitivity, we also measured plasma FFA levels in all dietary groups. FFA levels were significantly higher in offspring exposed to a sucrose diet at any stage of life. Interestingly, offspring weaned on a CD from SRD-fed dams (SRD–CD) also exhibited a significant difference compared with levels in offspring fed the SRD after weaning (Table 4).

Plasma and liver triglyceride levels, triglyceride secretion rate and fractional removal rate of fat emulsion

An increased level of plasma Tg was observed in offspring fed a post-weaning SRD regardless of the dam's diet compared with the CD–CD group. This result was associated with high levels of

Table 3 Body weight, energy intake and liver, epididymal and retroperitoneal tissue weights of rats at the end of the experimental period[a]

Group	Body weight (g)	Energy intake (kJ per rat per day)	Liver weight	Epididymal weight (g per 100 g body weight)	Retroperitoneal weight (g per 100 g body weight)
CD–CD	424.8 ± 7.5	257.5 ± 2.1	3.22 ± 0.04	1.31 ± 0.06[b]	1.08 ± 0.08[c]
CD–SRD	442.5 ± 11.6	264.7 ± 27.9	3.29 ± 0.08	1.73 ± 0.03[a]	1.69 ± 0.17[a]
SRD–SRD	425.0 ± 4.8	254.2 ± 13.2	3.58 ± 0.08	1.76 ± 0.03[a]	1.63 ± 0.08[a]
SRD–CD	419.5 ± 8.9	258.9 ± 12.5	3.35 ± 0.08	1.78 ± 0.09[a]	1.35 ± 0.09[b]
2 × 2 ANOVA					
Pregnancy + lactation (P + L)	NS	NS	NS	S	NS
Post-weaning (PW)	NS	NS	NS	NS	S
(P + L) × (PW)	NS	NS	NS	S	NS
Residual mean square	548.7	1521.9	0.054	0.027	0.108

[a] Values are expressed as mean ± SEM, $n = 6$ at each treatment. Values in a column that do not share the same superscript letters were significantly different ($p < 0.05$) when one variable at a time was compared by Tukey's test. S: significant; NS: not significant.

Table 4 Plasma free fatty acids (FFAs), glucose and insulin levels, glucose tolerance test (IVGTT) and insulin tolerance test (ITT) of rats at the end of the experimental period[a]

Group	FFA (μmol L^{-1})	Glucose (mmol L^{-1})	Insulin (μU mL^{-1})	IVGTT		ITT	
				K_g[b] (10^{-2} min^{-1})	ΔGlucose 0–60[c] min (mmol min^{-1})	K_{ITT}[d] (10^{-2} min^{-1})	ΔGlucose 0–60[e] min (mmol min^{-1})
CD–CD	400 ± 19[c]	6.10 ± 0.10[b]	84.46 ± 7.75	2.47 ± 0.12[a]	8.26 ± 0.30	2.02 ± 0.15[a]	4.97 ± 0.09
CD–SRD	722 ± 26[a]	7.17 ± 0.34[a]	89.55 ± 8.68	1.83 ± 0.10[b]	8.27 ± 0.21	0.98 ± 0.23[b]	5.57 ± 0.36
SRD–SRD	726 ± 68[a]	7.71 ± 0.21[a]	93.72 ± 8.31	1.63 ± 0.08[b]	9.81 ± 0.58	1.26 ± 0.23[b]	5.38 ± 0.53
SRD–CD	502 ± 25[b]	7.05 ± 0.16[a]	80.28 ± 5.52	1.91 ± 0.11[b]	8.62 ± 0.41	1.14 ± 0.20[b]	5.49 ± 0.63
2 × 2 ANOVA							
Pregnancy + lactation (P + L)	NS	S	NS	S	NS	NS	NS
Post-weaning (PW)	S	S	NS	S	NS	S	NS
(P + L) × (PW)	NS	NS	NS	NS	NS	S	NS
Residual mean square	5631	0.224	357.22	0.052	0.678	0.183	0.892

[a] Values are expressed as mean ± SEM, $n = 6$ at each treatment. Values in a column that do not share the same superscript letters were significantly different ($p < 0.05$) when one variable at a time was compared by Tukey's test. S: significant; NS: not significant. [b] Rates of glucose disappearance were calculated from the slopes of the regression lines obtained with log-transformed glucose values after glucose administration. [c] Glucose responses during the acute glucose challenge were used to calculate the incremental blood glucose values integrated over the 60 min period after the injection of glucose (ΔG 0–60). [d] Rates of glucose disappearance were calculated from the slopes of the regression lines obtained with log-transformed glucose values after insulin administration. [e] Glucose responses during the acute insulin challenge were used to calculate the decrease of blood glucose values integrated over the total period after the injection of glucose (ΔG 0–60).

VLDL-Tg secretion rate and liver Tg content and a significant decrease in K_2 of intravenous fat emulsion ($p < 0.05$ CD–SRD and SRD–SRD *vs.* CD–CD, Table 5). Offspring weaned on a CD from SRD-fed dams (SRD–CD group) exhibited the same pattern on plasma Tg and K_2 compared with the CD–SRD and SRD–SRD groups. Moreover, in this particular group, the liver Tg content and VLDL-Tg secretion rate were significantly different ($p < 0.05$) in both offspring from CD fed dams weaned on a CD diet and offspring weaned on an SRD diet, regardless of the dams' diet.

Hepatic enzyme activities involved in lipid metabolism

As shown in Fig. 1, when offspring exposed to the sucrose-rich diet independent of the dams' diet were compared with those from the CD–CD group, *de novo* lipogenesis enzyme activities (ACC, FAS, ME and G-6-PDH) significantly increased. Conversely, mitochondrial CPT I activity, which is related to fatty acid oxidation, was significantly reduced in the offspring that consumed sucrose at any period of life compared with those belonging to the CD–CD group.

Discussion

The present study provides new information concerning the impact of a maternal sucrose-rich diet during pregnancy and lactation on the glucose and lipid metabolism of the offspring weaned onto either a control or a sucrose-rich diet for a long period of time. The major new findings in the offspring exposed to a sucrose-rich diet at any stage of life (CD–SRD, SRD–SRD or SRD–CD groups) are as follows: (1) an increase in adipose tissue weight (both epididymal and retroperitoneal) was recorded without changes in the final body weight; (2) dyslipidemia was the result of increased very low density lipoprotein (VLDL)-Tg secretion rate and decreased Tg clearance; (3) the hepatic steatosis (elevated Tg content) was associated with increased activity of key enzymes involved in liver *de novo* lipogenesis (ACC, FAS, ME, and G-6-PDH) and a significant decrease in the activity of the enzyme involved in the mitochondrial fatty acid oxidation (CPT I). Moreover, all the above results were more pronounced when sucrose was present after weaning. (4) Increased plasma glucose levels without changes in insulin levels were accompanied by a deterioration of IVGTT and ITT.

In agreement with previous results, offspring from SRD dams were smaller at birth and exhibited higher weight gain at the end of the weaning period.[17] Other studies regarding the effect of a sucrose or fructose diet during pregnancy in rats have reported a reduction[10] or no reduction[11,32] of fetal or new-born weight, depending on differences in the strain of rat or quantity of carbohydrate present in the diet.[33] Pup growth during lactation is reported to be directly related to maternal intake.[34] The present study suggests that maternal sucrose intake, particularly during lactation, affects the metabolism of offspring, resulting in increased body weight. Similar results were previously reported by Ghusain-Choueiri and Rath.[35]

The consumption of excess amounts of sucrose/fructose in a normal energy intake is linked to visceral adiposity, metabolic syndrome, type II diabetes.[8,36,37] In this regard, the adiposity

Table 5 Plasma and liver triglyceride content, very-low density lipoprotein (VLDL)-Tg secretion rate and fractional removal rate of fat emulsion (K_2) of rats at the end of the experimental period[a]

Group	Plasma triglyceride (mmol L^{-1})	Liver triglyceride (μmol per g wet tissue)	VLDL-Tg secretion (nmol per min per 100 g body weight)	K_2 (% min^{-1})
CD–CD	0.62 ± 0.05^{b}	7.73 ± 0.50^{c}	164.28 ± 4.25^{c}	10.66 ± 0.90^{a}
CD–SRD	1.36 ± 0.17^{a}	19.49 ± 2.09^{a}	227.87 ± 14.38^{a}	5.92 ± 0.33^{b}
SRD–SRD	1.31 ± 0.14^{a}	18.97 ± 1.14^{a}	223.18 ± 13.16^{a}	6.72 ± 0.50^{b}
SRD–CD	1.20 ± 0.11^{a}	13.93 ± 0.61^{b}	189.50 ± 4.61^{b}	6.15 ± 0.26^{b}
2 × 2 ANOVA				
Pregnancy + lactation (P + L)	NS	NS	NS	S
Post-weaning (PW)	S	S	S	S
(P + L) × (PW)	S	S	NS	S
Residual mean square	0.13	9.44	566.5	2.076

[a] Values are expressed as mean ± SEM, $n = 6$ at each treatment. Values in a column that do not share the same superscript letters were significantly different ($p < 0.05$) when one variable at a time was compared by Tukey's test. S: significant; NS: not significant.

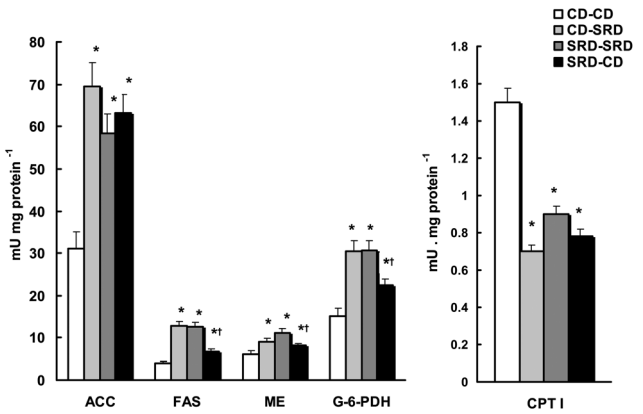

Fig. 1 Liver acetyl-CoA carboxylase (ACC), fatty acid synthase (FAS), malic enzyme (ME), glucose-6-P-dehydrogenase (G-6-PDH) and carnitine palmitoyltransferase I (CPT I) activities in male offspring at the end of the experimental period. CD–CD: offspring on a CD from dams fed a CD; CD–SRD: offspring on a SRD from dams fed a CD; SRD–SRD: offspring on a SRD from dams fed a SRD; SRD–CD: offspring on a CD from dams fed a SRD. Data are mean ± SEM of six animals per group. * $p < 0.05$ CD–CD *vs.* CD–SRD, SRD–SRD and SRD–CD; † $p < 0.05$ SRD–CD *vs.* SRD–SRD and CD–SRD.

depicted in the groups of rats where SRD was present at any stage of life without changes in the total body weight or energy intake was the first difference observed when the time of offspring life was extended up to 150 days. Previous reports[8,26] well documented the effect of a sucrose-rich diet on adipose tissue weight in adult rats feeding on the diet for 90 to 120 days. Thus, it is not surprising that CD–SRD-fed rats yielded similar results. On the SRD–SRD group the predictive adaptive response hypothesis described by Gluckman and Hanson[38] seemed not to be present. Moreover, offspring from the SRD–CD group also showed increased adiposity. Similar results were observed later in life in offspring weaned on a control diet from both high carbohydrate supplemented-fed dams during the last weeks of pregnancy[39] and westernized "junk food" fed-dams.[40] Moreover, Ghusain-Choueiri and Rath[35] and Sedova *et al.*[41] showed that the high maternal sucrose diets fed during pregnancy and nursing periods enhanced adiposity in neonate mice at the age of 12 days and male rats at weaning, respectively.

The impaired glucose homeostasis observed in rats fed an SRD after weaning and up to 150 days of life from CD or SRD fed dams resembles that reported in offspring at 100 days of life[17] and adult rats fed an SRD.[7,8,18] The significant increases of plasma FFA could also contribute to the altered glucose homeostasis observed in these dietary groups. IR was observed in Wistar rats fed a very high fructose diet (60%) during the intrauterine and post-natal periods (90 days of age).[14] Moreover, regarding the SRD–SRD group, the present results show that the possible predictive adaptive protection on glucose tolerance and insulin sensitivity is not induced by the sucrose-fed dams, at least under the present experimental conditions. Regarding the SRD–CD group, a clear derangement in glucose homeostasis is observed. Interestingly, in this dietary group the increased adiposity was associated with levels of FFA slightly above those observed in the CD–CD group. In this regard, a normal i.v. glucose tolerance test and enhanced insulin secretion or insulin and leptin resistance were reported in offspring weaned on a control diet from high carbohydrate supplemented-fed dams during lactation or during the last weeks of pregnancy respectively.[42,39]

The presence of sucrose at any stage of life (SRD–SRD, SRD–CD or CD–SRD groups) induced enhanced liver Tg content. The intracellular pooling of hepatic Tg reflects the balance between free fatty acid flux, fatty acid oxidation, *de novo* lipogenesis and VLDL-Tg secretion. Increased free fatty acid level in offspring exposed to a SRD suggests an enhanced release of adipose tissue lipolytic products which in turn could increase liver Tg synthesis and release into circulation under the form of VLDL-Tg. Indeed, free fatty acid flux is proposed as the major contributor to the availability of Tg for VLDL-assembly.[43] On the other hand, liver steatosis produced by sucrose feeding would also be the consequence of a combined increase *de novo* lipogenesis and diminished fatty acid oxidation. This hypothesis fits well with the enhanced activities of lipogenic enzymes (ACC,

FAS, ME, G-6-PDH) and the decreased activities of mitochondrial enzyme CPT I. The fructose moiety of the sucrose diet has proved to be related to the up-regulation of the gene expression of hepatic fatty acid synthesis through the transcription factor SREBP-1 and ChREBP.[44] In contrast, the mRNA contents and protein mass levels of PPARα as well as the gene expression and activities of key enzymes of fatty acid oxidation were significantly decreased in adult rats given a sucrose/fructose diet for different periods of time.[45,23] Moreover, the hypertriglyceridemia observed in the progeny where sucrose was present at any stage of life could be the result of the impaired clearance of the triglyceride-rich lipoprotein (decreased K_2) associated with the increased VLDL-Tg secretion from the liver. Regarding the SRD–SRD group, again the predictive adaptive response was not present, at least under the present experimental conditions. In contrast, the phenotypic changes from SRD–CD offspring are consistent with the evidence that maternal sucrose diet predisposes offspring to an array of metabolic disorders. In this context, Ching *et al.*[46] showed that maternal fructose exposure resulted in dyslipidemia, hepatic lipid accumulations and increased hepatic mRNA expression of ACC2 without modification of SREBP1 mRNA in offspring that consumed a control diet up to 14 weeks of life. Indeed, in fetuses from fructose fed mothers (10% in drinking water), Rodríguez *et al.*[11] reported a higher hepatic triglyceride content compared to fetuses from control mothers associated with higher expression of genes related to lipogenesis and a lower expression of fatty acid catabolism genes. Moreover, the authors proposed that the diminished maternal leptin response to fasting and refeeding and the impairment in the transduction of the leptin signal in the fetus would be responsible for their hepatic steatosis. Independent of the after-weaning diet, the levels of leptin in adult offspring from dams fed an SRD are still not known.

In brief, this study emphasizes that maternal high-sucrose feeding during critical window of life, like pregnancy and suckling period, are important determinants in the long-term programming of health and diseases. Moreover, this work signals a specific point in lifetime to achieve interventional strategies that would primarily prevent non-communicable chronic diseases at their earliest beginnings.

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