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Lipogenic enzyme activities and glucose uptake in fat tissue of dyslipemic, insulin-resistant rats: Effects of fish oil

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Abstract

Objective: The purposes of the present work were twofold: (1) investigate same mechanisms involved in the development of fat cell hypertrophy in the experimental model of dyslipidemia and whole-body insulin resistance induced in rats chronically fed a sucrose-rich diet (SRD); and (2) analyze the possible beneficial effect of fish oil on these mechanisms.

Methods: For 6 mo, male Wistar rats received a sucrose-rich diet (62.5% w/w sucrose, 8% corn oil) or a control diet in which sucrose was replaced by starch. After this period, the sucrose-fed animals were divided randomly into two groups: the first one continued with the same diet up to 8 mo and the second one received the same diet, but with corn oil replaced by 7% fish oil + 1 % corn oil. Rats were fed with this diet for the next 2 mo.

Results: Although an enlarged fat cell lipolysis and an impaired insulin-stimulated glucose uptake were present in the fat cells of SRD-fed rats, an increase of several key enzymes of the novo lipogenesis could be one of the possible mechanisms involved in visceral adiposity. The addition of dietary fish oil restored or improved the above abnormalities.

Conclusion: This study shows possible mechanisms conditioning the influence of nutrients on the development and management of dyslipidemia, insulin sensitivity, and fat cell accretion, all abnormalities present in the metabolic syndrome.

Keywords: Adipose tissue lipogenesis; Glucose uptake; n-3 fatty acid; Sucrose diet; Dyslipidemia; Insulin resistance

Introduction

Accretion of body weight, visceral adipose tissue mass, and insulin resistance (IR) are the core features of the metabolic syndrome. Associated abnormalities that include dyslipidemia, abnormal glucose metabolism, and hypertension predispose individuals to cardiovascular disease and type-2 diabetes [1]. Although the pathogenesis of this syndrome is complex and so far incompletely understood, the interaction of genetic factors, overweight, sedentary lifestyle, and diet composition are known to contribute to its development.

Previous work from our laboratory demonstrated that normal Wistar rats fed a sucrose-rich diet (SRD) for a long period of time (up to 40 wk) developed several of the metabolic abnormalities observed in the metabolic syndrome such as dyslipidemia (increased plasma levels of triacylglycerol [Tg] and free fatty acids [FFA]), moderate hyperglycemia, normoinsulinemia, and whole-body IR. At this point, the SRD-fed rats were slightly overweight, with enlargement of visceral adipose tissue mass. In addition, hypertrophy of adipocyte from the epididymal fat pad, with a clear alteration in their cell size distribution, was observed [2].

Triglyceride stored in adipose tissue originates either from the esterification of FFAs provided mainly from the diet or from de novo synthesis. The activity of the lipogenic pathway in adipose tissue is highly dependent on nutritional conditions [3]. Fatty acid synthase (FAS) and acetyl-coenzyme A carboxylase (ACC) play a central role in de novo lipogenesis in mammals. In this regard, Kim et al. [4] demonstrated that in rats, starvation significantly reduced the level of adipose tissue FAS and ACC mRNA, whereas refeeding with

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a high carbohydrate diet induced a several-fold increase in the expression of both enzymes. Moreover, the adipose tissue of rats fed a sucrose or fructose-rich diet showed a significant increase of both malic enzyme (ME) and glucose-6-phosphate dehydrogenase (G-6-PDH) activities [5,6]. In addition, in obese fa/fa Zucker rats, Lopez Soriano et al. [7] demonstrated a significant increase of both adipose tissue lipogenic rate and lipoprotein lipase (LPL) activity expressed as total white fat pad when compared with age-matched lean controls. An increased activity of LPL in the epididymal fat pad was also observed by Deshaies [8] in Wistar rats fed a SRD for a short period of time (4 wk).

On the other hand, a defective insulin-mediated glucose uptake and metabolism in adipose tissue was observed in animal models of genetic obesity such as OLEFT rats [9] or in normal rats in which a fat-rich diet induced obesity [10,11]. Besides, impaired insulin sensitivity was also observed in adipose tissue of rats fed either a fructose- or sucrose-rich diet for a short period of time (3–6 wk) [12–14]. Interestingly, Soria et al. [15] demonstrated that under the same experimental conditions, these animals were not overweight and the adipocyte cell size and distribution were similar to their age-matched controls.

At present, the mechanism/s underlying the development of fat cell hypertrophy associated with an increase of plasma FFA, which might suggest an increased adipose tissue lipolysis- after chronic administration (up to 40 wk) of a SRD, are still not completely elucidated.

On the other hand, there is accumulated evidence of the beneficial role of dietary fish oil (FO), rich in 20:5 n-3 and 22:6 n-3 polyunsaturated fatty acids, on animal and human health, especially of their protecting role against the adverse symptoms of the metabolic syndrome [16]. In this vein, it has been shown that FO prevents the onset of IR and dyslipidemia in rats fed a high-fat or high-sucrose diet [16]. Regarding the effect of FO on adipose tissue, Raclot et al. [17] and Haug et al. [18] showed that feeding ω-3 polyunsaturated fatty acids (PUFAs), mainly 22:6 ω-3, decreased mRNA levels of FAS and LPL in retroperitoneal adipose tissue of rats fed a high-fat diet. Benhizia et al. [19] showed that FO compared with lard or corn oil is more effective in decreasing the activity and mRNA levels of FAS in the white fat pad of rats. Besides, Peyron-Caso et al. [20] showed that the presence of dietary ω-3 prevented adiposity induced by 3 wk of sucrose feeding. Moreover, we have recently demonstrated that FO as dietary fat led to a markedly reduced fat pad mass and improved the altered cell size distribution in epididymal fat pad of rats chronically fed a SRD. This was accompanied by normalization of whole body peripheral IR and dyslipidemia [2].

In the present work, rats chronically fed a SRD (up to 40 wk) were used as a model of dyslipidemia, IR, and visceral adiposity. In these rats we studied: (1) the behavior of several key enzymes involved in de novo lipogenesis and its possible relationship to the accretion of adipose tissue mass; (2) in isolated adipocyte, basal lipolysis as an estimation of hormone-sensitive lipase (HSL) and triglyceride lipase (TGL) activities, as well as basal- and insulin-stimulated glucose uptake; and (3) the effect of dietary FO on the parameters mentioned above, because, as we previously described, the addition of this dietary oil improved the visceral adiposity of rats fed long term a SRD.

Materials and methods

Male Wistar rats initially weighing 180 to 190 g and purchased from the National Institute of Pharmacology (Buenos Aires, Argentina) were maintained under controlled environmental conditions (temperature, 22 ± 1 °C; 12-h light/dark cycle: light on 0700–1900 h; humidity and air flow conditions). They were initially fed a standard nonpurified diet (Ralston Purina, St. Louis, MO, USA) containing by weight (g/100 g): 63 starch (corn, sorghum, wheat, oats, and barley); 22.5 protein; 3.5 fat; 6 fiber; 1 vitamin mixture; and 4 salt mixture. After 1 wk of acclimation, the rats were randomly divided into two groups. The experimental group ($n = 40$) received a semisynthetic SRD (sucrose 62.5 g/100 g, corn oil 8 g/100 g). The control group ($n = 20$) received the same semisynthetic diet, but with sucrose replaced by corn starch (62.5 g/100 g; high starch diet [CD]). The experimental group received the SRD for 6 mo. At this time, the rats were randomly subdivided into two subgroups. The rats of the first subgroup continued the SRD for up to 8 mo of feeding. The second subgroup (SRD + FO) received the SRD in which the source of fat (corn oil, 8 g/100 g) had been replaced by FO (7 g of cod liver oil/100 g plus 1 g/100 g of corn oil) from months 6 to 8 (Table 1). The control group received the CD throughout the experimental period. Cod liver oil was purchased from ICN, Biomedical Pharmaceutical (Costa Mesa, CA, USA). The SRD without the addition of FO used from months 6 to 8 and the CD were balanced for the cholesterol and vitamins D and A present in the FO. All diets were isoenergetic, providing approximately 16.3 kJ/g of chow, and the animals had free access to food and water. Diets were prepared every day by adding the oils to the base mixture containing the other nutrients. The oils and base mixture were stored separately at 4 °C until preparation of the diet. FO was kept under nitrogen atmosphere during storage. Dietary fats were analyzed by capillary gas chromatography, as previously described [2]. The weight of each animal was recorded twice a wk during the experimental period. In a separate experiment, the individual caloric intake and weight gain of at least 8 animals in each group were assessed twice a wk. On the day of the experiment, food was removed at 0700 h (end of the dark period) and experiments were performed between 0800 and 1000 h. Six animals from each dietary group were used for the determination of plasma metabolite levels and epididymal fat pad enzymes. In sets of 6 animals from each group, basal- and insulin-stimulated glucose uptakes were analyzed in isolated adipocytes. The last 8 animals per group were used for adipocyte triglyceride content and basal lipolysis determination. In all the assays

Table 1
Composition of experimental diets[1*]

Diet ingredients	CD		SRD		SRD + FO	
	% by weight	% of calories	% by weight	% of calories	% by weight	% of calories
Casein free vitamin	17.0	17.5	17.0	17.5	17.0	17.5
Salt mix[†]	3.5		3.5		3.5	
Vitamin mix[‡]	1.0		1.0		1.0	
Choline chloride	0.2		0.2		0.2	
Methionine	0.3		0.3		0.3	
Cellulose	7.5		7.5		7.5	
Corn starch	62.5	64.0				
Sucrose			62.5	64.0	62.5	64.0
Corn oil	8.0	18.5	8.0	18.5	1.0	2.3
Fish oil					7.0	16.2

* Diets are based on AIN-93 M diet. CD, control diet; SRD, sucrose-rich diet; SRD + FO, SRD + fish oil.

[†] Salt mix is based on salt mix AIN-93 M (in g/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.

[‡] Vitamin mix is based on vitamin mix AIN-93 M (in g/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, 2,500 Units; vitamin E (%00 IU/g), 15.00; vitamin A (500,000 IU/g) 0.80; vitamin D3 (400,000 IU), 0.25; vitamin K, 0.075.

used from isolated adipocytes, fat cell size, volume, and number were determined.

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 epididymal and retroperitoneal adipose tissues were totally removed and rapidly weighed. The epididymal fat pad was immediately processed to obtain the isolated adipocytes or frozen and stored at the temperature of liquid nitrogen. The experimental protocol was approved by the Human and Animal Research Committee of the School of Biochemistry, University of Litoral (Santa Fe, Argentina).

Analytic methods

Plasma glucose, Tg, and FFA levels were determined by spectrophotometric methods and insulin by immunoreactive assays, as previously described [2,15]. The insulin assay was calibrated against rat insulin standard (Novo Nordisk, Copenhagen, Denmark).

Preparation of isolated adipocytes. Fat cell size, volume and fat cell number determination

The removed epididymal fat pads were rinsed in isotonic saline solution at 37 °C and the adipocytes were isolated according to the method of Rodbell [21], with minor modifications as previously described [15]. Cell diameters and fat pad cell number and volume were determined as previously described [2,15].

Basal lipolysis of the fat cells

Aliquots of diluted epididymal fat cells in the isolation buffer were placed into plastic vials (1×10^5 cells/mL) and incubated in a shaking Dubnoff water bath (60 cycles/min) at 37 °C for 1 h under an atmosphere of O_2:CO_2 95:5. Under these conditions, there was a time-dependent increase in lipolysis during 60 min [15]. Therefore, this time period was chosen for the incubation. Basal lipolysis was assayed by measuring glycerol release into the incubation medium over 1 h at 15-min intervals as described by Robdell et al. [21]. The infranadant (three aliquots of 200 μL) was removed from each incubation mixture for the measurement of glycerol by the enzymatic method of Wieland [22], as previously described [2,15].

FAS activity

Epididymal adipose tissue samples were homogenized in an ice-cold buffer containing 0.25 mol/L of sucrose, 1 mmol/L of dithiothreitol, and 1 mmol/L of ethylenediaminetetraacetic acid (EDTA), pH 7.4. Cytosolic fractions were obtained by centrifugation at $100{,}000 \times g$ for 1 h at 4 °C. FAS activity in the cytosolic fraction of adipose tissue was immediately assayed in duplicate by measuring malonyl CoA-dependent oxidation of nicotinamide adenine dinucleotide phosphate (NADPH) at 37 °C [23]. One unit of enzyme activity represents 1 μmol of NADPH oxidized per minute at 37 °C.

ACC activity

ACC activity was assayed by measuring the incorporation of (^{14}C) bicarbonate into malonyl-CoA as described by

Halestrap et al. [23]. Briefly, epididymal adipose tissue samples were homogenized at 0 to 4 °C in potassium phosphate buffer (50 mmol/L), pH 7.3, containing 2 mmol/L of EDTA, and 5 mmol/L of reduced glutathione (GSH). The homogenate was immediately centrifuged at 15000 × *g* for 2 min, and the enzymatic activity assayed in the infranadant. The initial ACC activity was measured 5 min after the enzymatic isolation. To determine the total enzymatic activity, ACC samples were preincubated at 30 °C for 30 min in the presence of 20 mmol/L of potassium citrate. Assays were initiated by adding a sample of infranadant to 100 mmol/L of (tris [hydroxymethyl] amino-methane)-hydrochloride (Tris-HCl), pH = 7.4, containing 5 mol/L of adenosine triphosphate (ATP), 10 mmol/L of magnesium chloride ($MgCl_2$), 0.5 mmol/L of EDTA, 1 mmol/L of GSH, 15 mmol/L of potassium biocarbonate [^{14}C] ($KH^{14}CO_3$) (specific radioactivity: 0.4 μCi/μmol), and 0.15 mmol/L of acetyl-CoA and incubated for 3 min at 30 °C. The reaction was stopped with 5 mmol/L of HCl. An aliquot (0.4 mL) was evaporated in a scintillation vial at 85 °C. The radioactivity contained in the residue was determined by liquid scintillation counting. One unit of ACC catalyzes the formation of 1 μmol of malonyl-CoA per min.

ME, G-6-PDH, and LPL activities

ME activity was measured according to Wise et al. [24]. Briefly, epididymal adipose tissue samples were homogenized (20% wt/vol) in ice-cold 0.25 mol/L of sucrose solution. Homogenates were then centrifuged for 10 min at 40.000 × *g* and 0 to 3 °C. The resulting aqueous supernatant fractions were used for the assay of the rate of NADPH formation by spectrophotometric measurement at 340 nm and 37 °C. The activity of G-6-PDH was measured in epididymal adipose tissue according to Cohen et al. [5], as previously described [15]. LPL activity was analyzed as described previously by Soria et al. [15] and was expressed as pktal (picomol of substrate transformed per second) per gram of fresh tissue.

Basal- and insulin-stimulated glucose uptake

Isolated adipocytes (10^6 cells per milliliter) were incubated in the absence and presence of insulin (10 nmol/L), for 30 min at 37 °C, according to Ciaraldi et al. [25]. At the end of the incubation period, a 50-μL aliquot of the cell suspension was added to 20 μL of 3-O- (^{14}C) methylglucose (0.5 μCi/tube) in 0.1 mmol/L 3-O-methylglucose. The reaction was terminated after 4 s by the rapid addition of 11 mL of chilled 0.9% sodium chloride (NaCl) containing 0.3 mmol/L phloretin-0.5% EtOH. Silicone oil was layered over the suspension and cells centrifuged at 1000 × *g* using a Beckman Microfuge B (Beckman Coulter, Fullerton, CA, USA) for 10 s. Cells were collected with absorbent pipe cleaners and the radioactivity determined by liquid scintillation counting. The portion of cellular uptake resulting from diffusion and trapping of the label in the extracellular water space was measured by performing parallel reactions with (^3H) inulin as substrate. All measurements were done in triplicate and values of transport were corrected for the (^3H) inulin value.

Statistical analysis

Sample sizes were calculated on the basis of measurements previously made in our laboratory with rats fed a CD or a SRD [2,15,26,27] based on 80% power. Results were expressed as mean ± SEM. Statistical comparisons were done transversely between different dietary groups. 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 [28]. Differences with P values < 0.05 were considered statistically significant. In all cases, the interclass correlation coefficients were ≥ 0.73.

Reagents

Enzymes for assays, substrates, and coenzymes were purchased from Sigma Chemical Co. (St. Louis, MO, USA) or from Boehringer Mannheim Biochemical (Indianapolis, IN, USA). $KH^{14}CO_3$, 3-O (^{14}C)methyl glucose and (^3H)insulin were purchased from Amersham Biosciences Inc. (Sunnyvale, CA, USA). Cod liver oil was purchased from ICN (Costa Mesa, CA, USA). All other chemicals were reagent grade.

Results

Body weight and energy intake

Body weight and energy intake were carefully monitored in the three dietary groups of rats throughout the experimental period. As we have previously shown [26], an increase ($P < 0.05$) in body weights (approximately 18%) and energy intakes occurred in rats chronically fed a SRD during 6 mo compared with rats fed a CD. These differences were still present when the SRD was extended till mo 8 of feeding. FO in the SRD diet did not modify body weight of the SRD-fed rats. However, in spite of a similar energy intake between SRD and SRD + FO–fed rats at 8 mo, the weight gain was moderately decreased in the latter group (Δ 6 to 8 mo) (Table 2).

Plasma metabolites and insulin levels

Basal levels of plasma Tg, FFA, and glucose were higher in rats fed the SRD for 8 mo compared with age-matched controls fed a CD. Similar values were obtained from rats fed a SRD during 6 mo (data not shown). All these variables returned to control values in the SRD-fed rats in which FO replaced corn oil for the last 2 mo of feeding. No statistical

Table 2
Body weight gain and energy intake in rats fed a control (CD), sucrose-rich (SRD) or SRD + fish oil (SRD + FO) diet

Diet	Body weight (g)		Energy intake (kJ/d)	Diet	Body weight (g)	Weight gain (g)	Energy intake (kJ/d)
	Initial	6 mo	Initial to 6 mo		8 mo	Months 6–8	Months 6–8
CD	188.3 ± 4.5*	405.3 ± 10.3†	283.7 ± 12.0†	CD	441.26 ± 4.2†	35.6 ± 5.0*	281.3 ± 6.2†
SRD	191.2 ± 4.0*	470.1 ± 7.2*	351.0 ± 11.9*	SRD	504.0 ± 9.5*	31.8 ± 5.4*	347.0 ± 11.8*
				SRD + FO	481.6 ± 7.0*	16.3 ± 5.0†	333.4 ± 6.5*

Values are expressed as mean ± SEM, $n = 20$. Values in each column that do not share the same superscript symbols are significantly different ($P < 0.01$) when one variable at a time was compared by the Newman-Keuls test. When FO replaced corn oil in the SRD, rats consumed SRD for 6 mo and a SRD diet containing FO for the last 2 mo of the experiment.

differences in plasma insulin levels were observed at the end of the experimental period between the three dietary groups. Values (mean ± SEM, $n = 6$) were Tg (mmol/L): 0.53 ± 0.06 in the CD; 2.37 ± 0.05 in the SRD; 0.56 ± 0.04 in the SRD + FO ($P < 0.05$, CD and SRD + FO versus SRD). FFA (μmol/L): 319.2 ± 24.3 in the CD; 885.8 ± 36.2 in the SRD; 305.2 ± 41.2 in the SRD + FO ($P < 0.05$, CD and SRD + FO versus SRD). Glucose (mmol/L): 6.40 ± 0.16 in the CD; 8.48 ± 0.12 in the SRD; 6.57 ± 0.20 in the SRD + FO ($P < 0.05$, CD and SRD + FO versus SRD). Insulin (pmol/L): 377.6 ± 28.4 in CD; 368.5 ± 36.7 in SRD; 372.5 ± 28.1 in SRD + FO.

Epididymal and retroperitoneal adipose tissue weight

The moderate overweight observed in rats fed a SRD (Table 3) was accompanied by an increase of visceral adiposity. Both epididymal and retroperitoneal fat tissue weights were significantly increased ($P < 0.05$) when compared with age-matched controls fed a CD. A significant reduction of both fat tissue weights was observed after FO administration, either expressed as total weight or relative to body weight (Table 3). A significant increase of cell volume in epidydimal adipocytes associated with a significant reduction of the adipose cell number per gram of tissue was observed in the SRD-fed rats. These parameters were improved when FO replaced corn oil in the SRD from the last 2 mo of the experimental period. However, the total cell number was similar in the three dietary groups (data not shown). Figure 1 depicts the high positive correlation between adipocyte Tg content and epididymal fat tissue weight in the three dietary groups of rats. In the SRD-fed rats, both parameters were significantly higher ($P < 0.05$) compared with those of the CD group. The presence of FO in the SRD for the last 2 mo of the diet significantly improved both adipocyte Tg content and fat pad tissue weight. However, these parameters are still higher than those recorded in the CD-fed animals.

Table 3
Epididymal and retroperitoneal adipose tissue weights of rats fed a CD, SRD, or SRD + FO diet

Diets	CD	SRD	SRD + FO
Epididymal fat			
Total weight (g)	7.19 ± 0.39‡	13.89 ± 0.60*	10.51 ± 0.61†
Relative weight (g/100 g body weight)	1.60 ± 0.10‡	2.79 ± 0.16*	2.19 ± 0.10†
Adipocyte cell volume (pl)	259.10 ± 12.6‡	439.9 ± 12.2*	332.1 ± 14.5†
Retroperitoneal fat			
Total weight (g)	6.10 ± 0.42‡	12.87 ± 0.46*	10.02 ± 0.63†
Relative weight (g/100 g body weight)	1.34 ± 0.12‡	2.60 ± 0.11*	2.00 ± 0.14†

Values are expressed as mean ± SEM, $n = 6$. Values in a line that do not share the same superscript symbols are significantly different ($P < 0.05$) when one variable at a time was compared by the Newman-Keuls test.

ACC, FAS, G-6PDH, ME, and LPL activities

The activities of enzymes related to de novo lipogenesis were measured in the epididymal adipose tissue of the three dietary groups. SRD-fed rats showed a significant increase ($P < 0.05$) of ACC, FAS, G-6-PDH, and ME activities, expressed as units or munits per total fat weight when compared with the control group fed a CD. Moreover LPL activity was also significantly higher in the adipose tissue of SRD-fed rats. A similar behavior was observed when all the enzyme activities were expressed as mU/cell number × 10^6 (data not shown). When dietary FO replaced corn oil as the source of fat in the SRD, a reduction of the ACC and ME activities

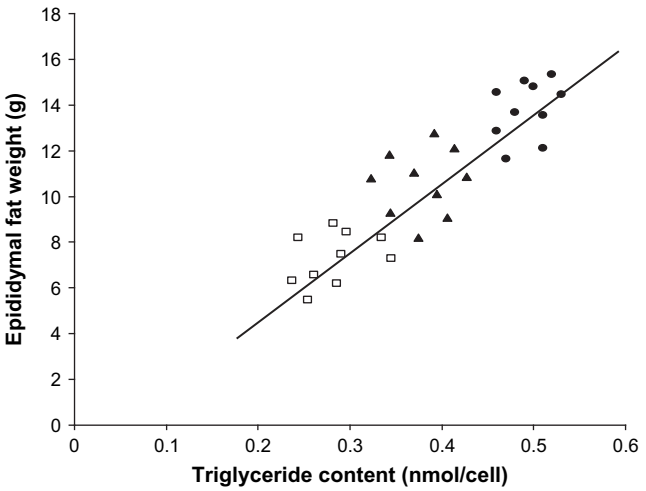

Fig. 1. Correlation between adipocyte triglyceride content and epididymal fat tissue weight of rats fed a CD, SRD, or SRD + FO diet. □, CD; ▲, SRD + FO; ●, SRD; $n = 30$; $r = 0.957$; $P < 0.05$.

Table 4
Lipogenic enzyme activities in epididymal adipose tissue of rats fed a CD, SRD, or SRD + FO diet

Diets	Acetyl-CoA carboxylase	Fatty acid synthase	Glucose-6-phosphate dehydrogenase	Malic enzyme	Lipoprotein lipase
	(mU/total fat weight)		(U/total fat weight)		(pktal/total fat weight)
CD	159.15 ± 18.4†	817.9 ± 121.5‡	1.47 ± 0.14†	1.08 ± 0.08†	17809 ± 1478‡
SRD	274.5 ± 36.5*	1768.9 ± 171.6*	2.50 ± 0.28*	1.95 ± 0.16*	45476 ± 2138*
SRD + FO	193.60 ± 6.90†	1248.1 ± 106.5†	0.90 ± 0.14‡	1.40 ± 0.18†	21857 ± 716†

Values are expressed as mean ± SEM, $n = 6$. Values in a column that do not share the same superscript symbols are significantly different ($P < 0.05$) when one variable at a time was compared by the Newman-Keuls test.

was observed, which reached values similar to those recorded in CD fed rats. However, FAS and LPL activities were still significantly higher than those in the CD group. On the other hand, G-6-PDH activity was significantly decreased, reaching values lower than those observed in the CD-fed animals (Table 4).

Basal and insulin-stimulated 3-O-methylglucose uptake in adipocytes from epididymal fat tissue

Figure 2 shows basal and insulin-stimulated 3-O-methylglucose uptake in isolated adipocytes from the three experimental groups. No differences in basal glucose uptake between the three dietary groups were recorded. On the other hand, insulin-stimulated 3-O-methylglucose uptake was significantly lower in adipocytes of SRD compared with age-matched control-fed rats. The addition of FO to the SRD for the last 2 mo of the experimental period completely restored the sensitivity of adipocyte to the insulin-stimulated 3-O-methylglucose uptake.

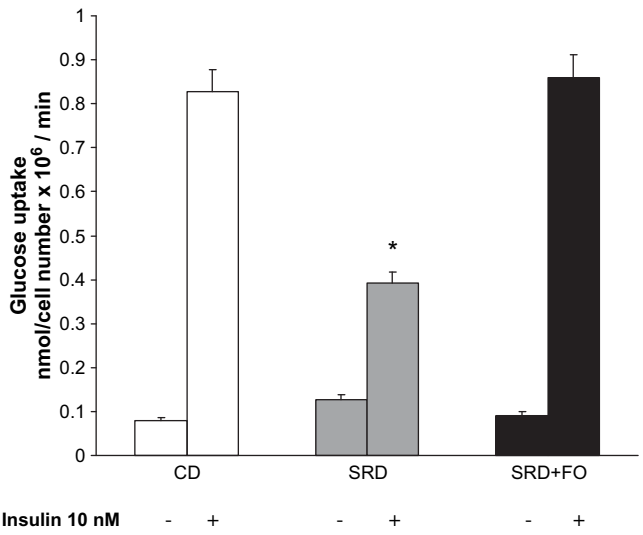

Fig. 2. Basal and insulin-stimulated 3-O-methylglucose uptake in isolated adipocytes from epididymal fat tissue of rats fed a CD, SRD, or SRD + FO diet. Values are expressed as mean ± SEM, $n = 6$. Adipocytes were stimulated with 0 or 10 nM of insulin. After stimulation, they were incubated with 3-O- (^{14}C) methylglucose (0.2 μCi / tube) in 0.1 mM 3-O-methylglucose. For further details on the methodology see Materials and Methods. *$P < 0.05$ SRD versus CD and SRD + FO.

Basal fat cell lipolysis

Basal lipolysis was measured in isolated epididymal adipocytes, as an estimation of both HSL and TGL activities. In enlarged fat cells of SRD-fed rats, basal lipolysis increased almost four-fold compared with age-matched controls fed a CD. When the source of fat—corn oil—was replaced by cod liver oil in the SRD group, basal lipolysis significantly decreased, although values remained slightly higher than in the CD-fed rats. Values were as follows (μmol glycerol/cell number × 10^6/hr, $n = 8$): 0.60 ± 0.03 in the CD; 2.70 ± 0.46 in the SRD; 1.10 ± 0.10 in the SRD + FO ($P < 0.05$ SRD versus CD and SRD + FO and $P < 0.05$ SRD + FO versus CD).

Discussion

The present work focused on some mechanisms underlying the accretion of adipose tissue mass, which developed in an experimental model of dyslipidemia and IR induced in rats by chronic administration of a SRD. Besides, we also explored the possible beneficial effect of dietary substitution of the source of fat—corn oil by FO—on these mechanisms.

The major new findings observed in the hypertrophied fat cells of SRD-fed rats are the following: (1) in epididymal fat pad: an increased activity of several key enzymes involved in de novo lipogenesis (FAS, ACC, ME, G-6-PDH); (2) in isolated adipocytes: an increased basal lipolysis was accompanied by a reduced insulin-stimulated glucose uptake; and (3) the presence of FO in the SRD acted to improve the signs of adiposity as well as the lipogenic enzyme activities. FO restored the altered capacity of insulin-stimulated glucose uptake. Moreover, FO was able to reverse the abnormal glucose homeostasis and dylipidemia without detectable changes in plasma insulin levels.

Adipose tissue metabolism is influenced by the size of the constituent cells in both rats and humans [15]. Our results show that chronic administration of SRD induces an increased cell volume and Tg storage that correlates with fat pad mass. The balance between fat synthesis and fat breakdown determines adipocyte fat accumulation. Both processes are tightly controlled by several factors (e.g., enzyme activities, nutritional conditions, and hormones). The present work reports an increased activity of the key enzymes related to de novo lipogenesis in the epididymal fat of animals chronically

fed a SRD. In agreement with these results, Cohen el al. [5] and Blakely et al. [6] reported increased G-6-PDH, 6-P-gluconate dehydrogenase, and NADP-malate dehydrogenase activities in adipose tissue of rats fed a sucrose or fructose diet. Moreover, the expression and enzymatic activity of G-6-PDH were significantly elevated in the white adipose tissue of several obese mice models, including db/db, ob/ob, as well as in diet-induced obesity [29].

The stimulation of adipose lipogenesis by carbohydrates occurs rapidly. In this regard, when control rats were tube-fed three fructose meals every 8 h for 24 h, they showed an increased FAS activity and synthesis [3]. However, Peyron-Caso et al. [20] showed that when a sucrose diet was administered during 3 wk, no change in FAS activity was observed. Interestingly, these animals were hyperinsulinemic and not overweight. In the present study, rats fed a SRD for 8 mo showed a significant increase of both FAS and ACC activities when they were expressed by total fat pad mass or by total cell number. A difference between our results and those of Peyron-Caso could be the duration of the SRD feeding because, as mentioned before, we previously demonstrated that the metabolic and hormonal milieu significantly changes according to the length of feeding [16].

FAS and ACC also show a complex gene regulatory mechanism. In the experimental model, gene regulation of fatty acid synthesis in adipose tissue through SREBP-1c is still controversial. In this regard, Wagner et al. [30] suggest that in adipose tissue of LPL-deficient mice, there is an induction of SREBP1 expression and processing, which in turn activates the adipose tissue lipogenic program. On the other hand, Sekiya et al. [31] suggest that in the adipocytes, unlike in hepatocytes, increments in nuclear SREBP1c are not accompanied by the transactivation of lipogenic genes; thus, SREBP1c is not committed to the regulation of adipose tissue lipogenesis. To the best of our knowledge, at present there are no data regarding the role of SREBP1 in the adipose tissue of a dyslipemic, IR animal model induced by long-term sucrose feeding.

LPL is another enzyme that is highly expressed in adipose tissue and also provides nonesterified fatty acid substrate for Tg synthesis. The present work shows an increased LPL activity in the epididymal adipose tissue of rats chronically (8 mo) fed a SRD. Similar results were observed by Deshaies et al. [8] in rats fed a SRD for a short period of time (4 wk). Moreover, no relationship between plasma insulin levels and LPL activity was recorded. This suggests that the level of circulating insulin after a high-sucrose diet did not determine *per se* the extent of stimulation of LPL in adipose tissue [8]. In this vein, a recent study reported no relationship between adipose tissue LPL mRNA or LPL activity and insulin sensitivity in IR subjects [32].

As is already known, there is a link between elevated circulating FFA levels and the development of IR. Our results show an increased basal lipolysis in the adipocytes of SRD-fed rats compared with age-matched controls fed a CD. A positive correlation between adipocyte HSL activity and fat-cell size was observed in obese rats [33]. Moreover, lipolysis in fat cells could also be influenced by the translocation of lipolytic enzymes (HSL and TGL) to the surface of a lipid droplet or by the amount of perilipin associated with the lipid droplet [34,35]. Although we only estimate the lipolytic activities (HSL or TGL) through the levels of glycerol release, we cannot discard the possibility that those mechanisms could be involved in this process.

Insulin has a central role in increasing glucose uptake and activating glycolytic enzymes in the adipose tissue. Although a similar basal glucose uptake from isolated adipocytes of SRD-fed rats was observed compared with that of age-matched controls, the insulin-stimulation of glucose uptake was impaired. On the other hand, although no differences in glucose transport were observed by Luo et al. [36] in rats fed a SRD for a short period of time (4 wk), other reports showed an altered adipocyte cell capacity for glucose transport and metabolism when sucrose is present in the diet [13,14]. Thus, even in the presence of both processes, altered hormone-stimulated glucose uptake and increased lipolytic activity—which suggest diminished insulin sensitivity—a long-term sucrose diet enhances lipogenic enzyme activities in the adipose tissue. This, in turn, leads to the accretion of adipose tissue mass.

The capacity of FO to limit adipocyte size and hypertrophy of visceral adipose tissue is well documented in rats [37]. In the present work, a significant reduction of adipocyte cell volume was recorded in SRD-fed rats, where FO was the source of fat. This was accompanied by a normalization of dislipidemia and whole body IR. The reversion of dyslipidemia by FO possibly contributes to the reduction of lipid stores and adipocite hypertrophy. Regarding the action of FO on lipogenic enzymes in adipose tissue, Raclot et al. [17] and Haug et al. [18] showed diminished mRNA levels and enzyme activities of FAS and LPL in normal rats fed a fat diet rich in FO. Moreover, ACC and FAS mRNA expressions were suppressed by feeding rats a diet enriched with n-3 PUFAs [38]. Changes in cell size and fat pad mass induced by FO were also observed in isolated adipocytes from Sprague-Dawley rats fed a SRD for a short period of time [20]. The presence of dietary FO in the SRD diet corrected the in vitro–enhanced basal lipolysis and significantly reduced the adipocyte cell size. Similarly to our results, Rustan et al. [39] showed a decreased basal and stimulated lipolysis from epididymal and perirenal adipocytes of rats fed a high-fat diet in the presence of PUFAs. Interestingly, adipose tissue mass was also reduced. Besides, Tsujita et al. [33] observed a positive correlation between adipocyte cell size and basal lipolysis in obese animals. It is well known that n-3PUFAs activate peroxisomal proliferators activated receptors (PPAR). The PPAPγ2 isoform expression in adipose tissue controls the expression of genes involved in adipogenesis and lipid and glucose metabolism [20]. Although no data have been reported so far concerning the PPARγ2 protein mass in adipose tissue of this experimental animal model, we cannot discard the possibility that this could be a feasible mechanism involved in the reduction of adipocyte cell size.

The substitution of the source of fat in the diet (corn oil by FO) in SRD reverses the impaired insulin-stimulated glucose uptake in isolated adipocytes from the epididymal fat pad. In this vein, Vrana et al. [40] showed increased insulin effect on glucose utilization in adipose tissue of sucrose-fed rats when FO was supplemented daily. Moreover, Peyron-Caso et al. [41] showed an increased insulin-stimulated glucose transport, oxidation, and incorporation into lipids in isolated adipocytes when FO was present in the SRD for the same period of time. Besides, insulin action is positively correlated with fatty acid insaturation index in membrane phospholipids of rats' adipocytes [41]. An increased Glut4 protein and mRNA levels in SRD supplementation with FO for 3 wk was observed by Peyron-Caso et al. [42]. The fatty acid composition of membrane phospholipids in insulin target tissue (e.g., adipose tissue, skeletal muscle, etc.) is an important factor that influences insulin sensitivity through its effect on insulin receptors and in glucose transport [43]. Although we did not evaluate the fatty acid composition of adipose tissue membrane phospholipids, we previously demonstrated an increased n-3 fatty acid content in the skeletal muscle phospholipids of rats fed a SRD + FO [44]. In these animals, FO restores whole-body insulin sensitivity [2]. On the other hand, Liao et al. [45] showed the activation of PPARγ-enhanced basal- and insulin-stimulated glucose transport in 3T3-L1 adipocytes. Thus, another possible mechanism by which FO could affect glucose uptake might be through activation of PPARγ.

Finally, feeding rats a SRD for a long term induced morphological and metabolic changes in fat pad mass that might contribute to whole-body insulin insensitivity and lipotoxicity. The hypolipidemic effect of FO was able to improve and/or reverse these abnormalities. Thus, this animal model proves to be useful to study the mechanisms conditioning the influence of nutrients on the development and management of these metabolic diseases.

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