Increased leptin storage with altered leptin secretion from adipocytes of rats with sucrose-induced dyslipidemia and insulin resistance: effect of dietary fish oil

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Abstract

This study examined the effect of long-term feeding a high-sucrose diet (SRD) on the modulation of rat adipocyte's leptin secretion and storage. For this purpose, we analyzed (*a*) basal and insulin-stimulated leptin release and the role of isoproterenol and palmitate on insulin-stimulated leptin secretion, (*b*) the correlation between leptin and glycerol released, (*c*) the relationship between leptin contents and adiposity, and (*d*) the effect of fish oil (FO) administration on the above parameters. Wistar rats were fed an SRD for 6 months. Whereas half the animals continued with SRD up to month 8, the other half was fed an SRD in which FO partially replaced corn oil from months 6 to 8. Total leptin release was reduced both basally and under insulin stimulation in SRD-fed rats. However, the ratio of leptin released after hormone stimulation to basal leptin levels was similar in the 3 dietary groups. Isoproterenol inhibited insulin-stimulated leptin release in the 3 groups, but the percentage was lower in the SRD. Palmitic acid mimicked the effect of isoproterenol. Leptin release from adipocyte of SRD-fed rats negatively correlated with glycerol release. Leptin store increased in fat pads of SRD and positively correlated with adiposity. Fish oil reduced leptin content and fat pad hypertrophy, and normalized basal lipolysis, leptinemia, and glucose homeostasis. This suggests that enhanced lipolysis and altered insulin sensitivity could play a role in the decrease of leptin released in SRD-fed rats. This is consistent with the reversion of all the alterations after FO administration.
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1. Introduction

Insulin resistance (IR), overweight, and intraabdominal fat deposition (visceral adiposity) are central features of the metabolic syndrome. This syndrome that has a high prevalence all over the world also includes a constellation of abnormalities such as hypertension, dyslipidemia, glucose intolerance, and type 2 diabetes mellitus, among other disturbances [1]. The interactions of both genetic and environmental factors (eg, sedentary lifestyle and diet composition) are known to contribute to its development. An experimental model that resembles several aspects of the metabolic syndrome can be induced in rats by feeding

them high-sucrose or high-fructose diets [2,3]. In this regard, studies from our laboratory have reported that rats chronically fed (up to 15-40 weeks) with a diet rich in sucrose (SRD) developed a steady state of hypertriglyceridemia, elevated plasma free fatty acid (FFA) levels, moderate hyperglycemia, and whole-body IR. This was accompanied by moderate overweight with sustained increase of visceral adiposity [3-5]. In addition, high blood pressure and cardiomyocyte dysfunction were also observed [6,7]. Thus, the long-term administration of SRD is able to develop most of the features present in the metabolic syndrome.

The adipose tissue is an active endocrine organ secreting molecules commonly referred to as *adipokines* and includes several cytokines, hormones, and growth factors. Leptin, a peptide hormone encoded by the *ob* gene that is produced and secreted by white adipocyte, plays a role in the regulation of food intake, energy expenditure, and adiposity, as well as immune and endocrine systems (for review, see

references [8-11]). Leptin transcription and secretion are modulated by numerous factors (eg, insulin, glucocorticoids, FFA, catecholamines) [8]. The leptin gene expression is subject to nutritional regulation [12]. Glucose and insulin are involved in the regulation of leptin secretion under physiologic and in vitro conditions. Starvation or dietary restriction decreases both leptin transcription in adipose tissue and plasma level. These changes are closely related to decreased plasma insulin levels, activation of the sympathetic nervous system, increased lipolysis, and FA levels [13]. Refeeding, or insulin administration, reverses the decrease of plasma leptin and *ob* gene expression in adipocytes of rodents and humans [13]. The effects of insulin to increase leptin expression and secretion [14] and the transcriptional activity of the leptin promoter [15] seem to be mediated by the insulin effect to increase adipocyte glucose utilization. However, little or no effects of insulin were found in some studies [16,17].

Plasma leptin is also influenced by the nature of dietary fatty acids. Peyron-Caso et al [18] demonstrated that the presence of n-3 polyunsaturated fatty acids (PUFAs) from fish oil (FO) in the diet of SRD-fed rats increased leptin levels and prevented dyslipidemia, adiposity, and impaired glucose homeostasis induced by 3 weeks of feeding an SRD. Besides, eicosapentaenoic acid (EPA) (20:5, n-3) has been reported to stimulate leptin expression and secretion in 3T3-L1 adipocytes [19]. In contrast, Reseland et al [20] reported that dietary n-3 PUFAs decreased leptin messenger RNA (mRNA) expression in vivo and in vitro; and Cammissotto et al [21] observed an inhibitory effect of medium- and long-chain fatty acids (including EPA) on insulin-stimulated leptin secretion in isolated white adipocytes of normal rats.

We have recently shown a decrease in plasma leptin levels without changes in their gene expression despite overweight and visceral adiposity in dyslipemic, long-term SRD-fed, IR rats [5]. In these animals, we previously demonstrated, using isolated adipocytes, a high increase in lipolysis and a reduced antilipolytic effect of insulin [22]. Moreover, insulin-stimulated glucose uptake was impaired [23]. Interestingly, it was shown that by shifting the source of dietary fat (corn oil to FO) for 2 months, increased plasma leptin levels reversed the preexisting state of IR and dyslipidemia (normalized plasma FAs and triglyceride levels) and improved adiposity [5]. Some factors could be implicated in the decrease of plasma leptin levels in the face of adiposity in a long-term SRD feeding. Among them, it is possible that both an intracellular increase of FAs generated by an activated lipolysis as well as a continuous exposure of adipose tissue to high plasma FAs levels could be involved in the reduced leptinemia. In this regard, it has been shown that both protein and mRNA levels of leptin were decreased by triacsin and by long-chain monounsaturated and polyunsaturated fatty acids in cultured rat adipocyte of normal rats [24]. Given this information, we considered that it was worthwhile to extend our previous knowledge studying on isolated adipocytes of rats the effect of long-term (8 months)

feeding of an SRD on (*a*) basal and insulin-stimulated leptin release and the role of the isoproterenol (*β*-agonists) and palmitate on insulin-stimulated leptin secretion, (*b*) the correlation between the levels of basal leptin release and glycerol release (as an index of lipolysis), and (*c*) the relationship between leptin content (reflecting the size of preformed leptin stores) in white adipose tissue and adipose tissue fat accretion. Besides, because the addition of dietary n-3 PUFAs decreases adiposity and normalizes leptinemia in long-term SRD-fed rats, as previously described, we investigated the effects of FO on all the parameters mentioned above.

2. Materials and methods

2.1. Animals and diets

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°C ± 1°C; 12-hour light/dark cycle, light on 7:00 AM to 7:00 PM; humidity and airflow conditions). After 1 wk of acclimation, the rats were randomly divided into 2 groups (Table 1). The experimental group received a purified high-sucrose diet ([SRD] sucrose, 62.5 g/100 g; corn oil, 8 g/100 g), whereas the control group received the same purified diet, but with sucrose replaced by cornstarch (62.5 g/100 g) (high-starch diet [CD]). The experimental group received the SRD for 6 months. At this time, rats were randomly subdivided into 2 subgroups. The rats of the first subgroup continued the SRD up to 8 months of feeding. The second subgroup (SRD + FO) (Table 1) 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 per 100 g plus 1 g/100 g of corn oil) from months 6 to 8. The control group received the CD throughout the experimental period.

Table 1
Composition of experimental diets

Diet ingredients	CD		SRD		SRD + FO[a]	
	% 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[b]	3.5		3.5		3.5	
Vitamin mix[c]	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	
Cornstarch	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
FO					7.0	16.2

Diets are based on the AIN-93M diet.
  [a] The FO used is cod liver oil.
  [b] Salt mix (AIN-93M-Mx [5]).
  [c] Vitamin mix (AIN-93-Vx [5]).

Cod liver oil was purchased from ICN Biomedical, Biomedical Research Products (Costa Mesa, CA). The composition of the diets is given in Table 1. 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 were consumed by rats ad libitum. Diets were prepared every day by adding the oils to the base mixture containing the other nutrients. The oils and base mixture were separately stored at 4°C until preparation of the diet. Fish oil was kept under nitrogen atmosphere during storage. Dietary fats were analyzed by capillary gas chromatography, as previously described [22]. The weight of each rat was recorded twice each week during the experimental period. In a separate experiment, the individual caloric intakes and weight gains of at least 8 animals in each group and subgroup were assessed twice a week. At the end of the 8-month dietary period, food was removed at end of the dark period (7:00 AM); and experiments were performed between 7:00 and 9:00 AM. 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; plasma was either immediately assayed or stored at −20°C. Epididymal and retroperitoneal adipose tissues were totally removed and rapidly weighed. The epididymal fat pad was immediately processed to obtain the isolated adipocytes or minced (to mix different parts of a pad), immediately used or frozen in liquid nitrogen, and stored at −80°C. The experimental protocol was approved by the Human and Animal Research Committee of the School of Biochemistry, University of Litoral (Santa Fe, Argentina).

2.2. Preparation of isolated adipocytes

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 as previously described [5,22,23], with minor modifications. Briefly, fat pads were placed in Krebs-Ringer bicarbonate (KRB) buffer (120 mmol/L NaCl, 4.75 mmol/L KCl, 2.5 mmol/L CaCl$_2$, 1.2 mmol/L KH$_2$PO$_4$, 1.2 mmol/L SO$_4$Mg, 25 mmol/L NaHCO$_3$, 5.5 mmol/L glucose, 20 mmol/L HEPES, and 1% fatty acid–free bovine serum albumin [BSA], pH = 7.4 [1% KRB]). The minced epididymal tissue was incubated in 1% KRB containing 1 mg/mL collagenase at 37°C for 15 to 20 minutes with shaking frequency of 150 cycles per minute. At the end of incubations, cells were filtered through a 500-μm nylon filter and diluted with 5 mL of 1% KRB. The floating cells were washed 4 times with 1% KRB.

2.3. Determination of fat cell volume and fat cell number

One fraction of washed cells was used for the determination of fat cell size and number as we previously described [5,22,23].

2.4. Determination of leptin secretion in isolated adipocytes

Another fraction of washed cells was preincubated at 37°C for 15 minutes in 1% KRB (shaking frequency of 40 cycles per min) and washed 2 times with warm (37°C) KRB containing 0.1% fatty acid–free BSA (because albumin strongly binds extracellular fatty acids) according to Cammissotto et al [21]. Finally, the cells were incubated under the same conditions for 2 hours in the absence or presence of 10 nmol/L insulin, 1×10^{-6} mol/L isoproterenol, or 1 mmol/L palmitic acid at a concentration of 3 to 5×10^5 cells per milliliter. The adipocytes were then allowed to float, and the infranatants were frozen al −20°C for leptin concentrations. Leptin concentrations in the infranatants were determined by radioimmunoassay using a kit available from Linco Research (St Charles, MO). The assays were done in triplicates according to the manufacturer. Leptin releases were expressed as nanograms per 10^6 cells × 2 hours. Because of the higher variability in basal leptin secretion between adipocytes cultured from different rats, the experimental results from each adipocyte suspension prepared from a single animal were analyzed in relation to a control animal.

2.5. Determination of basal lipolysis

For the study of basal lipolysis, aliquots of diluted fat cells were incubated in Krebs-Henseleit phosphate buffer (pH = 7.4) containing 4% BSA essentially free of fatty acids in shaking Dubnoff water bath (60 cycles per minute) at 37°C for 1 hour under an atmosphere of 95% O$_2$–5% CO$_2$ as previously described [22]. At the end of incubations, aliquots of infranatants were removed from the incubation mixture for measurement of glycerol release by enzymatic method [22].

2.6. Determination of tissue leptin content

Fresh epididymal adipose tissue samples were homogenized in TES buffer (20 mmol/L Tris-HCl, 1 mmol/L EDTA, 225 mmol/L sucrose, 0.1 mmol/L phenylmethylsulfonyl fluoride, and protease inhibitor: 0.036 mmol/L pepstatin, 1% Triton X-100), pH = 7.4, using a ground-glass homogenizer according to Peyron-Caso et al [18] and Russell et al [25]. Homogenates were centrifuged at 12 000g (10 minutes, 4°C), and the leptin content of the cytosolic phase was measured as mentioned above. Data are expressed as leptin concentration of epididymal adipose tissue as nanograms per gram of tissue or adjusted for body weight.

2.7. Analytic methods

Plasma glucose, triacylglycerol, and FFA levels were determined by spectrophotometric methods and insulin by immunoreactive assays as previously described [5,22,26]). The insulin assay was calibrated against rat insulin standard (Novo Nordisk, Copenhagen, Denmark). Plasma leptin was determined by radioimmunoassay kit (Linco Research).

2.8. Statistical analysis

Samples sizes were calculated on the basis of measurements made previously in our laboratory with rats fed either a CD or an SRD [3-5,22,23], considering an 80% power. Results were expressed as mean ± SEM. Statistical comparisons were done transversely between different dietary groups at the end of the experimental study. The statistical significance between groups was determined by 1-way analysis of variance with 1 factor (diet) followed by the inspection of all differences between pairs of mean by the Newman-Keuls test [27]. Differences having P values < .05 were considered to be statistically significant. In all cases, the interclass correlation coefficients were at least 0.73.

3. Results

3.1. Body weight gain and energy intake

In the 3 dietary groups of rats, body weight and energy intake were carefully monitored throughout the experimental period. Similarly, as previously shown [5,23], a significant increase (P < .05) in both body weight and energy intake was recorded in rats chronically fed an SRD for 6 months compared with the CD-fed group. These differences were still present when the SRD was fed for 8 months. However, the presence of FO in the SRD developed a moderate reduction of weight gain compared with both SRD and CD groups (Table 2).

3.2. Plasma lipids, glucose, and insulin levels

In agreement with previous results from our laboratory [5,23], at the end of the dark period (7:00 AM), plasma triglyceride, FFA, and glucose concentrations were higher in rats consuming SRD for 8 months compared with age-matched controls fed a CD. All these variables returned to control values in the group of SRD rats in which FO replaced corn oil during the last 2 months of the experimental period. No statistical differences in plasma insulin levels were observed at the end of the feeding period among the 3 dietary groups (Table 3).

3.3. Effect of insulin, isoproterenol, and palmitic acid on leptin secretion in isolated adipocytes

Fig. 1 shows that, in the absence of insulin, the amount of leptin released from isolated adipocytes of SRD-fed rats

Table 3
Plasma metabolites and insulin concentration in rats fed CD, SRD, or SRD + FO diet

Diet[a]	Triglyceride (mmol/L)	FFA (μmol/L)	Glucose (mmol/L)	Insulin (pmol/L)
CD	0.48 ± 0.08[b]	355.0 ± 32.0[b]	6.45 ± 0.07[b]	375.0 ± 30.0
SRD	2.30 ± 0.05[a]	850.0 ± 48.0[a]	8.20 ± 0.10[a]	369.0 ± 39.0
SRD + FO	0.63 ± 0.04[b]	405.0 ± 41.0[b]	6.58 ± 0.18[b]	365.0 ± 28.0

[a] Rats in CD and SRD were fed their respective diet for 8 months. Rats in SRD + FO were fed the SRD for 6 months and SRD + FO from months 6 to 8 (for more details, see "Materials and methods"). Values are expressed as mean ± SEM. Six animals were included in each experimental group. Values in each column that do not share the same superscript letter are significantly different (P < .05) when one variable at a time was compared by the Newman-Keuls test.

was significantly (P < .05) lower compared with that recorded from CD-fed rats. As expected, the addition of insulin (10 nmol/L) to the incubation medium significantly stimulated leptin secretion (P < .05) from the CD-fed group. Adipocytes from rats fed an SRD were also sensitive to the stimulatory effect of insulin. However, although the amount of leptin expressed as absolute values was significantly lower in this dietary group, the increase of leptin released above the basal levels under the stimulus of the hormone was similar to that observed in the rats fed a CD (ca 60%). Besides, the addition of FO in the SRD was able to increase leptin released in either the absence or presence of insulin, reaching values similar to those of the control group (CD) (Fig. 1). In the SRD-fed rats, the lower leptin secretion could be metabolically associated with the increases of lipolysis. Therefore, the adipocytes were incubated with insulin in the presence of 10 μmol/L of isoproterenol (β-agonists) or 1 mmol/L of palmitic acid. Isoproterenol significantly decreased the leptin release in all dietary groups. The absolute values of leptin release were similar in all groups of rats. However, the percentage of inhibition of leptin secretion was quite different if each dietary group was compared with their own values obtained after insulin stimulation. Thus, almost 3-fold and 2-fold decreases of leptin release were observed in adipocytes from CD and SRD + FO, respectively, whereas these values reached only 1-fold in rats fed an SRD. Palmitic acid mimicked the inhibitory effect of isoproterenol on insulin-stimulated leptin release (Fig. 1). Besides, it is important to consider that we recently demonstrated [23]

Table 2
Body weight gain and energy intake in rats fed CD, SRD, or 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 6th mo		8 mo	6th to 8th mo	6th to 8th mo
CD (n= 8)	184.2 ± 7.2	407.0 ± 19.4[b]	274.0 ± 16.6[b]	CD (n = 8)	438.7 ± 13.1[b]	33.8 ± 4.9[a]	272.5 ± 10.7[b]
SRD (n= 16)	190.0 ± 4.7	474.9 ± 11.0[a]	343.5 ± 15.5[a]	SRD (n = 8)	507.5 ± 17.6[a]	32.7 ± 4.8[a]	346.0 ± 17.5[a]
				SRD + FO (n = 8)	483.2 ± 6.7[a]	15.8 ± 5.2[b]	330.5 ± 11.3[a]

Values are expressed as mean ± SEM. Values in each column that do not share the same superscript letter are significantly different (P <.05) when one variable at a time was compared by the Newman-Keuls test.
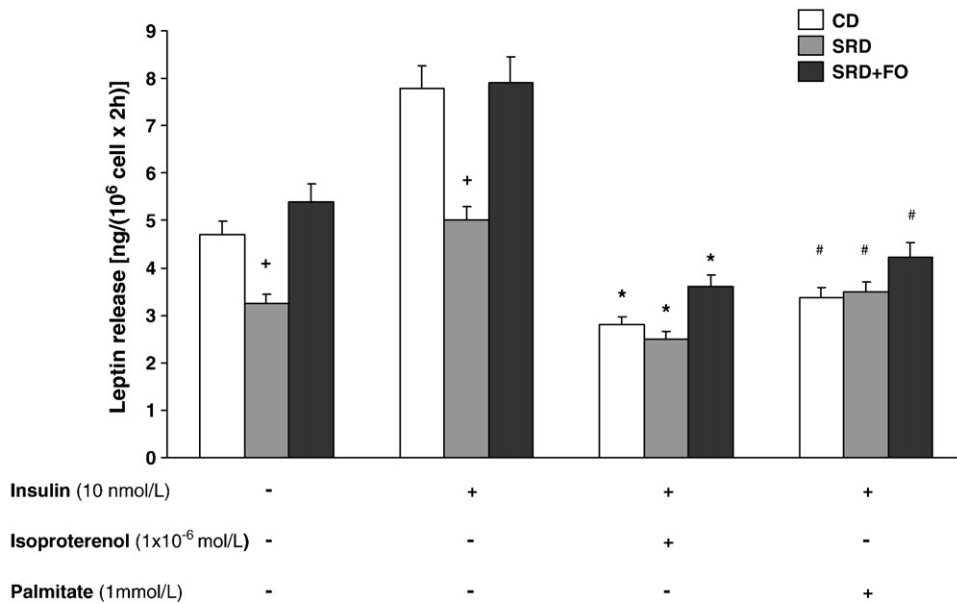
Fig. 1. Effects of insulin, isoproterenol, and palmitic acid on leptin release in isolated adipocytes of rats fed a control (CD), sucrose-rich (SRD), or SRD + FO diet. Adipocytes were incubated in the absence or presence of insulin (10 nmol/L), isoproterenol (10^{-6} mol/L), and palmitic acid (1 mmol/L) as described in "Materials and methods." Values are expressed as mean ± SEM (n = 8). $^{+}P < .05$ SRD vs CD and SRD + FO. $^{*}P<.05$ CD, SRD, and SRD + FO in the presence of insulin and isoproterenol vs CD, SRD, and SRD + FO, respectively, incubated with insulin alone. $^{#}P < .05$ CD, SRD, and SRD + FO in the presence of palmitic acid and insulin vs CD, SRD, and SRD + FO, respectively, incubated with insulin alone.

a reduction of insulin-stimulated glucose uptake in isolated adipocytes from rats fed an SRD, during the same period. Glucose uptake returned to normal values after FO administration. Values were as follows: mean ± SEM nanomoles per cell number × 10^6 per minute: CD, 0.82 ± 0.09; SRD, 0.39 ± 0.07: SRD + FO, 0.86 ± 0.07; $P< .05$ CD and SRD + FO vs SRD. Moreover, no differences in basal glucose uptake between the 3 dietary groups were recorded (data not shown) [23].

3.4. Correlation between basal glycerol release and leptin release

Fig. 2 shows the high negative correlation (r^2 = -0.775, $P < .001$) between basal glycerol release (as an index of lipolysis) and leptin release. In the enlarged fat cells of SRD-fed rats, basal glycerol release was significantly increased compared with control rats fed a CD, whereas leptin release was significantly lower. When the source of fat—corn oil—was replaced by FO in the SRD for the last 2 months of the experimental period, both basal glycerol and leptin releases reached values similar to those observed in the CD-fed animals. Fat cell volume was significantly higher in the SRD-fed group and was reduced after FO administration. Values were as follows: mean ± SEM, picoliters (n = 6): CD, 274.4 ± 15.1; SRD, 562.3 ± 29.7; SRD + FO, 376.5 ± 20.1; $P< .001$ CD and SRD + FO vs SRD and $P < .01$ CD vs SRD + FO. Total cell number of epididymal fat pad was similar in the 3 dietary groups (data not shown).

3.5. Relationship between total epididymal fat pad leptin contents and the epididymal fat pad weight

Fig. 3 depicts a high correlation (r^2 = 0.948, $P < .001$) between total adipose tissue leptin contents and epididymal fat pad weight in the 3 dietary groups. The results show that,
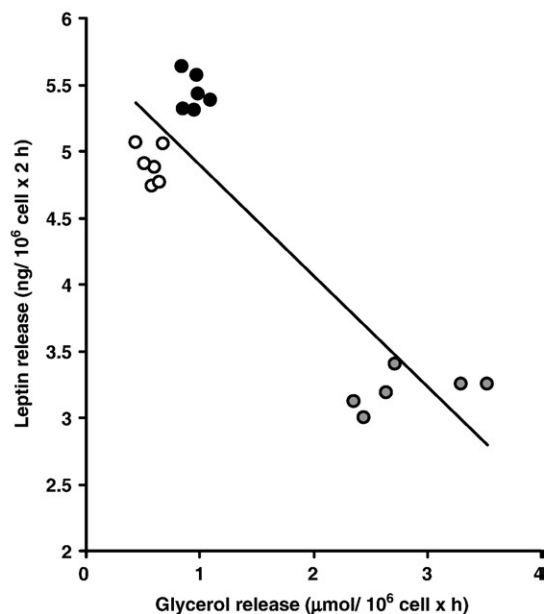

Fig. 2. Correlation of leptin release and glycerol release of rats fed a control (CD), sucrose-rich (SRD), or SRD + FO diet. ○, CD; ◖, SRD; ●, SRD + FO. Leptin release negatively correlated with basal fat cell lipolysis (glycerol release) (n = 18, r^2 = 0.775, $P < .001$).
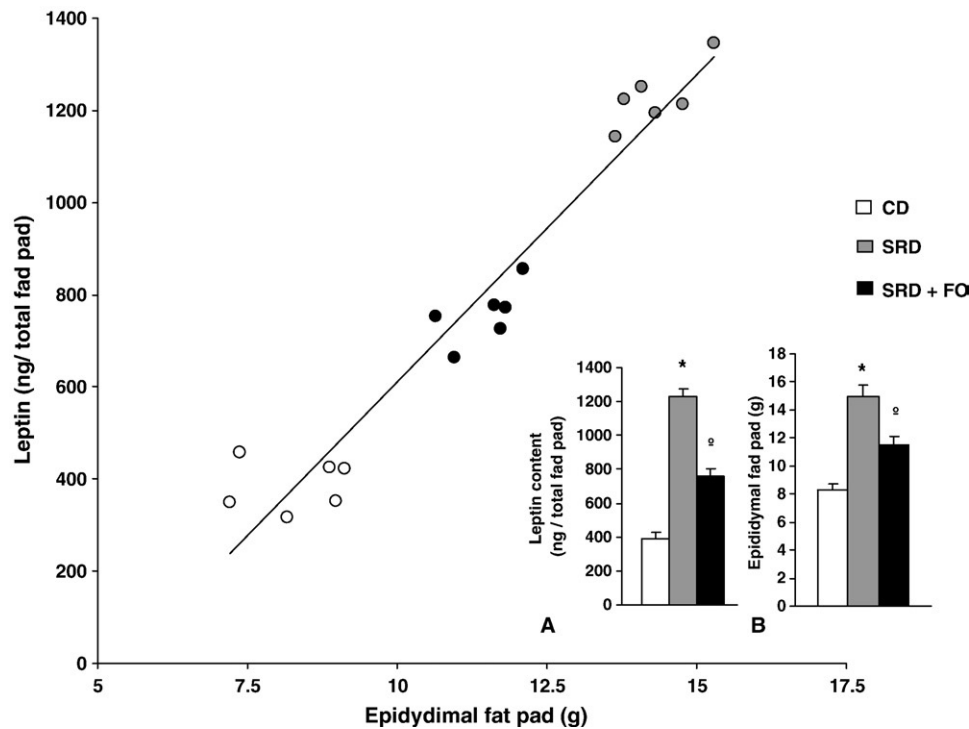
Fig. 3. Correlation of adipose tissue leptin content and epididymal fad pad weight of rats fed a control (CD), sucrose-rich (SRD), or SRD + FO diet. ○, CD; ◖, SRD; ●, SRD + FO. Adipose tissue leptin content positively correlated with epididymal fat pad weight (n = 18, r^2 = 0.948, P < .001). Mean values ± SEM of adipose leptin content (A) and epididymal fat pad weight (B) of rats fed CD, SRD, and SRD + FO. n = 6 from each dietary group. *P < .05 SRD vs CD and SRD + FO. °P < .05 SRD + FO vs CD.

in rats fed an SRD, the increase of leptin concentration in the epididymal fat pads correlates with the increases of fat pad accretion. The administration of FO in the SRD significantly reduces fat pad weight and leptin contents, although the values are still higher than those found in the CD. Inserts A and B depict the mean ± SEM values of total fat pad leptin concentrations and epididymal fat pad weight of the 3 dietary groups. Moreover, confirming a previous report [5], the plasma leptin levels were significantly lower in the SRD-fed rats even in the presence of both increased adiposity and tissue leptin storage. Fish oil was able to normalize plasma leptin levels, which reached similar values to those of the CD-fed rats. Values were as follows: (nanograms per milliliter, mean ± SEM, n = 7) CD, 14.2 ± 1.80; SRD, 9.6 ± 0.93; SRD + FO, 16.8 ±2.2; P< .05 SRD vs CD and SRD + FO. Moreover, a negative correlation between plasma leptin levels and adipose tissue mass (r^2 = −0.834, P < .001) was observed.

4. Discussion

Rats chronically fed a sucrose-rich diet develop dyslipidemia, IR, visceral obesity accompanied by a decrease of plasma leptin levels, impaired glucose uptake, and increased adipose tissue lipolysis [5,23]. Expanding our previous research, this study examined the effect of long-term feeding of high-sucrose diet on the modulation of

rat adipocyte leptin secretion as well as leptin adipose tissue storage. Besides, we explored the effect of dietary FO on leptin secretion and storage.

The major new findings provided by the present study are the following: (a) Leptin release expressed as absolute values was reduced both basally and under the stimulus of insulin in isolated adipocytes from long-term SRD-fed rats. However, the ratio of leptin released after insulin stimulation to basal leptin levels was similar in the 3 dietary groups. The administration of dietary FO normalized basal and insulin-stimulated leptin release (Fig. 1). Isoproterenol significantly inhibited the secretion of leptin under the stimulus of insulin in all dietary groups, but the percentage of inhibition was lower in rats fed an SRD. Palmitic acid mimicked the inhibitory effect of isoproterenol. (b) Isolated adipocyte from SRD-fed rats showed a strong negative correlation between basal glycerol release (as an index of lipolysis) and leptin release. Both parameters reached values similar to those of the control group after FO administration. (c) Leptin store significantly increased in epididymal fat of SRD-fed rats and was positively correlated with fat pad accretion. Fish oil reduced both leptin concentration and fat tissue weight, although the values recorded were still higher than those observed in CD-fed rats. Besides, and confirming previous results, in the SRD group, the administration of FO was able to reduce adipocyte cell volume and basal lipolysis, normalizing plasma FFA, triglyceride, and leptin levels as

well as glucose homeostasis without changes in circulating insulin levels.

Plasma leptin and FFA levels reciprocally change with nutritional and physiologic conditions. Studies in vitro using isolated adipocytes of normal rats have shown that insulin stimulated leptin secretion, whereas β-agonist and other agents activating adenylate cyclase exerted a strong inhibitory effect on insulin-stimulated leptin secretion and concomitantly stimulated lipolysis and fatty acid release [26]. The present results show that the acute effect of insulin alone and in the presence of isoproterenol or palmitate on leptin release from isolated adipocytes of rats fed a CD is in agreement with the above observations. A different picture emerges from isolated adipocytes of SRD-fed rats. In these animals, the total amount of leptin release, both basal and under the stimulus of insulin, was significantly reduced. The increases of basal glycerol release indicate enhanced lipolysis. Moreover, we previously demonstrated a significant increase of glycerol release in isoproterenol-stimulated lipolysis in adipocyte of rats chronically fed an SRD as compared with age-matched controls fed a CD [22].Thus, the strong negative correlation between the release of basal leptin and glycerol levels observed in this dietary group suggests that an intracellular increase of fatty acid generated as a consequence of an enhanced lipolysis could play a role in the modulation of leptin secretion. Besides, the addition of palmitic acid significantly decreases insulin-stimulated leptin release. Accordingly, Cammissotto et al [21] demonstrated in vitro in adipocyte of normal rats that medium- and long-chain fatty acids, independent of the degree of insaturation, acutely suppress the stimulatory effect of insulin on leptin secretion.

Besides lipid metabolism, the glucose transport and metabolism are important factors in the regulation of leptin expression and secretion. Cammissotto et al [28] recently observed that glycolytic substrates are necessary to maintain both basal and insulin-stimulated leptin secretion. Mueller et al [14] showed that the increase in insulin-stimulated leptin secretion was more closely related to the amount of glucose taken up by the adipocytes than to the insulin concentration per se. Moreover, the inhibition of glucose transport or phosphorylation and glycolysis cause a suppression of leptin release in cultured rat adipocytes [14]. In adipocytes from SRD-fed rats, we recently demonstrated a substantial reduction of both insulin-stimulated glucose uptake and the antilipolytic action of insulin [22,23]. As mentioned above, leptin release under the stimulus of insulin, expressed as absolute amount, was significantly lower in SRD-fed rats. However, the ratio of leptin released after the hormone stimulation was similar to that recorded in the CD and SRD + FO groups. Therefore, these findings suggest that the sensitivity of the leptin secretory pathway to insulin could be involved in the decrease in insulin-stimulated leptin secretion from the adipocytes of SRD-fed rats.

On the other hand, controversial results (inhibition-stimulation) regarding in vitro effects of EPA on leptin gene expression and secretion have been previously reported [19,20]. Eicosapentaenoic acid has been shown to stimulate leptin mRNA expression and secretion in 3T3-L1 adipocytes [19].The EPA-induced increased basal leptin secretion was highly correlated with the increased glucose utilization and inversely related to anaerobic glucose metabolism to lactate [29]. However, other studies have reported inhibitory effects of n-3 PUFAs on leptin expression and secretion in different cell lines [20,21]. The present results show an increase of the total amount of leptin released in both the absence or the presence of insulin from isolated adipocytes of rats fed an SRD + FO that reached values that remained within the physiologic range. This was accompanied with a decrease of intracellular lipolysis (glycerol release was similar to those of CD group). Interestingly, we recently demonstrated a normalization of both insulin-stimulated glucose uptake and the antilipolytic action of the hormone in the adipose tissue of SRD + FO–fed rats [22,23]. Thus, the addition of dietary FO decreasing adipocyte intracellular lipolysis and increasing glucose uptake (previous results) [23] might contribute to normalize leptin secretion in SRD-fed rats.

Parrish et al [30] demonstrated that dietary FO modifies the lipid structure of rat adipocyte plasma membrane by increasing PUFAs that would increase the fluidity of the membrane. Although our study did not analyze the fatty acid composition of adipose tissue plasma membrane, we have previously demonstrated an increase in n-3 EPA and docosahexaenoic acid as well as the n-3/n-6 ratio in the skeletal muscle and microsomal membrane of liver of SRD-fed rats after FO administration [31,32]. Therefore, these changes could be involved in the mechanisms related to the effects of dietary PUFAs on the improvement of adipose tissue insulin sensitivity and decreased lipolysis.

Newly synthesized leptin may be stored, secreted, or degraded intracellularly before being secreted [33]. Nutritional states and hormonal stimulus could affect any of these processes to regulate leptin release [12]. Larger adipocytes contain a greater amount of leptin compared with smaller ones, both in rats and in humans [12,34]. In agreement, the present results show, in enlarged fat cells from long-term SRD-fed rats, a significant increase of leptin concentration that highly positively correlates with adipose tissue mass. These findings show that leptin production is proportional to fat cell size and that it is not impaired in rats fed an SRD. Thus, sucrose feeding appears to exclusively reduce leptin secretion. In this regard, the addition of FO that reduces fat cell hypertrophy without changes in cell number significantly decreased leptin store in the fat pad of the SRD group. It is worth noticing that all the changes mentioned above in the SRD-fed rats, either in the presence or in the absence of dietary FO, appeared without any modification of adipose tissue *ob* mRNA expression [5].

Studies addressing the leptin content in adipose tissue particularly in the SRD insulin-resistant model are few. Peyron-Caso et al [18] found that both 3 and 6 weeks of sucrose diets induced a parallel increase in both plasma

leptin and adiposity. However, by increasing the length of sucrose feeding from 3 to 6 weeks, leptin started to increase its accumulation within the adipose tissue when compared with rats fed a CD. The above observation and the present data suggest that the dissociation between the increase in both leptin store and adipose tissues weight, and the decrease in plasma leptin levels initially began after 6 weeks of high-sucrose administration and was completely expressed after long-term sucrose feeding.

On the other hand, a different behavior of plasma leptin levels is observed in rats fed chronically a high-fat diet. In these animals, obesity, with concomitant adipocyte IR and increased lipolysis, positively correlated with increased leptinemia. However, Ainslie et al [35] demonstrated a decrease in plasma leptin levels with an increase of fat pad weight in rats fed a high-fat diet for a short period (4 weeks), suggesting that the effect of dietary fat on leptin may be dependent on the length of feeding and the type of fat consumed that could have varying effects on lipolysis and adipose tissue glucose uptake. Therefore, it is possible that different factors and/or mechanisms could participate in the regulation of leptin release when IR and adiposity are induced in rats by either high-sucrose or high-fat diets. At present, we are unaware of any published data concerning the mechanisms [36] that could be involved in the impaired leptin secretion from adipose tissue of SRD-fed rats. However, the present results suggest that the enhanced lipolysis and insulin insensitivity that worsens with the length of feeding could participate in this process. In this direction, the addition of dietary FO that significantly reduced intracellular lipolysis and normalized IR [23] was able to increase leptin release and significantly reduce leptin store within the epididymal fat pads.

Finally, caution is warranted before extrapolating these results to humans. However, Teff et al [37] recently demonstrated that women, with normal weight and consuming high fructose (as beverages) compared with glucose, had lower circulating leptin and insulin concentration and an increase of both ghrelin and triglycerides levels. Because leptin, insulin, and possibly ghrelin function as key signals to the central nervous system in the long-term regulation balance, the authors suggested that the prolonged consumption of diets high in fructose could lead to increased caloric intake and contribute to weight gain and obesity. The results described by the work of Teff et al in humans are appealing because the behavior of some parameters is similar to the behavior of those found in dyslipidemic, insulin-resistant rats fed a high-sucrose diet.

Acknowledgment

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