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Dietary soy protein improves adipose tissue dysfunction by modulating parameters related with oxidative stress in dyslipidemic insulin-resistant rats

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ABSTRACT

The present study investigates the benefits of the dietary intake of soy protein on adipose tissue dysfunction in a rat model that mimics several aspects of the human metabolic syndrome. Wistar rats were fed a sucrose-rich diet (SRD) for 4 months. After that, half of the animals continued with SRD until month 8 while in the other half, casein protein was replaced by isolated soy protein for 4 months (SRD-S). A reference group consumed a control diet all the time. In adipose tissue we determined: i) the activities of antioxidant enzymes, gene expression of Mn-superoxide dismutase (SOD) and glutathione peroxidase (GPx), and glutathione redox state ii) the activity of xanthine oxidase (XO), ROS levels and the gene expression of NAD(P)H oxidase iii) the expression of the nuclear factor erythroid-2 related factor-2 (Nrf2). Besides, adiposity visceral index, insulin sensitivity, and tumor necrosis factor-α (TNF-α) in plasma were determined. Compared with the SRD-fed rats, the animals fed a SRD-S showed: activity normalization of SOD and glutathione reductase, improvement of mRNA SOD and normalization of mRNA GPx without changes in the expression of the Nrf2, and improvement of glutathione redox state. These results were accompanied by a normalization of XO activity and improvement of both the ROS production as well as TNF-α levels in plasma. Besides, adipocyte size distribution, adiposity visceral index and insulin sensitivity improved. The results suggest that soy protein can be a complementary nutrient for treating some signs of the metabolic syndrome.

1. Introduction

Oxidative stress has been implicated in the pathogenesis of several metabolic diseases as well as in the co-morbidity of diabetes mellitus and atherosclerosis [1]. This alteration can occur as consequence of a general increase in reactive oxygen species (ROS) generation, a depression of the antioxidant systems, or both [2]. The adipose tissue is especially susceptible to the damage of oxidative stress [3].

Dietary interventions may play an important role in the prevention or improvement of metabolic disorders such as obesity, type 2 diabetes, insulin resistance, dyslipidemia, nonalcoholic fatty liver disease, among others [4,5]. Clinical studies and experimental animals have shown that soy protein has antiatherosclerotic, antidiabetic and hypolipidemic effects [6–9]. Bioactive peptides and isoflavones (genistein, glycitein, daidzein) from soy protein also contribute to these beneficial effects [7,10–12]. One of the potential mechanisms mediating these effects might be the reduction of oxidative stress [13–15]. In this regard, Lee et al. [16] showed that the administration of isolated soy protein increased the activity of hepatic antioxidant enzymes superoxide dismutase (SOD), catalase (CAT) and gluthatione peroxidase (GPx) associated with reduced levels of lipid peroxidation in streptozotocin-induced diabetic rats. The characteristic features of the human Metabolic Syndrome phenotype (visceral adiposity,

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impaired glucose homeostasis, insulin resistance, type 2 diabetes, dyslipidemia, hypertension) can be induced in rats by the administration of high-fructose/sucrose or high-fat diet [17–19]. In this regard, Sreeja et al. [20] showed that the substitution of soy protein for casein reduced ROS generation and prevented the expression of proinflammatory cytokines such as tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6) and plasminogen activator inhibitor-1 in the liver of Wistar rats fed a high-fructose diet. In addition, genistein has also shown anti-inflammatory and anti-oxidants effects in animal models [21,22].

Recently, in dyslipemic insulin resistant rats chronically fed a sucrose-rich diet (SRD), we found a deterioration of the adipose tissue function in the presence of hypertrophied adipocytes associated with an increase of lipoperoxidation, depletion of enzymatic and nonenzymatic cellular antioxidant defenses and dysregulation of cytokines [23]. Previous studies from our laboratory have demonstrated that the replacement of dietary casein by isolated soy protein in SRD-fed rats reduces adipocyte hypertrophy and basal lipolysis, corrects the inhibitory effect of the high-sucrose diet upon the antilipolytic action of insulin and normalises or improves enzyme activities involved in de novo lipogenesis in adipose tissue [24]. However, to the best of our knowledge, no study has been published focusing on whether the dietary soy protein exerts its beneficial effect on adipose tissue dysfunction through mechanisms that involve improvement or normalization of the oxidative stress status in the SRD-fed rats model. Therefore, the aim of the present study was to assess in the epididymal fat pad the effect of dietary isolated soy protein on the following: (i) enzymatic and nonenzymatic antioxidant defense system, (ii) gene expression of antioxidant enzymes manganese-SOD, GPx and of the nuclear factor-erythroid 2-related factor-2 (Nrf2) involved in redox homeostasis, (iii) ROS levels, xanthine oxidase (XO) activity and NAD(P)H oxidase p47phox subunit (p47NOX) expression, (iv) adipocyte size distribution and visceral adiposity index. Besides, TNF-α plasma levels and insulin sensitivity were examined.

2. Methods

2.1. Animals and diets

Male Wistar rats initially weighing 170–185 g and purchased from the National Institute of Pharmacology (Buenos Aires, Argentina) were maintained in the animal room under controlled temperature (22 ± 1 °C), humidity and air flow conditions, with a fixed 12 h light–dark cycle (light 07.00–19.00 h). They were initially fed a standard non-purified diet (Ralston Purina, St Louis, MO, USA). After 1 week of the acclimatization period, they were randomly divided into two groups of rats (control and experimental) and were housed individually. The experimental group (n = 28 rats) received a purified SRD containing by weight (g/100 g): 62.5 sucrose, 18 casein-free vitamins, 7 corn oil, 7.5 cellulose, 3.5 salt mixture (AIN-93M-MX), 1 vitamin mixture (AIN-93-VX), 0.2 choline chloride and 0.3 DL-methionine. The control group (n = 14 rats) received the same purified diet but with sucrose replaced by cornstarch (high-starch diet, control diet (CD)). Both groups received each diet for 4 months after which period the SRD group of rats was randomly subdivided into two subgroups (n = 14 rats). The rats in the first subgroup continued on the SRD up to 8 months of feeding. The second subgroup, SRD with soy protein (SRD-S), received the SRD in which the source of protein (18 g casein/100 g) had been replaced by isolated soy protein (MP Biomedicals, Solon, OH, USA) for the next 4 months. The control group was fed the CD throughout the experimental period.

Table 1
Composition of the experimental diets (based on the AIN-93 diet).

Diet ingredients	CD		SRD		SRD-S	
	% w/w	% energy	% w/w	% energy	% w/w	% energy
Cornstarch	62.5	65	–	–	–	–
Sucrose	–	–	62.5	65	62.5	65
Casein-free vitamin	18	19	18	19	–	–
Soy protein[a]	–	–	–	–	18	19
Corn oil	7	16	7	16	7	16
Vitamin mix[b]	1		1		1	
Cellulose	7.5		7.5		7.5	
Salt mix[c]	3.5		3.5		3.5	
Choline bitartrate	0.2		0.2		0.2	
DL-Methionine	0.3		0.3		0.3	

CD, control diet; SRD, sucrose rich diet; SRD-S, SRD with soy protein.
[a] Isolated soy protein (MP Biomedicals, Solon, OH, USA).
[b] AIN-93-VX.
[c] AIN-93M-MX.

Details on the composition of the diets are given in Table 1. Diets were isocaloric, providing approximately 16.3 kJ/g of food, and were available ad libitum. Diets were prepared every week. The composition of the soy protein isolated was described previously [25]. The weight of each animal and the energy intake were recorded twice per week throughout the experimental time in all groups and subgroups of rats. At the end of the experimental period, food was removed at 07.00 h (end of the dark period), and unless otherwise indicated, experiments were performed between 07.00 and 09.00 h. At least six rats from the three dietary groups were used in each procedure. Rats from the three dietary groups were anesthetized with intraperitoneal sodium pentobarbital (60 mg/ kg body weight). Blood samples were obtained from the jugular vein collected in tubes containing sodium EDTA as anticoagulant and rapidly centrifuged. The plasma was either immediately assayed or stored at −80 °C. The epididymal adipose tissue (e-WAT) was totally removed, weighed and an aliquot immediately used for the preparation of isolated adipocytes and to determine adipocyte cell size diameter and cell number as previously described [18]. Another fraction of epididymal adipose tissue was frozen and stored at the temperature of liquid N_2. Retroperitoneal and omental adipose tissues were removed and weighed. The visceral adiposity index (%) was calculated as previously described [26]. The experimental protocol was reviewed and approved by the Human and Animal Research Committee of the School of Biochemistry, University of Litoral, Santa Fe, Argentina CAI +D (#50120110100293-2012), and adequate procedures were taken to minimize the pain or discomfort of the rats.

2.2. Analytical methods

Commercially available analytical kits were employed to determine plasma glucose, triglyceride (TAG) and uric acid 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 as previously described [23]. The immunoreactive insulin assays were calibrated against the rat insulin standard (Novo Nordisk, Copenhagen, Denmark). Plasma tumor necrosis factor-α (TNF-α) was measured using commercial ELISA kits (Thermo Scientific, Rockford, USA; Legend max™ Biolegend® Inc., San Diego, USA). The minimum detectable limit was 4.2 pg/mL, with an intra- and inter-assay CV of less than 7.4% and 10.5% respectively. All determinations were performed in triplicate.

2.3. Euglycemic clamp studies

Whole body peripheral insulin sensitivity was measured using the euglycemic-hyperinsulinemic clamp technique as described elsewhere [27]. Briefly, after 5 h of food deprivation, 6 rats from each dietary group were anesthetized and an infusion of highly purified porcine neutral insulin (Actrapid, Novo Nordisk, Denmark) was administered at 0.8 U/(kg h) for 120 min. Glycemia was maintained at euglycemic level by infusing glucose (200 g/L) at a variable time. The glucose infusion rate (GIR) during the second hour of the clamp study was taken as the net steady state of the whole body glucose.

2.4. Lipid peroxidation and protein carbonyl groups determination in plasma

Lipid peroxidation in plasma was estimated by measuring thiobarbituric acid reactive substances (TBARS) according to D'Alessandro et al. [23]. Protein carbonyl formation, a marker of protein oxidative damage, was measured spectrophotometrically following to Ferreira et al. [28].

2.5. Determinations in epididymal fat tissue

2.5.1. Reactive oxygen species (ROS)

ROS production was quantified by means of the dichlorodihydrofluorescein diacetate ($DCFH_2$-DA) method as recently described [28]. $DCFH_2$-DA can be converted to dichlorofluorescein (DCF) by ROS-dependent oxidation, therefore acting as a direct indicator of oxidative stress. Results were normalized by protein concentration (fluorescence intensity/mg protein) and expressed relative to the control group. Protein concentration was quantified by the Bradford assay (Bio-Rad reagent).

2.5.2. Glutathione redox state

Total glutathione [reduced (GSH) + oxidized form (GSSG)] and GSSG were assayed by the enzymatic recycling procedure in the presence of glutathione reductase as previously described [23]. The redox state was calculated as [(oxidized form/total forms) $\times$ 100], with total forms = (oxidized form + reduced form).

2.5.3. Antioxidant and oxidant enzyme activities

Epididymal adipose tissue catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GR) and xanthine oxidase (XO) activities were determined as described recently [23]. The protein content was measured by the Bradford assay (Bio-Rad reagent).

2.5.4. RNA isolation and semi-quantitative RT-PCR

GPx, SOD, p47NOX subunit of NADP(H) oxidase and Nrf2 mRNA levels of epididymal fat tissue were determined by RT-PCR as previously described [28]. Total RNA was isolated from adipose tissue samples using Trizol reagent (Invitrogen) and following the manufacturer's indications. 10 μg of total RNA were reversed transcribed, using random primer hexamers (Biodynamics, SRL) and ImProm-II reverse transcriptase (Promega), at 37 °C during 1 h. PCR amplification was carried out using specific oligonucleotide primers as described in Table 2. A cDNA aliquot (1/10 of the RT reaction product) was amplified with a PCR master mix, using Taq DNA polymerase (Invitrogen). PCR products were analyzed on 2% agarose gels, containing GelRed (Genbiotech) and photographed under UV transilumination to visualize the bands. Bands intensities were quantified using NIH Image J software (Image Processing and Analysis in Java from http://rsb.info.nih.gov/ij/). Relative amounts of mRNA were expressed as the ratio of band intensity for the target genes relative to that for 28S rRNA.

2.6. Statistical analysis

Results were expressed as mean values with their standard error. Statistical comparisons were made transversely between different dietary groups. Data were tested for variance using Levene's test and normality by Shapiro-Wilk's test. Variables that were not normally distributed were transformed (using log 10 function) prior to the statistical analyses. The statistical significance between groups (CD, SRD and SRD-S) was determined by one-way ANOVA, with one factor (diet) followed by the inspection of all differences between pairs of means by the Scheffe's test [29,30]. When appropriate, the statistical significance between two groups (CD and SRD) was determined by Student's *t*-test. Differences having *P* values lower than 0.05 were considered to be statistically significant (SPSS 17.0 for Windows, SPSS INC, Chicago, Illinois). All *P* values reported were two-sided.

3. Results

3.1. Body weight, energy intake, epididymal tissue weight, fat pad morphology and adiposity visceral index

Body weight and energy intake were carefully monitored in all groups of rats throughout the experimental period. As shown in an early publication of our group [25] and confirmed in the present work, increases in body weight and energy intake were comparable in rats fed the CD or SRD during the first 4 months of the feeding period of their respective diets (see Table insert in Fig. 1). However, a significant increase in both parameters was observed in the rats that continued with the SRD up to 8 months. Besides, in agreement with previous results, when the source of dietary casein in the SRD was replaced by soy for the last 4 months on diet (SRD-S), both energy intake and body weight remained similar to those observed in the control-diet fed rats [24,25]. Furthermore, a significant decrease in the accretion of epididymal fat weight was observed in the SRD-S group [24]. In agreement with previous publications [23], Fig. 1 depicts both a clear differentiation into adipocyte size distribution from epididymal fat pad as an increase in cell diameters in the SRD fed rats compared to the control group. A significant reduction of adipocyte cell size diameter was recorded in the SRD-S group although the values were still higher than those observed in the CD-fed rats. However, no differences in the total cell number expressed as cell number x 10^6 per total fat weight were observed in all dietary groups. Values were as follows: mean ± SEM (n = 6); CD: 22.4 ± 0.8; SRD: 22.1 ± 1.3; SRD-S:

Table 2
Sequences of the primers used to amplify different genes by RT-PCR and sizes of the fragments generated.

Gene name	Forward primer sequences (5′-3′)	Reverse primer sequences (5′- 3′)	Size (bp)
SOD	AGCTGCACCACAGCAAGCAC	TCCACCACCCTTAGGGCTCA	191
GPX	CCACCGTGTATGCCTTCTCC	ACCGGGGACCAAATGATGTA	424
p47 phox	AGGGAACGCTCACCGAGTACT	TCTTTGGCCGTCAGGTATGTC	160
Nrf2	CGGCATTTCACTGAACACAAGT	TGGCTGTGCTTTAGGTCCATT	160
28S	GTGAAAGCGGGGCCTCACGATCC	TACTGAGCAGGATTACCATGGC	200

	CD	SRD	SRD-S
Initial body weight (g)	194.0 ± 6.2 (8)	189.4 ± 2.3 (16)	-
Body weight at month 4 (g)	421.5 ± 10.2 (8)	425.4 ± 9.7 (8)	424.4 ± 7.6 (8)
Body weight at month 8 (g)	472.1 ± 5.9^{b} (8)	545.7 ± 7.7^{a} (8)	489.0 ± 8.0^{b} (8)
Energy intake months 0 - 4 (kJ/d)	260.0 ± 4.6 (8)	263.5 ± 3.7 (16)	-
Energy intake months 4 - 8 (kJ/d)	262.9 ± 5.5^{b} (8)	351.4 ± 8.9^{a} (8)	270.0 ± 4.9^{b} (8)
Epididymal fat weight (g)	8.08 ± 0.46^{c} (8)	15.1 ± 0.55^{a} (8)	12.2 ± 0.56^{b} (8)

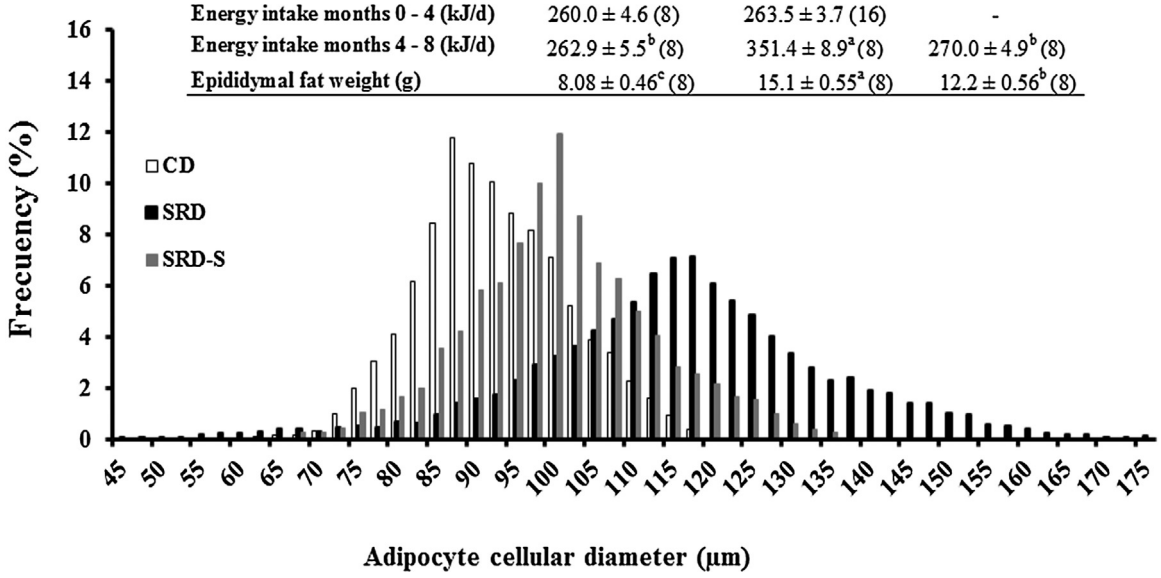

Fig. 1. Representative histogram showing the distribution of adipocytes cell diameters isolated from the epididymal depots of rats fed a control diet (CD, *white bar*), a sucrose-rich diet (SRD, *black bar*) or SRD with soy protein (SRD-S, *gray bar*).
The histogram was constructed by sizing at intervals of 2.5 µm, 100 adipocytes from each individual rat. Six animals were included in each experimental group. Bars represent the mean of the cell measured (percent) that falls within a given size indicated.
In the table, values are expressed as mean ± SEM; at least six animals were included in each experimental group. Values in a line that do not share the same superscript letter differ ($P < 0.05$) when one variable at a time was compared (One way ANOVA followed by Scheffe's test).

20.8 ± 0.5. On the other hand, the SRD-fed rats showed a high increase of the visceral adiposity index which was significantly reduced after soy protein administration. Values were as follows: mean ± SEM (n = 6); CD: 3.58 ± 0.19; SRD: 6.49 ± 0.35; SRD-S: 5.41 ± 0.23 ($P < 0.05$ SRD vs DC and SRD-S; $P < 0.05$ SRD-S vs DC).

3.2. Plasma metabolites, insulin, glucose infusion rate (GIR), TBARS, protein carbonyl groups and TNF-α levels

In agreement with previous results from our laboratory [24,25], at the end of the dark period (07.00 h), plasma levels of TAG, FFA and glucose were significantly higher in rats consuming SRD for 8 months compared with age-matched controls fed a CD. All these variables returned to control values when the casein present in the SRD rats was replaced by soy protein during the last 4 months of the experimental period. Moreover, the increase of plasma uric acid recorded in the SRD values reached was similar to that observed in CD. No statistical differences in plasma insulin levels were observed at the end of the feeding period between the dietary groups (Table 3). A significant reduction ($P < 0.01$) of the GIR (whole-body peripheral insulin sensitivity) was observed in the SRD-fed rats compared to age-matched controls fed a CD. Although GIR significantly increased after soy protein administration, values were still lower ($P < 0.05$) from those recorded in the CD-fed rats (Table 3).

Both plasma TBARS (an estimation of lipid peroxidation) and protein carbonyl group were significantly higher in the SRD-fed group when compared to the CD-fed rats. Interestingly, these parameters returned to control levels in the SRD-S group (Table 3). Besides, dietary soy protein improved plasma TNF-α levels although values were still higher than those observed in the CD-fed rats (Table 3).

Table 3
Plasma metabolites, insulin, glucose infusion rate (GIR), biomarkers of oxidative stress and inflammatory cytokines in rats fed a control diet (CD), sucrose-rich diet (SRD) or the SRD with soy protein (SRD-S).

	CD	SRD	SRD-S
Plasma metabolites and insulin levels			
Triglyceride (mmol/L)	0.69 ± 0.06^{b}	2.06 ± 0.15^{a}	0.65 ± 0.05^{b}
Free fatty acids (µmol/L)	318.2 ± 4.2^{b}	782.0 ± 10.5^{a}	327.0 ± 5.6^{b}
Glucose (mmol/L)	6.29 ± 0.07^{b}	8.31 ± 0.08^{a}	6.47 ± 0.11^{b}
Uric acid (µmol/L)	241.7 ± 33.1^{b}	379.6 ± 33.2^{a}	206 ± 17.7^{b}
Insulin (µU/mL)	70.5 ± 4.1	74.7 ± 5.6	79.2 ± 6.0
GIR [µmol/(kg min)]	62.7 ± 2.0^{a}	28.4 ± 1.2^{c}	48.9 ± 1.1^{b}
Plasma biomarkers of oxidative stress			
TBARS (nmol/mL)	2.58 ± 0.30^{b}	4.91 ± 0.41^{a}	2.10 ± 0.30^{b}
Protein carbonyl groups (nmol/mL)	33.8 ± 1.0^{b}	39.9 ± 1.5^{a}	32.0 ± 0.9^{b}
Plasma inflammatory cytokine			
TNF-α (pg/mL)	5.39 ± 0.66^{c}	12.9 ± 0.49^{a}	8.62 ± 0.42^{b}

Values are expressed as mean ± SEM; at least six animals were included in each experimental group. Values in a line that do not share the same superscript letter were significantly different ($P < 0.05$) when one variable at a time was compared by Scheffe's test.

3.3. Enzymatic and non-enzymatic antioxidant defense

Fig. 2 shows the activities and mRNA expression levels of antioxidant enzymes GPx and SOD in the three dietary groups. The activity and mRNA expression of GPx (Fig. 2A and B) were significantly lower in the SRD group compared to rats fed a CD. The addition of soy protein to the SRD did not modify the lower GPx activity although GPx mRNA levels returned to normal values. Interestingly, the total SOD activity was reduced although Mn-SOD mRNA expression was higher in the SRD group (Fig. 2C and D).

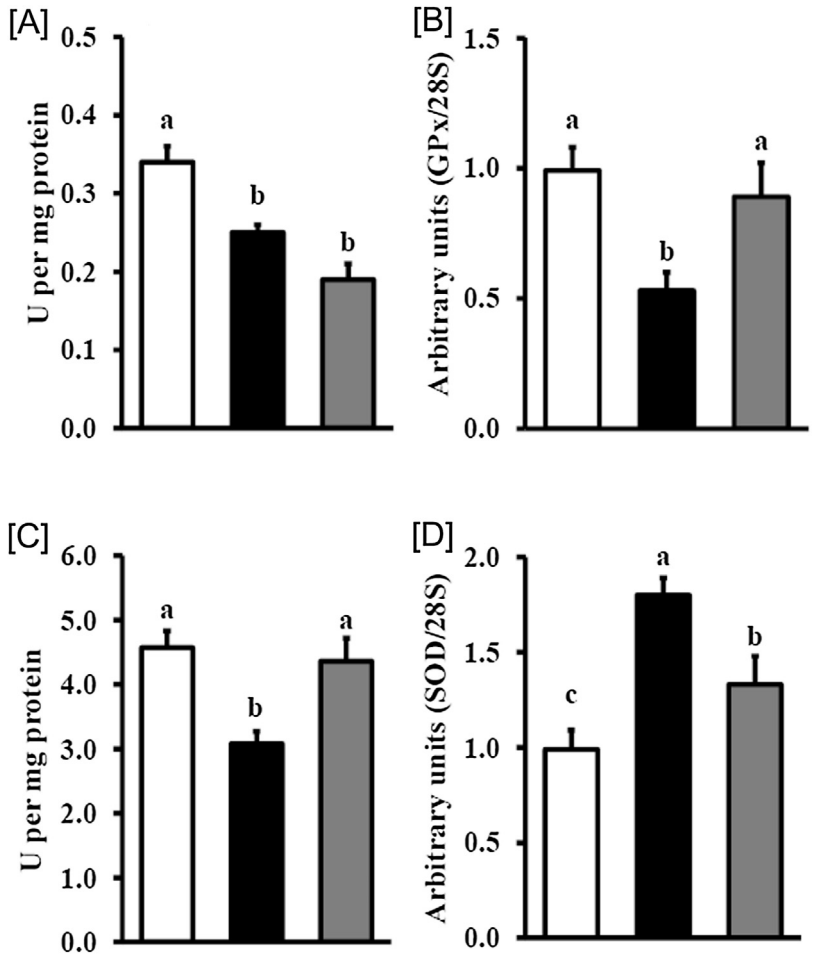

Fig. 2. Glutathione peroxidase (GPx) activity [A], GPx mRNA expression [B], superoxide dismutase (SOD) activity [C] and Mn-SOD mRNA expression [D] in epididymal fat pad in rats fed a control diet (CD, *white bar*), a sucrose-rich diet (SRD, *black bar*) or a SRD with soy protein (SRD-S, *gray bar*).
GPx and SOD transcript levels were measured by RT-PCR, normalized against 28S rRNA and expressed in arbitrary units relative to the CD group. Values are expressed as mean ± SEM (n = 6), with their standard errors represented by vertical bars. Values that do not share the same superscript letter were significantly different ($p < 0.05$) when a single variable at a time was compared (One way ANOVA followed by Scheffe's test).

Dietary soy protein normalizes the total SOD activity in the epididymal fat pads of SRD-fed rats. Regarding Mn-SOD mRNA expression, even though it was improved in the SRD-S group, the levels were still higher than those of the CD-fed rats.

Furthermore, soy protein normalized the decreased enzymatic activity of GR recorded in the SRD fed group. Values were as follows: mean ± SEM (n = 6), (mU/mg prot); CD: 23.0 ± 1.2; SRD: 16.1 ± 1.4; SRD-S: 20.7 ± 1.9 ($P < 0.05$ SRD vs DC and SRD-S). However, it did not modify the lower activity of CAT observed in the rats fed a SRD. Values were as follows: mean ± SEM (n = 6), (U/mg prot); CD: 2.61 ± 0.12; SRD: 1.76 ± 0.07; SRD-S: 1.81 ± 0.17 ($P < 0.05$ SRD and SRD-S vs DC).

In addition, dietary soy protein significantly increased the reduced redox state of glutathione observed in the SRD-fed rats, although the levels were still lower than those observed in the CD-fed rats. Values were as follows: mean ± SEM (n = 6); 44.7 ± 6.2% in rats fed a CD; 24.5 ± 3.5% in the SRD group and 36.1 ± 5.8% in SRD-S group ($P < 0.05$ SRD vs DC and SRD-S; $P < 0.05$ SRD-S vs DC).

3.4. Biomarkers of oxidative stress in adipose tissue

Soy substitution in place of casein significantly decreased the ROS levels in adipose tissue of SRD rats. Although, values reached by the SRD-S group were still above those recorded in the age-matched control rats fed a CD. Values -expressed as arbitrary units- were as follows, mean ± SEM (n = 6): 1.0 ± 0.05 in rats fed a CD; 1.80 ± 0.03 in the SRD group and 1.34 ± 0.03 in the SRD-S group ($P < 0.05$ SRD vs DC and DRS-S; $P < 0.05$ SRD-S vs DC). Furthermore, the increased activity of pro-oxidative enzyme XO recorded in the adipose tissue of SRD-fed rats was completely normalized in the group of animals fed dietary soy. Values were as follows: mean ± SEM, (n = 6), (U per total fat weight); CD: 2.71 ± 0.34; SRD: 4.60 ± 0.44 and SRD-S: 2.02 ± 0.4 ($P < 0.05$ SRD vs DC and SRD-S).

NADPH oxidase is a key factor in enzymatic cellular ROS production. We examined the expression of p47NOX subunit of NAD(P)H oxidase in adipose tissue in all dietary groups. No changes in the mRNA abundance of p47NOX subunit were recorded in the three dietary groups. Values of p47NOX mRNA/28S abundance expressed as arbitrary units relative to controls were as follows: mean ± SEM (n = 4); CD: 1.01 ± 0.05; SRD: 1.17 ± 0.12; SRD-S: 0.86 ± 0.09.

3.5. Nrf2 expression in epididymal fat pads

Despite the changes observed in the expression of antioxidant enzymes (SOD, GPx), similar Nrf2 mRNA levels were recorded in all groups of animals (Fig. 3).

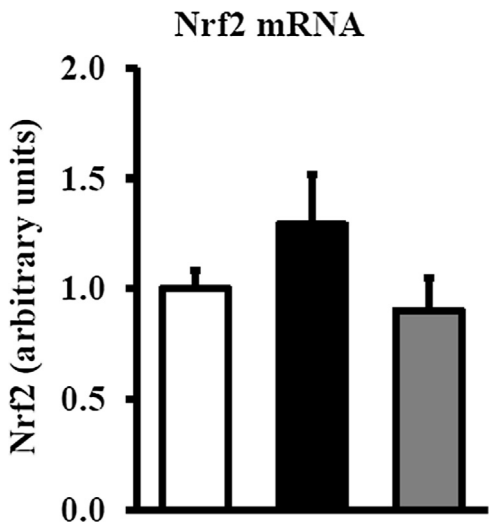

Fig. 3. Nrf2 expression in epididymal fat pad in rats fed a control diet (CD, white bar), a sucrose-rich diet (SRD, *black bar*) or a SRD with soy protein (SRD-S, *gray bar*). Nrf2 transcript levels were measured by RT-PCR, normalized against 28S rRNA and expressed in arbitrary units relative to the CD group. Values are expressed as mean ± SEM (n = 4), with their standard errors represented by vertical bars.

4. Discussion

The present study focuses on the effect of dietary soy protein upon the reversion/improvement of deteriorated antioxidant defenses and oxidative stress involved in adipose tissue dysfunction of a dyslipemic insulin-resistance rat model. Studies with rodent models of diabetes and dyslipidemia, demonstrated that the administration of soy protein increases antioxidant defenses and reduces oxidative stress in tissues (eg. liver, heart muscle) [16,20,31]. Nowadays there is no evidence linking soy protein with antioxidant enzymes activities in adipose tissue. Different reports including ours, have shown that the administration of high-fructose/sucrose or high fat diet induced dysfunctional enlarged adipose tissue with impaired antioxidant defenses [23,32,33]. Manganese superoxide dismutase (Mn-SOD) and glutathione peroxidase (GPx) are two major enzymes that are responsible for ROS detoxification in mitochondria. The present data showed that the replacement of dietary casein by soy protein induced different behaviors in the activities of antioxidant enzymes, while SOD and GR reached normal values, no changes in CAT and GPx activities were observed. Furthermore, soy protein returned to normal values the mRNA GPx levels and improved the impaired expression of Mn-SOD. The effect of dietary soy protein on the expression of Mn-SOD and GPx possibly was not mediated by Nrf2 since mRNA levels of this transcription factor were not modified in either dietary groups. Interestingly, a lack of correlation between the activity and expression of SOD was observed in the SRD-fed rats. In this regard, our results show an increase of mitochondrial Mn-SOD expression and a decreased total enzyme activity in the SRD-fed rats. It is noteworthy that the measure of SOD activity in this work does not discriminate between Cu-Zn-SOD and Mn-SOD isoforms. However, we did not rule out the possibility that post-translational modifications and/or changes in individual SOD activities could be involved in the lack of correlation between activity and gene expression in the SRD-fed rats. Additionally a limitation of this study arises from the semi-quantitative RT-PCR analysis used for the gene expression determinations. On the other hand, the overexpression of Mn-SOD increases the production of hydrogen peroxide (H_2O_2) [34]. Therefore, H_2O_2 is a fine-tuning regulator of the nuclear factor kB (NF-kB)-process. [35]. Although in the present study NF-kB was not evaluated, we cannot discard the possibility that part of adipose tissue dysfunction in the SRD-fed rats could take place through the mechanism mentioned above. The beneficial effects of soy protein observed in our work also could be mediated by NF-kB. In this vein, Sreejja et al. [20] demonstrated that soy protein, when included in a fructose diet instead of casein, prevents the activation of NF-kB and c-Jun N-terminal kinase (JNK) and reduces oxidative stress in liver. In addition, Choi et al. [36] showed that genistein can also suppress NF-kB activation improving oxidative stress and antioxidant defenses in murine macrophage cell line RAW 264.7.

Elevated ROS production accompanied hypertrophied adipocytes [3]. Furukawa et al. [1] reported that increased oxidative stress in accumulated fat is an important pathogenic mechanism of obesity-associated metabolic syndrome. Interestingly, elevated plasma uric acid levels have also been positively associated with visceral fat area [37]. In addition, Tsushima et al. [38] in obese mice model (ob/ob) fed a high fat-high sucrose diet showed that epididymal tissue can secrete uric acid and increase uric acid plasma level accompanied by increased activities of XO and xanthine oxide reductase (XOR). Moreover it has been shown that under high-fructose conditions XO is also an important oxidoreductase that contributes to intracellular ROS production. [39]. Recently we reported that elevated XO activity accompanied the adipocyte enlargement in rats chronically fed a SRD [23]. In this regard, our present data shows that soy protein was able to normalize XO activity and plasma uric acid levels and reduce the ROS in WAT. This was accompanied by a significant reduction of adipocyte size. In this vein, in the SRD-fed rats, the reduction of both ROS and uric acid plasma levels by soy protein could have been in part via restoring the normal XO activity in epididymal fat pad. Another important ROS source in adipose tissue is NAD(P)H oxidase. Although NAD(P)H oxidase p47NOX subunit expression remained unchanged in all experimental groups, we cannot discard the possibility that changes in their activity could also contribute to reduce the ROS levels in adipose tissue. On the other hand, an increase in the markers of systemic oxidative stress (lipid peroxidation or protein carbonylation products) has also been associated with obesity and metabolic syndrome [1,40,41]. In this vein our data shows that soy protein was able to normalize systemic oxidative stress associated with the reduction of visceral adiposity index. Sreeja et al. [20] reported that soy protein in diet (20%) reduces molecules related to lipoperoxidation and nitro-oxidative stress in the liver of Wistar rats fed a high-fructose diet.

Oxidative stress is closely related to inflammation [42]. Inflammatory cytokines, such as TNF-α, leads to inhibition of insulin signaling and insulin resistance in adipocytes. TNF-α induces insulin resistance by activation of mitogen-activated protein kinases (MAPK) such as extracellular signal-related kinase (ERK) and JNK and transcription factors such as NF-kB and activator protein1 (AP-1). Together, these proteins may inhibit peroxisome proliferator-activated receptor γ (PPARγ) activity or decrease the insulin receptor substrate 1 (IRS-1) signaling affecting insulin sensitivity [42]. Moreover, TNF-α and oxidative stress together contributes to the amplification of the inflammatory cascade through MAPK and NF-κB [43]. Different studies in rodents including our own have reported lower adipocytes size in rats fed a high sucrose/fat diet when soy protein was administered [24,44]. Iritani et al. [45] showed that dietary soy protein in a saturated high fat diet improved insulin sensitivity in adipose tissue as well as in liver. In the present work, the reduction of fat pad mass recorded in the SRD-S group was accompanied by a significant improvement of insulin resistance and a decrease of plasma TNF-α. On the other hand, experimental evidence suggests that soy protein improves insulin resistance and lipid levels by activating PPARγ [46,47]. Moreover, the high expression of PPARγ, has been associated with a decrease in systemic inflammation accompanied

by a decrease in adipocyte expression of proinflammatory factors [48]. Recently, we demonstrated that soy protein was able to improve the relative abundance of PPARγ in hypertrophic adipocytes of rats chronically fed a SRD [24]. As mentioned above soy protein also contains isoflavones and it has been demonstrated that genistein supresses the hypertrophy of adipocytes through down-regulation of genes associated with adipogenesis in C57BL/6J high fat-fed mice [49]. Additionally, Sakamoto et al. [50] observed in C57BL/6J mice fed a high fat-high sucrose diet that the consumption of daidzein reduces adipocyte size concomitant with a modulation of cytokine gene expression (TNF-α, adiponectin and MCP-1), and improves in glucose metabolism and activation of PPARγ. In this regard, Sreeja et al. [20] observed that the substitution of soy protein for casein reduced TNF-α, IL-6, and PAI-1 expression associated with reduced protein abundance of inflammatory kinases JNK and IKKβ in the liver of fructose-fed rats. The isolated soy protein used in the present study contains isoflavones (175 mg/kg of protein) [25]. Therefore, we cannot discard the possibility that cooperative interventions between isoflavones, protein and other components of the isolated soy protein could contribute to our findings. Besides, the normalization of dyslipidemia observed in SRD-S decreases the availability of plasma FFA, the latter could also contribute to improve insulin sensitivity and adipose tissue dysfunction.

5. Conclusion

In brief, the present study provides new data on the beneficial effects of soy protein to improve or reverse adipose tissue deteriorated antioxidant defense and oxidative damage as well as visceral adiposity against the detrimental effects of sucrose in a dyslipemic, insulin resistant rat model. Finally, these results suggest that soy protein can be a complementary nutrient for treating some signs of the metabolic syndrome.

Authors' **contributions**

Paola G. Illesca and Silvina M. Álvarez contributed equally to this study and share first authorship.

Conceived and designed the experiments: MED, YBL.

Performed the experiments: PGI, SMA, DAS, MRF, MED.

Analyzed the data: PGI, SMA, DAS, MRF, MSG, YBL, MED.

Wrote the paper: MED. PGI contributed to the writing the manuscript. YBL reviewed the manuscript.

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Conflict of interest

The authors declare that there is no conflict of interest.

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