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Dietary Salba (*Salvia hispanica* L) improves the altered metabolic fate of glucose and reduces increased collagen deposition in the heart of insulin-resistant rats

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ABSTRACT

This study reports the effects of dietary Salba (chia) seeds on the mechanisms underlying impaired glucose metabolism in the heart of dyslipemic insulin-resistant rats fed a sucrose-rich diet (SRD). Wistar rats were fed a SRD for 3 months. Afterwards, half the animals continued with the SRD; in the other half's diet chia seeds replaced corn oil (CO) for three months (SRD + chia). In the control group, corn starch replaced sucrose. The replacement of CO by chia seeds in the SRD restored the activities of key enzymes involved in heart glucose metabolism decreasing fatty acid oxidation. Chia seeds normalized insulin stimulated GLUT-4 transporter, the abundance of IRS-1 and pAMPK, changed the profile of fatty acid phospholipids, reduced left-ventricle collagen deposition and normalized hypertension and dyslipidemia. New evidence is provided concerning the effects of dietary chia seeds in improving the altered metabolic fate of glucose in the heart of dyslipemic insulin-resistant rats.

1. Introduction

The Metabolic Syndrome (MS) increases the risk of Type 2 diabetes and cardiovascular disease. This syndrome, which is a combination of metabolic disorders influenced by genetic and environmental factors, now reaches epidemic proportions in the world [1]. It is known that changes in lifestyle (diets and physical activity) could play an important role in the rise of MS and associated cardiovascular pathologies.

Cardiac energy metabolic shifts occur as a normal response to diverse physiological and dietary conditions and as a component of the pathophysiological processes that accompany heart disease, cardiac muscle being a target tissue of insulin. An altered insulin-stimulated cardiac glucose uptake has been described in animal models of diabetes and obesity [2,3]. It has been demonstrated that increased fatty-acid uptake and oxidation inhibit glucose oxidation in the heart. Altered glucose metabolism is mediated at least by inhibition of several glycolytic steps (e.g.: glucose transport, phosphorylation and oxidation, etc.) [4]. Modifications in the regulation of some key proteins involved in energy metabolism such as insulin receptor β (IRβ), IRS-1, GLUT-4, and the metabolic sensor of cellular energy status AMP-activated protein kinase (AMPK), are associated with cardiac dysfunction [5]. Moreover, the impairment of insulin signaling has been linked to intramuscular lipid accumulation in the heart [6]. In the heart of Wistar rats treated with a small dose of Streptozotocin and fed with a high fat-high fructose diet, Ménard et al. [7] demonstrated a reduction of Glut4 mRNA expression while both mitochondrial carnitine palmitoyltranferase 1 (CPT1) activity and CD36 mRNA expression increased. The heart of Zucker diabetic fatty rats showed an increase of fibrous tissue accompanied by cardiac lipotoxicity. Moreover, a reduction of pyruvate dehydrogenase complex (PDHc) activity was also observed [2,8].

On the other hand, it is well known that feeding rats with dietary high sucrose, fructose and/or high fat induces metabolic and physiological alterations, mimicking several aspects of MS in humans such as dyslipidemia, insulin resistance, altered glucose homeostasis, visceral adiposity and hypertension [9–11]. In this regard, our group has shown that the metabolic abnormalities described above lead to deep alterations of myocardial substrate utilization in rats chronically (3 months) fed a sucrose–rich diet (SRD). The heart of this animal model displays lipotoxicity, impaired glucose uptake and decrease of glucose oxidation [12,13].

Several epidemiological, clinical and animal studies demonstrated that marine or algae-derived very long chain n-3 PUFAs, particularly EPA (20:5,n-3) and DHA (22:6,n-3), exert cardio protective effects playing a role against the adverse effects of MS [14]. Moreover, dietary

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ALA (18:3,n-3) intake -which derives from vegetable sources- is associated with a reduced incidence of coronary heart disease [15]. ALA could be a valuable source of long-chain n-3 PUFAs (EPA, DPA, DHA) via elongase/desaturase activities [16]. One of the botanical oil sources rich in ALA is the seed of *Salvia hispanica* L., commonly known as chia seed, which also contains considerable amounts of proteins, fiber, minerals and antioxidant activity due to the phenolic compound in the seed or oil, mainly quercetin and myricetin among others [17,18]. Salba, a variety of chia seed, has a nutrient profile that is highly standardized with a high degree of reproducibility [19]. In subjects with well controlled type 2 diabetes Vuksan et al. [20] demonstrated that the consumption of the Salba grain improves cardiovascular risk factors by reducing blood pressure, inflammatory and coagulation markers. Ho et al. [21] showed that both ground and whole and Salba seeds are equally effective in attenuating blood glucose levels in dose dependent manner when incorporated in bread in healthy individuals. We recently demonstrated in SRD-fed rats -a well stable rat model of dyslipidemia and insulin resistance- that dietary Salba seed (chia) reduces the availability of plasma lipid flux, normalizes dyslipidemia and glucose homeostasis and was able to improve heart lipotoxicity (decreased triglyceride, long-chain acyl CoA -LC-acylCoA- and diacylglycerol) normalizing the protein level of membrane fatty acid transporter (FAT/CD36) [22]. However, the beneficial effects of dietary Salba seeds (chia) on the mechanisms involved in the altered metabolic fate of glucose in the heart of these rats have been scarcely and only partially investigated. Therefore, we considered it worthwhile to further explore whether dietary chia seeds could improve/reverse the mechanisms underlying impaired glucose metabolism in the heart of dyslipemic insulin resistant rats fed a SRD. To achieve this goal we analyzed: a) Metabolites and key enzyme activities involved in glucose phosphorylation and oxidative glucose disposal. b) Glucose transporter: basal and under insulin stimulation and the protein mass level of the early proximal insulin signaling step IRS-1. c) The protein mass level of total and phosphorylated AMPK -a master switch for cellular energy levels that regulate fatty acids and glucose metabolism-. d) Left ventricle collagen distribution and hydroxyproline level as an estimation of fibrous tissue. Additionally, blood pressure and plasma lipid levels were measured. We also analyzed the composition of fatty acids phospholipids of heart tissue after dietary chia seeds. This study was conducted in rats fed a SRD for 6 months in which a stable dyslipidemia, insulin resistance, hypertension and impaired non oxidative and oxidative glucose metabolism in the heart muscle, were present before corn oil was replaced by Salba seeds (chia) as dietary fat for the last 3 months of the experimental period in half of the animals.

2. Materials and methods

2.1. Animals and diets

Male Wistar rats (n = 72) weighing 170–180 g purchased from the National Institute of Pharmacology (Buenos Aires, Argentina) were maintained with unrestricted access to water and food under controlled temperature (22 ± 1 °C), humidity, and airflow conditions with a fixed (12 h) light-dark cycle (lights on from 07:00 to 19:00 h). They were initially fed a standard non-purified diet (Raltson Purina, St Louis, MO, USA). Adequate measures were taken to minimize the pain or discomfort of the rats. Animal experiments complied with the National Institutes of Health guide for the Care and Use of Laboratory Animals (National Academy of Science, NIH Publication 6–23, revised 1985) and were evaluated and approved by the Human and Animal Research Investigation Committee of the School of Biochemistry, University of Litoral, Argentina (FONCyT-PICT #945/2012).

2.2. Dietary manipulations

After 1 week of acclimation, rats were randomly divided into two groups: control (n = 24) and experimental (n = 48) and were housed individually. The control group (CD, reference group) received a semi-synthetic diet containing corn starch (60% energy), protein (17% energy) and corn oil (CO) as source of fat (23% energy) throughout the experimental period (6 months) (control diet, reference group). The experimental group received the same semi-synthetic diet, but with corn starch (60% energy) replaced by sucrose as the carbohydrate source [sucrose-rich diet (SRD)]. After 3 months of treatment, the animals in the SRD-group were randomly divided into two subgroups. The rats in the first subgroup (n = 24) continued with the SRD up to 6 months of feeding. The second subgroup (n = 24) (SRD + chia) received the SRD in which the source of fat (corn oil, 23% energy) had been replaced by (Salba seed (chia) 22.8% energy plus 0.2% energy of corn oil) as the source of dietary fat for the next 3 months. The fiber, vitamin mix and salt mix contents of each diet were similar. The carbohydrate, protein, fiber, and salt mix contents in the feed of the SRD + chia group were balanced with the CD and SRD groups, according to the amount of these nutrients present in the Salba seeds (chia). Details of the composition of each diet are given in Table 1. The fatty acid composition of each experimental diet (g/Kg of food) is shown in Table 2. The preparation and handling of the diets have been reported elsewhere [23]. All diets provided approximately 17 kJ/g of food. The body weight of each animal was recorded twice per week throughout the experimental period in all groups and subgroups of rats. In a separate experiment, the individual caloric intake and weight gain of six animals in each group

Table 1
Composition of experimental diets.

Diet ingredients[a]	CD		SRD		SRD + chia	
	% w/w	% Energy	% w/w	% Energy	% w/w	% Energy
Carbohydrates						
Corn starch	58.0	60.0	2.5	2.6	–	–
Sucrose	–	–	55.5	57.4	55.5	57.4
Chia seed[b]	–	–	–	–	2.5	2.6
Fat						
Corn oil	10.5	23.0	10.5	23.0	0.1	0.2
Chia seed	–	–	–	–	10.4	22.8
Protein						
Casein (vitamin free)	16.3	17.0	16.3	17.0	8.6	9.0
Chia seed	–	–	–	–	7.7	8.0

[a] The diets are based on the AIN-93M diet, contained by weight: salt mix 3.5% (AIN-93Mx); vitamin mix 1% (AIN-93Vx); choline chloride 0.2%; methionine 0.3%; fiber 10–11%.

[b] Chia seed (*Salvia hispanica* L, variety Salba): 362 g/Kg diet. Chia composition (g/100 g chia seed): carbohydrate 37.45; insoluble fiber 81% of total of carbohydrates; fat 30.23; protein 21.19. Mineral composition (mg/100 g chia seed): Na 103.15; K 826.15; Ca 589.60; Fe 11.90; Mg 77.0; P 604.0; Zn 5.32; Cu 1.66; Mn 1.36.

Table 2
Fatty acid composition of experimental diets.

Fatty acids[a]	CD and SRD g/kg of diet	SRD + chia
16:0	10.92	6.96
18:0	2.73	2.42
18:1 n-9	33.71	7.39
18:2 n-6	54.10	19.85
18:3 n-3	0.80	67.26
20:1 n-9	0.47	0.36
Total saturated	13.65	9.38
Monounsaturated	34.18	7.75
Polyunsaturated		
n-6	54.10	19.85
n-3	0.80	67.26
n-6/n-3	67.62	0.295

[a] Other minor fatty acids have been excluded.

and subgroup were assessed twice a week. At the end of the experimental period, food was removed at 07:00 h and unless otherwise indicated, experiments were performed between 07:00 and 9:00 h.

2.3. Analytical methods

Blood pressure was measured in the three dietary groups in conscious animals during the experimental period using a CODA™ Monitor of tail-cuff non-invasive blood pressure system (Kent Scientific Corporation, Torrington, CT, USA) as previously described [22]. Rats from all dietary groups were anaesthetized with intraperitoneal sodium pentobarbital (60 mg/kg body weight). Blood samples were obtained from the jugular vein. Plasma triglycerides, free fatty acids, glucose and immunoreactive insulin were determined as previously described [22]. The heart muscle was totally removed, weighed and the left ventricle was separated. The tissue was immediately frozen and stored at temperature of liquid N_2. The frozen muscle powder was used to determine glucose-6-phosphate (glucose-6-P) and glycogen contents and hexokinase, PDHc and M-CPT1 enzyme activities as described elsewhere [22,24]. Heart muscle lipids were extracted according to the procedure described by Folch et al. [25] and total phospholipids were separated by thin layer chromatography; fatty acid composition of total phospholipids was determined by gas liquid chromatography of their methyl esters, as previously described [9,26].

2.4. Determination of GLUT4 protein mass level in the heart muscle (clamp studies)

Whole body peripheral insulin sensitivity was measured using the euglycemic-hyperinsulinemic clamp technique as described elsewhere [22,23]. 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. The protein mass levels of GLUT4 were determined at the beginning and at the end of the clamp. Plasma membrane fractions from heart muscles were prepared as previously described [22]. Total proteins samples were resolved by SDS-PAGE, transferred to PVDF membranes and probed with a specific antibody (polyclonal antibody anti-GLUT4) and with horseradish peroxidase-linked secondary antibody (Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA) followed by chemiluminescence detection according to the manufacturer's instruction (Pierce Biotechnology, Rockford, IL, USA). The intensity of the bands was quantified by the National Institutes of Health (NIH) imaging software (Bethesda, MD, USA). The relationship between the amount of the sample subjected to immunoblotting and the signal intensity observed was linear under the conditions described above. The protein levels were normalized to actin.

2.5. Determination of IRS-1, AMPK and pAMPK(Thr 172) protein mass levels in the heart muscle

Frozen heart powder was homogenized in lysis buffer (100 mM Tris pH 7.4, 1% Triton X-100, 100 mM sodium pyrophosphate, 100 mM sodium fluoride, 10 mM sodium vanadate, 10 mM EDTA, and 5 μL/100 mg wet tissue of protease inhibitor cocktail (Sigma-Aldrich Co., St Louis, MO, USA) and centrifuged at 4 °C as reported by Bezerra et al. [27]. Total protein of supernatants was resolved on SDS-PAGE, transferred to PVDF membranes, probed with primary polyclonal antibody anti-IRS-1, anti-AMPK or anti-pAMPK (Thr172) and with horseradish peroxidase-linked secondary antibody and quantified as described above.

2.6. Determination of hydroxyproline content in left ventricles

Hydroxyproline content was analyzed according to the procedure of Neuman and Logan [28] with slightly modifications. Briefly, left ventricle tissue was hydrolyzed with 6 N HCl at 120 °C for 24 hs. Hydroxyproline content in the resultant hydrolysate was determined by oxidation with 6% v/v H_2O_2 followed by a colorimetric reaction with 5% p/v p-dimethylaminobenzaldehyde. The intensity of the red product was measured at 540 nm. The amount of hydroxyproline in unknown samples was calculated using a standard curve of hydroxyproline.

2.7. Histological analysis of collagen in left ventricles

Immediately after removal, left ventricles were fixed in 4% buffered formaldehyde for 24 h and then dehydrated and embedded in paraffin wax. Cross-sections (5 μm) of mid-ventricular wall were cut and stained using Masson's Trichrome method. 3 slides per tissue specimen were prepared and examined with a magnification of 400× (Olympus BH2, Japan) and the collagen deposition was measured within total area using ImageJ 1.49 v Software (Wayne Rasband, National Institutes of Health, USA). 3 random non-overlapping fields per slide were taken to avoid biased analyses. Perivascular collagen was excluded from this analysis. Results were expressed as percentage of interstitial collagen with respect to total area [29].

2.8. Statistical analysis

Sample sizes were calculated according to measurements previously made in our laboratory with rats fed a CD or SRD considering an 80% power [22,24,26] as described by Glantz [30]. Results were expressed as mean with their standard errors. 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 + chia) was determined by one-way ANOVA with one factor (diet) followed by the inspection of differences between pairs of mean by Newman Keul's post hoc test [31]. When appropriate, the statistical significance between two groups (CD and SRD) was determined by Student's *t*-test. Differences with P values < 0.05 were considered to be statistically significant. Statistical analyses were performed using GraphPad Prism version 5.00 for Windows (San Diego, CA, USA). All reported P values are 2-sided.

3. Results

3.1. Body weight, energy intake, heart and left ventricle weights, blood pressure, plasma metabolites and insulin levels

As previously demonstrated [22] and confirmed in the present work, the SRD and SRD + chia-fed rats showed a significant increase of body weights at the end of the feeding period (Table 3). Energy intake was also increased in SRD and SRD + chia groups compared to CD-fed rats during the last 3 months of the experimental period. The present study shows an increase in heart and left ventricle weights either in the SRD or SRD + chia compared with CD-fed rats. However, no difference was observed between the three dietary groups when both left ventricles and heart weights were expressed relative to 100 g of body weight. Besides, chia seed normalized systolic blood pressure and plasma metabolites without changes in insulin levels (Table 3).

3.2. Metabolites concentration and enzyme activities

Fig. 1 depicts a significant decrease of glucose-6-P and glycogen concentration within the cardiac muscle of SRD-fed rats. This was accompanied by a high reduction of hexokinase and PDHa -the active form of PDHc- activities. The present data show that in the SRD + chia group, neither parameter differed from the CD group at the end of the experimental period. Besides, total PDHc activity expressed relative to milligrams of a soluble protein remained unchanged between the groups (data no shown).

Table 3
Body weight, energy intake, heart and left ventricle weights, systolic blood pressure, plasma metabolites and insulin levels of rats fed a control diet (CD), sucrose-rich diet (SRD) or SRD with chia seed (SRD + chia).

	CD	SRD	SRD + chia
Final body weight (g)	465.0 ± 7.0[b]	522.5 ± 11.0[a]	496.0 ± 11.5[a]
Energy intake (kJ/d)			
Initial-3 month[1]	278.1 ± 9.2	281.1 ± 9.8	
3–6 month	280.6 ± 7.8 [b]	356.2 ± 7.2 [a]	349.1 ± 4.8 [a]
Heart muscle			
Total weight (g)	1.26 ± 0.01[b]	1.34 ± 0.02[a]	1.31 ± 0.02[a]
Relative weight (g/ 100 g body weight)	0.272 ± 0.009	0.255 ± 0.004	0.257 ± 0.006
Left ventricle			
Total weight (g)	0.873 ± 0.02[b]	0.940 ± 0.01[a]	0.972 ± 0.01[a]
Relative weight (g/100 body weight)	0.189 ± 0.007	0.179 ± 0.003	0.192 ± 0.006
Systolic Blood Pressure (mmHg)	118.2 ± 3.4[b]	135.8 ± 2.5[a]	119.2 ± 1.0[b]
Plasma			
Triglyceride (mM)	0.78 ± 0.06[b]	2.32 ± 0.1[a]	0.79 ± 0.08[b]
FFA (μM)	340.3 ± 19[b]	714.5 ± 25[a]	366.3 ± 28[b]
Glucose (mM)	6.5 ± 0.1[b]	8.4 ± 0.1[a]	6.7 ± 0.1[b]
Insulin (pmol/L)	435.3 ± 19	457.0 ± 22	469.2 ± 41

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

[1] From initial to 3 months 6 animals for CD and 12 animals for SRD.

3.3. Protein mass level of GLUT-4 at the beginning and at the end of clamp studies

The immunoblotting of lysate heart tissue revealed a single 45 kDa band consistent with GLUT-4. Each gel contained equal number of samples from rats fed a CD, SRD and SRD + chia at the beginning (0 min) and at the end (120 min) of the euglycemic-hyperinsulinemic clamp (Fig. 2, top panel). After the densitometry of immunoblots, the GLUT-4 of the CD groups at the beginning of the clamp was normalized to 100%, and both SRD and SRD + chia at the beginning, and also the three dietary groups at the end of the study, were expressed relative to this. At the beginning no differences in the relative abundance of the total plasma membrane GLUT-4 protein was observed between all groups. Under insulin stimulation the translocation of GLUT-4 to the plasma membrane significantly increased ($P < 0.05$) in CD-fed rats, while the increase was lower (11%) in the SRD-fed group. Dietary chia seed significantly increased (72%; $P < 0.05$), the GLUT-4 protein mass reaching values above those recorded in the CD-fed rats (Fig. 2, bottom panel). This was accompanied by a normalization of GIR which reached values similar to those of rats fed a CD (table inserted in Fig. 2).

3.4. IRS-1 protein mass level

The inmunoblotting of lysate heart tissue revealed a single 180 kDa band consistent with IRS-1. Each gel contained equal number of samples from the CD, SRD and SRD + chia groups (Fig. 3, top panel). After

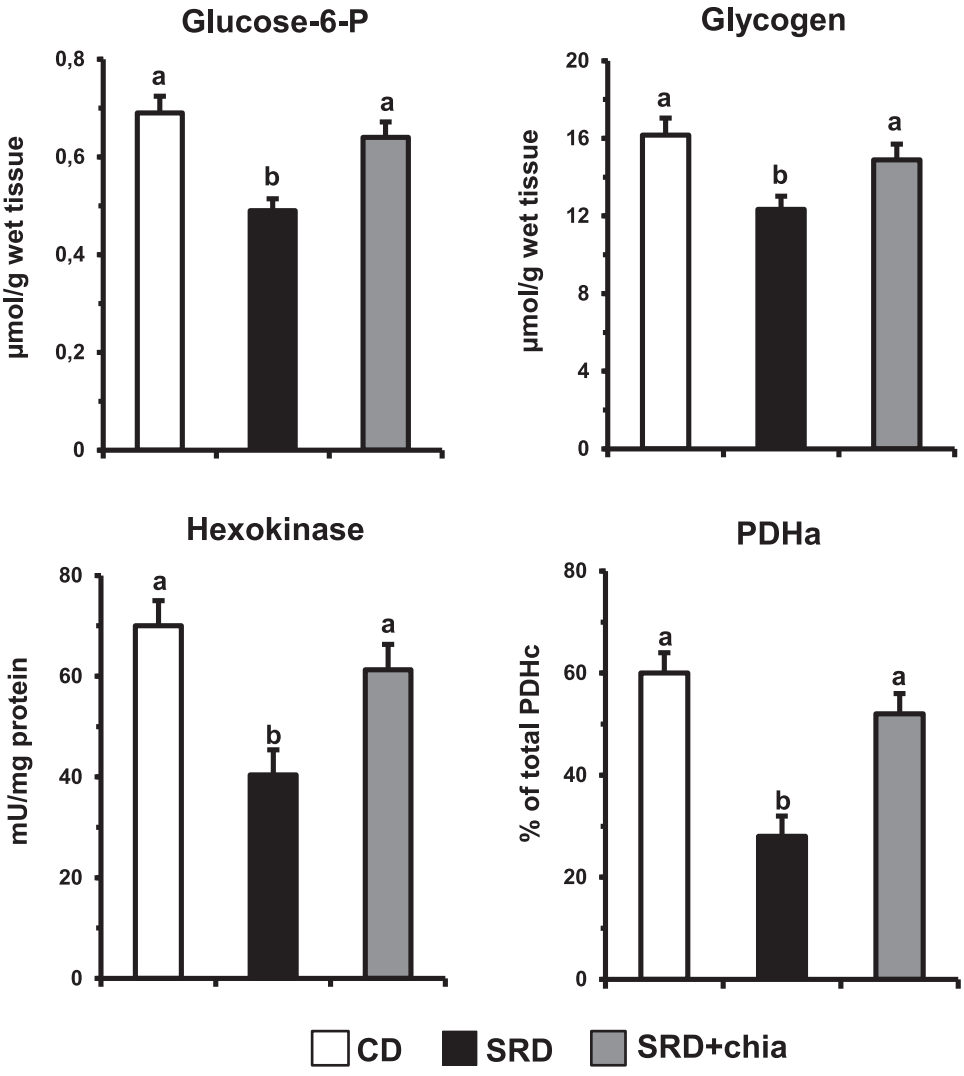
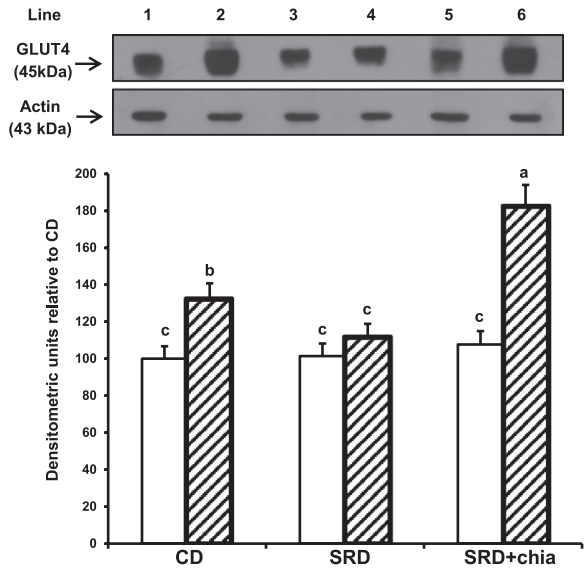


Fig. 1. Glucose-6-P and glycogen concentrations and hexokinase and PDHa activities in the heart muscle of rats fed a control diet (CD), sucrose-rich diet (SRD) or SRD with chia seed (SRD + chia). Values are mean ± SEM (6 animals per group) with their standard error depicted by vertical bars. Values that do not share the same letter are significantly different, $P < 0.05$ (ANOVA followed by the Newman Keul's test).



	CD	SRD	SRD+chia
Glucose infusion rate (GIR) µmol / (min x kg)	53.0 ± 2.9[a]	31.4 ± 1.6[b]	50.6 ± 2.7[a]

Fig. 2. Heart muscle protein mass level of GLUT-4 at the beginning and under insulin stimulation at the end of the clamp studies in rats fed a control diet (CD), sucrose-rich diet (SRD) or SRD with chia seed (SRD + chia). Top panel: a representative immunoblot of GLUT-4 of heart muscle from CD, SRD or SRD + chia. Molecular marker is shown on the right. Lane 1, CD 0 min; lane 2, CD 120 min; lane 3, SRD 0 min; lane 4, SRD 120 min; lane 5, SRD + chia 0 min; lane 6, SRD + chia 120 min. Bottom panel: densitometric immunoblot analysis of GLUT-4 of heart muscle from CD, SRD or SRD + chia at the beginning (*square* 0 min) and at the end (*lined square* 120 min) of clamp studies. Values are mean ± SEM (6 animals per group), with their standard errors depicted by vertical bars and expressed as percentage relative to CD at 0 min of the clamp. Values that do not share the same letter are significantly different, $P < 0.05$ (ANOVA followed by the Newman Keul's test). In the table values are expressed as mean ± SEM (6 animals per group). Values in a line that do not share the same superscript letter are significantly different ($P < 0.05$) when one variable at a time was compared by one-way ANOVA followed by the Newman Keul's test.

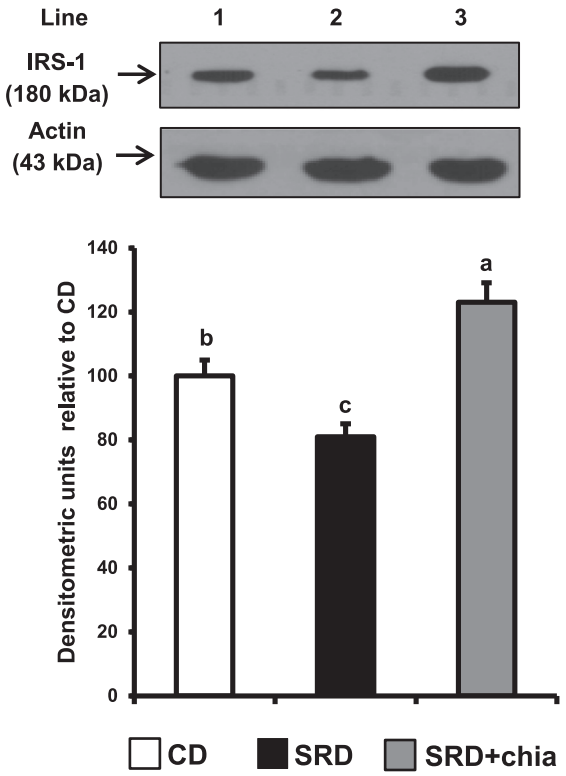


Fig. 3. Heart muscle protein mass level of IRS-1in rats fed a control diet (CD), sucrose-rich diet (SRD) or SRD with chia seed (SRD + chia). Top panel: a representative immunoblot of IRS-1 of heart muscle from CD, SRD or SRD + chia. Molecular marker is shown on the right. Lane 1, CD; lane 2, SRD; lane 3, SRD + chia. Bottom panel: densitometric immunoblot analysis of IRS-1 of heart muscle from CD, SRD or SRD + chia. Values are mean ± SEM (6 animals per group), with their standard errors depicted by vertical bars and expressed as percentage relative to the CD. Values that do not share the same letter are significantly different, $P < 0.05$ (ANOVA followed by the Newman Keul's test).

the densitometry of immunoblots, the IRS-1 from the CD group was normalized to 100% and the level of IRS-1 from the SRD and SRD + chia was expressed relative to this. The qualitative and quantitative analyses of the Western blot showed that the relative abundance of IRS-1 at basal conditions (without insulin stimulation) was significantly decreased ($P < 0.05$) in the hearts of the SRD group compared with rats fed a CD. The addition of chia seed to the SRD-fed rats significantly increased ($P < 0.05$) the protein mass level of IRS-1, reaching values even higher than those recorded in the CD-fed rats (Fig. 3, bottom panel).

3.5. AMPK and pAMPK protein mass levels

The immunoblotting of lysate heart tissue revealed a single 63 kDa band consistent with the AMPK and pAMPK. Each gel contained an equal number of samples from CD, SRD and SRD + chia groups (Fig. 4a and b top panel). After the densitometry of immunoblots, both the AMPK and pAMPK of the CD group were normalized to 100% and the levels of AMPK and pAMPK from the SRD and SRD + chia groups were expressed relative to this. The qualitative and quantitative analysis of the Western blot showed no differences in the relative abundance of AMPK protein mass between the three dietary groups while a significant increase ($P < 0.05$) of pAMPK was recorded in the heart of the SRD-fed group. When corn oil was replaced by dietary chia seed as a dietary fat in the diet (SRD + chia), a significant reduction ($P < 0.05$) of pAMPK protein mass level was observed. In this group, values were similar to those of the CD-fed rats (Fig. 4a and b, bottom panel). Besides, the significant increase ($P < 0.05$) of the pAMPK/AMPK protein mass ratio observed in the SRD-fed rats was completely normalized in the SRD + chia group reaching values similar to the CD-fed rats (Fig. 4c).

Moreover, similarly to our previous report [22], an increase of the M-CPT1 activity was observed in the heart tissue of SRD-fed rats that significantly decreased after chia administration. Values were as follows: mean ± SEM (n = 6), nmol/min x mg protein, CD 8.0 ± 0.8; SRD 23.9 ± 2.0; SRD + chia 16.1 ± 1.9, $P < 0.05$ SRD vs CD and SRD + chia and $P < 0.05$ SRD + chia vs CD.

3.6. Fatty acid composition of heart tissue phospholipids

Table 4 depicts the heart muscle tissue fatty acid membrane phospholipids and the ratios of n-3 PUFAs to total fatty acids, n-3 to n-6 and n-3 to total saturated fatty acids. Compared with the CD-fed rats, a significantly increase of saturated fatty acids can be observed in the SRD group, while polyunsaturated as well as n-3PUFAs/total fatty acids and n-3PUFAs/total saturated fatty acids ratios significantly decreased in the latter group. The n-3/n-6 ratio was similar in both groups. Chia seeds significantly decreased saturated fatty acids compared with SRD, while an enhancement of n-3 and n-6 PUFAs was observed due to an increase of ALA, 22:6 n-3 and 20:4 n-6. Besides, n-3/total saturated fatty acids were increased in the chia seed group reaching values similar to those of the CD-fed rats.

3.7. Interstitial collagen deposition and hydroxyproline level

Compared to the CD group, the histological analysis of the left ventricle of rats fed a SRD showed both a significant increase of

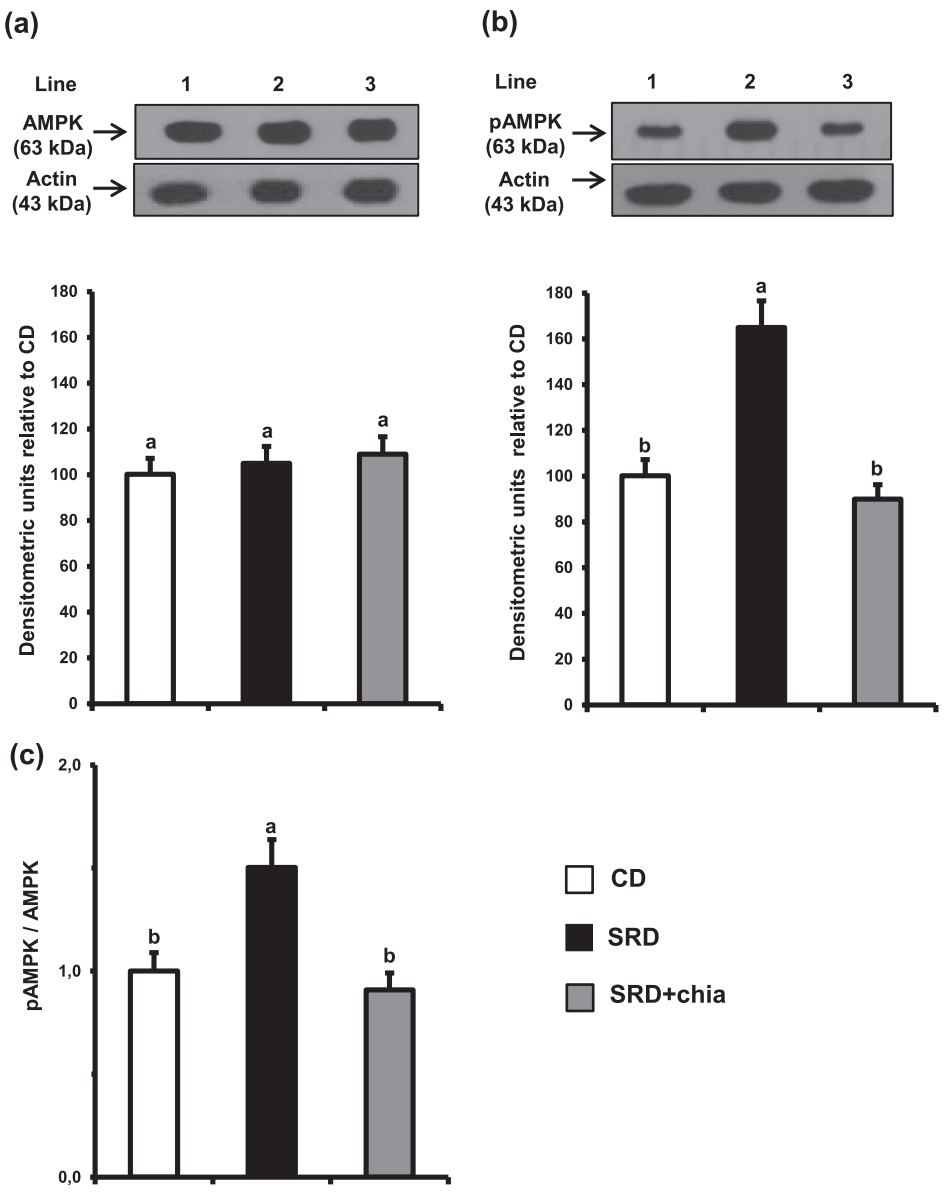


Fig. 4. Heart muscle protein mass level of AMPK and pAMPK (Thr172) in rats fed a control diet (CD), sucrose-rich diet (SRD) or SRD with chia seed (SRD + chia). a: Top panel: a representative immunoblot of AMPK of heart muscle from CD, SRD or SRD + chia. Molecular marker is shown on the right. Lane 1, CD; lane 2, SRD; lane 3, SRD + chia. Bottom panel: densitometric immunoblot analysis of AMPK of heart muscle from CD, SRD or SRD + chia. b: Top panel: a representative immunoblot of pAMPK (Thr 172) of heart muscle from CD, SRD or SRD + chia. Molecular marker is shown on the right. Lane 1, CD; lane 2, SRD; lane 3, SRD + chia. Bottom panel: densitometric immunoblot analysis pAMPK of heart muscle from CD, SRD or SRD + chia. In Fig. a and b values are mean ± SEM (6 animals per group), with their standard errors depicted by vertical bars and expressed as percentage relative to the CD. Values that do not share the same letter are significantly different, $P < 0.05$ (ANOVA followed by the Newman Keul's test). c: pAMPK-AMPK ratio of rats fed control diet (CD), sucrose-rich diet (SRD) or SRD with chia seed (SRD + chia). Values are mean ± SEM (6 animals per group), with their standard errors depicted by vertical bars and expressed as percentage relative to the CD. Values that do not share the same letter are significantly different, $P < 0.05$ (ANOVA followed by the Newman Keul's test).

interstitial collagen deposition and collagen fractional area per visual field (%) (Fig. 5a marked I and II and Fig. 5b). Hydroxyproline concentration was also higher at the end of the experimental period in the cardiac muscle of the SRD group (Fig. 5c). This metabolite was just increased after 3 months on the SRD. Values were as follows: mean ± SEM (n = 6), μg/g wet tissue CD 426.4 ± 10.0; SRD 595.7 ± 26.0; $P < 0.05$. Interstitial collagen deposition (Fig. 5a marked III) as well as collagen fractional area and hydroxyproline level were significantly improved after chia seed supplementation (Fig. 5b and c), reaching values similar to those recorded in the CD fed group.

4. Discussion and conclusion

This study provides new information focusing on the possible beneficial effects of dietary chia seed supplementation in improving and/or reversing the underlying mechanism involved in the altered metabolic fate of glucose in the heart muscle of rats rendered dyslipidemic and insulin resistant by feeding them a SRD.

Insulin resistance in heart muscle has been linked to intramuscular accretion of fatty acids. This metabolite could interfere with the activation of insulin signaling pathways involved in the recruitment of GLUT-4 to the surface. In this regard, our data show that at basal state the protein mass levels of GLUT-4 in the heart of the SRD group did not differ from that recorded in either the control or SRD + chia groups. A reduction of insulin stimulation cell surface recruitment of GLUT-4 was observed in the heart of SRD-fed rats as compared with the CD group. However, the stimulus of the hormone upon GLUT-4 recruitment in the SRD + chia-fed rats was even higher than that observed in the control group. Desrois et al. [32] demonstrated a reduction of glucose uptake, and total protein GLUT-4 and IRS-1 protein levels in the heart of Goto-Kakuzaki rats, a model of spontaneous Type 2 diabetes mellitus. Bonen et al. [6] found an inverse correlation between fatty acids transporter FAT/CD36 and GLUT-4 protein mass expression in the heart muscle of lean and ZDF rats. They suggest a possible link of impaired GLUT-4 trafficking to the plasma membrane with both the increase of plasmalemmal FAT/CD36 and the rate of fatty acids transport and lipid accumulation. In this regard, we have recently demonstrated [22] that dietary chia seed was able to reverse the increased protein mass levels of FAT/CD36 and the impaired insulin stimulated FAT/CD36 translocation to the plasma membrane, decreasing lipid accretion (triglyceride, LC-acylCoA, diacylglycerol) in the cardiac muscle of SRD-fed rats. This could favor GLUT-4 normalization. The reduction of LC-acylCoA levels within the heart of rats of the SRD + chia group may also contribute to improve the impaired glucose phosphorylation since the

Table 4
Fatty acid composition of heart phospholipids (g/100 g total fatty acids) of rats fed a control diet (CD), sucrose-rich diet (SRD) or SRD with chia seed (SRD + chia).

Fatty acids[1]	CD	SRD	SRD + chia
16:0	20.5 ± 1.1[b]	29.7 ± 2.0[a]	16.1 ± 0.8[c]
16:1	0.8 ± 0.1[a]	0.2 ± 0.02[c]	0.5 ± 0.03[b]
18:0	31.0 ± 2.3[b]	39.9 ± 3.0[a]	33.6 ± 2.2[a,b]
18:1	6.2 ± 0.4	5.3 ± 0.3	5.8 ± 0.3
18:2 n-6	10.2 ± 0.5[a]	6.6 ± 0.2[b]	11.4 ± 0.7[a]
18:3 n-3	Tr	Tr	3.1 ± 0.2
20:3 n-6	4.0 ± 0.2[a]	2.2 ± 0.1[b]	4.1 ± 0.1[a]
20:4 n-6	11.3 ± 0.7[a]	6.0 ± 0.2[b]	11.9 ± 0.6[a]
20:5 n-3	0.9 ± 0.05	0.8 ± 0.1	0.9 ± 0.2
22:5 n-3	5.0 ± 0.1	4.1 ± 0.3	4.0 ± 0.3
22:6 n-3	9.6 ± 0.4[a]	4.3 ± 0.1[b]	8.2 ± 0.5[a]
Saturated	51.50 ± 2.5[b]	69.60 ± 3.6[a]	49.70 ± 2.3[b]
Monounsaturated	7.0 ± 0.4[a]	5.50 ± 0.3[b]	6.30 ± 6.30[a]
Polyunsaturated	41.0 ± 1.0[a]	24.0 ± 0.4[b]	43.9 ± 1.1[a]
n-6	25.5 ± 0.9[a]	14.80 ± 0.3[b]	27.40 ± 0.9[a]
n-3	15.5 ± 0.4[a]	9.20 ± 0.3[b]	16.2 ± 0.7[a]
n-3/total	0.16 ± 0.004[a]	0.09 ± 0.005[b]	0.16 ± 0.008[a]
n-3/n-6	0.61 ± 0.03	0.62 ± 0.02	0.59 ± 0.03
n-3/total saturated	0.30 ± 0.02[a]	0.13 ± 0.01[b]	0.33 ± 0.02[a]

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

[1] Minor fatty acids made content up to 100%.

activity of the hexokinase as well as glucose-6-P concentration reached values similar to those recorded in the CD-fed group. In this vein, Thompson et al. [33] demonstrated "in vitro" that increased levels of acylCoA reduced the activity of hexokinase in the skeletal muscle of normal rats by allosteric inhibition. Besides, the increased uptake and oxidation of fatty acids (M-CPT1activity was increased) in the SRD group reduced the activity of PDHc. This, in turn, impaired glucose oxidation as observed in the heart muscle of diabetic rats [34]. The normalization of both PDHc and the improvement of M-CPT1 activities after dietary chia seeds could be one of the possible mechanisms involved in the improvement of glucose oxidation in the heart muscle of the SRD + chia-fed rats.

In addition, increased intracellular lipids have been shown to inhibit the action of insulin by the activation of serine/threonine kinases and phosphorylation of IRS-1 [35]. In this regard, a significant reduction of IRS-1 protein mass was observed in the heart of SRD-fed rats induced by a high-sucrose feeding. Chia seed markedly increased the amount of protein mass of IRS-1 and as previously demonstrated normalized lipid accretion [22]. Our results do not provide data about all the possible mechanism/s underlying the beneficial effect of chia seed that could involve ALA and/or other components of the seed on insulin action in the cardiac muscle of SRD-fed rats. Besides, we are unaware of other studies concerning this topic. However, some clues have been provided by recent publications. For instance in the SRD fed rats dietary chia seed normalized dyslipidemia by mechanisms that included hepatic up-regulation of PPARα and their target enzymes CPT-1 and FAO reducing "de novo lipogenesis" [36]. A significant increase of liver PPARα, CPT-1 and ACOX1 expressions with a hepatic accretion of n-3 PUFAs was also recorded in normal rats fed chia oil [37]. Besides, dietary chia seed normalized lipotoxicity, whole body insulin resistance and improved the mechanisms underlying the impaired glucose metabolism in the skeletal muscle of SRD fed rats [23]. From the above findings, we could suggest that the normalization of dyslipidemia, glucose homeostasis and heart lipotoxicity, induced by dietary chia seed leads to a normalization of the altered protein mass level of IRS-1 and GLUT-4 translocation and insulin resistance. These factors in turn could contribute to the mechanisms involved in the improvement of the oxidative and non-oxidative pathways of glucose and the impaired balance of heart fuel utilization.

On the other hand, AMPK plays an important role in muscle fuel preference and flexibility. Longnus et al. [38] showed that AMPK activation inhibits IRS-1 associated PI3K activity, and AMPK activates atypical PKC and extracellular signal-regulated kinase in the heart. Insulin decreases pAMPK [39]. Inhibition of AMPK activity is also associated with an increase of acetyl CoA carboxylase activity and a decrease of mitochondrial fatty acid oxidation [40]. Our data show a significant increase of pAMPK protein mass level and the pAMPK/AMPK ratio in the heart muscle of SRD-fed animals. It has been proposed that the activation of AMPK could also stimulate GLUT-4 transport and glycolysis [39]. However, we do not observe these effects in the SRD group. Dietary chia seed was able to completely normalize AMPK activity. Recently, Samovski et al. [41] showed a close coordination between AMPK and FAT/CD36 in the regulation of FA metabolism. In myocytes and other cell types, they observed that FAT/CD36 maintains basal AMPK quiescence at low levels of FA. However, an increase of FA induced FAT/CD36 signaling activate AMPK, which disinhibits β oxidation recruiting more FAT/CD36 to the sarcolemma. Although these mechanisms mentioned above could be involved in the effect of dietary chia seed on the behavior of this "cellular fuel gauge", we cannot discard the possibility that a coordinate action between the behavior of AMPK, FAT/CD36 and a decrease of fatty acid availability induced by chia seed might also contribute to the improvement of heart substrate utilization. Further studies are needed to evaluate this issue.

Increases in fibrillar collagen have been observed in the left cardiac ventricle of both animals and humans with hypertension [42]. The present data show that the hypertension developed in the SRD-fed rats is accompanied by a significant increase of both left ventricle interstitial collagen deposition and hydroxyproline concentration, suggesting that the heart of these animals developed left ventricular fibrosis. By contrast, hypertension, collagen deposition and hydroxyproline levels were markedly reduced after dietary chia seed administration. In this regard, Poudyal et al.[11,43], working with rats fed a high fat-high fructose diet, have recently shown that the administration of chia seeds or oil improved hypertension and heart left ventricular dimensions, contractility, volume, stiffness and markedly reduced inflammation and collagen deposition. The mechanism/s by which dietary chia seed, ALA and/or other components present in the chia seed (e.g. antioxidants like quercetin, fiber, among others) could normalize hypertension and collagen deposition in dyslipidemic insulin-resistant SRD-fed rats are still not fully elucidated. Ogawa et al. [44] demonstrated that the low pressure mechanism of dietary ALA in spontaneous hypertensive rats (SHR) may be involved in the reduction of angiotensin-converting enzyme activity and mRNA expression levels in the aorta. Besides, ALA protects against cardiac injury and remodeling induced by β-adrenergic overstimulation [45]. Lei Yan et al. [46] showed that the administration of quercetin dose-dependently to SHR rats decreases hypertension, ameliorates cardiac hypertrophy and collagen deposition by enhanced PPARγ expression and suppresses activation protein-1 signaling pathway. Panchal et al. [47], in rats fed a high fat-high fructose diet supplemented with quercetin for 8 weeks, showed decreased blood pressure along with attenuation of changes in structure and function of the heart compared to rats fed the same diet without quercetin, suggesting mechanisms involving reduction of inflammation and oxidative stress. In this regard, we have recently demonstrated that dietary chia decreases plasma inflammatory cytokines (TNFα, IL-6) and improves adipose tissue dysfunction through amelioration of oxidative stress in the SRD + chia group [26]. Therefore, we do not discard the possibility that cooperative effects of ALA, quercetin, other antioxidants, fibers and other components of chia seed [48] might contribute to our findings.

In addition, our data demonstrate that the replacement of corn oil by chia seed was able to increase the incorporation of both ALA and DHA in the fatty acids phospholipids of cardiac muscle of SRD-fed rats. Similarly, Poudyal et al. [49] showed an increase of ALA, DHA and DPA in the cardiac muscle of rats fed a high fat-high fructose diet supplemented with chia seed compared to those without the addition of chia.

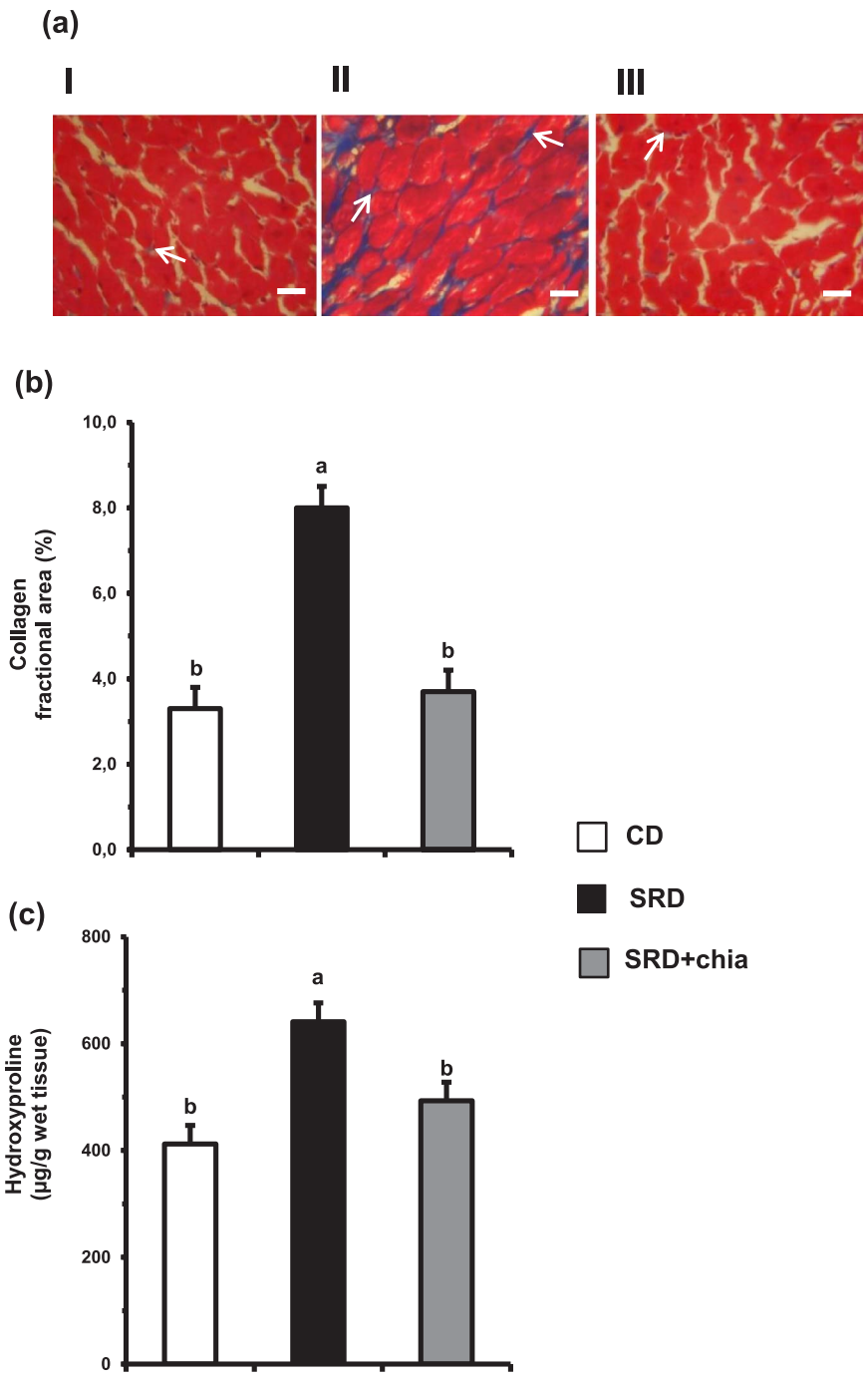


Fig. 5. Collagen and hydroxyproline contents in left ventricle of rats fed a control diet (CD), sucrose-rich diet (SRD) or SRD + chia seed (SRD + chia). a: Masson´s Trichrome-stained histological sections (at 400× magnification) of left ventricle of rats fed a CD (I), SRD (II) or SRD + chia (III). Arrows show collagen deposition. Bar represents 20 µm. b: Quantitative analysis of interstitial collagen of left ventricle of rats fed a CD, SRD or SRD + chia. Values are mean ± SEM (6 animals per group), with their standard errors depicted by vertical bars. Values that do not share the same letter are significantly different, $P < 0.05$ (ANOVA followed by the Newman Keul's test). c: Hydroxyproline content in left ventricle of rats fed a CD, SRD, or SRD + chia. Values are mean ± SEM (6 animals per group), with their standard errors depicted by vertical bars. Values that do not share the same letter are significantly different, $P < 0.05$ (ANOVA followed by the Newman Keul's test).

Further, an increase of ALA and EPA was also observed in the heart muscle phospholipids of rats fed during 3 weeks chia oil (10% w/w) compared to those fed sunflower oil as a dietary source of fat [50]. Our results show, in the heart of SRD + chia fed rats, that an increase of PUFAs in the cardiac phospholipids induced a significant rise in membrane unsaturation (PUFAs/saturate FA ratio was higher in SRD + chia compared to SRD: 0.88 vs 0.34, respectively). This suggests an increase in membrane fluidity, and it is well known that the degree of unsaturation in the membrane phospholipids is associated with improved insulin stimulated glucose uptake and sensitivity [51]. Thus, the changes observed in the fatty acids profile in the heart muscle phospholipids of the SRD + chia group might be another possible mechanism responsible for the normalization of insulin sensitivity and its action and hypertension. Besides, it was shown in rats fed high fructose–high fat, high sucrose as well as control diets that dietary chia seed or chia oil administration changes the fatty acids profile increasing n-3 PUFAs in the heart muscle and others tissues [11,37]. This might activate the peroxisome proliferator activated receptor α (PPAR α) that could modulate among others cellular redox state given a cito-protective effects [52].

In brief, expanding our previous results, this study provides new information on the beneficial effects of dietary chia seed to improve some key mechanisms collectively involved in the altered metabolic fate of glucose in the heart muscle of the dyslipemic insulin-resistant rat model. This nutritional manipulation was also effective in reducing left ventricle collagen deposition and in reversing hypertension. In this context, the decrease of plasma lipid flux availability contributes to normalized dyslipidemia and insulin resistance protecting the cardiac muscle to lipotoxicity. Caution is warranted before extrapolating these results to humans, in particular considering the different quantities of

chia seed that have been used (0.36–0.72 g daily/kg of body weight in human-average body weight 70 kg versus 20 g daily/kg body weight in rats) [20]. However, Vuksan et al. [19] demonstrated that consumption of the whole grain Salba in healthy subjects improved postprandial glycemia and increased satiety. Besides, in type 2 diabetes patients treated with calorie restricted diet and pharmacological standard care, these authors showed [53] that Salba-chia seed consumption reduces visceral adiposity, improves low-grade body inflammation and increased adiponectin secretion, suggesting the beneficial use of chia seed as a complementary therapy for treating some signs of the metabolic syndrome.

Conflict of interest

The authors declare that there is no conflict of interest.

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References

[1] K.D. Bruce, M.A. Hanson, The developmental origins, mechanisms, and implications of metabolic syndrome, J. Nutr. 140 (2010) 648–652.
[2] Y.T. Zhou, P. Grayburn, A. Karim, M. Shimabukuro, M. Higa, D. Baetens, L. Orci, R.H. Unger, Lipotoxic heart disease in obese rats: implications for human obesity, Proc. Natl. Acad. Sci. USA 97 (2000) 1784–1789.
[3] P.J. Randle, E.A. Newsholmet, P.B. Garland, Regulation of glucose uptake by muscle 8, Biochem. J. 93 (1964) 652–665.
[4] L. Hue, H. Taegtmeyer, The Randle cycle revisited: a new head for an old hat, AJP Endocrinol. Metab. 297 (2009) E578–E591.
[5] F.A. Neves, E. Cortez, A.F. Bernardo, A.B.M. Mattos, A.K. Vieira, T.O. Malafaia, A.A. Thole, A.C. Alessandra, É.P. Garcia-Souza, R. Sichieri, A.S. Moura, Heart energy metabolism impairment in western-diet induced obese mice, J. Nutr. Biochem. 25 (2014) 50–57.
[6] A. Bonen, G.P. Holloway, N.N. Tandon, X. Han, J. McFarlan, J.F.C. Glatz, J.J.F.P. Luiken, Cardiac and skeletal muscle fatty acid transport and transporters and triacylglycerol and fatty acid oxidation in lean and Zucker diabetic fatty rats, Am. J. Physiol. Regul. Integr. Comp. Physiol. 297 (2009) R1202–R1212.
[7] S.L. Ménard, E. Croteau, O. Sarrhini, R. Gélinas, P. Brassard, R. Ouellet, M. Bentourkia, J.E. van Lier, C. Des Rosiers, R. Lecomte, A.C. Carpentier, Abnormal in vivo myocardial energy substrate uptake in diet-induced type 2 diabetic cardiomyopathy in rats, Am. J. Physiol. Endocrinol. Metab. 298 (2010) E1049–E1057.
[8] J.C. Chatham, A.M. Seymour, Cardiac carbohydrate metabolism in Zucker diabetic fatty rats, Cardiovasc Res. 55 (2002) 104–112.
[9] G.J. Hein, A.M. Bernasconi, M.A. Montanaro, M. Pellon-Maison, G. Finarelli, A. Chicco, Y.B. Lombardo, R.R. Brenner, Nuclear receptors and hepatic lipidogenic enzyme response to a dyslipidemic sucrose-rich diet and its reversal by fish oil n-3 polyunsaturated fatty acids, Am. J. Physiol. Endocrinol. Metab. 298 (2010) E429–E439.
[10] A. Chicco, J.C. Basabe, L. Karabatas, N. Ferraris, A. Fortino, Y.B. Lombardo, Troglitazone (CS-045) normalizes hypertriglyceridemia and restores the altered patterns of glucose-stimulated insulin secretion in dyslipidemic rats, Metabolism 49 (2000) 1346–1351.
[11] H. Poudyal, S.K. Panchal, L.C. Ward, J. Waanders, L. Brown, Chronic high-carbohydrate, high-fat feeding in rats induces reversible metabolic, cardiovascular, and liver changes, Am. J. Physiol. - Endocrinol. Metab. 302 (2012) E1472–E1482.
[12] A. Chicco, A. Soria, P. Fainstein-Day, R. Gutman, Y.B. Lombardo, Multiphasic metabolic changes in the heart of rats fed a sucrose-rich diet, Horm. Metab. Res. 26 (1994) 397–403.
[13] M. Montes, A. Chicco, Y.B. Lombardo, The effect of insulin on the uptake and metabolic fate of glucose in isolated perfused hearts of dyslipemic rats, J. Nutr. Biochem. 11 (2000) 30–37.
[14] D.B. Jump, C.M. Depner, S. Tripathy, Omega-3 fatty acid supplementation and cardiovascular disease, J. Lipid Res. 53 (2012) 2525–2545.
[15] L. Djoussé, D.K. Arnett, J.S. Pankow, P.N. Hopkins, M.A. Province, R. Curtis Ellison, Dietary linolenic acid is associated with a lower prevalence of hypertension in the NHLBI family heart study, Hypertension 45 (2005) 368–373.
[16] G. Barceló-Coblijn, E.J. Murphy, Alpha-linolenic acid and its conversion to longer chain n-3 fatty acids: benefits for human health and a role in maintaining tissue n-3 fatty acid levels, Prog. Lipid Res. 48 (2009) 355–374.
[17] R. da S. Marineli, E.A. Moraes, S.A. Lenquiste, A.T. Godoy, M.N. Eberlin, M.R. Marostica, Chemical characterization and antioxidant potential of Chilean chia seeds and oil (Salvia hispanica L.), LWT - Food Sci. Technol. 59 (2014) 1304–1310.
[18] M.A. Valdivia-Lopez, Chia (Salvia hispanica): a review of native Mexican seed and its nutritional and functional properties, Adv. Food Nutr. Res. 75 (2015) 53–75.
[19] V. Vuksan, A. Jenkins, A. Dias, A. Lee, E. Jovanovski, A. Rogovik, A. Hanna, Reduction in postprandial glucose excursion and prolongation of satiety: possible explanation of the long-term effects of whole grain Salba (Salvia hispanica L.), Eur. J. Clin. Nutr. 64 (2010) 436–438.
[20] V. Vuksan, W. Dana, J.L. Sievenpiper, A.L. Jenkins, A.L. Rogovik, R.P. Bazinet, E. Vidgen, A. Hanna, Supplementation of conventional therapy with the novel grain salba (Salvia hispanica L.) improves major and emerging cardiovascular risk factors in type 2 diabetes, Diabetes Care 30 (2007) 2804–2810.
[21] H. Ho, A.S. Lee, E. Jovanovski, A.L. Jenkins, R. DeSouza, V. Vuksan, Effect of whole and ground Salba seeds (Salvia hispanica L.) on postprandial glycemia in healthy volunteers: a randomized controlled, dose-response trial, Eur. J. Clin. Nutr. 67 (2013) 786–788.
[22] A. Creus, M.R. Ferreira, M.E. Oliva, Y.B. Lombardo, Mechanisms involved in the improvement of lipotoxicity and impaired lipid metabolism by dietary α-linolenic acid rich salvia hispanica L (Salba) seed in the heart of dyslipemic insulin-resistant rats, J. Clin. Med. 5 (2016) 1–18.
[23] M.E. Oliva, M.R. Ferreira, A. Chicco, Y.B. Lombardo, Dietary salba (salvia hispanica L) seed rich in alpha-linolenic acid improves adipose tissue dysfunction and the altered skeletal muscle glucose and lipid metabolism in dyslipidemic insulin-resistant rats, Prostaglandins Leukot. Essent. Fat. Acids 89 (2013) 279–289.
[24] M.E. D'Alessandro, A. Chicco, Y.B. Lombardo, Dietary fish oil reverses lipotoxicity, altered glucose metabolism, and nPKCε translocation in the heart of dyslipemic insulin-resistant rats, Metabolism 57 (2008) 911–919.
[25] J. Folch, M. Lees, G.H.S. Stanley, A simple method for the isolation and purification of total lipids from animal tissues, J. Biol. Chem. 226 (1957) 497–509.
[26] M.R. Ferreira, S.M. Alvarez, P. Illesca, M.S. Gimenez, Y.B. Lombardo, Dietary Salba (Salvia hispanica L.) ameliorates the adipose tissue dysfunction of dyslipemic insulin-resistant rats through mechanisms involving oxidative stress, inflammatory cytokines and peroxisome proliferator-activated receptor γ, Eur. J. Nutr. (2016) 1–12.
[27] R.M. Bezerra, M. Ueno, M.S. Silva, D.Q. Tavares, C.R. Carvalho, M.J. Saad, A high fructose diet affects the early steps of insulin action in muscle and liver of rats, J. Nutr. 130 (2000) 1531–1535.
[28] R.E. Neuman, M.A. Logan, The determination of hydroxyproline, J. Biol. Chem. 184 (1950) 299–306.
[29] M.J. Black, A. D'Amore, A. Auden, L. Stamp, T. Osicka, S. Panagiotopoulos, G. Jerums, Chronic type 1 diabetes in spontaneously hypertensive rats leads to exacerbated cardiac fibrosis, Cardiovasc. Pathol. 19 (2010) 361–370.
[30] S.A. Glantz, Pimer of Biostatistics, 6th ed., McGraw Hill, New York, 2005.
[31] G.W. Snedecor, W.G. Cochran, Statistical Methods Applied to Experiments in Agriculture and Biology, 6th ed., Iowa State University Press, Ames, 1967.
[32] M. Desrois, R.J. Sidell, D. Gauguier, L.M. King, G.K. Radda, K. Clarke, Initial steps of insulin signaling and glucose transport are defective in the type 2 diabetic rat heart, Cardiovasc Res. 61 (2004) 288–296.
[33] A.L. Thompson, G.J. Cooney, Acyl-CoA inhibition of hexokinase in rat and human skeletal muscle is a potential mechanism of lipid-induced insulin resistance, Diabetes 49 (2000) 1761–1765.
[34] M.J. Holness, M.C. Sugden, Regulation of pyruvate dehydrogenase complex activity by reversible phosphorylation, Biochem. Soc. Trans. 31 (2003) 1143–1151.
[35] S. Gray, J.K. Kim, New insights into insulin resistance in the diabetic heart, Trends Endocrinol. Metab. 22 (2011) 394–403.
[36] A.S. Rossi, M.E. Oliva, M.R. Ferreira, A. Chicco, Y.B. Lombardo, Dietary chia seed induced changes in hepatic transcription factors and their target lipogenic and oxidative enzyme activities in dyslipidaemic insulin-resistant rats, Br. J. Nutr. (2012) 1–11.
[37] D. González-Mañán, G. Tapia, J.G. Gormaz, A. D'Espessailles, A. Espinosa, L. Masson, P. Varela, A. Valenzuela, R. Valenzuela, Bioconversion of α-linolenic acid to n-3 LCPUFA and expression of PPAR-alpha, acyl coenzyme A oxidase 1 and carnitine acyl transferase I are incremented after feeding rats with α-linolenic acid-rich oils, Food Funct. 3 (2012) 765–772.
[38] S.L. Longnus, C. Ségalen, J. Giudicelli, M.P. Sajan, R.V. Farese, E. Van Obberghen, Insulin signalling downstream of protein kinase B is potentiated by 5′AMP-activated protein kinase in rat hearts in vivo, Diabetologia 48 (2005) 2591–2601.
[39] J.R.B. Dyck, G.D. Lopaschuk, AMPK alterations in cardiac physiology and pathology: enemy or ally? J. Physiol. 574 (2006) 95–112.
[40] J. Gamble, G.D. Lopaschuk, Insulin inhibition of 5' adenosine monophosphate-activated protein kinase in the heart results in activation of acetyl coenzyme A carboxylase and inhibition of fatty acid oxidation, Metabolism 46 (1997) 1270–1274.

[41] D. Samovski, J. Sun, T. Pietka, R.W. Gross, R.H. Eckel, X. Su, P.D. Stahl, N.A. Abumrad, Regulation of AMPK activation by CD36 links fatty acid uptake to b-oxidation, Diabetes 64 (2015) 353–359.
[42] K.T. Weber, Fibrosis and hypertensive heart disease, Curr. Opin. Cardiol. 15 (2000) 264–272.
[43] H. Poudyal, S.K. Panchal, L.C. Ward, L. Brown, Effects of ALA, EPA and DHA in high-carbohydrate, high-fat diet-induced metabolic syndrome in rats, J. Nutr. Biochem. 24 (2013) 1041–1052.
[44] A. Ogawa, Y. Suzuki, T. Aoyama, H. Takeuchi, Dietary alpha-linolenic acid inhibits angiotensin-converting enzyme activity and mRNA expression levels in the aorta of spontaneously hypertensive rats, J. Oleo Sci. 58 (2009) 355–360.
[45] A. Folino, A.E. Sprio, F. Di Scipio, G.N. Berta, R. Rastaldo, Alpha-linolenic acid protects against cardiac injury and remodelling induced by beta-adrenergic over-stimulation, Food Funct. 6 (2015) 2231–2239.
[46] L. Yan, J.D. Zhang, B. Wang, Y.J. Lv, H. Jiang, G.L. Liu, Y. Qiao, M. Ren, X.F. Guo, Quercetin inhibits left ventricular hypertrophy in spontaneously hypertensive rats and inhibits angiotensin II-induced H9C2 cells hypertrophy by enhancing PPAR-γ expression and suppressing AP-1 activity, PLoS One 8 (2013) (e72548 1-14).
[47] S.K. Panchal, H. Poudyal, L. Brown, Quercetin ameliorates cardiovascular, hepatic, and metabolic changes in diet-induced metabolic syndrome in rats, J. Nutr. 142 (2012) 1026–1032.
[48] V. Vuksan, L. Choleva, E. Jovanovski, A.L. Jenkins, F. Au-Yeung, A.G. Dias, H.V.T. Ho, A. Zurbau, L. Duvnjak, Comparison of flax (Linum usitatissimum) and Salba-chia (Salvia hispanica L.) seeds on postprandial glycemia and satiety in healthy individuals: a randomized, controlled, crossover study, Eur. J. Clin. Nutr. (2016) 1–5.
[49] H. Poudyal, S.K. Panchal, J. Waanders, L. Ward, L. Brown, Lipid redistribution by α-linolenic acid-rich chia seed inhibits stearoyl-CoA desaturase-1 and induces cardiac and hepatic protection in diet-induced obese rats, J. Nutr. Biochem. 23 (2012) 153–162.
[50] B.R. Valenzuela, R.C. Barrera, M. Gonzalez-Astorga, C.J. Sanhueza, B.A. Valenzuela, G.O. Tapia, Alpha linolenic acid (ALA) from Rosa canina, sacha inchi and chia oils may increase ALA accretion and its conversion into n-3 LCPUFA in diverse tissues of the rat, Food Funct. 5 (2014) 356–367.
[51] L.H. Storlien, A.B. Jenkins, D.J. Chisholm, W.S. Pascoe, S. Khouri, E.W. Kraegen, Influence of dietary fat composition on development of insulin resistance in rats. Relationship to muscle triglyceride and omega-3 fatty acids in muscle phospholipid, Diabetes 40 (1991) 280–289.
[52] F. Echeverría, M. Ortiz, R. Valenzuela, L.A. Videla, Long-chain polyunsaturated fatty acids regulation of PPARs, signaling: relationship to tissue development and aging, Prostaglandins Leukot. Essent. Fat. Acids 114 (2016) 28–34.
[53] V. Vuksan, A.L. Jenkins, C. Brissette, L. Choleva, E. Jovanovski, A.L. Gibbs, R.P. Bazinet, F. Au-Yeung, A. Zurbau, H.V.T. Ho, L. Duvnjak, J.L. Sievenpiper, R.G. Josse, A. Hanna, Salba-chia (Salvia hispanica L.) in the treatment of overweight and obese patients with type 2 diabetes: a double-blind randomized controlled trial, Nutr. Metab. Cardiovasc. Dis. 27 (2017) 138–146.