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Could post-weaning dietary chia seed mitigate the development of dyslipidemia, liver steatosis and altered glucose homeostasis in offspring exposed to a sucrose-rich diet from utero to adulthood?

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ABSTRACT

The present work analyzes the effects of dietary chia seeds during postnatal life in offspring exposed to a sucrose-rich diet (SRD) from utero to adulthood. At weaning, chia seed (rich in α–linolenic acid) replaced corn oil (rich in linoleic acid) in the SRD. At 150 days of offspring life, anthropometrical parameters, blood pressure, plasma metabolites, hepatic lipid metabolism and glucose homeostasis were analyzed. Results showed that chia was able to prevent the development of hypertension, liver steatosis, hypertriglyceridemia and hypercholesterolemia. Normal triacylglycerol secretion and triacylglycerol clearance were accompanied by an improvement of *de novo* hepatic lipogenic and carnitine-palmitoyl transferase-1 enzymatic activities, associated with an accretion of n-3 polyunsaturated fatty acids in the total composition of liver homogenate. Glucose homeostasis and plasma free fatty acid levels were improved while visceral adiposity was slightly decreased. These results confirm that the incorporation of chia seed in the diet in postnatal life may provide a viable therapeutic option for preventing/mitigating adverse outcomes induced by an SRD from utero to adulthood.

1. Introduction

Clinical and experimental studies have suggested that long-chain n-3 polyunsaturated fatty acids (n-3 PUFAs), especially those derived from marine sources, such as eicosapentaenoic (EPA, 20:5 n-3) and docosahexaenoic (DHA, 22:6 n-3) fatty acid, have the ability to prevent several metabolic disorders included in chronic non-communicable diseases [1,2]. The primary precursor of EPA and DHA is the essential α-linolenic acid (ALA, 18:3 n-3) derived from plant sources. Studies in adult rats have reported that dietary fats rich in ALA such as linseed or perilla oil, compared to those rich in linoleic acid (LA, 18:2 n-6), decrease serum lipid concentration and improve insulin sensitivity and glucose tolerance by changing insulin response in target tissues [3–5]. The seed of *Salvia hispanica* L, commonly known as chia seed, is one of the most important natural sources of ALA. The seed -considered as a functional food regarding its physiological active compounds- contains around 40% fat, 60% of which is composed of ALA and 20% of LA. Chia seed has gained recent attention not only for its fatty acids composition but also for the high amount of insoluble fiber, vegetable protein, minerals, and polyphenols such as caffeic acid, chlorogenic acid and quercitine, with high antioxidant activity [6]. The beneficial effects of feeding either chia oil or seed on plasma lipid metabolites as well as the hepatic accretion of n-3 PUFA that modulates the fatty acid metabolism and antioxidant response were reported in normal rats [7–10]. Moreover, in an adult dyslipemic insulin-resistant rat model, different studies have described the capability of dietary chia seed in normalizing/improving altered glucose homeostasis, dyslipidemia, hypertension and liver steatosis [11–14].

The metabolic syndrome (MS) -included in the non-communicable diseases- is a cluster of interrelated risk factors such as dyslipidemia, altered glucose homeostasis, insulin resistance, hypertension and central obesity that promote the development of type 2 diabetes and cardiovascular diseases and has emerged as a worldwide health problem. At present, strong evidence suggests that predisposition to the development of MS begins in utero as part of a broader life course perspective [15]. Different insults, including a deficient nutrition during the intrauterine environment as well as an excess of energy like “junk food” or high-fat diet during pregnancy and/or lactation have also linked with the development of exacerbated adiposity, dyslipidemia, hypertension and insulin resistance in the adult offspring [16–18]. Regarding the impact of a maternal sucrose feeding in utero and during suckling, Samuelsson et al. [19] described altered glucose homeostasis in the female offspring weaned on a control diet at 3 months of age. In 100-day-old offspring from dams fed a sucrose-rich diet (SRD) during

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pregnancy and lactation, D'Alessandro et al. [20] reported several metabolic changes including dyslipidemia, altered glucose tolerance and insulin tolerance, among others. Moreover, when the life time is extended up to 150 days, these changes are exacerbated accompanied by an increase in the weight of adipose tissues regardless of the weaning diet [21].

Even though the alterations induced by the early nutritional programming have been considered as irreversible changes, some studies have reported that reducing postnatal hostile exposures represents a potential opportunity to mitigate the adverse intrauterine effects under the "two-hit hypothesis" [22,23]. In this context, a postnatal supplementation with EPA and DHA from birth to adulthood rescued glucocorticoid-programmed hypertension, dyslipidemia, inflammatory state and can limit adverse fetal programming effects on the adipose tissue of adult offspring [24,25]. Moreover, Hou et al. [26] showed that a 6% fish oil diet during the post-suckling period prevented programmed excess of adipose accumulation and insulin resistance in the model of early postnatal overfed rats by reducing the litter size after post-natal day 3. In this line, and to the best of our knowledge, there is no report regarding the effect of chia seed as second hit on the prevention or amelioration of programmed outcomes induced by maternal SRD as first hit.

Taking into account the effect of dietary chia seed described above, we hypothesized that changes in the dietary fat source at weaning may also provide a viable option to mitigate adverse outcomes induced by an SRD during the fetal and post-natal periods. With this aim, we investigated the partial substitution of corn oil (rich in LA) by chia seed (rich in ALA) as a fat source in the SRD and its effects on the anthropometrical parameters, adiposity, hypertension, dyslipidemia, hepatic lipid metabolism and glucose homeostasis of adult offspring from SRD-fed dams.

2. Methods

2.1. Animal models and diets

Female Wistar rats (200–230 g), purchased from the Centro de Experimentaciones Biológicas y Bioterio, (Esperanza, Argentina), were housed in a colony room under a 12 h light–dark cycle and constant temperature (22 °C) and humidity. The experimental protocol was approved by the Human and Animal Research Committee of the Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral, Santa Fe, Argentina (Ethical Approval number 00293). Adequate measures were taken to minimize the pain or discomfort of the rats. Female rats were mated with male rats of the same strain and the day spermatozoids appeared in vaginal smears was considered Day 1 of gestation. Before and during mating, rats were fed a standard powdered rodent commercial diet -as reference diet- (RD) (GEPSA, Argentina) containing (% w/w): carbohydrate (corn, sorghum, wheat, oats, barley) 42; protein 24; fat 6; fiber 7; minerals and vitamins 8; water 13; digestible energy 15.33 kJ g^{-1} as stated by the manufacturer. Pregnant rats were transferred to individual cages. Through gestation and lactation, one group of rats (n=16) was fed a home-made sucrose rich diet (SRD) containing (% w/w): carbohydrate (sucrose) 62.5; protein (casein free of vitamins) 17.5; fat (corn oil, rich in 18:2 n-6 linoleic acid) 7; fiber (cellulose) 8.5; minerals (salt mix) 3.5; vitamins (vitamin mix) 1.0. Other components of the SRD are in agreement with what was recommended by the final report of the American Institute of Nutrition [27]. The preparation and handling of the SRD has been reported elsewhere [11–14,20,21]. Another group of rats (n=8) was fed the RD described above. During gestation, dams were weighed three times a week.

At birth, pups were weighed and litter size was reduced to eight pups, with an equal number of male and female pups whenever possible. The pups were kept with their own mother until weaning. At that time (21 days post-partum), the male offspring of SRD-fed dams were weighed, randomized and assigned to either an SRD or an SRD where Salba (*Salvia hispanica* L) a variety of chia seed (20.0 g per 100 g of food) was incorporated as the source of dietary fat (SRD +Chia). According to this, offspring born from SRD dams are referred to as SRD-SRD (n=30) or SRD-SRDC (n=30) respectively. The male offspring born to RD dams fed an RD diet after weaning are referred to as RD–RD (reference group). All offspring were fed their respective diet until 150 days of age.

Table 1
Composition of experimental diets.

Ingredient (% energy)		SRD[a]	SRD+Chia[a]
Carbohydrates			
Starch		1.5	
Sucrose		63.7	63.7
Chia seed			1.5
Fat			
Maize oil		16.8	2.3
Chia seed[b]			14.5
Protein			
Casein (vitamin-free)		18	13.5
Chia seed			4.5
Energy (kJ/g of food)		15.75	15.75
Fatty acid Profile (g/kg of food)[c]	**RD[d]**	**SRD**	**SRD+chia**
16:0 Palmitic acid	11.80	7.28	5.03
18:0 Stearic acid	11.84	1.82	1.95
18:1 n-9 Oleic acid	26.94	22.50	7.31
18:2 n-6 Linoleic acid	5.68	36.05	16.39
18:3 n-3 α-Linolenic acid	0.12	0.52	39.13
20:1 n-9 Eicosanoic acid	0.09	0.31	0.23
Total saturates	23.64	9.10	6.98
Monounsaturates	27.00	2.56	7.54
Polyunsaturates			
n-6	5.68	36.05	16.39
n-3	0.12	0.52	39.13
n-6/n-3	47.33	69.32	0.42

[a] The home-made experimental diets are based on the AIN93 recommendations. Both diets contained by weight: Salt mix 35% (AIN93), vitamin mix 1% (AIN93), choline chloride 0.2%, methionine 0.3%, fiber 12%. The SRD+Chia was balanced in the fiber and salt mix according to the amount of each one in the chia seed provided by the manufacturer.

[b] Chia seed (Salba; *Salvia hispanica* L): 200 g/kg diet. Chia composition (g/100 g chia seed): carbohydrate 37.45, insoluble fiber 8.1% of total carbohydrate, 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.

[c] Other minor fatty acids have been excluded.

[d] Rodent commercial diet (% w/w): carbohydrate (corn, sorghum, wheat, oats, barley) 42; protein 24; fat 6; fiber 7; minerals and vitamins 8; water 13; digestible energy 15.33 kJ g^{-1} as stated by the manufacturer.

The content of carbohydrate, proteins, fiber, vitamin and mineral mix in the SRD diet was balanced taking into account the amount of these nutrients presents in the chia seed. The composition of chia seed was provided by the supplier (Agrisalba S.A. Buenos Aires, Argentina). Table 1 details the ingredients of both experimental diets. The amount of chia seed used in this study was in agreement with that previously described with at weaning offspring studies [7,8].

The present study was conducted in male offspring to avoid the effects of different sexual hormones on the lipid metabolism. Throughout the experimental period, dams and offspring had free access to food and water and were kept under controlled room conditions, as described above. Food intake and the body weight of offspring were monitored weekly, starting at post-weaning diet up to 150 days of life. At this time, food was removed at 07.00 h and, unless stated otherwise, experiments were performed between 07.00 and 09.00 h. At least six rats from each dietary group were used in each experiment. Rats were anaesthetized with sodium pentobarbital (60 mg/kg, i.p.). In one group of animals, in vivo experiments were conducted as described below. In another group, blood samples were obtained from the jugular vein in tubes containing ethylenediaminete-

traacetic sodium salt as anticoagulant and centrifuged at 2700*g* for 10 min at room temperature. The plasma obtained was either immediately assayed or stored at −20 °C until use. The liver was removed from the second group of rats, weighed, frozen and stored at −80 °C until use. Heart, epididymal, retroperitoneal and omental adipose tissues were also removed and weighed. Visceral adiposity index was calculated by the sum of epididymal, retroperitoneal and omental fat weights divided by body weight×100 [12].

2.2. Determination of blood pressure (BP)

Systolic and diastolic BP was measured by tail-cuff method, non-invasive pressure measurements (CODA™, Kent Scientific Corporation, Torrington, CT, USA). The measurements were conducted as indicated by the company protocol. All animals were accustomed to the procedure for 7 days before the BP measurement session. At least 8 readings were taken from each animal per session and averaged to get a single session value. At 150 days, we took the average BP values from eight offspring of each treatment [13].

2.3. Anthropometrical determinations and carcass composition

The abdominal circumference, thoracic circumference and body length were determined in anaesthetized rats as previously described [28]. The body weight and body length were used to determine the body mass index (BMI). Visceral organs and adipose tissues were removed in pre- shaved rats from each dietary group. The carcass weight as well as fat, water, protein and ash compositions were determined as previously described [29].

2.4. Analytical methods

A commercially available analytical kit was employed to determine plasma triacylglycerol (TAG) and glucose concentration by spectrophotometric methods (Wiener Lab., Rosario, Santa Fe, Argentina). Plasma free fatty acids (FFAs) were determined using an acyl-CoA oxidase based colorimetric kit (Wako NEFA-C, Wako Chemicals, Neuss, Germany). The liver TAG content was determined using the spectrophotometric method as previously described [11–14,20,21].

2.5. Lipid analysis

Lipids were extracted from the different diets and liver homogenate according to the procedure reported by Folch et al. [30]. Samples were transformed into methyl esters by employing 10% BF3/methanol at 80 °C for 45 min; the fatty acid composition of total lipids was determined by gas liquid chromatography of their methyl esters as previously described [28,31].

2.6. Liver enzymatic activity assays

Cytosolic liver fractions were used for the assay of the different liver enzymatic activities [14,20,21]. The acetyl-CoA carboxylase (ACC) activity was measured using a NADH-linked assay [32]. The fatty acid synthase (FAS) activity was assessed by measuring malonyl-CoA-dependent NADPH oxidation at 37 °C [33]. The activity of malic enzyme (ME) was determined by measuring the rate of NADPH formation by spectrophotometric measurement [34]. Glucose-6-phosphate dehydrogenase (G-6-PDH) was investigated following the increase of NADPH absorption [35]. The activity of carnitine-palmitoyl transferase-1 (CPT-1) was determined spectrophotometrically using the method described by Karlic et al. [36].

2.7. Triacylglycerol secretion rate (TAGSR)

The TAGSR was evaluated in fasting rats (16–18 h) by blocking the removal of plasma TAG with Triton W*R* 1339 (600 mg/kg body weight) dissolved in 0.9% NaCl. The TAGSR was calculated from the linear increase of TAG versus time [11,20,21].

2.8. Intravenous fat tolerance test (IVFTT)

The IVFTT (TAG clearance) was performed in rats fasted for 16–18 h by injecting 0.1 mL/100 g body weight, i.v., 10% Intralipid (Sigma-Aldrich, St Louis, MO, USA), a soybean oil fat emulsion. The first-order rate constant (K_2) of elimination of fat emulsion from the bloodstream (fractional removal rate) was calculated by the least squares method [11,20,21].

2.9. Intravenous glucose tolerance test (IVGTT)

An IVGTT was performed on anaesthetized rats fasted for 16–18 h after the administration of glucose solution (0.5 g glucose/kg body weight). The methodology of the IVGTT has been described in detail elsewhere [21]. A constant for blood glucose removal (Kg) during the glucose tolerance test and the area under the curve (AUC) during the IVGTT was calculated as previously described [20,21].

2.10. Insulin tolerance test (ITT)

Whole-body insulin action was determined by an ITT in anaesthetized rats (5 h fasted) after the injection of i.p. insulin (0.75 U/kg body weight, Humulin; Lilly, Indianapolis, IN, USA). The methodology of the ITT has been described in detail elsewhere [20,21]. A constant for blood glucose removal (K_{ITT}) during the insulin test and the AUC during the ITT was calculated as previously described [20,21].

2.11. Statistical analysis

Sample sizes were calculated on the basis of measurements previously made with rats fed either an RD or an SRD [20,21,28] considering 80% power. Results were expressed as the mean ± SEM. Statistical analyses were performed with SPSS version 17.0 (SPSS, Chicago, IL, USA). The data were subjected to a one-way ANOVA, followed by Tukey's post-hoc test or, when appropriate, the statistical significance of differences was determined using Student's *t*-test. Means with P values < 0.05 were considered statistically different.

3. Results

3.1. Body weight, energy intake, anthropometry, tissues weight and adiposity

Confirming previous results [20,21], female weight gain during pregnancy and the number of pups at birth were slightly but significantly decreased in the SRD-group compared with the RD-group. Moreover, SRD neonates displayed a rapid catch-up in growth and were heavier than RD neonates at the time of weaning (data not shown).

Table 2 shows similar values in food and energy intake, body weight, body length, abdominal and thorax circumferences as well as BMI in all groups at the end of the experimental period. Besides, there were no significant differences in liver and heart weight between any of the groups. The presence of chia seed in the SRD after weaning (SRD-SRDC group) slightly improves visceral adiposity. Nevertheless, individual adipose tissues weights were still significantly higher than those of the RD-RD group. Moreover, the high fat content recorded in SRD-SRD carcass composition was slightly but not significantly decreased in the SRD-SRDC group. This change was at the expense of water content, without differences in total carcass weight. Results are expressed as mean ± SEM (n=6), carcass fat content (% wet weight): RD-RD: 11.71 ± 0.60, SRD-SRD: 18.40 ± 0.97, SRD-SRDC: 16.00 ±

Table 2
Energy intake, anthropometric measurements, organ weight and visceral adiposity obtained from different experimental groups RD-RD, SRD-SRD and SRD-SRDC.

	Groups		
	(a) RD-RD	**(b) SRD-SRD**	**(c) SRD-SRDC**
Energy Intake (kJ/day)	300 ± 11	294 ± 10	285 ± 10
Total body weight (g)	441 ± 13	462 ± 18	436 ± 7
Body length (cm)	25.1 ± 0.47	24.3 ± 0.29	24.1 ± 0.35
Thorax circumference (cm)	17.6 ± 0.60	17.2 ± 0.12	17.5 ± 0.80
Abdominal circumference (cm)	21.1 ± 0.47	21.1 ± 0.29	21.2 ± 0.16
BMI (g/cm^2)	0.74 ± 0.10	0.78 ± 0.02	0.77 ± 0.03
Liver weight (g/100 g body weight)	3.40 ± 0.06	3.36 ± 0.06	3.25 ± 0.10
Heart weight (g/100 g body weight)	0.25 ± 0.01	0.26 ± 0.01	0.26 ± 0.01
Epididymal fat pad weight (g/100 g body weight)	2.39 ± 0.06^{bc}	2.77 ± 0.09^{a}	2.71 ± 0.05^{a}
Retroperitoneal fat pad weight (g/100 g body weight)	2.50 ± 0.11^{bc}	4.50 ± 0.20^{a}	4.16 ± 0.11^{a}
Omental fat pad weight (g/100 g body weight)	1.24 ± 0.04^{bc}	1.84 ± 0.07^{a}	1.77 ± 0.11^{a}
Visceral Adiposity Index (%)	6.08 ± 0.16^{bc}	9.53 ± 0.29^{ac}	8.33 ± 0.23^{ab}

Values represent mean ± SEM (n=6 rats/experimental group). RD-RD: offspring from dams fed a reference diet (RD) and fed the RD after weaning; SRD-SRD: offspring from SRD-fed dams fed an SRD after weaning; SRD-SRDC: offspring from SRD-fed dams fed an SRD+Chia after weaning. Statistical significance was assessed at $P < 0.05$ by one-way ANOVA followed by Tukey´s post hoc test. Values sharing the same letter in each row are not statistically significant.

1.00; carcass water content (% wet weight): RD-RD: 61.62 ± 0.63, SRD-SRD: 56.32 ± 0.70, SRD-SRDC: 58.00 ± 0.08 ($P < 0.05$ RD-RD vs SRD-SRD and SRD-SRDC in both fat and water content). No differences were observed in total carcass wet weight, carcass protein and ash content (data not shown).

3.2. Blood pressure, plasma lipid levels, TAG secretion rate and intravenous fat tolerance test

The SRD-SRD offspring exhibited hypercholesterolemia and increased systolic and diastolic BP ($P < 0.05$) compared with offspring from RD-fed dams (Fig. 1) while in the SRD-SRDC these parameters reached values similar to those observed in the RD-RD group. On the other hand, the increased levels of plasma FFAs recorded in the SRD-SRD group were significantly decreased when chia seed was present in the diet after weaning, although values are still higher than those in the RD-RD group. Values (mmol/L) are expressed as mean ± SEM (n=6): RD-RD: 364 ± 16; SRD-SRD: 775 ± 46 and SRD-SRDC: 595 ± 58 ($P < 0.01$ SRD-SRD and SRD-SRDC vs RD-RD and $P < 0.05$ SRD-SRDC vs SRD-SRD).

Plasma TAG level, TAGSR and the first-order rate constant (K_2) of elimination of fat emulsion from the bloodstream during the IVFTT are also depicted in Fig. 1. The hypertriglyceridemia -associated with high TAGSR and a significant decrease in K_2 of intravenously injected fat emulsion- in the SRD-SRD group were completely prevented when chia seed was incorporated into the diet after weaning, reaching values indistinguishable from those of RD-RD offspring.

3.3. Fatty acid composition of total liver homogenate

Table 3 summarizes the fatty acid composition of total liver homogenate expressed as percentage of total fatty acid content in offspring from the different experimental groups at 150 days of life. Total saturated fatty acid was similar between the groups, although different proportions of individual fatty acid were observed. The elongase activity index estimated from the relationship between 18:0/16:0 fatty acid was significantly improved when chia seed was present from weaning (0.96 ± 0.05 in RD-RD; 0.46 ± 0.08 in SRD-SRD and 0.70 ± 0.01 in SRD-SRDC group, $P < 0.05$ SRD-SRD and SRD-SRDC vs RD-RD and $P < 0.05$ SRD-SRDC vs SRD-SRD). An inverse correlation was observed between elongase activity index and liver TG content between the groups (r=0.841 $P < 0.01$). The high SCD-1 activity index -estimated from the relationship between 18:1 n-9 to 18:0 fatty acid- observed in the SRD-SRD offspring was significantly decreased in the presence of chia seed after weaning (0.50 ± 0.04 in RD-RD, 1.81 ± 0.10 in SRD-SRD and 0.84 ± 0.06 in the SRD-SRDC group, $P < 0.05$ SRD-SRD and SRD-SRDC vs RD-RD and $P < 0.05$ SRD-SRDC vs SRD-SRD). A positive correlation was observed between the SCD-1 activity index and liver TG content (r=0.947 $P < 0.01$). Furthermore, chia seed consumption from weaning produced an increase in the hepatic content of 18:3 n-3, 20:5 n-3 and 22:6 n-3 fatty acids and a consequent decrease of total n-6 fatty acid compared with SRD-SRD and RD-RD ($P < 0.05$). These results are reflected in the remarkably low ratios of n-6/n-3 in SRD- offspring weaned with chia seed until 150 days of life.

3.4. Liver TAG content, de novo lipogenic and Carnitine-palmitoyl transferase-1 enzymes activities

As indicated in Table 4, the increased liver TAG content present in SRD-SRD was significantly improved in the SRD-SRDC offspring, although the values remained still significantly higher than those recorded in the RD-RD group. The presence of chia seed in the after-weaning diet prevented the increase of ACC enzyme activity and improved FAS, G-6-PDH and ME enzymes activities. In addition, for rats of all groups the hepatic lipogenic enzymes activities and hepatic triglyceride content were directly correlated (r=0.959 $P < 0.001$ for FAS; r=0.579 $P < 0.006$ for ACC; r=0.774 $P < 0.001$ for G-6-PDH and r=0.941 $P < 0.001$ for ME). Conversely, the increased mitochondrial CPT-1 activity observed in the SRD-SRD group was completely normalized by the presence of chia in the post-weaning diet.

3.5. Basal glucose levels, insulin sensitivity and glucose tolerance test

The moderate hyperglycemia depicted in the SRD-SRD offspring was not present in the SRD-SRDC group. Values (mmol/L) expressed as mean ± SEM (n=6) are as follows: RD-RD: 6.00 ± 0.21, SRD-SRD: 7.40 ± 0.26 and SRD-SRDC: 6.59 ± 0.40 ($P < 0.05$ for SRD-SRD vs RD-RD and SRD-SRDC).

Fig. 2a shows plasma glucose levels during the ITT. The table inserts in Fig. 2a display the constant for blood glucose removal (K_{ITT}) during the test. The reduced K_{ITT} values ($P < 0.05$) exhibited in the SRD-SRD group were completely normalized when chia was present in the after-weaning diet (SRD-SRDC group). Moreover, glucose values integrated over a 60-min period after insulin injection (ΔG_{0-60}) were lower in the SRD-SRD and returned to control values in SRD-SRDC offspring (Fig. 2b).

Blood glucose profiles during IVGTT are shown in Fig. 3a. The table insert in Fig. 3a shows that the glucose disappearance rates (Kg values) decreased significantly ($P < 0.05$) in offspring from SRD-dams independently of the presence of chia seed in the after-weaning diet. However, incremental glucose values integrated over a 60-min period after glucose injection (ΔG_{0-60}) were similar in all groups (Fig. 3b).

4. Discussion and conclusion

4.1. Discussion

The association between adverse intrauterine and post-weaning exposure to a high refined sugar diet was reported as not having a predictive adaptive response in glucose and lipid homeostasis in the adult offspring [20,21]. In order to elucidate if an early post-weaning

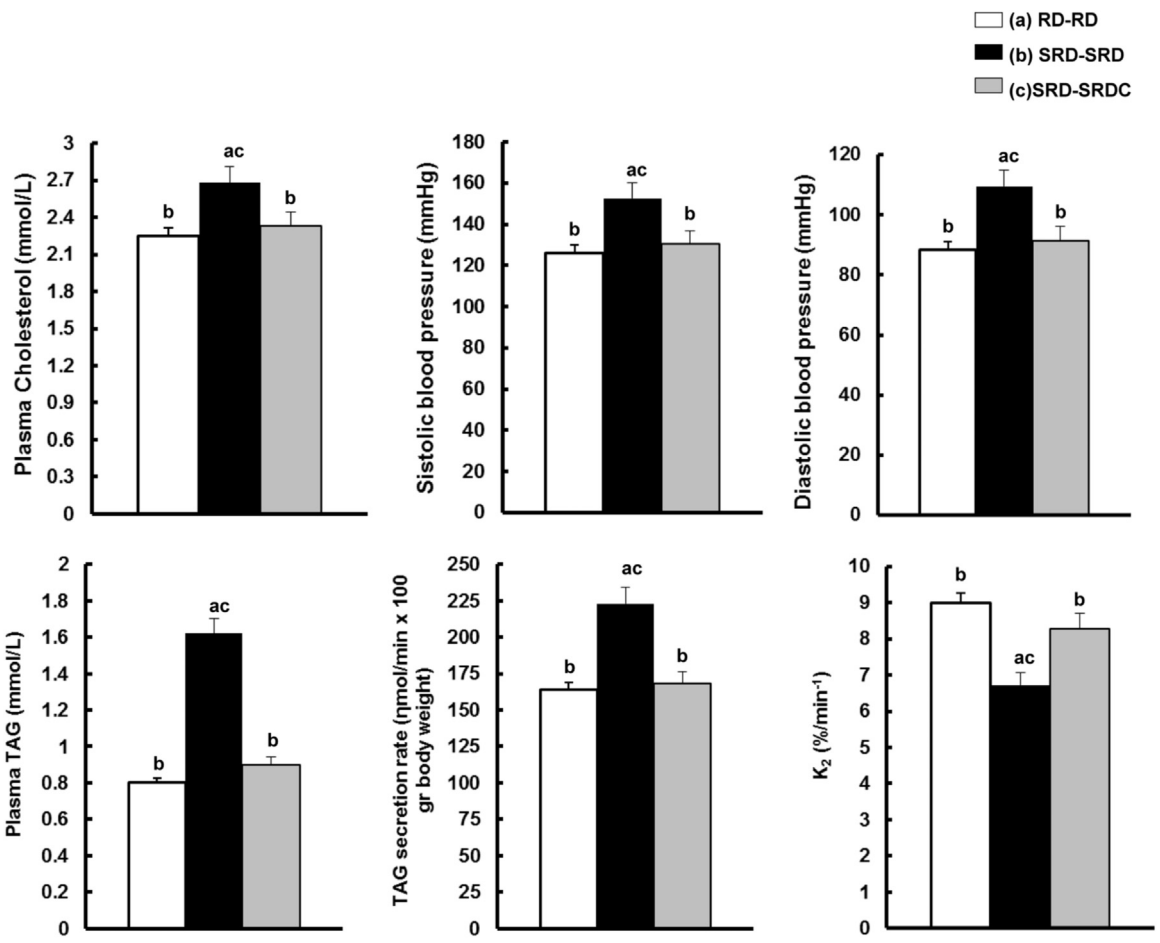

Fig. 1. Systolic and diastolic blood pressure, plasma cholesterol and TAG levels, TAG secretion rate (TAGSR) and fractional removal rate of fat emulsion (K_2) obtained from different experimental groups RD-RD, SRD-SRD and SRD-SRDC. The identification of the different groups is shown in Table 2. Values represent mean ± SEM (n=6 rats/experimental group). Significant differences between the groups are indicated by the letter identifying each group ($P < 0.05$).

management of diet composition is able to mitigate these abnormalities, we changed the source of fat in the diet at weaning. The main findings of this study were that the chia seed included in the post-weaning diet was able to prevent the development of liver steatosis, hypertriglyceridemia and hypercholesterolemia and improved plasma FFA levels. Moreover, an important accretion of n-3 PUFAs was observed in fatty acid composition of total liver homogenate. These results were associated with normal TAGSR and TAG clearances and with normalized/improved *de novo* hepatic lipogenic and CPT-1 enzymatic activities. Hypertension was also prevented. The incorporation of chia seed was also able to ameliorate glucose homeostasis: normal plasma glucose levels and K_{ITT} but an altered Kg. Finally, it was able to slightly improve visceral adiposity without changes in total body weight. All the results were recorded although sucrose was present in offspring from utero to 150 days of life.

The dietary LA: ALA relationship plays an important role in plasma lipid levels. In the present study, this ratio is 0.42. The maximum hypotriglyceridemic effects in rats were observed with a ratio of 0.33, suggesting that the effect of ALA may be due to an increase of long-chain PUFA in membrane phospholipids [37,38]. The effects of chia seeds preventing the development of hypertriglyceridemia were similar to those previously reported in a similar experimental model when fish oil was the source of fat in the post-weaning diet [28]. Moreover, the hepatic TAGSR and plasma TAG clearance observed in the SRD-SRDC group seems to be the main factor of hypertriglyceridemia prevention. Very low density lipoprotein assembly and secretion is a substrate-dependent process that is highly regulated by the availability of hepatic TAG [39] and this content reflects a balance between the uptake of circulating fatty acids, hepatic fatty acid synthesis and oxidation.

Conversion from ALA to EPA and DHA in mammals is low and gender dependent. However recent studies have demonstrated that the administration of chia oil or another vegetable oil-based diet [9–11] produce a significant increase of long-chain PUFAs in plasma and different tissues. The results reported in the present study (high levels of ALA, DHA and EPA on total liver homogenate) when chia seed was present from weaning, are in agreement with those reported by Rincón-Cervera et al. [10], and suggest an endogenous conversion of ALA to n-3 LCPUFA with a concomitant reduction of total n-6 PUFA. In this context the intense competition between LA and ALA for the same desaturating and elongating enzymes activities is well known. Wu et al. [40] observed that endogenous n-3 PUFAs synthesis from the precursor ALA is regulated more by substrate levels than gene expression of the synthetic enzymes. It is also important to remark that the presence of chia seed from weaning improves the elongases and desaturases indexes and their relationship with liver TAG. This is in agreement with the ability of polyunsaturated fatty acids to suppress expression of both sterol regulatory element–binding protein 1c [41] and SCD1 [42] and to activate the genes involved in hepatic fatty acid oxidation [2,28,31].

Moreover, the hepatic TAG levels observed in the SRD-SRDC offspring are coincident with the *de novo* lipogenic and mitochondrial CPT-I enzymatic activities. FAS and ACC play a central role in lipogenesis and the inhibitory potency of n-3 PUFAs on these enzymes seems to vary depending upon the degree of fatty acids unsaturation and chain length [43,44]. On the other hand, the reduced activity of G-6-PDH and ME -linked to the NADPH synthesis- is associated with the

Table 3
Fatty acid composition of total liver homogenate obtained from different experimental groups RD-RD, SRD-SRD and SRD-SRDC.

	Fatty acid composition (g/100 g FAME)		
	Groups		
	(a) RD-RD	(b) SRD-SRD	**(c) SRD-SRDC**
Fatty acid			
14:0	0.33 ± 0.04^{bc}	1.10 ± 0.30^{a}	0.71 ± 0.13^{a}
16:0	17.93 ± 0.39^{bc}	26.74 ± 3.09^{a}	21.95 ± 0.81^{a}
17:0	0.74 ± 0.02	Traces	Traces
18:0	17.14 ± 0.61^{b}	11.57 ± 0.88^{ac}	15.31 ± 0.67^{b}
24:0	0.79 ± 0.04^{bc}	0.29 ± 0.05^{ac}	2.47 ± 0.20^{ab}
∑ SFA	**36.93 ± 1.48**	**39.79 ± 2.44**	**40.44 ± 1.34**
16:1 n-7	0.71 ± 0.17^{bc}	4.30 ± 0.88^{a}	3.79 ± 0.37^{a}
18:1 n-9	8.52 ± 0.35^{bc}	20.78 ± 0.62^{ac}	12.65 ± 0.58^{ab}
18:1 n-7	3.21 ± 0.06	3.52 ± 0.13	3.37 ± 0.20
∑MUFA	**12.44 ± 0.47^{bc}**	**28.61 ± 0.42^{ac}**	**19.81 ± 0.97^{ab}**
18:2 n-6	20.50 ± 0.41^{bc}	15.24 ± 0.73^{a}	13.30 ± 0.48^{a}
18:3 n-6	0.15 ± 0.02^{bc}	0.26 ± 0.02^{ac}	0.52 ± 0.08^{ab}
20:2 n-6	0.92 ± 0.03^{b}	0.40 ± 0.02^{a}	Traces
20:3 n-6	0.67 ± 0.02^{bc}	0.40 ± 0.04^{ac}	1.00 ± 0.03^{ab}
20:4 n-6	22.81 ± 0.67^{bc}	13.53 ± 1.71^{ac}	6.58 ± 0.16^{ab}
22:5 n-6	0.51 ± 0.02	0.51 ± 0.09	Traces
∑ LCPUFA n-6	**45.56 ± 0.31^{bc}**	**30.34 ± 2.58^{ac}**	**20.89 ± 0.61^{ab}**
18:3 n-3	0.62 ± 0.03^{bc}	0.33 ± 0.08^{ac}	7.21 ± 0.40^{ab}
20:5 n-3	0.33 ± 0.02^{b}	Traces	6.04 ± 0.30^{a}
22:5 n-3	0.23 ± 0.02^{b}	1.12 ± 0.18^{a}	Traces
22:6 n-3	4.21 ± 0.13^{bc}	1.74 ± 0.29^{ac}	5.22 ± 0.21^{ab}
∑LCPUFA n-3	**5.40 ± 0.13^{bc}**	**3.20 ± 0.42^{ac}**	**18.52 ± 0.78^{ab}**
Total LCPUFA	**50.92 ± 0.43^{bc}**	**33.54 ± 2.99^{a}**	**39.8 ± 1.51^{a}**
LCPUFA n6/n3 ratio	**8.45 ± 0.15^{c}**	**9.83 ± 0.66^{c}**	**1.13 ± 0.02^{ab}**

Values are expressed as g fatty acid/100 g of total fatty acid methyl esters (FAME) and represent the mean ± SEM (n=6 rats/experimental group). Statistical significance was assessed at $P < 0.05$ by one-way ANOVA followed by Tukey´s post hoc test. Values sharing the same letter in each row are not statistically significant. The identification of the different groups is shown in Table 2.

Table 4
Liver triacylglycerol content, oxidative and "de novo" lipogenic enzymes activities obtained from different experimental groups RD-RD, SRD-SRD and SRD-SRDC.

	Groups		
	(a) RD-RD	**(b) SRD-SRD**	**(c) SRD-SRDC**
Triacylglycerol (μmol/g tissue)	8.19 ± 0.41^{bc}	22.45 ± 1.35^{ac}	13.80 ± 0.69^{ab}
Carnitine-Palmitoyl Transferase-1 (mU/mg protein)	1.53 ± 0.03^{b}	0.86 ± 0.05^{ac}	1.60 ± 0.16^{b}
Acetyl-CoA Carboxylase (mU/mg protein)	27.62 ± 2.14^{b}	55.00 ± 5.48^{ac}	$30,11 \pm 2.33^{b}$
Fatty acid synthase (mU/mg protein)	4.51 ± 0.44^{bc}	15.27 ± 1.40^{ac}	9.09 ± 0.36^{ab}
Glucose-6-P dehydrogenase (mU/mg protein)	15.07 ± 0.70^{bc}	30.86 ± 2.62^{ac}	23.53 ± 1.65^{ab}
Malic enzyme (mU/mg protein)	6.32 ± 0.40^{bc}	16.02 ± 0.68^{ac}	13.38 ± 0.60^{ab}

Values represent mean ± SEM (n=6 rats/experimental group). Statistical significance were assessed at $P < 0.05$ by one-way ANOVA followed by Tukey´s post hoc test. Values sharing the same letter in each row are not statistically significant. The identification of the different groups is shown in Table 2.

fatty acid synthesis rates. Moreover, the high correlation between lipogenic enzymatic activities and liver TAG content is consistent with the concept that the activities of these enzymes were reflected by the concentration of TAG in liver and plasma [3]. Furthermore, it is important to take into account that both fructose (moiety of sucrose) and n-3 PUFAs are involved in the regulation of different transcription factors with a final impact on lipid metabolism [2,45]. Hepatic steatosis

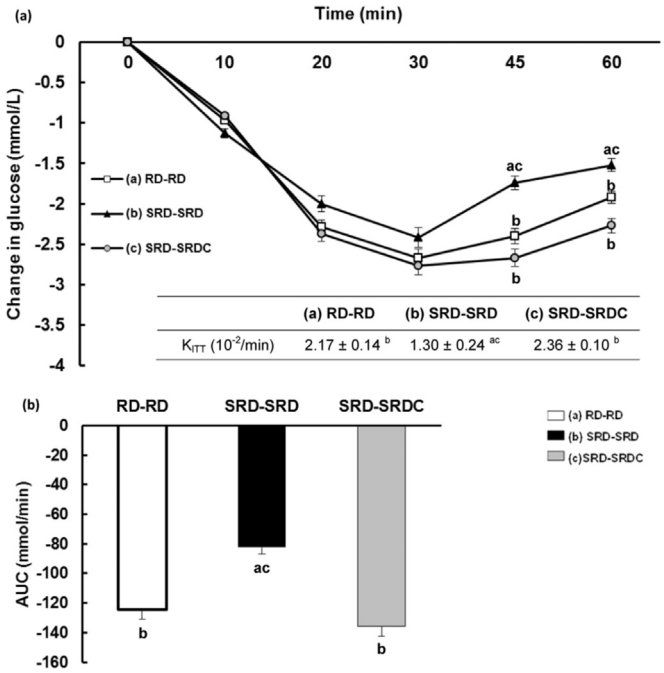

Fig. 2. (a) Intravenous insulin tolerance test in male offspring obtained from different experimental groups RD-RD (▢), SRD-SRD (▲) and SRD-SRDC (⬭). The identification of the different groups is shown in Table 2. Rates of glucose disappearance (K_{ITT}) were calculated from the slopes of the regression lines obtained with log-transformed glucose values after insulin administration. (b) Glucose responses during the acute insulin challenge were used to calculate the decrease of blood glucose values integrated over the total period after the injection of insulin ($AUC=\Delta G_{0-60}$ min). Significant differences between the groups are indicated by the letter identifying each group ($P < 0.05$).

in fetuses from fructose-fed dams were associated with a higher expression of genes related to lipogenesis (SREBP, ACC_2) and a lower expression of fatty acid oxidation genes (PPAR) [46–48]. A previous study from our group showed that fat source substitution (corn oil for fish oil) in the SRD diet from weaning was able to overcome the decreased liver PPARα protein mass levels in offspring born from SRD-dams, suggesting that n-3 PUFA could consequently trigger the activation of CPT-1 [28]. The effects of ALA on the regulatory mechanism and molecular interaction with genes are only beginning to be described [49]. Moreover, at present we are unaware of any information regarding the regulatory mechanism of chia seed in attenuating disease processes after fetal programming has occurred.

Extending previous studies on the effect of sucrose-rich diet from utero to adult life, we observed a high BP in addition to hypercholesterolemia in the SRD-SRD group. Again, it suggests that the predictive adaptive hypothesis is not applied under these experimental conditions. This may imply an irreversible, adverse effect of the maternal diet on the pathway of BP control as suggested by Khan et al. [50] in offspring of fat-fed dams raised on the same diet. On the contrary, the observation that both hypercholesterolemia and hypertension never developed in SRD-SRDC suggests that the post weaning treatment completely suppress the effects of sucrose exposure in utero and suckling period, although sucrose was also present after weaning. These observations are consistent with the well-known effects of n-3 PUFAs in adult rats. In this regard, Creus et al. [13] reported a normalization of blood pressure in dyslipemic insulin resistant rats by feeding chia seed. Moreover, similar results were reported by Poudyal et al. [51] suggesting that the hypotensive effect of chia seed in rats fed high-fructose high-fat diet was associated with increasing docosapentaenoic acid (DPA) and DHA contents in cardiac phospholipids. Furthermore, studies in vitro showed that a protein fraction of chia has the capacity to act as antioxidant and could be considered as a

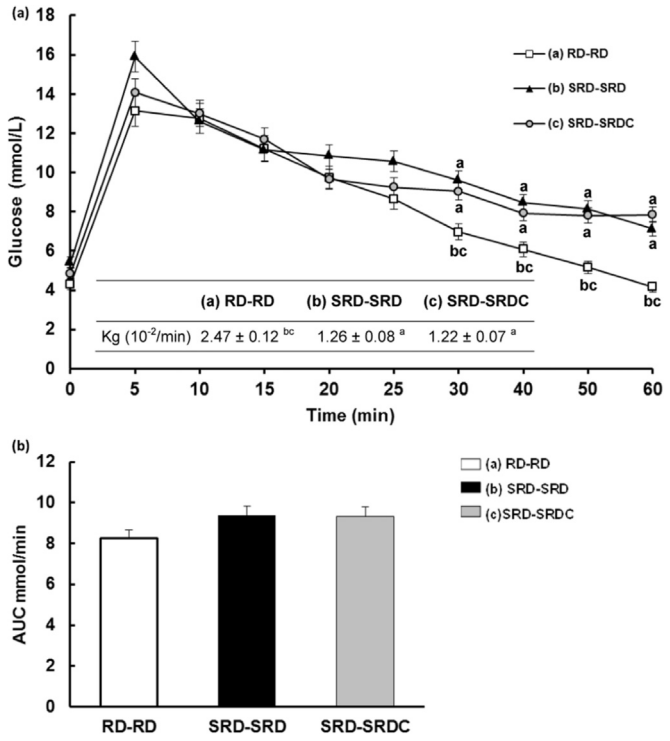

Fig. 3. (a) Intravenous glucose tolerance test in male offspring obtained from different experimental groups RD-RD (▢), SRD-SRD (▲) and SRD-SRDC (⬭). The identification of the different groups is shown in Table 2. Rates of glucose disappearance (Kg) were calculated from the slopes of the regression lines obtained with log-transformed glucose values after glucose administration. (b) Glucose responses during the acute glucose challenge were used to calculate the incremental blood glucose values integrated over the total period after the injection of glucose (AUC=ΔG_{0-60} min). Significant differences between the groups are indicated by the letter identifying each group ($P < 0.05$).

novel hypotensive source [52]. At this point, it is important to notice that Yamamoto et al. [53] showed an antihypertensive effect of quercetin (flavonoid included in chia seed) in rats fed a high-fat high-sucrose diet, suggesting that the increased nitric oxide availability is one of the main factors of quercetin effect on blood pressure.

The capacity of n-3 PUFAs to limit adiposity was reported by different studies [2,45]. When fish oil was the source of fat at weaning, a significant improvement in visceral adiposity and plasma FFAs was observed in adult offspring from SRD-dams [28]. Between the long-chain n-3 PUFAs, EPA and DHA seem to exert a more pronounced effect than their precursor ALA [54]. The effect of chia seed on the SRD-SRDC offspring was not an unexpected finding. Studies in adult animal models also showed similar patterns. da Silva Marineli et al. [55] reported that the long-term consumption of chia seed (133 g/kg of diet vs 200 g/kg of diet in our study) did not reduce body weight gain and abdominal adiposity but improved FFA levels in rats fed a high-fat high-sucrose diet from weaning. Regarding adult animal models, the administration of chia seed was able to reduce adiposity without changes in total body weight [11–14]. Moreover, in obese rats, chia oil did not reduce total body fat but could improve visceral adiposity inducing lipid redistribution [51].

The development of insulin resistance and impaired glucose handling are closely related to circulating lipids and visceral adiposity. The observation that basal hyperglycemia and altered K_{ITT} never developed in those offspring maintained on chia seed from weaning suggested a positive effect on glucose homeostasis. However, the clear derangement of IVGTT (decreased Kg) recorded when those offspring were exposed to a glucose challenge suggested a state of impaired glucose management. An interesting finding is that plasma FFAs in the SRD-SRDC are still higher compared to those of the reference group. On the other hand, an impaired glucose homeostasis as well as increased levels of plasma FFAs was also observed when offspring from SRD-dams were fed an RD after-weaning [21]. The above observations suggest that although dietary chia seed could prevent the development of increased basal glucose levels, it was unable to prevent the impaired i.v. glucose challenge. Further studies are definitely needed to clarify this matter.

4.2. Conclusion

The predisposition to the development of the different disorders included in the MS begins in utero as part of a broader life course perspective. In this context, the present study provides new information regarding the possible beneficial effect of dietary chia seed given to offspring exposed to a nutritional challenge (high sucrose diet) from utero to adulthood. The presence of chia seed from weaning was able to mitigate and/or prevent the altered lipid metabolism and glucose homeostasis in adult offspring although visceral adiposity was slightly modified. While these results cannot be extrapolated to humans, they shed light on the convenience of early detection and the potential ways for preventing lipid and glucose-related metabolic diseases in adult offspring by addressing the post-weaning diet as a second hit to mitigate the adverse outcomes induced in utero and lactation as first hit.

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