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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-