

Proceeding Paper

Chia Seed Oil Intake: Is It Beneficial for Preventing Cardiovascular Risk Factors? [†]

Susana Jerez ^{1,2,*}, Analía Medina ², Gabriela Alarcón ¹, Liliana Sierra ² and Mirta Medina ²

¹ Instituto Superior de Investigaciones Biológicas (INSIBIO-CONICET-UNT), San Miguel de Tucuman 4000, Argentina; gabydel.jesus@gmail.com

² Facultad de Ciencias Naturales e IML-UNT, San Miguel de Tucuman 4000, Argentina; analiamed@csnat.unt.edu.ar (A.M.); lilianasierra@csnat.unt.edu.ar (L.S.); medmirta@csnat.unt.edu.ar (M.M.)

* Correspondence: sjerez@herrera.unt.edu.ar

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Abstract: Cold-pressed chia seed oils (ChO) are known for their health-promoting characteristics due to their high content of omega-3 α -linolenic acid (ω -3 ALA). We investigated the influence of ChO supplementation as a functional food on animal models of the cardiovascular risk factors hypercholesterolemia and metabolic syndrome (MS). Dietary intervention with ChO (equivalent to 4.8 g ALA per day) was found to improve vascular dysfunction and mitigate the rise in plasma triglyceride (TG) levels under hypercholesterolemic conditions. However, impaired glucose tolerance was found in control ChO-treated animals. In order to verify whether the effects of chia seed are the same as that of ChO, we replaced ChO with an equivalent amount of seed. Glucose intolerance was found once again. For this reason, we carried out a new study in which ChO intake was reduced to 3 g ALA per day, and no alterations were observed in such conditions. Thus, dietary intervention with ChO equivalent to 3 g ALA intake per day was chosen to analyze the effects on the alterations that characterize high-fat diet-induced MS. ChO supplementation lowered the ω -6/ ω -3 ratio, TG, blood pressure and improved endothelial function. Nevertheless, ChO worsened the high-fat diet's deleterious effects on visceral abdominal fat, fasting glucose and glucose tolerance. Our results support the view that dietary guidelines for treating patients with hypercholesterolemia or MS must be carefully planned in such a way that the incorporation of ChO into the diet should be controlled and nutritional background be considered.

Keywords: chia oil; endothelial function; glucose intolerance; hypercholesterolemia; metabolic syndrome; rabbits



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1. Introduction

A cardiovascular risk factor is defined as a measurable characteristic that is causally associated with increased cardiovascular disease (CVD) frequency and that is a significant independent predictor of an increased risk of presenting with the disease. Traditional CVD risk factors are modifiable and non-modifiable. Modifiable CVD risk factors are those that can be reduced or controlled with altered behavior: tobacco use, hypertension, diabetes mellitus, hypercholesterolemia, physical inactivity and obesity. Diet is a key modifiable risk factor in the prevention and risk reduction of CVD. Nutritional interventions include ω -3 fatty acids, which were associated with reduced cardiovascular mortality and other cardiovascular outcomes.

Chia seeds have oil contents ranging from 25% to 40%, in which 60% is n-3 alpha-linolenic acid (18:3, ALA) and 20% is n-6 linoleic acid (18:2, LA) [1]. Of all the known food sources, chia contains the highest concentration of these fatty acids. The beneficial effects of chia seed on risk factors for CVD have been widely studied [2]. However, there is little evidence regarding the effects of chia seed oil (ChO). Han et al. [3] reported that ChO

prevents high-fat diet-induced hyperlipidemia and oxidative stress in mice. Recently, Enes et al. [4] found that ChO is able to improve glucose tolerance and restore the energy fuel system in the livers of rats fed a high-fat diet. Furthermore, ChO supplementation changes body composition and activates the insulin signaling cascade in the skeletal muscle tissue of obese animals [5].

Endothelial dysfunction marks a stage of atherosclerosis and is an important prognostic marker for CVD. It has been reported that endothelial functionality is positively related to the proportion of ALA in plasma. Furthermore, several studies demonstrate that ALA is an endothelial protective factor [6]. In this sense, we demonstrate the beneficial effects of dietary intervention with ChO on endothelial function by using a rabbit model of hypercholesterolemia [7] and metabolic syndrome (a cluster of CVD risk factors including obesity, excessive visceral fat storage, dyslipidemia, hypertension and insulin resistance). However, some undesirable side effects on insulin resistance were found [8].

Thus, the aim of the present work was to discuss the effects of dietary interventions with ChO on animal models of CVD risk factors induced by high cholesterol or high fat diets.

2. Materials and Methods

2.1. Animals and Diets

Male hybrid rabbits initially weighing 850–1000 g were housed individually in gridded cages on a constant 12-h light/dark cycle under controlled temperature and conditions. After one week acclimation period, animals were randomized and separated in groups ($n = 8$ each): fed on regular rabbit chow (CD); fed on CD supplemented with either 10% (CD-ChO¹⁰) or 3% of ChO (CD-ChO³); fed on CD supplemented with 224.10 g/kg of chia seed (CD-Ch); fed on CD supplemented with 1% cholesterol (HD); fed on CD supplemented with 8% lard and 10% corn oil (HFD); fed on HD supplemented with 10% ChO (HD-ChO); and fed on HFD in which 3% of the oil source (corn oil) was replaced with ChO (HFD-ChO). Details of the diets are shown in Table 1. Rabbits were fed 180 g of the appropriate dietary treatment per day for 6 weeks. Daily energy intake from fat was similar in HFD and HFD-Ch. The dose of ALA was of 4.9 g (CD-ChO¹⁰ and CD-Ch) and 3 g/day (CD-ChO³), all in agreement with the American Heart Association recommendations.

Table 1. Composition of experimental diets (g/100 g diet).

	CD	CD-ChO ¹⁰	CD-ChO ³	CD-Ch	HD	HD-ChO ¹⁰	HFD	HFD-ChO ³
Carbo hydrate	34 ± 2	34 ± 2	34 ± 2	43.4 ± 6	34 ± 2	34 ± 2	34 ± 2	34 ± 2
Protein	15 ± 3	15 ± 3	15 ± 3	18.7 ± 1	15 ± 3	15 ± 3	15 ± 3	15 ± 3
Fiber	15 ± 2	15 ± 2	15 ± 2	23 ± 2	15 ± 2	15 ± 2	15 ± 2	15 ± 2
Total fat	3.1 ± 0.1	13.1 ± 1.6	6.2 ± 0.3	9.9 ± 0.2	3.0 ± 0.2	13.2 ± 1.4	21 ± 1.7	21 ± 1.4
Fatty acids								
Total SFA	21.4 ± 0.1	16.1 ± 0.9	16.9 ± 0.2	-	19.2 ± 0.6	18.1 ± 0.2	23.9 ± 0.9	22.6 ± 0.2
Total MUFA	17.6 ± 0.1	21.1 ± 0.5	20.0 ± 0.3	-	15.6 ± 0.1	15.2 ± 0.3	22.4 ± 0.5	22.3 ± 0.5
Total PUFA	56.7 ± 0.5	64.3 ± 0.4	64.0 ± 0.4	-	66.6 ± 0.9	66.7 ± 0.5	53.9 ± 0.1	58.7 ± 0.5
Total calories (Kcal)	250	332	275	359	250	332	410	411

SFA: saturated fatty acid; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids. CD: rabbits fed a control diet CD; CD-ChO¹⁰: fed a CD supplemented with 10% chia oil; CD-ChO³: fed a CD supplemented with 3% chia oil; CD-Ch: fed a CD supplemented with 224 g/kg chia seed; HD: fed a CD supplemented with 1% cholesterol; CD-ChO¹⁰: fed a HD supplemented with 10% chia oil; HFD: fed a CD supplemented with 10% corn oil and 8% lard; HFD-ChO³: fed a CD supplemented with 3% chia oil, 7% corn oil and 8% lard.

2.2. Clinical and Biochemical Parameters

An intraperitoneal glucose tolerance test (GTT) was performed two days before the end of the 6 weeks of feeding [8]. At the end of the dietary intervention, food was withdrawn for 12 h and animals were anesthetized. Mean arterial blood pressure (MAP) and heart rate (HR) were measured directly in the carotid artery through a catheter connected to a pressure transducer (Gould-Statham P23, Oxnard, CA, USA) and recorded using a data acquisition system (Biopac MP100, Aero Camino, Goleta, CA, USA). After MAP measurement, blood

samples were collected through the catheter inserted in the carotid artery. Then, using surgical techniques, a midline incision was made in the rabbit and adipose tissues from the abdominal areas were collected and weighed. The visceral abdominal fat (VAF) was expressed as a percentage of the total body weight: $(\text{fat weight}/\text{animal weight}) \times 100$. Plasma total cholesterol (TC), HDL-C, low density lipoprotein (LDL-C), TG, and FG were measured by using colorimetric reactions with commercial kits (Wiener, Rosario, Argentina).

The triglyceride-glucose index (TyG index) is a simple marker that has a high correlation with the degree of insulin resistance measured by hyperinsulinemic-euglycemic clamp studies. The TyG index was calculated as $\ln(\text{fasting triglycerides (mg}\cdot\text{dL}^{-1}) \times \text{fasting glucose (mg}\cdot\text{dL}^{-1})$.

Fatty acids from plasma were measured by gas chromatography according to previous work [8].

2.3. Vascular Function Assessment

Isometric contractions from aortic rings were measured by using force-displacement transducers (Grass Technologies, West Warwick, RI, USA) and were recorded under an initial tension of 2 g, which had been found to be the optimal tension for KCl-induced contraction (96 mM). The endothelial function was evaluated by testing the relaxation induced by a concentration response curve (CRC) to acetylcholine (Ach, 10^{-8} – 5×10^{-6} M) in aortic rings pre contracted with phenylephrine (Phe) 5×10^{-6} M.

2.4. Statistical Analyses

CRC were fitted using a nonlinear interactive fitting program (GraphPad Prism 3.0; GraphPad Software Inc., San Diego, CA, USA). Agonist potencies were calculated as pEC50 (negative logarithm of the molar concentration of agonist producing 50% of the maximum response), and maximum response was expressed as Rmax (maximum effect elicited by the agonist). Investigators were blinded to treatment until data analysis. Results are reported as mean \pm standard error of the mean (SEM).

The Shapiro–Wilks goodness-of-fit test was used to test for normal distribution. Statistically significant differences were calculated by one- or two-way analysis of variance (followed by Duncan's post-test) or unpaired Student's *t*-test; $p < 0.05$ was considered statistically significant.

3. Results and Discussion

3.1. Clinical and Biochemical Parameters

Independent of the differences in caloric intake, animal body weights did not differ significantly among the groups (Table 2). As was previously reported [2], TC, TG, MAP and TyG index were higher in rabbits fed a HD than CD group, and ChO 10% supplementation normalized TG and MAP. Unexpectedly, the control group, CD-ChO¹⁰, showed glucose intolerance and higher levels of FG and TyG index compared with the CD group. These results disagree with those of other authors [4,5]. Several studies find that dietary supplement with chia seed improves glucose tolerance and insulin sensitivity. Chicco et al. [9] report that dietary chia seed improves adiposity and normalizes hypertriglyceridemia and insulin resistance in an experimental model of dyslipidemia and insulin resistance. Poudyal et al. [10] show that a chia seed-supplemented high carbohydrate-high fat diet does not reduce total body fat but induces lipid redistribution away from the abdominal area and improves glucose tolerance and insulin sensitivity. However, all these studies were performed with the chia seed, the composition of which is different than ChO [1]. Chia seeds contain high levels of fiber, and this would be an advantage with respect to ChO. Thus, in a recent study, we replaced the 10% of ChO by chia seed in such a way that the contents of ALA is equivalent. An increase in VAF and glucose intolerance were found in this group. We hypothesized that ALA intake was too high and would be responsible for such alterations. Thus, ChO intake was reduced (4.9 g to 3 g per day) and no differences were found in the CD-ChO³ group as compared with the CD group. Therefore, we aimed

to study the effects of dietary intervention with 3% ChO on a rabbit model of metabolic syndrome induced by HFD. According to Alarcon et al. [8], TG, FG, VAF, glucose tolerance test and the n-6/n-3 fatty acid ratio were higher and HDL-C was lower in the HFD than in the CD group (Table 2). Although the replacement of 3% corn oil with ChO into the HFD lowered the TG levels and the n-6/n-3 fatty acid ratio, it failed to improve HDL-C levels. In addition, ChO worsened the deleterious effects of the HFD on VAF, FG and glucose intolerance.

Table 2. Clinical and biochemical parameters from rabbits fed a control diet (CD); fed a CD supplemented with 10% chia oil (CD-ChO¹⁰); fed a CD supplemented with 3% chia oil (CD-ChO³); fed a CD supplemented with 224 g/kg chia seed (CD-Ch); fed a CD supplemented with 1% cholesterol (HD); fed a HD supplemented with 10% chia oil (CD-ChO¹⁰); fed a CD supplemented with 10% corn oil and 8% lard (HFD); and fed a CD supplemented with 3% chia oil, 7% corn oil and 8% lard (HFD-ChO³).

	CD	CD-ChO ¹⁰	CD-ChO ³	CD-Ch	HD	HD-ChO ¹⁰	HFD	HFD-ChO ³
BW	1.9 ± 0.1	1.9 ± 0.6	1.8 ± 0.6	2.0 ± 0.6	2.1 ± 0.4	1.8 ± 0.1	2.0 ± 0.4	1.9 ± 0.2
VAF	1.0 ± 0.2	1.3 ± 0.2	1.4 ± 0.4	2.1 ± 0.1 *	0.70 ± 0.3	0.93 ± 0.2	2.3 ± 0.1 *	3.4 ± 0.3 *
FG	113 ± 3	132 ± 6 *	105 ± 23	109 ± 11	115 ± 4	139 ± 8 *	126 ± 6 *	148 ± 11 *
Glu-60	183 ± 5	200 ± 16	170 ± 35	205 ± 27	194 ± 10	210 ± 14	248 ± 17 *	277 ± 14 *
Glu-120	138 ± 3	161 ± 9 *	140 ± 19	160 ± 19 *	138 ± 6	171 ± 11 *	172 ± 9 *	241 ± 21 *
TC	59 ± 60	53 ± 16	49.8 ± 12	95.5 ± 38	872 ± 114 *	783 ± 278 *	78 ± 5	81 ± 5
LDL-C	29 ± 8	24.6 ± 4.0	23.5 ± 3.8	24 ± 1.4	666 ± 99 *	705 ± 291	47 ± 7	42 ± 7
HDL-C	51 ± 7	25 ± 4 *	24 ± 2 *	51 ± 2	164 ± 45 *	128 ± 47 *	24 ± 3 *	20 ± 0.4 *
TG	113 ± 14	120 ± 29	122 ± 15	65 ± 2.4	222 ± 33 *	102 ± 2	192 ± 22 *	104 ± 9
TyG	8.3 ± 0.2	9.5 ± 0.1 *	8.6 ± 0.2		10.2 ± 0.5 *	9.3 ± 0.2 *	9.3 ± 0.3 *	8.9 ± 0.1 *
MAP	56.0 ± 2.6	61 ± 6	44.6 ± 4.6	48.3 ± 2.4	73 ± 2 *	64.8 ± 4.6	57 ± 5	45 ± 2
HR	265 ± 25	224 ± 5	249 ± 27	253 ± 5	226 ± 11	253 ± 7	282 ± 27	325 ± 44
n-6/n-3	9.2 ± 0.2	4.0 ± 0.3	4.2 ± 0.1	-	7.6 ± 0.2	3.4 ± 0.1	35 ± 0.2 *	12 ± 1.3

BW: body weight (kg); VAF: visceral abdominal fat (%); FG: fasting glucose (mg/dL); Glu-60: glucose level 60 min post intraperitoneal injection of glucose (mg/dL); Glu-120: glucose level 120 min post intraperitoneal injection of glucose (mg/dL); TC: total cholesterol (mg/dL); TG: triglycerides (mg/dL); MAP: mean arterial blood pressure (mmHg); HR: heart rate (bpm); * statistically different from CD.

3.2. Vascular Function

Relaxation to Ach in blood vessels is a validated marker of endothelial function. Endothelium-dependent relaxation to Ach was lower in aortic rings from the HD (37 ± 4%) and HFD (44 ± 6%) group than those from the CD group (68 ± 9%). Dietary intervention with ChO partially normalized Ach relaxation in the HD-ChO group (51 ± 5%) and significantly improved endothelial function from HFD-ChO (60 ± 4%). These results confirm the beneficial effect of ALA on endothelial function.

4. Conclusions

Many authors claim the benefit of chia seed on CVD risk factors. However, the effects of dietary interventions with ChO have been little explored. Results from our studies show that incorporation of ChO into the diet has beneficial and harmful effects. The beneficial effects include the reduction of TG levels and MAP and the improvement of endothelial function. However, ChO may induce glucose intolerance, and even more importantly, it may worsen the HFD-induced deleterious effects on VAF and FG. The experimental animal models were previously characterized, and they mimic the CVD risk factors in human beings. Therefore, our results support the view that dietary guidelines for treating patients with CVD risk factors must be carefully planned in such a way that before the incorporation of ChO into the diet, the nutritional background should be considered.

Institutional Review Board Statement: All animal care and use programs were performed according to the Guide for the Care and Use of Laboratory Animals (NIH Publication 86 to 23, revised 1985), and approved by the Institutional Animal Care and Use Committee (CICUAL-UNT; protocol code 021/2019).

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