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Ground chia seed and chia oil effects on plasma lipids and fatty acids in the rat

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Abstract

There is considerable evidence suggesting that regular consumption of ω-3 fatty acids prevents cardiovascular diseases, including atherosclerosis and thrombosis. Most studies, however, have been carried out with fish oils that are rich in eicosapentaenoic acid and docosahexaenoic acid fatty acids, but α–linolenic fatty acid, which is a precursor of eicosapentaenoic acid and docosahexaenoic acid, has received little attention. The purpose of the present study was to assess the effect chia seed, which is the highest known vegetative source of α–linolenic fatty acid, has on plasma total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein, triacylglycerol content, and fatty acid profile when fed to rats. Twenty-four male Wistar rats were fed ad libitum 3 diets containing equal energy levels derived from corn oil (T_1), chia seed (T_2), or chia oil (T_3) for 4 weeks. At the end of the feeding period, 6 rats from each treatment were used for blood analyses. Blood samples were analyzed for total cholesterol, HDL, low-density lipoprotein, triacylglycerol content, and fatty acid composition. Rats fed chia showed a significant decrease in serum triacylglycerol content, 3 and 2.5 times lower than the control for T_2 and T_3, respectively. There was a significant increase in serum HDL cholesterol content, 21.8% and 51% for T_2 and T_3, respectively, with the increase between chia diets being significant ($P < .05$). Total cholesterol was significantly lower for the T_2 diet compared with the T_3 diet. Serum fatty acid composition showed a significantly higher α–linolenic acid content and an improved ratio of ω-6/ω-3 fatty acids for T_2 and T_3 compared with T_1. In summary, the chia diets dramatically decreased triacylglycerol levels and increased HDL cholesterol and ω-3 fatty acid contents in rat serum. These findings suggest that α-linolenic–rich chia oil may be an alternative to ω-3 sources for vegetarians and people allergic to fish and fish products.

Keywords: Chia; Fatty acids; Linolenic; Rats; ω-3; Cholesterol

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1. Introduction

Coronary heart disease (CHD) is the single most common cause of death in the United States, the United Kingdom, and other Western industrialized countries [1]. There is a consensus among scientists that dietary changes during the last century, which have included an increased intake of total lipids and saturated and polyunsaturated ω-6 fatty acids, have led to the high incidence of CHD [2,3].

There is increasing evidence from animal, epidemiological, and clinical studies that consuming lipids rich in ω-3 fatty acids is important in reducing CHD [4,5]. Early evidence of this appeared in the late 1970s from epidemiological studies conducted in populations consuming large quantities of fish that are rich in the very long polyunsaturated ω-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). At that time, it was generalized that the ω-3 α–linolenic acid was only a precursor of the long-chain fatty acids EPA and DHA, hence the reason for setting a low α-linolenic requirement [6]. Results from a number of recent epidemiological and controlled studies on α–linolenic acid in humans and animals suggest that this fatty acid has an important role for ω-3 status. These studies show that the consumption of α–linolenic acid is important, and it may reduce the risk for cardiovascular diseases [7-12].

Chia, along with corn, beans, and amaranth, was a core component in the diet of many pre-Columbian civilizations in America, including the Mayan and Aztec populations [13]. Chia contains the richest botanical source of α–linolenic acid known but does not contain any of the antinutritional compounds (total linamarin, linustatin, and neolinustatin) and vitamin B_6 antagonist factors [14-16], which are found in flax [17-19]. Flax is the most common source of α–linolenic acid commercially available; however, its antinutritional factors have significantly limited its use as a food or feed [20-24].

Chia seed was used successfully to increase the ω-3 content of eggs, poultry meat, and cow's milk; however, the effect of chia seed or its oil on serum lipids has not been reported. The objective of the present study was to compare the effect of equivalent dietary intakes of α–linolenic acid from chia seed and oil on serum total cholesterol (TC), low-density lipoprotein (LDL), high-density lipoprotein (HDL), triacylglycerol content, and fatty acid composition when fed to rats.

2. Methods and materials

2.1. Animals

Male Wistar rats, 4 weeks of age, were obtained from the Department of Nutrition vivarium in the School of Pharmacy and Biochemistry at the University of Buenos Aires, Argentina. The rats weighed 35 to 48 g at the beginning of the trial and were randomized into 3 groups containing 6 rats each, such that mean body weight per group was approximately equal. The animals were housed individually in stainless steel cages and maintained at 21°C $\pm$ 1°C with a 12-hour light/dark cycle. Water and food were available ad libitum. Each group of rats was fed an assigned diet for 4 weeks; during that period, food consumption and body weights were recorded. At the end of the experimental period, after a 4-hour fast, body weights were recorded. Animals were anesthetized with ethyl ether and exsanguinated by venous puncture. Blood samples were collected and used to determine serum lipids and fatty acid composition. Livers were excised and frozen at −20°C until weighed. All procedures were in accordance with the guide for care and use of laboratory animals established by the Department of Nutrition, School of Pharmacy and Biochemistry, University of Buenos Aires.

2.2. Diets

The experimental diets were isocaloric (16.76 Kj/g of diet) and contained 20% protein (Table 1). These were prepared according to the American Institute of Nutrition guidelines [25,26]. The 3 diets were as follows: no chia (T_1), 15% ground chia seed (T_2), or 5% chia oil (T_3). The 2 chia diets were formulated to provide equal quantities of α–linolenic acid from the chia. The chia seeds and chia oil were provided by Functional Products SA (Salta, Argentina) and Applied Resources Inc (Gilbert, Ariz), respectively.

2.3. Laboratory analyses

Serum TC, triacylglycerols (TG), high-density lipoprotein cholesterol (HDL-C), and LDL cholesterol concentrations were measured by enzymatic methods using Wiener kits (Enzimatic Cholestat AA, Color Triacylglycerols GPO/PAP AA, Monofase Cholesterol HDL AA, and Cholesterol LDL precipitating reagent; Wierner Laboratories, Rosario, Argentina) in an Alcyon Analyzer (AL-ISE; St Mathieu de Treviers, France). Serum lipids

Table 1
Composition of the experimental diets, control (T_1), ground chia (T_2), and chia oil (T_3), fed to rats

	T_1	T_2	T_3
Ingredient			
Calcium caseinate[a] (g/kg)	225	190	225
Chia seeds ground (g/kg)	–	150	–
Chia oil (g/kg)	–	–	51.9
Corn oil[b] (g/kg)	70	18.1	18.1
Minerals[c] (g/kg)	35	35	35
Vitamins[c] (g/kg)	10	10	10
Choline chloride (mL)	7.1	7.1	7.1
Dextrin[d] (g/kg)	653	590	653
Diet			
Metabolizable energy (kcal/kg)	4040	3906	4040
Crude protein (g/kg)	200	200	200
Palmitic (g/kg)	8.5	5.8	5.8
Stearic (g/kg)	1.5	1.9	1.9
Oleic (g/kg)	19.3	8.4	8.4
Linoleic (g/kg)	39.9	20.2	20.2
α-Linolenic (g/kg)	0.6	33.4	33.4
Linoleic/α-linolenic	66.5	0.6	0.6

[a] 88.8% of protein.
[b] Molinos Rio de la Plata SA, Buenos Aires, Argentina.
[c] Mineral and vitamin premixes AIN-93 (American Institute of Nutrition guidelines [25,26]).
[d] Maltodextrina G C15, Food SA, Buenos Aires, Argentina.

were extracted according to the method of Folch et al [27] and converted to fatty acid methyl esters using the IRAM 5-560II method [28]. Fatty acid methyl esters were separated and quantified by an automated gas chromatograph (Model 6890, GC; Hewlett Packard Co, Wilmington, Del) equipped with flame ionization detectors and a 30 m $\times$ 530 μm inner diameter capillary column (Model HP-FFAP; Hewlett Packard Co).

2.4. Statistical analyses

One-way analysis of variance was used to determine treatment effects. When the F value was significant, means were analyzed using Duncan multiple range test (CoStat Pro Version 6.204; CoHort Software, Monterey, Calif).

3. Results

3.1. Animals

All animals appeared healthy after the 4-week experimental period. Initial body weight, final body weight, food intake, and liver weight are presented in Table 2. There were no significant differences among groups for initial body weight. Rats fed the chia diets were heavier than those fed the control diet. Final body weight and food intake were significantly greater for rats fed both chia diets than rats fed the control diet. No significant differences between the chia seed and chia oil diets were detected. Feed efficiency (body weight gain/feed intake) was significantly higher for T_2 and T_3 compared with the T_1. Liver weight was not significantly affected by diet; however, relative liver weight (100$\times$liver weight/body weight) for the rats fed the control diet was significantly higher than for the rats fed the chia diets.

3.2. Cholesterol, HDL, LDL, and triacylglycerol determinations

Serum TC, HDL, LDL, and triacylglycerol contents are presented in Table 3. Total cholesterol was lower in the serum of rats fed chia seed than in the rats fed either the control diet or chia oil. High-density lipoprotein cholesterol was higher in the serum from animals fed both chia diets than in the serum from the animals fed the control diet. Rats fed chia had a

Table 2
Body weights and food intake of rats

	T_1	T_2	T_3	$LSD_{.05}$
Body weight, initial (g)	41.9^{a}	40.5^{a}	41.1^{a}	5.12
Body weight, final (g)	206.1^{b}	237.9^{a}	236.1^{a}	22.31
Food intake (g/d)	13.7^{b}	15.4^{a}	14.9^{a}	1.17
Feed efficiency (%)	40^{b}	43^{a}	43^{a}	2
Liver weight (g)	11.01^{a}	11.11^{a}	11.00^{a}	1.47
Relative liver weight*	5.3^{a}	4.7^{b}	4.7^{b}	0.38

Values (means of 8 rats per group) in rows with different superscript letters are significant ($P < .05$). Refer to Table 1 for diet descriptions. LSD indicates least significant difference for mean separation.

* 100 $\times$ liver weight/body weight.

Table 3
Serum cholesterol, LDL, HDL, and triacylglycerol levels in rats

	T_1	T_2	T_3	$LSD_{.05}$
Cholesterol	108.11^{a}	95.88^{b}	105.54^{a}	7.77
Triacylglycerols (mg/dL)	206.83^{a}	69.67^{b}	82.50^{b}	27.08
HDL	26.19^{c}	31.92^{b}	39.56^{a}	4.16
LDL	29.59^{a}	30.60^{a}	27.88^{a}	7.30
LDL/HDL	1.15^{a}	$0.97^{a,b}$	0.70^{b}	0.27
TG/HDL	8.04^{a}	2.22^{b}	2.00^{b}	1.13
TC/HDL	4.17^{a}	3.03^{b}	2.67^{b}	0.37
TC/LDL	3.83^{a}	3.19^{a}	3.92^{a}	0.94

Values (means of 6 rats per group) in rows with different superscript letters are significant ($P < .05$). Refer to Table 1 for diet descriptions.

significantly lowered ratio of TC/HDL compared with the control. The serum ratio of LDL/HDL for rats fed chia seed appeared to decrease but not significantly. Addition of chia to the diet of rats significantly decreased serum triacylglycerol content compared with the control group. No significant difference in serum LDL or in the ratio of TC/LDL was found among the treatments.

3.3. Fatty acid analysis

Serum fatty acid composition, as a percentage of total fatty acid content, is presented in Table 4. Palmitic and stearic fatty acid percentages were not significantly different among the

Table 4
Serum fatty acid composition (area %) of rats

	T_1	T_2	T_3	LSD
Palmitic	32.75^{a}	30.12^{a}	32.75^{a}	5.49
Palmitoleic	3.24^{a}	1.08^{b}	1.33^{b}	1.32
Stearic	19.12^{a}	21.48^{a}	21.30^{a}	4.81
Oleic	19.17^{a}	10.28^{b}	12.24^{b}	4.87
Linoleic	21.28^{a}	20.65^{a}	18.33^{a}	2.87
α-Linolenic	0.21^{b}	7.13^{a}	7.73^{a}	2.82
AA	8.02^{a}	4.78^{b}	3.73^{b}	1.76
DHA	1.41^{b}	4.12^{a}	2.36^{b}	1.36
ω-3	1.61^{b}	11.24^{a}	10.09^{a}	2.69
ω-6	29.30^{a}	25.43^{b}	22.07^{c}	3.31
SFA/ω-3	33.77^{a}	4.80^{b}	5.86^{b}	11.24
SFA	46.73^{b}	$51.60^{a,b}$	54.05^{a}	6.69
MUFA	22.41^{a}	11.36^{b}	13.57^{b}	1.19
PUFA	30.92^{b}	36.67^{a}	32.16^{b}	4.08
SAT/PUFA	1.53^{a}	1.43^{a}	1.70^{a}	0.35
ω-6/ω-3	20.59^{a}	2.34^{b}	2.39^{b}	5.51
AA/DHA	6.55^{a}	1.24^{b}	1.76^{b}	2.03
PAL/PUFA	1.06^{a}	0.82^{b}	1.02^{c}	3.17

Values (means of 6 rats per group) in rows with different superscript letters are different ($P < .05$). Refer to Table 1 for diet descriptions. AA indicates arachidonic acid; SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PAL, palmitic acid.

treatments. Total saturated fatty acids, calculated as the sum of palmitic and stearic acids, were higher for the chia diets; however, this was only significant for T_3. The monounsaturated fatty acids oleic (which was the greater percentage) and palmitoleic tended to decrease in serum of rats fed chia (Table 4). Total monounsaturated fatty acid content, calculated as the sum of palmitoleic and oleic acids, was significantly lower in serum of rats fed chia diets compared with the control rats. The polyunsaturated ω-6 fatty acid (linoleic) content was not different among treatments. The percentage of arachidonic acid, a metabolite of linoleic acid, was lower in serum of rats fed chia. Total ω-6 polyunsaturated fatty acids (PUFAs), calculated as the sum of linoleic and arachidonic acids, were significantly lower in serum for rats fed both chia diets compared with control diet. In addition, serum from rats fed chia oil contained significantly less total ω-6 fatty acids compared with those fed ground chia seed.

Addition of chia to the diet significantly increased the serum α–linolenic acid content. Furthermore, the dietary ground chia treatment increased DHA content, which is an elongation and desaturation product of α–linolenic acid. Total ω-3 fatty acid content, calculated as the sum of α-linolenic and DHA, was significantly higher in serum of rats fed both chia diets. Both ground chia seed and chia oil fed to rats resulted in lower serum ratios of ω-6/ω-3, arachidonic-DHA, and saturated (SFA)/ω-3 compared with the values for the control diet. The ratio of saturated-PUFA was not significantly different among treatments but the ratio of palmitic acid–PUFA was lower in serum of rats fed chia compared with the control group (Table 4).

4. Discussion

Our study showed that rats fed chia gained more body weight than did those fed corn oil and that food intake was greater in those groups. The effect of fat type on diet palatability, intake, nutrient absorption, and metabolism demonstrated in laboratory animals [29-31] might be responsible for the improved feed use observed in rats fed chia.

Chia oil reduced serum triacylglycerols levels in rats by 66% and 60% for diets T_2 and T_3, respectively. A number of studies have demonstrated that dietary ω-3 fatty acids lower the level of plasma triacylglycerols in rats [32-35]. In rats, feeding 15% menhaden oil in the diet (14% EPA and 9% DHA) resulted in a 66% reduction in triacylglycerols [35]. The decrease observed with chia was greater than that found with diets containing 15% DHA oil [34], 20% fish oil (10% EPA and 33% DHA), and 12.6% DHA-rich fish oil [36], which reduced triacylglycerol level by 45%, 43%, and 20%, respectively. As in human studies, most rat investigations evaluated fish oils that are rich in long-chain ω-3 fatty acids (EPA and DHA); few have examined vegetable sources such as α-linolenic, but none evaluated chia seed or oil. Our results with chia are different from those observed when rats were fed α–linolenic acid provided by a 20% perilla oil–enriched diet and a 20% safflower oil–enriched diet [33]; a 15.2% perilla oil diet, and a 15.2% borage–enriched diet [37]; a 20% matthiola (*Matthiola incana*) oil–enriched diet, a 20% coconut oil–enriched diet, or a 20% sunflower oil–enriched diet [38]; and a 15% flaxseed oil–enriched diet and a 15% palm oil– or 15% safflower–enriched diet [39]. In all cases, these supplements failed to significantly decrease serum or plasma triacylglycerol levels.

Compared with the rats fed the control diet, a significantly higher serum HDL (22% and 51% for T_2 and T_3, respectively) was found when chia was fed. The increase is similar to a previous report [35] that 15% menhaden oil in the diet fed to rats lowered serum triacylglycerol but elevated serum HDL levels compared with rats fed 15% olive oil. The increase in HDL found in rats fed chia is different from that obtained when other sources of α–linolenic fatty acid were fed to rats. For example, Ihara-Watanabe et al [37] found significantly ($P < .05$) less HDL in the serum of rats fed perilla oil than in serum from rats fed borage oil, and Wiesenfeld et al [40] reported significantly lower serum HDL levels in rats fed 20% or 40% flaxseed diets compared with rats fed 14% soybean oil.

The lack of a significant difference in total serum cholesterol between T_1 and T_3, but not between T_1 and T_2, could be associated with the fiber content of the ground chia seed. Chia seed has 60.9% total dietary fiber, on a dry basis, with 7% being soluble and 55.9% being insoluble [14]. The hypocholesterolemic effect of dietary fiber in rats [41] and other rodents [42] has been reported, as well as a source effect [43,44]. Some researchers have reported that consumption of soluble fiber provided by seeds such as psyllium and guar, which are similar to that of the soluble fiber in chia, produced a reduction not only in serum TC but also in HDL-C [45]. The ratio of TG/HDL, LDL/HDL, TC/HDL, TC/LDL, and TG/HDL tended to be improved by feeding ground chia seed and chia oil to the rats. These ratios are considered important predictors of a cardiovascular health in the human.

No difference was found in serum linoleic acid content among the treatments, but a significantly lower amount of arachidonic acid was observed in rats fed the chia treatments compared with the control diet. As expected, serum level of α–linolenic fatty acid was higher in rats fed the chia treatments, as was the elongation product DHA. Chia led to an increased amount of the ω-3 fatty acids in rat serum (7- and 6.3-fold increases for ground chia seed and chia oil, respectively). Other studies showed significant increases in ω-3 fatty acids in tissues of animals fed chia seeds [35-38].

The results of this study showed that chia, which contains α–linolenic acid, resulted in increased HDL-C, decreased triacylglycerol content, and lower ratios of TG/HDL and TC/HDL. Chia feeding increased the ω-3 fatty acid content and lowered the ratio of ω-6/ω-3 without an adverse effect on rat growth. This study suggests that chia lipids could be an alternative to fish for non–fish-eating consumers such as vegetarians and people with fish allergies to benefit from ω-3 fatty acids. Further research is necessary, however, to understand the nutrition and health potential of chia.

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