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Effects of defatted amaranth (*Amaranthus caudatus* L.) snacks on lipid metabolism of patients with moderate hypercholesterolemia

Efeitos de snack de amaranto desengordurado (Amaranthus caudatus L.) no metabolismo lipídico de pacientes com hipercolesterolemia moderada

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Abstract

We evaluated the effects of defatted amaranth (*Amaranthus caudatus* L.) snacks on plasma lipids in moderate hypercholesterolemic patients. Twenty-two subjects [30–65 years old], 11 males, with total cholesterol (TC) ≥ 240 mg.dL⁻¹, low-density cholesterol (LDL-c) 160–190 mg.dL⁻¹ and plasma triglycerides (TG) < 400 mg.dL⁻¹ were randomized in a double blind clinical trial to receive an amaranth snack (50 g/day) or equivalent corn snack (placebo) for 2 months. There were no differences between amaranth and placebo on TC and LDL-c, and TG respectively: -8.4 and -5.7% ($p = 0.17$); -12.3 and -9.7% ($p = 0.41$) and -0.6 and -7.3% ($p = 0.47$). However, amaranth snacks significantly reduced high-density cholesterol (HDL-c): -15.2 vs. -4% ($p = 0.03$). In conclusion, the intake of 50 g of extruded amaranth daily during 60 days did not significantly reduce LDL-c in moderate hypercholesterolemic subjects; furthermore there was a significant reduction in HDL-c. Studies with greater number of subjects and greater quantity of this food are necessary to test the effects of amaranth on lipid metabolism in humans.

Keywords: cholesterol; amaranth; functional food; HDL-c; dislipidemia.

Resumo

Avaliámos os efeitos de *snacks* feitos com amaranto desengordurado (*Amaranthus caudatus* L.) no metabolismo lipídico de pacientes com hipercolesterolemia moderada. O ensaio clínico contou com vinte e dois voluntários [30–65 anos, 11 homens, com colesterol total ≥ 240 mg.dL⁻¹, LDL-c 160–190 mg.mL⁻¹, e triglicérides plasmáticos (TG) < 400 mg.mL⁻¹] aleatoriamente distribuídos em dois grupos que receberam um *snack* de amaranto (50 g/dia) ou um placebo equivalente (*snack* de milho) por dois meses. Não houve diferenças significativas entre o amaranto e o placebo no colesterol total, LDL-c e TG, cujos valores foram, respectivamente, -8,4 e -5,7% ($p = 0,17$); -12,3 e -9,7% ($p = 0,41$) e -0,6 e -7,3% ($p = 0,47$). Entretanto, o consumo de *snacks* de amaranto reduziu significativamente o HDL-c: -15,2 vs. -4% ($p = 0,03$). Concluímos que o consumo de 50 g de amaranto extrusado por 60 dias não foi suficiente para reduzir significativamente LDL-c em pacientes com hipercolesterolemia moderada; além disso, observou-se significativa redução de HDL-c. Estudos com maior número de participantes e maior quantidade de amaranto são necessários para se testarem os seus efeitos no metabolismo humano.

Palavras-chave: colesterol; amaranto; alimento funcional; HDL-col.; dislipidemia.

1 Introduction

Amaranth is a pseudo cereal native to Andean countries and has been consumed in that region since the pre-Columbian era. Interest in its widespread consumption for human nutrition has grown recently due to favorable reports of amaranth nutritive value and health benefits. In addition to its promising nutritional qualities, amaranth may also be valuable for celiac patients (TOSI; CIAPPINI; MASCIARELLI, 1996), diabetic (CHATURVEDI et al., 1997), hypercholesterolemic subjects (MAIER; TURNER; LUPTON, 2000) and coronary heart disease and hypertension patients (MARTIROSYAN et al., 2007). Among several possible processing methods, extrusion is the most interesting from the nutritional and economical point of view because it produces a stable product with all nutritive aspects preserved or enhanced (BRESSANI; SANCHEZ-MARROQUIN; MORALES, 1992; MENDOZA; BRESSANI, 1987).

Extrusion cooking has been optimized for the production of amaranth snacks that presented a high acceptability as compared with commercial brands (CHÁVEZ-JÁUREGUI; SILVA; ARÊAS, 2000; CHÁVEZ-JÁUREGUI et al., 2003).

The nutritional evaluation of these snacks showed that they exhibit a high nutritional value in terms of protein biological value, bioavailability of minerals, and high content of fiber (CHÁVEZ-JÁUREGUI; SILVA; ARÊAS, 2000; FERREIRA; ARÊAS, 2004). Amaranth's cholesterol-lowering properties have been investigated in animal models and humans using differently processed amaranth of various species and cultivars (BERGER et al., 2003; CHATURVEDI; SAROJINI; DEVI, 1993; DANZ; LUPTON, 1992; GRAJETA, 1999; MAIER; TURNER; LUPTON, 2000). In an experiment with hypercholesterolemic

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rabbits, Plate and Arêas (2002) observed a reduction of 50 and 18% of total cholesterol when the animals were fed diets with extruded amaranth and amaranth oil. Further study demonstrated that protein is the major responsible for this hypocholesterolemic effect (MENDONÇA et al., 2009). This fact opened the possibility of using whole amaranth or its protein as a functional food for dislipidemia treatment and possible atherosclerosis prevention. However, the effect of amaranth consumption in plasma lipids has not been clearly shown in humans. The purpose of this study was to evaluate the effects of extruded amaranth snack on plasma lipids of moderate hypercholesterolemic subjects.

2 Materials and methods

2.1 Clinical trial

Study subjects were recruited among the patients of the Heart Institute (InCor), University of São Paulo Medical School Hospital, Lipid Clinic Section. Twenty two moderate hypercholesterolemic patients (11 males) aged 30–65 years, with TC ≥ 240 mg.dL⁻¹ and LDL-c levels 160–190 mg.dL⁻¹ and TG < 400 mg.dL⁻¹ were included. The exclusion criteria were previous history of diabetes, thyroid, liver, and coronary artery diseases, as well as use of lipid lowering drugs in the last 6 weeks and smoking. The subjects followed a step I American Heart Association diet for at least 3 months. The study was approved by the Ethical Committee of the InCor (Instituto do Coração - SP, Brazil – a heart disease treatment center), and a written informed consent was obtained. The subjects' baseline data are shown in Table 2. The subjects were randomized in a double blind fashion to receive either an amaranth (50 g/day) or a corn snack (placebo) for 2 months.

2.2 Snack production

Amaranth (*Amaranthus caudatus* L.) CAC-43A, Oscar Blanco variety was provided by the germ plasma bank of the "Centro de Investigación en Cultivos Andinos (CICA)" of the National University of San Antonio Abad del Cusco – Peru. The grain was milled in stainless steel knife mill (Mod. Termomatic, Marconi - SP, Brazil) and defatted with n-hexane in soxhlet apparatus to a lipid concentration of 0.16%. The amaranth snacks developed in this study were extruded as described by Chávez-Jáuregui, Silva and Arêas (2000). The conditions were: a 20 mm laboratory single screw extruder with 20:1 L/D ratio, screw of 4:1 compression ratio, screw speed 200 rpm, feed moisture 15%, feed material at 70 g/minute, temperature at the metering zone 150 °C, and die of 3 mm. The expansion ratio of the product was typically 3.5 to 4. The amaranth and corn

snacks were flavored with pizza or bacon, 15% canola oil, and 0.9% of salt and they were packed in bags without labeling. In order to retain the flavor, canola oil was chosen because it does not induce hypercholesterolemia as compared to partially hydrogenated vegetable fat.

2.3 Proximate analysis

The proximate composition of the amaranth and corn snacks was determined by conventional methods for protein and ash, as described by AOAC (ASSOCIATION..., 1990). Lipid extraction was performed using the dry column method in accordance with Marmer and Maxwell (1981). Carbohydrate was estimated by difference from 100.

2.4 Fatty acid composition

Ten grams of milled snacks were used to analyze the fatty acid composition, and an aliquot containing 50 mg of lipid obtained using the dry column method was extracted as described previously (MARMER; MAXWELL 1981). The fatty acid esterification was carried out in accordance with the method developed by Hartman and Lago (1973). The fatty acid methyl esters were analyzed by gas chromatography using a Chrompack CP9002 and a fused silica capillary column (CP-Sil 88; 50 m \times 0.25 mm I.D. 0.20 μ m film).

2.5 Clinical analyses

Blood samples were collected after 12-hours fasting for the determination of plasma lipids. Commercial enzymatic methods were used for total and HDL-cholesterol and triglycerides. The LDL-c levels were determined through the Friedewald, Fredrick, and Levi (1972) equation for TG lower than 400.0 mg.dL⁻¹: [LDL-cholesterol = total cholesterol - (HDL-cholesterol + triglyceride/5)]. The content of very low-density cholesterol (VLDL-c) was evaluated by the formulae: VLDL-cholesterol = TG/5 for TG lower than 400.0 mg.dL⁻¹. Data are expressed as mean \pm Standard Deviation (SD).

2.6 Statistical analyses

The statistical analyses were performed using the Stata version 7 (Stata Corp, College Station, TX). The comparison of nonparametric data was performed by the Wilcoxon signed rank test. The level of significance was set at $p < 0.05$.

3 Results and discussion

The proximate analysis and fatty acid composition of the amaranth snack are shown in Table 1. The snack contained

Table 1. Proximate composition of amaranth and corn snacks (g.100 g⁻¹) and percentage of fatty acid composition.

Material	Protein	Lipids	Carbohydrate	Ash	Kcal			
Amaranth	14.18	14.10	67.00	4.72	451.62			
Corn	6.33	14.80	76.60	2.31	464.92			
Percentage of fatty acid composition								
	C _{16:1}	C _{18:0}	C _{18:1}	C _{18:2}	C _{18:3}	C _{23:0}	C _{20:5}	C _{24:6}
Amaranth snack	13.04	2.42	36.50	28.62	4.59	1.57	11.67	1.59

Table 2. Clinical and biochemical characteristics.

	Baseline (n =11)		60 days (n =11)		% Change		P placebo vs. amaranth
	Placebo	Amaranth	Placebo	Amaranth	Placebo	Amaranth	
Age (years)	50.9 ± 13.9	47.9 ± 8.2	-	-	-	-	0.54
Sex (M/F)	5/6	6/5	-	-	-	-	-
Waist circumference (cm)	97.4 ± 9.1	93.5 ± 6.9	96.9 ± 9.5	93.0 ± 5.4	-0.5	-0.5	0.54
Body mass index (Kg.m ⁻²)	28.6 ± 3.2	26.9 ± 2.7	28.7 ± 3.4	26.8 ± 2.6	-0.4	-0.4	0.50
Total cholesterol (mg.dL ⁻¹)	261.0 ± 22.5	250.0 ± 21.0	246.0 ± 29.6	229.0 ± 25.0	-5.7	-8.4	0.17
HDL-cholesterol (mg.dL ⁻¹)	53.0 ± 14.9	46.0 ± 16.0	51.0 ± 10.0	39.0 ± 10.0	-4.0	-15.2	0.03
LDL-cholesterol (mg.dL ⁻¹)	175.0 ± 22.0	171.0 ± 8.0	158.0 ± 23.0	150.0 ± 21.0	-9.7	-12.3	0.41
VLDL-cholesterol (mg.dL ⁻¹)	34.0 ± 16.0	36.4 ± 21.0	38.0 ± 18.0	34.0 ± 19.0	-11.8	-6.6	0.47
Triglycerides (mg.dL ⁻¹)	164.0 ± 80.0	171.0 ± 94.0	152.0 ± 29.0	170.0 ± 95.0	-7.3	-0.6	0.47

Values are means ± standard deviation.

a higher proportion of unsaturated fatty acids such as 49.5% monounsaturated, and 46.5% polyunsaturated fatty acid and only 4.0% of saturated fatty acid, as a consequence of the use of canola oil in the snack flavoring. Both snacks, the amaranth and placebo, were consumed by 100% of subjects during the study and were well accepted and tolerated. Table 2 shows the plasma lipids of the subjects at baseline and after 60 days. The total amount of protein intake of all individuals in the amaranth group amounted ca. 7 g/day. Positive effect of soya consumption on cholesterol decrease was observed when at least 25 g protein/day was consumed by 70 kg subjects (REYNOLDS et al., 2006) for the observed effects of soya on lipid metabolism. Other crops also need about the same concentration of protein for the cholesterol-lowering effect to be observed (FROTA et al., 2008). Mendonça et al. (2009) found that protein is the component responsible for nearly all the hypocholesterolemic effects observed with amaranth consumption. Although this protein intake was not achieved in the present study, the total cholesterol decreased more in subjects of the amaranth group than in the placebo groups, even with this lower protein consumption. However, this difference was not significant ($p = 0.17$). Similarly, There were no significant differences ($p < 0.05$) between the effects of amaranth and placebo on total, LDL-c, and triglyceride concentrations (Table 2). On the other hand, amaranth significantly reduced HDL-c ($p = 0.03$). No difference was found among the groups regarding Body Mass Index evolution and abdominal waist, which remained constant during the assay. Differently from animal studies, a snack containing amaranth did not differ from placebo in reducing LDL-c levels in moderate hypercholesterolemic subjects. These results might be explained by possible differences of lipid metabolism in rabbits, rats, chicken, and humans due to the moderate hypercholesterolemia present in the subjects. We cannot rule-out the hypocholesterolemic effect of amaranth in humans based on the present results. They probably result either from the low protein concentration (7 g/day) or small sample (11 subjects) used in this study. The reduction of HDL-cholesterol is an indication that amaranth affects lipid metabolism in humans. Previously reported reductions in LDL-c in rabbits were obtained when 50% of food ingested by those animals were extruded amaranth (PLATE; ARÊAS, 2002). The intake level obtained in the present work was possibly not sufficient to reduce significantly LDL-c levels in those subjects. Therefore, higher amaranth concentrations to achieve at least

25 g protein/day must be tested in human subjects in order to detect any possible effect of this food on total and LDL-c. One important finding of our study was the significant reduction in HDL-c plasma levels with amaranth consumption. This implicates in possible deleterious consequences since low HDL-c is an independent risk factor for atherosclerosis. Similarly, lower HDL-c levels were also found in amaranth fed rabbits (PLATE; ARÊAS, 2002), and it has not been clearly understood yet. HDL-c reduction in both rabbits and humans could be explained by several mechanisms. A reduced methionine/lysine ratio is observed in amaranth, similarly to what is observed in soy. The low methionine/lysine ratio observed in soy has been associated with the reduction in HDL-c secretion in rats (MORITA et al., 1997). This reduction might be also related to the possible effects of amaranth protein reducing cholesterol levels and to the higher sensitivity of HDL-c to this reduction. However, the exact mechanism must be further elucidated.

4 Conclusion

In conclusion, the consumption of 50 g of extruded amaranth daily (ca 7 g of protein/day) for 60 days did not significantly reduce total or LDL-c in moderate hypercholesterolemic subjects. It was observed, however, a significant reduction in HDL-c following this consumption. A reduction in total cholesterol in the subjects that consumed amaranth snack for 60 days was also observed although this reduction was not significant ($p = 0.17$). Further studies with greater number of subjects and higher intake of this food are necessary to test the effects of amaranth on lipid metabolism in humans.

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