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Effects of Intake of Processed Quinoa Seeds on Lipid Profile in Patients with Coronary Heart Disease

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Running Title: Intake of Quinoa Seeds on Lipid Profile

Abstract: Background: Quinoa (*Chenopodium quinoa Willd*) is a gluten-free pseudocereal with high biological value protein, low glycemic carbohydrates, phytosterols and omega-3 and omega-6 fatty acids. It originated in the Andean region and has been consumed for thousands of years. Our objective was to assess blood cholesterol levels in coronary heart disease (CHD) patients in an outpatient clinic after the intake of processed quinoa seeds. Methods: This is a prospective study with twenty-seven patients, aged between 48 and 70 years (64.0 ± 8.4 yo), who were treated during 120 to 200 days. Blood samples were collected before and after the intake of quinoa seeds to determine the lipid profile of the group. Results: The results showed beneficial effects of the intake of quinoa with a significant reduction in total cholesterol (P=0.0008), triglycerides (P=0.001) and LDLc (P=0.008). Conclusions: The incorporation of quinoa in the eating routine can be considered beneficial in the prevention and control of risk factors for cardiovascular diseases, considered the major causes of death worldwide.

Keywords: Quinoa; Dyslipidemia, Coronary Heart Disease, Biochemical Markers

Introduction

Quinoa (*Chenopodium quinoa Willd*) is a plant in the Amaranthacea family, which is extremely tolerant and resistant to climatic stress. It originated in the Andean regions of South America and has been cultivated for over 7,000 years. Quinoa is an excellent source of protein, as it contains mainly lysine, an essential amino acid not usually available in cereals [1]. With an unusual composition and exceptional balance between protein, oil and fat, quinoa is an extraordinary example of functional food, which aims to reduce the risk of various diseases [2].

Therefore, cereals and pseudocereals, such as quinoa, have been extensively investigated for their protective effect in cardiovascular diseases [3]. Some studies have demonstrated the beneficial effects associated with the intake of this crop food on the control of blood pressure, diabetes, obesity and lipid profile. In relation to quinoa, this effect has been attributed to its composition of soluble and insoluble fibers, vitamin E, zinc, iron, magnesium and phytosterols, among other compounds [4].

According to the World Health Organization (WHO), quinoa is considered as unique due to its very high nutritional value. It was rated as the best food of plant origin for human consumption by the United States Academy of Sciences and selected by the National Aeronautics and Space Administration (NASA) to integrate the astronauts' diet into long-duration space flights [5].

With the discovery of its properties, quinoa began to arouse worldwide interest. In America, it can be found from Canada to the south of Chile, with different names, such as “quinoa” or “quinua” in

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Peru, Chile, Argentina and Bolivia, and "suba" [6] in Colombia.

Quinoa stands out as an important source of protein for humans due to its digestibility and essential amino acids composition [7,8].

The ability of the various nutrients found in pseudocereals to reduce the risk of coronary heart disease (CHD) has not been fully clarified, mainly due to their numerous consumption patterns in different populations. However, in the late 1990s, five large prospective cohort studies with 207,000 participants demonstrated an inverse association between intake of grains and cereal fiber and risk of cardiovascular disease [9].

Thus, this study aimed to prospectively assess the effects of the intake of processed quinoa seeds by CHD patients on lipid profile and glycemic control.

Casuistic and Methods

After the studied had been approved by the Research Ethics Committee of the Medical School of São José do Rio Preto (Process Number 136/2009), all 27 participants were informed about the study and confirmed their willingness to participate by signing an Informed Consent Form. All patients had hyperlipidemia and were treated at a University Hospital's Cardiology Outpatient Clinic. They were also advised on the use and consumption of processed quinoa as food supplement in the first and follow-up sessions.

The following biochemical markers were tested: total cholesterol (TC), low density lipoprotein cholesterol (LDLc), high density lipoprotein cholesterol (HDLc), very low density lipoprotein cholesterol (VLDLc), triglycerides (TG) and blood glucose before the quinoa intake period (Phase 1), at the end of the intake period (180 days on average, 60-300 days) (Phase 2), and after the end of the intake period (Phase 3).

In this study, the following values were adopted according to the Brazilian Guidelines on Obesity [10]: eutrophic individuals with body mass index (BMI) of 18.5-24.9 kg/m^2; overweight individuals with BMI of 25-29.9 kg/m^2; and obese individuals with BMI of 30-39.9 kg/m^2.

The reference values used for CT, LDLc, HDLc, VLDLc, TG and blood glucose were 200 mg/dl, 130 mg/dl, 40 mg/dl, 50 mg/dl, 150 mg/dl and 100 mg/dl, respectively. The results were compared using absolute and relative data with the Chi-Square test, Mann-Whitney test and paired t-test. The significance level was set at $P<0.05$.

Results

Twenty-seven patients were studied (59% males), where 35% were eutrophic, 45% were overweight and 20% were obese. Regarding blood glucose levels, 62% of patients were normoglycemic and 38% of them were hyperglycemic. All patients were on combined drug therapy for the treatment of heart diseases.

The patients consumed the pseudocereal for approximately 120 to 200 days followed by the laboratory analysis of biochemical markers. After a period of 90 to 200 days without pseudocereal intake, laboratory analysis of these markers was once again conducted in eutrophic, overweight and obese CHD patients.

Tables 1 to 4 show the laboratory analysis of biochemical markers in the total group, in relation to Body Mass Index (BMI), gender and cut-off levels before the intake of the pseudocereal, respectively.

Table 1. Results of the laboratory analysis of biochemical markers in the total group during Phases 1, 2 and 3 of intake of processed quinoa seeds

	Phase 1	Phase 2	Phase 3	P- Value
Blood glucose	140.8±55.1	114.1±42.1	120.1±46.2	0.1246
Triglycerides	216.9±124.8	132.2±55.2 #	160.2±83.5	# 0.0033
Total Chol.	203.4±51.7	158.1±49.1 #	155.7±49.1##	#0.0022;##0.004
HDLc	51.9±17.1	50.4±12.9	56±23.2	0.5241
VLDLc	39.9±25.5	35.5±14.9	34.3±12.2	0.5209
LDLc	118.2±50.4	92.9±24.4 #	102±22.0	# 0.0244

= comparison between Phase 1 of intake of quinoa vs Phase 2 of intake of quinoa
= comparison between Phase 1 of intake of quinoa vs Phase 3 of intake of quinoa

There was a significant reduction in levels of triglycerides, total cholesterol and LDLc between Phase 1 and Phase 2 (P=0.0033; P=0.0022; P=0.0244, respectively); while the same was observed for TC in Phase 3 (P=0.004) (Table 1). Subgroup analysis also demonstrated a significant reduction of blood glucose and triglycerides in obese patients between Phase 1 and Phase 2 (P=0.0497, P=0.0166, respectively), and triglycerides and VLDLc in eutrophic between Phase 1 and Phase 3 (P=0.0424, P=0.0182, respectively) (Table 2).

Table 2. Results of the laboratory analysis of biochemical markers in the total group during Phases 1, 2 and 3 of intake of processed quinoa seeds, in relation to BMI

		Phase 1	Phase 2	Phase 3	P- Value
Blood	Eutrophic	134±62.0	96.4±7.4	106±10.1	0.1646
	Overweight	124.9±38.3	134±58.4	134.3±65.8	0.9113
Glucose	Obese	161.5±64.8	108.9±34.0#	115.7±37.1	# 0.0497
	Eutrophic	234.8±112.7	148.1±71.7	160.7±69.7	0.1603
Triglycerides	Overweight	217.3±168.0	131.3±67.21	162.7±121.0	0.3173
	Obese	204±88.4	121.9±23.5#	157.4±47.4	# 0.0166
	Eutrophic	218.3±41.8	158.7±60.6	140.7±60.7##	## 0.0424
Total Chol.	Overweight	193.8±57.9	159.4±48.0	159.3±35.7	0.1998
	Obese	202.7±53.9	156.5±47.0	162.4±56.8	0.1225
	Eutrophic	58.1±16.0	56.7±9.9	63.7±12.2	0.5754
HDLc	Overweight	53.0±23.8	48.9±14.8	60.8±29.0	0.5189
	Obese	46.3±11.3	47.7±12.3	45.8±20.6	0.9595
	Eutrophic	35.8±23.9	40.9±13.9	42.6±12.8	0.7618
VLDLc	Overweight	46.7±33.6	36.9±19.1	29.3±13.2	0.2066
	Obese	34.8±17.2	30.3±9.8	33.2±6.5	0.7152
	Eutrophic	154.0±56.6	105.9±25.2	93.72±16.8##	## 0.0182
LDLc	Overweight	94.6±38.1	82.1±13.7	96.5±15.1	0.3874
	Obese	116.8±46.1	94.7±29.2	113.3±27.5	0.3371

= comparison between Phase 1 of intake of quinoa vs Phase 2 of intake of quinoa
= comparison between Phase 1 of intake of quinoa vs Phase 3 of intake of quinoa

In the comparison between the normoglycemic and hyperglycemic subgroups, the effect of quinoa was significant only in total cholesterol among normoglycemic patients (P=0.0019; P=0.0023) and HDLc among hyperglycemic patients (P=0.001; P=0.0018), maintaining a significant difference in Phase 3 (Table 3).

Table 3. Results of the laboratory analysis of biochemical markers in the total group during Phases 1, 2 and 3 of intake of processed quinoa seeds, according to blood glucose level

		Phase 1	Phase 2	Phase 3	P- Value
Triglycerides	Normogl.	223.9±35.4	138.4±60.3	181.3±28.9	0.11
	Hipergl.	215.4±39.5	139±62.6	179±35.5	0.2673
Total Chol.	Normogl.	233.4±43.1	149.4±35.0#	151.1±36.5##	#0.0019;##0.0023
	Hipergl.	180.8±34.8	160.2±57.2	168±47.2	0.6224
LDLc	Normogl.	133.1±46.0	102.6±10.1	100.8±13.8	0.084
	Hipergl.	105±50.1	88.6±35.3	108.8±30.7	0.4945
HDLc	Normogl.	58.9±13.1	55.2±10.5	67.1±16.3	0.2693
	Hipergl.	45.7±10.3	47.9±11.1#	54.8±28.8##	#0.001; ##0.0018

= comparison between Phase 1 of intake of quinoa vs Phase 2 of intake of quinoa
= comparison between Phase 1 of intake of quinoa vs Phase 3 of intake of quinoa

Regarding gender, the best result was the reduction of triglycerides and total cholesterol (P=0.0037; P=0.0037) among men, and LDLc (P=0.0258; P=0.0314) and HDLc (P=0.0004) among women (Table 4).

Table 4. Results of the laboratory analysis of biochemical markers in the total group during Phases 1, 2 and 3 of intake of processed quinoa seeds, according to gender (Males=M; Females=F).

		Phase 1	Phase 2	Phase 3	P-Value
Triglycerides	M	217.9±124.5	127.5±51.2 #	147.3±49.1	# 0.0094
	F	215.4±131.1	139±62.6	179±117.8	0.2673
Total Chol.	M	184.5±47.9	135.3±27.11#	135.343.6 ##	# 0.0037;## 0.0037
	F	231±45.6	191.3±55.9	185.1±44.7	0.0746
LDLc	M	97.9±36.1	85.6±20.0	100±21.4	0.2820
	F	147.8±54.1	103.5±27.2 #	104.9±23.6##	# 0.0258;# 0.0314
HDLc	M	61.8±17.9	54.7±13.8	59.4±20.36	0.5594
	F	151.1±45.4	153.1±25.8	155.5±21	# 0.0004

= comparison between Phase 1 of intake of quinoa vs Phase 2 of intake of quinoa
= comparison between Phase 1 of intake of quinoa vs Phase 3 of intake of quinoa

Discussion

A study with animal models analyzed the blood of rats fed with processed quinoa seeds and showed an effective reduction of total cholesterol and triglycerides, compared with controls [11].

In a recent study [12], 22 patients (18-45 years), who consumed quinoa cereal bars during 30 days, showed a significant reduction in total cholesterol levels among women, but not among men.

Hirose et al. [13] analyzed the crude extracts from quinoa seeds cultivated in Japan, which showed higher antioxidant effects than those from South America.

Variations in antioxidant activity of quinoa genotypes were already expected, since several factors (genetic and agro-technical processes and environmental conditions) may influence the presence of phenolic compounds [14]. Also, the agronomic, physical, nutritional and antioxidant properties of six different genotypes of quinoa cultivated in three different geographic zones of Chile were analyzed. Such contrasting features might be associated with sow-to-harvest period and sensibility to higher temperatures. In contrast, the crop harvested in the southern region stood out in terms of total dietary fiber, i.e., 12.08g of fiber per 100g of the analyzed seeds [15].

Another study assigned 13 patients with type 2 diabetes mellitus to follow two types of diet for six weeks: a diet containing moderate amounts of fiber (total, 24g, 8g soluble fiber and 16g of insoluble fiber), as recommended by the American Diabetes Association (ADA), and a high-fiber diet (total, 50g, 25g of soluble fiber and 25g of insoluble fiber). Both diets were prepared in a research kitchen and had the same macronutrient and energy content. The effects of both diets on glycemic control and plasma lipid concentrations were compared and a reduction on plasma lipid concentrations, triglyceride levels and LDLc concentrations could be observed. In conclusion, a high intake of dietary fiber, especially of the soluble type, above the level recommended by the ADA promotes the following benefits: improving glycemic control, decreasing hyperinsulinemia and reduced plasma lipid concentrations in patients with type 2 diabetes mellitus [16].

As a high-protein food, quinoa was added to dark chocolate bars in another study. The product showed increase in essential amino acids and was approved by 92% of the group of testers [17]. A rich-content protein beverage from mixing quinoa and lupine was also developed for children aged between 2 and 5 years with nutritional deficiencies. The beverage was stored for 90 days and subsequently tested. The results showed that the protein content was 1.36% [18].

Karlström et al. [19] studied the metabolic effects of an increased dietary content of cereal fiber in 14 type 2 diabetic individuals. These individuals received a diet containing 18.9g fiber/day during two consecutive periods of 3 weeks, and a significant reduction in mean blood glucose level could be observed.

Nsimba et al. [20] evaluated the antioxidant potential of extracts from quinoa and amaranth and found high levels of phenolic and non-phenolic compounds, which could justify such protective effect.

Quinoa stands out as an important source of protein for humans because of its digestibility and balanced essential amino acids composition. In experiments with rats, the analysis of protein efficiency coefficient, true digestibility and nitrogen balance showed similarity between the efficiency of the

protein in quinoa and milk protein [7]. This result could also be observed in a study with humans.

Jenkins et al. [21] assessed the effect of food products (breakfast cereals, breads, frozen pasta, cakes and biscuits) supplemented with Psylium (7.2g) and Beta-glucans (7.2, Oats), in 37 men and 31 postmenopausal and hypercholesterolemic women in a one-month period and found significant reductions in serum lipid concentrations, confirming the beneficial effect of dietary fiber intake.

A curious finding of the study is that quinoa is consumed in the Andean region, among other forms, as a fermented alcoholic beverage called "Chicha". The technique is over 1,000 years old and the preparation of the seeds begins with the germination. At this stage, the starch is broken and the substrate is made available for bacterial fermentation. The major natural bacteria found in quinoa are of the genus Lactobacillus (L. plantarum, L. fermentum and L. paralimentarius), which carry out lactic fermentation and have organic acids as the main end product of metabolism. The organic acids produced by the fermentation may delay gastric emptying [22].

Gewehr [23] prepared breads by replacing wheat flour with quinoa flakes. The composition was analyzed according to contents of amino acids, fibers, tocopherols and minerals, showing higher protein content, total fiber, and tocopherol. The increase in tocopherols in bread with 20% of quinoa improved the lipid profile of blood and liver fat of experimental animals, compared to white bread. Vannucchi & Jordão [24] report that tocopherols have antioxidant activities as they are incorporated into the lipid portion of cell membranes and play the role of protecting this structure from toxic compounds, radiation and free radicals, in addition to protecting cholesterol from oxidative damage.

All patients showed coronary heart diseases and hyperlipidemia; however, other risk factors may also be associated with cardiovascular complications. Thus, it could be observed that 55% of the patients had high blood pressure, 85% smoked regularly, 26% showed moderate alcohol consumption, and 63% of the patients had a sedentary lifestyle.

Dietary supplements have been referred to as adjuvants in an attempt to reduce the risk for coronary artery disease. In this study, the intake of processed quinoa aimed to reduce or control hyperlipidemia. It was found that 95% of the patients approved the taste of the pseudocereal and agreed to change their eating habits and include vegetables, fruits and legumes in their daily meals.

Several studies have demonstrated that the dietary intake of pseucereals provides beneficial effects in the studied populations [25]. Further studies show that fiber intake reduces the risk of cardiovascular diseases, hyperlipidemia, obesity and diabetes [4].

According to Berti et al. [26] the intake of quinoa, including soluble and insoluble fiber, may be considered nutraceutical as it reduces the levels of blood glucose, triglyceride and cholesterol. Our results in the present study demonstrate that quinoa can be used to reduce these types of hyperlipidemia.

Quinoa contains high amounts of vitamin E, zinc, manganese, phytosterols, as well as substances with hypocholesterolemic effects [27]. Antioxidants, polyphenols and flavonoids can also be found in processed quinoa. These substances are associated with the reduction of plasma lipids, blood glucose levels and cardiovascular diseases [28]. Matsuo [29] demonstrated that quinoa may have beneficial effects on reducing the production of antioxidant enzymes, in addition to decreasing LDLc oxidation and the risk of cardiovascular diseases.

Ando et al. [30] and Konish et al. [31] analyzed the minerals content of milled quinoa (sample of 100 g), and found higher levels of calcium (55.1 to 91.8 mg), phosphorus (360.2 to 411.0 mg), potassium (639.3 to 732.0 mg), magnesium (415.2 to 502.0 mg), iron (9.2 to 15.0 mg) and zinc (0.8 to 4.0 mg). Some studies have shown that quinoa seeds contain significantly higher amounts of these minerals when compared to most cereals commonly consumed in Brazil, such as wheat, maize, rice and oats [32-35]. Quinoa has a high concentration of lysine, unlike most pseudocereals [36,37].

The present study demonstrated that quinoa positively influences the reduction of lipidemic alterations and glycemic control, thus contributing to the reduction of risk for the development of cardiovascular diseases.

Conclusions

The use of processed quinoa seeds as food supplement for hyperlipemic patients allowed a significant reduction in the levels of blood glucose, total cholesterol, LDLc and triglycerides in specific subgroups and in specific phases, namely:

1. Significant reduction in levels of triglycerides, total cholesterol and LDLc between Phase 1 and

2. For total cholesterol, the effect was maintained in Phase 3.
2. Reduction in levels of blood glucose and triglycerides in obese individuals between Phase 1 and Phase 2, and triglycerides and VLDLc in eutrophic individuals between Phase 1 and Phase 3.
3. Significant reduction only in total cholesterol in normoglycemic patients and HDLc in hyperglycemic patients, maintaining a significant difference in the late stage.
4. Regarding gender, the best response was the reduction in levels of triglycerides and total cholesterol in male patients and LDLc in female patients.

The findings suggest that quinoa may be considered useful to reduce risk factors for cardiovascular disease. Long-term event studies should be conducted in order to confirm this hypothesis.

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Conflict of Interest Statement
The authors declare no conflict of interest. All authors have made substantial contributions to the manuscript, since the conception and design of the study, acquisition, analysis and interpretation of data, to drafting the article and for submission.

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