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Cowpea protein reduces LDL-cholesterol and apolipoprotein B concentrations, but does not improve biomarkers of inflammation or endothelial dysfunction in adults with moderate hypercholesterolemia

Karoline de Macêdo Gonçalves Frota[1], Raul Dias dos Santos[2], Valdenir Queiroz Ribeiro[3] and José Alfredo Gomes Arêas[4]

[1]Departamento de Nutrição, Universidade Federal do Piauí, Teresina-PI. [2]Instituto do Coração (InCor), Hospital das Clínicas, Faculdade de Medicina da Universidade de São Paulo, São Paulo-SP. [3]Empresa Brasileira de Pesquisa Agropecuária, Embrapa Meio Norte, Teresina-PI. [4]Departamento de Nutrição, Faculdade de Saúde Pública, Universidade de São Paulo, São Paulo-SP. Brazil.

Abstract

Introduction: **The risks of cardiovascular diseases, the leading cause of death in the world, can be reduced by diet. Cowpea protein has been shown to significantly reduce total cholesterol, LDL-cholesterol, and liver steatosis in hamsters.**

Objective: **The objective of this proof-of-concept study was to verify whether the consumption of cowpea protein improves lipid profile and biomarkers of inflammation and endothelial dysfunction in adults with moderate hypercholesterolemia.**

Methods: **In a randomized, double-blind, crossover design, 38 hypercholesterolemic subjects (LDL-cholesterol = 182.5 ± 2.7 mg/dL) consumed 25 g/day of cowpea protein isolate or 25 g/day of casein (control group) for 6 weeks each, separated by a 4-week washout interval. Fasting blood samples were collected at baseline and at the end of each diet period. Lipids (total cholesterol, LDL-cholesterol, triglycerides, HDL-cholesterol) were determined by enzymatic methods, apolipoproteins (apoA-I and apoB) by standardized immunoassays, inflammatory biomarkers (C-reactive protein) by turbidimetry, and biomarkers of endothelial dysfunction (intercellular adhesion molecule 1 and vascular cell adhesion molecule 1) by enzyme-linked immunosorbent assays.**

Results and discussion: **Consumption of cowpea protein significantly reduced total cholesterol (12 %), LDL-cholesterol (18.9 %), nonHDL-cholesterol (16 %) and apoB (14 %), and increased HDL-cholesterol (+2.7 %). No significant differences between treatment groups were observed for any of the serum inflammatory or endothelial dysfunction biomarkers.**

Conclusion: The present findings demonstrated the favorable effect of cowpea protein consumption on

Correspondence: Karoline de Macêdo Gonçalves Frota.
Departamento de Nutrição, Universidade Federal do Piauí,
Campus Ministro Petrônio Portela.
SG-13, Ininga – CEP: 64049-550. Teresina - PI, Brasil.
E-mail: karolfrota@ufpi.edu.br

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PROTEÍNA CAUPÍ REDUCE LAS CONCENTRACIONES DE LDL-COLESTEROL Y APOLIPOPROTEÍNA B, PERO NO MEJORA BIOMARCADORES INFLAMATORIOS Y DISFUNCIÓN ENDOTELIAL EN ADULTOS CON HIPERCOLESTEROLEMIA MODERADA

Resumen

Introducción: **Los riesgos de las enfermedades cardiovasculares, la principal causa de muerte en el mundo, pueden ser reducidos con la dieta. Proteína caupí en hámsters redujo el colesterol total, LDL-colesterol, así como la esteatosis hepática de manera significativa.**

Objetivo: **Este estudio de prueba de concepto fue verificar si el consumo de proteína de frijol mejora el perfil de lípidos y actúa sobre los biomarcadores de inflamación y disfunción endotelial en pacientes con hipercolesterolemia moderada.**

Métodos: **En un diseño aleatorio doble ciego cruzado, 38 sujetos con hipercolesterolemia (colesterol-LDL = 182,5 ± 2,7 mg/dL) consumieron 25 g / día de aislado de proteína de frijol o 25 g / día de caseína (grupo control) durante seis semanas cada uno, y un intervalo de lavado de cuatro semanas Se recogieron muestras de sangre en ayunas al comienzo y al final de cada período de dieta. Los lípidos (colesterol total, LDL-colesterol, triglicéridos, HDL-colesterol) se determinaron por métodos enzimáticos, apolipoproteínas (apoA-I y apoB) por inmunoensayos normalizados, biomarcadores de inflamación (proteína C reactiva) por turbidimetría y los biomarcadores de disfunción endotelial (molecule-1 de adhesión intercelular y de molécula-1 de adhesión celular vascular) por técnicas de ensayo de inmunoabsorción ligados a enzimas.**

Resultados y discusión: **El consumo de proteínas caupí redujo significativamente el colesterol total (12%), el colesterol LDL (18,9%), colesterol no HDL (16%), apoB (14%), y aumentó el colesterol HDL (2,7%). No se observaron diferencias significativas relacionadas con el grupo de tratamiento para cualquiera de los biomarcadores inflamatorios y de disfunción endotelial.**

Conclusión: Los presentes hallazgos demostraron el efecto favorable del consumo de proteína caupí en

proatherogenic serum lipids and apoB in subjects with moderate hypercholesterolemia, similar to what was observed in a previous studies on animals.

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Key words: *Cowpea*. *Plant proteins*. *Cholesterol*. *Apolipoproteins*. *Inflammation*.

lípidos séricos pro-aterogénicas y apoB en sujetos con hipercolesterolemia moderada, de manera similar a lo observado en un trabajo previo con los animales.

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Palabras clave: *Caupi*. *Proteínas de plantas*. *Colesterol*. *Apolipoproteínas*. *Inflamación*.

Abbreviations

CVD: cardiovascular disease.
LDL-C: low-density lipoprotein cholesterol.
TNFα: tumor necrosis factor α.
IL1: interleukin 1.
CRP: C-reactive protein.
VCAM1: vascular cell adhesion molecule 1.
ICAM1: intercellular adhesion molecule 1.
CHD: coronary heart disease.
CPI: cowpea protein isolate.
BMI: body mass index.
HDL-C: high-density lipoprotein cholesterol.
NonHDL-C: nonHDL cholesterol.
ApoA-I: apolipoprotein AI.
ApoB: apolipoprotein B.
SEM: standard error of the mean.
kg: kilogram.
cm: centimeter.
kJ: kilojoule.
g: gram.

Introduction

Cardiovascular diseases (CVD) are the leading cause of death and disability in the world[1]. In the United States, CVD account for 31.9% of all deaths[1]. A large body of evidence has established a direct and causal association between elevated blood cholesterol and increased CVD risk[2-4]. There is undisputable evidence that lowering cholesterol reduces morbidity and mortality due to CVD. The impact on CVD risk appears to be similar whether low-density lipoprotein cholesterol (LDL-C) is reduced by dietary or surgical intervention (ileal bypass), or by medications[2, 5].

Atherosclerosis is a multifactorial disease and etiological factors include low-grade subclinical inflammation and endothelial dysfunction[6]. Subclinical inflammation plays an important systemic role in the development of CVD. Inflammatory biomarkers such as tumor necrosis factor α (TNFα), interleukin 1 (IL1), and C-reactive protein (CRP) have been associated with the atherosclerotic process[7]. All of these factors induce the expression of cell adhesion molecules such as vascular cell adhesion molecule 1 (VCAM1) and intercellular adhesion molecule 1 (ICAM1) which, in turn, mediate the adhesion of leukocytes to the vascular endothelium[8].

Diet is an important modifiable risk factor for many types of heart disease[5]. Legume consumption has been associated with a lower risk of coronary heart disease (CHD) in observational epidemiological studies, and has been shown to decrease total cholesterol and LDL-C in clinical trials[9-11]. However, most studies evaluating the hypocholesterolemic effects of legume consumption specifically examined soybean rather than the many non-soy legumes which are more commonly consumed in Western countries[12]. In this respect, a strong hypocholesterolemic effect of cowpea protein has been demonstrated in a recent animal study[13].

The aim of this proof-of-concept study was to evaluate the effect of consumption of a ready-to-drink product made from cowpea protein on serum lipids and apolipoproteins in moderately hypercholesterolemic adults. The effect of ingestion of this protein on biomarkers of systemic inflammation (CRP) and endothelial dysfunction (VCAM1 and ICAM1) was also investigated.

Materials and methods

Study design

Forty-four eligible men and women were randomly assigned to one of two dietary treatments in a double-blind, placebo-controlled, crossover study. Treatment consisted of the consumption of either 25 g of a ready-to-drink product made from cowpea protein isolate (CPI) or a comparative control (25 g of casein). The test foods were offered in the form of a shake consumed daily for 6 weeks. After a 4-week washout period, the subjects received the opposite test food for an additional 6 weeks.

Written informed consent was obtained from the participants before the initial screening visit and before randomization. The study protocol was approved by the institutional review boards of Hospital das Clínicas, University of São Paulo Medical School, and of the Federal University of Piauí.

The participants of this study were men (30 to 70 years of age) and postmenopausal women (45 to 70 years of age) with mild or moderate hypercholesterolemia. The inclusion criterion was LDL-C concentration ≥ 160 and ≤ 190 mg/dL. The exclusion criteria were: lipid-altering drug therapy (an at least 8-week washout); CHD or its equivalent as defined by the NCEP

ATP-III[2]; uncontrolled hypertension (systolic blood pressure > 160 mm Hg or diastolic blood pressure > 100 mm Hg); significant endocrine (including diabetes mellitus and familial hypercholesterolemia), hepatic, renal, pancreatic, eating, and gastrointestinal disorders; clinically significant laboratory results including, but not limited to, plasma triglycerides ≥ 400 mg/dL and fasting glucose ≥ 126 mg/dL; extreme dietary habits; body mass index (BMI) > 35 kg/m^2; use of weight-loss medications or products; consumption of > 14 alcoholic drinks per week, and hormone replacement therapy within 6 months prior to the screening visit and throughout the study. The subjects were recruited during routine outpatient visits to the cardiology, gynecology and endocrine clinics of Hospital Getúlio Vargas and Centro Integrado Lineu Araújo, Teresina, Piauí, Brazil, between May and October 2010. The state of menopause was assumed if no vaginal bleeding had occurred in the last 12 months preceding the study. Functional foods or dietary supplements expected to alter lipid metabolism were not allowed after screening. The participants were advised to maintain their usual life and nutrition habits, to minimize differences in daily energy intake, to maintain their body weight, and to avoid the use of lipid-lowering drugs throughout the study. The physical characteristics and nutrient intake of the subjects were documented.

Study participants

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Test products and intervention

The treatments consisted of a CPI and casein (control) beverage powder (Linea Sucralose, Anapolis, Brazil) in two 30-g packets/day. The subjects were asked to incorporate two servings/day into their normal diet during the intervention period, one during breakfast and one in the late afternoon or early evening. During the CPI treatment phase (CPI shake), the subjects drank shakes similar to those consumed during the control phase (control shake: 25 g casein/day), except that the treatment phase products were formulated with cowpea protein (25 g CPI/day). The CPI was produced by Bremil Arroio do Meio Ltda. (Rio Grande do Sul, Brazil) by alkaline extraction of protein from defatted cowpea flour, centrifugation (10,000 x *g*) and acid (pH 4.5) precipitation of the protein, followed by centrifugation and spray drying of the precipitate[14]. The CPI shake had an average nutrient content of 108.3 kcal energy, 12.6 g protein, 12.1 g carbohydrate, 0.4 g fat, and 337.2 mg calcium. The casein shake had an average nutrient content of 106.01 kcal energy, 14.1 g protein, 10.1 g carbohydrate, 0.3 g fat, and 385.9 mg calcium. Two flavors were offered to the volunteers: strawberry and mixed fruit. The subjects perceived the CPI and control shakes as identical in appearance and taste. To ensure blinding, the study participants received their supplies in packages labeled with three digits without any other information. This code was kept secret from all participants, including the researchers. In the middle of each treatment period, staff contacted the participants by telephone to encourage compliance with consumption of the study product.

Laboratory tests

Blood samples (20 mL) were collected into tubes without anticoagulant after a 12-hour fast. The samples were immediately centrifuged at 1,100 x *g* for 15 minutes at 4 °C, serum was separated, and aliquots were stored at -80 °C until the time of analysis. Serum total cholesterol, triglycerides, and direct HDL-cholesterol (HDL-C) were measured by automated enzymatic methods using Roche Diagnostic kits (Sao Paulo, Brazil). LDL-C concentration (mg/dL) was calculated according to the Friedewald formula[15] for triglyceride levels < 400 mg/dL. NonHDL-cholesterol (NonHDL-C) was calculated by subtracting HDLC from total cholesterol. Apolipoproteins (apoAI and apoB) were measured by automated standardized immunoassays (Roche Diag-

nostics). Soluble adhesion molecule concentrations (sICAM1 and sVCAM1) were analyzed in duplicate in a single run using enzyme-linked immunosorbent assays (R & D Systems®, Minneapolis, MN, USA). Serum highly sensitive CRP concentrations were assessed by turbidimetry (BioTécnica®, Sao Paulo, Brazil) in a Cobas Mira Plus (Roche®) device according to manufacturer instructions. Glucose concentrations were measured by an enzymatic colorimetric method (Labtest, Belo Horizonte, Brazil).

Clinical and nutritional evaluation

A baseline questionnaire was administered to record demographic and lifestyle characteristics and medical history. Body weight, height and waist circumference were measured at baseline and at the end of each experimental period. BMI (kg/m^2) was calculated as body weight/height^2. Nutritional evaluation was performed by the application of three 24-h dietary recalls at baseline and at the end of each phase. Dietary composition was analyzed using the NutWin® nutrition support program (v. 1.5)[16]. Side effects and compliance were assessed using a symptom questionnaire, counts of returned unconsumed packets, and self-reported supplement use.

Statistical analysis

Data are reported as the mean ± standard error of the mean (SEM), except for CRP, sVCAM-1 and sICAM-1 which are expressed as means (95% confidence interval). Differences in serum lipids, apolipoproteins, inflammatory biomarkers, anthropometric measures and food consumption as a function of type and order of the ingested shakes were evaluated by three-factor repeated measures ANOVA, assuming structured correlations of symmetric components between assessments. When statistically significant results were obtained, multiple comparisons were performed by the Bonferroni method[17] to verify which treatment was the most significant and whether the ingestion order influenced the results. The difference in each lipid and apolipoprotein concentration (delta: end - beginning) was calculated for each shake. The percent variation [delta %: (end beginning)/beginning] was also calculated for lipids and apolipoproteins and related to each shake and ingestion order. The mean variations in each parameter according to shake are reported with their standard error, adopting a level of significance of 5%. The sample size was based on a predicted 21 mg/dL reduction in total cholesterol after consumption of CPI. The variability in the outcome parameter was estimated at 40 mg/dL and the standardized effect size was 0.52. The adopted statistical power was 80% ($\beta = 0.20$, $\alpha = 0.05$) and the calculated sample size was 35 subjects. All calculations were performed using the SAS software (Cary, NC, USA)[18].

Results

Forty-four subjects were initially assigned to the two groups for 6 weeks, including 23 in the casein group and 21 in the CPI group. After a 4-week washout period, the subjects received the opposite test food for an additional 6 weeks.

The CPI and casein shakes were well accepted by most participants. Among the 44 subjects who agreed to participate in the study, only six (13%) did not complete it. The reasons for study drop out were: missing some appointments (n =1); illness (n = 2); changes in the clinical exams (weight gain) (n = 1), and failure to consume the products (n = 2). Table I shows the baseline clinical and laboratory characteristics of the subjects who completed the study (n = 38).

Table II shows the anthropometric characteristics and daily dietary nutrient intake based on 24-h recalls before and after consumption of the casein and CPI shakes. The interventions did not result in significant changes in body weight, BMI or waist circumference during the study. The subjects consumed similar daily amounts of food during the study period. There were no significant differences in energy, macronutrient or saturated, monounsaturated and polyunsaturated fat intake.

Table III shows the concentrations of serum lipids and apolipoproteins before and after consumption of the test shakes, as well as the percent changes in each phase. The consumption of CPI resulted in a decrease in total cholesterol, LDL-C, nonHDL-C, and apoB concentrations (all p values < 0.001). There were significant, although small, increases in HDL-C ($p = 0.047$). No significant differences were observed in triglyceride or apoAI concentrations.

Table IV shows the mean net changes in serum lipids and apolipoproteins when consumption of casein was crossed over to CPI or vice-versa after the washout period. A significant net reduction in serum total cholesterol, LDL-C, non-HDL-C, and apoB was observed for CPI consumption, irrespective of ingestion order (all p values < 0.001). In contrast, HDL-C showed a net increase of 5.6% ($p = 0.044$).

The comparison of inflammatory and endothelial dysfunction biomarkers between the CPI and casein shakes is shown in table V. No significant changes in these biomarkers were observed during any phase of the study.

The main side effects observed after the consumption of cowpea protein were flatulence (10.5 %, n = 4), obstipation (7.9 %, n = 3), and an increase in stool softening (5.3 %, n = 2). The side effects observed after the consumption of casein protein were flatulence (7.9 %, n = 3), obstipation (2.6 %, n = 1) and an increase in stool softening (5.3 %, n = 2). No patient dropped out of the study due to side effects.

Table I
Baseline clinical and laboratory characteristics of the study participants (n = 38)

Characteristic	*Average*
Age, years	57.0 (1.7)
Women, n (%)	32 (84 %)
Current smoking, n (%)	3 (7.9 %)
Alcohol drinking, n (%)	9 (23.7 %)
Body weight, kg	66.7 (2.3)
Body mass index, kg/m^2	27.3 (0.6)
Waist circumference, cm	88.8 (1.6)
Total cholesterol, mg/dL	270.6 (21.5)
LDL-C, mg/dL	182.6 (15.9)
HDL-C, mg/dL	56.4 (9.8)
Triglycerides, mg/dL	157.3 (63.3)
Non-HDL-C, mg/dL	214.2 (19.9)
Apolipoprotein B, mg/dL	131.0 (3.0)
Apolipoprotein A-I, mg/dL	151.0 (3.0)
Fasting glucose, mg/dL	101.6 (12.6)
CRP, mg/L	1.62 (1.24, 2.00)
Soluble VCAM-1, ng/mL	231.3 (203.9, 258.7))
Soluble ICAM-1, ng/mL	117.0 (101.4, 132.6)

[1]Values are means (SEM), except for C-reactive protein (CRP), soluble vascular adhesion molecule 1 (VCAM-1), and soluble intercellular adhesion molecule 1 (ICAM-1) which are reported as means (95% confidence interval).

Discussion

This is the first study that investigated and showed favorable effects of CPI in reducing serum proatherogenic lipids and apoB in moderate hypercholesterolemic adults. Although Brazil is one of the largest producers of cowpea in the world[19], most studies investigating the effects of vegetable protein on plasma lipids and biomarkers have used soy protein[12, 20-21]. Our results reproduced the favorable effects of cowpea protein on plasma lipids demonstrated in an animal model[13]. Nevertheless, the consumption of CPI did not change biomarkers of subclinical inflammation or endothelial dysfunction. The consumption of CPI was well tolerated.

The most remarkable findings of this study were the consistent and robust reductions in LDL-C and nonHDL-C induced by the consumption of 25 g/day of cowpea protein. Additionally, a reduction in apoB concentrations was observed, suggesting that CPI consumption reduced not only cholesterol content, but also the concentration of proatherogenic serum lipoproteins. The present findings are similar to the effects of soy protein reported in humans[22, 23]. Cowpea protein also exerted favorable effects on HDL-C, although to a lesser extent.

The relative and absolute changes in proatherogenic serum lipids and apolipoproteins observed in the present study can be considered robust when these findings are compared to the results of three meta-analyses that evaluated the effect of soy protein supplementation on serum lipids. Anderson et al.[20] showed that an average soy protein intake of 47 g per day was associated with the following net changes in serum lipid concentrations compared to the concentrations achieved with the control diet: a 9.3% decrease in total cholesterol and a 12.9 % decrease in LDL-C. Reynolds et al.[21] observed an overall pooled net absolute effect of soy protein supplementation on serum lipids of 5.3 *m*g/dL for total cholesterol, 4.2 mg/dL for LDL-C, and 0.8 *m*g/dL for HDL-C. Anderson and Bush[24] showed that soy protein intake was associated with net reductions of 5.5% and 4.2% in serum LDL-C in parallel and crossover studies, respectively.

Reductions in LDL-C concentrations are one of the most powerful strategies to decrease CVD risk. The average reduction of 33 mg/dL in this lipid fraction found in the present study is of significant clinical importance. A recent meta-analysis including 170,000 subjects who received lipid-lowering therapy with statins showed that each 40 mg/dL decrease in LDL-C reduces the risk of major atherosclerotic events by 22%[25]. Furthermore,

Table II
Anthropometric characteristics and nutrient intake before and after consumption of the cowpea protein isolate and casein shakes (n = 38)

	Treatment group				
	Cowpea protein isolate		*Casein*		
	Baseline	*Week 6*	*Baseline*	*Week 6*	*P-value*
Anthropometric variable					
BMI, kg/m^2	27.3 (0.6)	27.2 (0.6)	27.1 (0.6)	27.2 (0.6)	0.91
Body weight, kg	66.9 (2.3)	66.7 (2.4)	66.3 (2.3)	66.6 (2.3)	0.73
Waist circumference, cm	88.4 (1.5)	88.5 (1.6)	88.2 (1.5)	88.3 (1.5)	0.67
Nutrient intake					
Energy, kcal	1,344.6(95.6)	1,400.3 (70.8)	1,388.3(84.55)	1,370.1(89.4)	0.47
Carbohydrate, g	198.5 (16.3)	181.8 (9.6)	184.7 (14.1)	171.1 (14.1)	0.08
Protein, g	71.7 (5.4)	92.3 (6.9)	80.4 (6.7)	90.7 (5.6)	0.73
Fat, g	34.8 (3.7)	33.7 (3.2)	41.9 (5.8)	34.5 (3.1)	0.34
Saturated fat, g	9.3 (1.2)	8.2 (1.0)	9.4 (0.9)	8.3 (0.9)	0.85
Polyunsaturated fat, g	7.3 (0.8)	6.7 (0.6)	7.0 (0.7)	7.2 (0.7)	0.18
Monounsaturated fat, g	10.3 (2.0)	8.7 (1.0)	9,1 (0,8)	9.4 (1.2)	0.50
Cholesterol, mg	138.2 (22.8)	187.9 (22.7)	178,6 (16,6)	165.7 (40.3)	0.01
Fiber, g	17.1 (2.2)	11.7 (1.2)	14,3 (1,8)	11.8 (1.6)	0.05

[1]Values are means (SEM). The P-value corresponds to the difference between treatments using repeated measures ANOVA.

Table III
Serum lipid and apolipoprotein concentrations before and after consumption of the cowpea protein isolate and casein shakes (n = 38)[1]

	Treatment group				
	Cowpea protein isolate		*Casein*		
Biomarker	*Baseline*	*Week 6*	*Baseline*	*Week 6*	*P-value*
TC, mg/dL	264.4 (3.8)	232.3 (4.4)	265.4(4.2)	254.3 (4.5)	< 0.001
TC, % change	-12.1 (1.1)		-3.7 (1.8)		< 0.001
LDL-C, mg/dL	174.9 (3.0)	141.9 (3.6)	174.5 (3.2)	165.0 (3.6)	< 0.001
LDL-C, % change	-18.9 (1.5)		-4.5 (2.5)		< 0.001
HDL-C, mg/dL	56.0 (1.4)	57.3 (1.5)	56.6 (1.6)	54.6 (1.5)	0.041
HDL-C, % change	2.7 (2.1)		- 2.8 (1.9)		0.047
TG, mg/dL	166.3(11.1)	163.5(14.3)	165.5(8.8)	173.0(9.7)	0.406
TG, % change	1.3 (5.5)		8.6 (5.7)		0.358
Non-HDL-C, mg/dL	208.3(3.5)	175.0(4.2)	208.8(3.9)	199.8(4.3)	< 0.001
Non-HDL-C, % change	-16.0 (1.5)		- 3.8 (2.0)		< 0.001
ApoA-I, mg/dL	153.0 (3.0)	153.0 (3.0)	151.0 (3.0)	149.0 (4.0)	0.509
ApoA-I, % change	0.6 (1.6)		- 1.3 (1.3)		0.349
ApoB, mg/dL	131.0 (3.0)	112.0(3.0)	128.0 (2.0)	125.0 (3.0)	< 0.001
ApoB, % change	-14.0 (1.5)		- 2.2 (2.1)		< 0.001

[1] Values are means (SEM). The P-value corresponds to the difference between baselines values and values obtained after 6 weeks using repeated measures ANOVA. TC: total cholesterol; TG: triglycerides.

Table IV
Net (mg/dL) and percent (%) changes in serum lipid and apolipoprotein concentrations in subjects ingesting cowpea protein isolate compared to those consuming casein (n = 38)[1]

Biomarker	*Cowpea protein isolate vs casein*	*P-value*
Total cholesterol, mg/dL	-21.05 (4.40)	< 0.001
Total cholesterol, %	-8.4 (1.7)	< 0.001
LDL-C, mg/dL	-23.53 (5.40)	< 0.001
LDL-C, %	-14.4 (2.5)	< 0.001
HDL-C, mg/dL	3.24 (1.56)	0.044
HDL-C, %	5.6 (2.7)	0.043
Non-HDL-C, mg/dL	-24.29 (4.27)	< 0.001
Non-HDL-C, %	-12.1 (2.1)	< 0.0001
ApoB, mg/dL	-15.55 (3.43)	< 0.001
ApoB, %	-11.7 (2.6)	< 0.001

[1]Variations in the parameters that differed between treatments. Multiple comparisons by the Bonferroni method ($p < 0.05$). Values are means (SEM).

Table V
Serum levels of C-reactive protein (mg/L), soluble intercellular adhesion molecule 1 (ng/mL) and soluble vascular cell adhesion molecule 1 (ng/mL) before and after consumption of the cowpea protein isolate and casein shakes (n = 38)[1]

	Treatment group				
	Cowpea protein isolate		*Casein*		
	Baseline	*Week 6*	*Baseline*	*Week 6*	*P-value*
CRP	1.96 (1.35, 2.57)	2.43 (1.68, 3.18)	1.91 (1.49, 2.33)	2.27 (1.71, 2.83)	0.838
sVCAM-1	246.1 (220.5, 271.7)	252.9 (219.7, 286.1)	246.3 (215.5, 277.1)	266.5 (234.2, 298.9)	0.401
sICAM-1	127.3 (111.3, 143.5)	137.9 (117.7, 158.2)	123.5 (105.1, 141.8)	138.6 (116.2, 161.0)	0.644

CRP: C reactive protein; sVCAM-1: soluble vascular cell adhesion molecule 1; sICAM-1: soluble intercellular adhesion molecule 1. [1]The P-value corresponds to the difference between baselines values and values obtained after 6 weeks using repeated measures ANOVA. Data are expressed as means (95% confidence interval).

there is evidence that each 1% decline in LDL-C over a period of 5 years results in a 1% reduction in CVD risk[26].

Non-HDL-C is an independent marker of CHD and stroke risk[27]. Evidence from a meta-analysis including more than 130,000 subjects indicates that a reduction in non-HDL-C exerts favorable effects on CVD risk even after adjustment for changes in LDL-C. These studies included different lipid-lowering strategies, such as diet, and showed that each 1% reduction in non-HDL-C decreased the risk of CVD by 1%[27]. Therefore, the reductions in non-HDL-C and apoB concentrations achieved with CPI in the present study may also be of clinical importance.

Healthy lifestyles, including dietary interventions that reduce serum cholesterol, are recommended as population strategies to reduce CVD risk[2]. The present study suggests that the consumption of cowpea protein, which can favorably modify the lipid profile and because it is widely available, may be an important strategy to reduce cholesterol and CVD risk in the population.

The cholesterol-lowering properties of soybeans have been hypothesized to reside in certain peptides or peptide fractions present in soy[28]. Like soy protein, cowpea protein may exert a protective effect against CVD by lowering LDL-C concentration. Cho et al.[28] reported that hydrolyzed soybean protein exhibited hypocholesterolemic effects by stimulating hepatic LDL receptor expression. In the case of cowpea protein which has shown marked effects on lipid parameters in both humans and experimental animals[13], peptides may be formed during processing and digestion. Marques et al.[29] identified peptides in the protein isolate from raw bean with a molecular mass of less than 3 kDa, which reduced the reaction velocity of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase by 89%. HMG-CoA reductase is the rate-limiting enzyme in hepatic cholesterol synthesis. For this

reason, this enzyme is the target of several drugs used to control high cholesterol levels[30]. However, other mechanisms could be involved in the hypocholesterolemic effect of cowpea protein.

Despite favorable effects on proatherogenic lipids and apoB, CPI did not affect the inflammatory and endothelial dysfunction biomarkers studied (CRP, sICAM1 and sVCAM1) when compared to control (casein). Similarly, recently published study showed that supplementation with soy or isolated soy protein does not alter CRP concentrations in humans[31].

The effects of legumes on biomarkers of inflammation and endothelial dysfunction are still unclear[31-34]. One study conducted over a period of 3 years on monkeys receiving 19% of calories derived from soy protein found a significant reduction in VCAM1 concentrations[35]. These controversial results regarding the effect of legumes on biomarkers of endothelial dysfunction suggest that these outcomes are influenced by other factors such as study duration and protein dose used. No effects of legume proteins on adhesion molecules were found in the present study and in three previous reports[31,33,34]. In two of these studies, the duration of the protocols was relatively short (only 6 and 8 weeks) and 26 to 40 g soy protein was offered[31,33]. In the other study[34], the protocol had a duration of 6 months, but only 15 g soy protein was offered. Long-term exposure associated with a representative amount of plant protein may be necessary to observe reductions in the serum concentrations of these biomarkers. These studies incorporated a similar amount of about 25 g of legume protein. A larger amount of plant protein may be necessary to observe some effect on adhesion molecules. Furthermore, the sample size of our study may have been too small to detect significant changes in biomarkers other than total cholesterol and its biologically related parameters (LDL-C, nonHDL-C, and apoB). In view of these limitations, we cannot rule out possible effects of cowpea protein on biomarkers of inflammation and endothelial dysfunction.

Despite the favorable effects of cowpea protein observed in the present study, further investigation is needed to confirm its effects on the blood lipid profile of humans. Furthermore, longer follow-up studies using different doses of CPI and including subjects with different degrees of dyslipidemia should be conducted.

In conclusion, in this proof-of-concept study, cowpea protein significantly reduced proatherogenic lipids and apoB in moderately dyslipidemic subjects. In view of its wide availability[19], cowpea protein may be an alternative to soy protein to reduce cholesterol levels and possibly to prevent CVD.

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Statement of Author's contributions to manuscript and conflict of interest

This manuscript is based on the PhD thesis of Karoline MG Frota, supervised by José AG Arêas and co-supervised by Raul D Santos. Valdenir Q Ribeiro was responsible for the statistical analysis and data interpretation. The authors declare no conflict of interest regarding this study.

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