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ORIGINAL ARTICLE

Effect of Hypoenergetic Diet Combined With Pumpkin Seed Flour Consumption on Obese Women

Anna Paula Soares de Carvalho,[1] Luciana Nicolau Aranha,[1] Larissa Almenara Soares,[1] Ronir Raggio Luiz,[1] José Firmino Nogueira Neto,[1] Gláucia Maria Moraes de Oliveira,[1] Glorimar Rosa[1]

Universidade Federal do Rio de Janeiro,[1] Rio de Janeiro, RJ – Brazil

Abstract

Background: Dietary treatment containing fiber-rich foods may contribute to lowering weight in obese women.

Objective: To investigate the effect of a hypoenergetic diet combined with pumpkin seed flour (PSF) consumption on diet quality, anthropometric indices, and glucose and lipid metabolism in obese women.

Methods: We conducted a randomized, double-blind, placebo-controlled, 90-day clinical trial with obese women, distributed into the following two groups: hypoenergetic diet + placebo (PG) and hypoenergetic diet + pumpkin seed flour (PSFG). A total of 100 participants were included in the PSFG (n = 47) and PG (n = 53). We evaluated neck circumference (NC); waist to height ratio; conicity index; fat mass (FM); lipid profile; blood concentrations of glucose and insulin; homeostatic model assessment for insulin resistance (HOMA-IR); quantitative insulin sensitivity check index (QUICKI); and blood pressure at baseline, 30, 60, and 90 days. Dietary analysis was determined by differences between diet quality indices before and after prescribing the experimental diet. Chi-squared, Student's t-tests and analysis for repeated measures were used, and values were considered significant at $p < 0.05$.

Results: The dietary pattern improved after 90 days in both groups. The PSFG presented lower NC ($p < 0.001$), FM ($p = 0.010$), triglycerides (TG) ($p = 0.025$), insulin ($p = 0.003$), and HOMA-IR ($p = 0.018$). The PG presented a lower diastolic blood pressure ($p = 0.004$) and low-density lipoprotein cholesterol (LDL-c) ($p = 0.056$).

Conclusion: A hypoenergetic diet combined with PSF consumption contributes to lowering NC, FM, HOMA-IR, TG, and insulin concentrations.

Keywords: Obesity; Cardiovascular Risk Factors; Caloric Restriction; Cucurbita.

Introduction

Obesity is a risk factor for cardiovascular diseases (CVD), which are the leading causes of death in Brazil and worldwide.[1] A study using Vigitel data from 2008 to 2015 showed increasing and alarming prevalence trends toward overweight and obesity among Brazilian women of reproductive age, and this increase occurred regardless of age, formal education, marital status, or race/ethnicity.[2]

Obesity treatment is based on modifying lifestyle, behavior, and dietary patterns, as well as on increasing physical activity. Dietary treatment is one of the main pillars for managing weight loss.[3] Hypoenergetic and nutritionally adequate diets contribute to lowering body weight, low-density lipoprotein cholesterol (LDL-c), and arterial pressure, thus contributing to lower cardiometabolic risk.[4] In evaluating diet quality, a consumption pattern rich in fruits, vegetables, and fibers, with less saturated fatty acids (SFA) and sugar, is directly related to decreased acute coronary events.[5]

Pumpkin (*Cucurbita* sp) is a fruit of the Cucurbitaceae family, which is widely cultivated worldwide; *Cucurbita maxima* is a species from South America.[6] Pumpkin seeds are generally considered to be agro-industrial waste and

Mailling Address: Glorimar Rosa
Programa de Pós-graduação em Medicina, Cardiologia. Universidade Federal do Rio de Janeiro. Rua Professor Rodolpho Paulo Rocco, 255, 8º andar, Sala 8 E 01. Postal code: 21941-913. Cidade Universitária, Rio de Janeiro, RJ - Brazil.
E-mail: glorimar@medicina.ufrj.br

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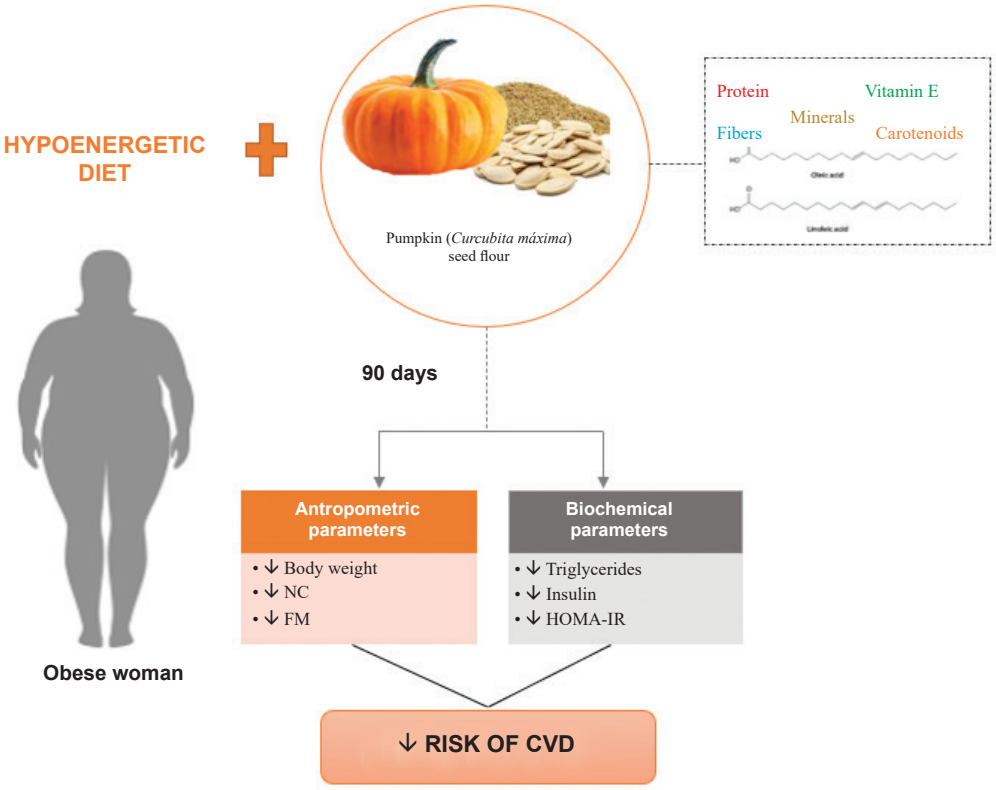

Central Illustration – The addition of pumpkin seed flour (PSF) to a hypoenergetic diet, for 90 days, reduced neck circumference, fat mass, HOMA-IR, triglycerides, and insulin concentrations.
NC: Neck circumference; FM: Fat mass; CVD: cardiovascular disease, HOMA-IR: Homeostatic model assessment - Insulin resistance.

discarded; however, they are good sources of proteins, fibers, minerals, essential fatty acids, vitamin E, carotenoids, and phytosterols, and their consumption as part of the usual human diet can be important for disease prevention and health promotion.[6-8] Studies in experimental animals have demonstrated the effects of pumpkin seeds on reducing body weight, glucose, and insulin,[9,10] as well as on improving lipid profiles and blood pressure.[11,12] However, in humans, studies are still limited.[13,14]

Therefore, this study aimed to investigate the effect of a hypoenergetic diet combined with pumpkin seed flour (PSF) consumption on diet quality, anthropometric indices, and glucose and lipid metabolism in obese women.

Individuals and methods

Ethical aspects

The study protocol was approved by the Research Ethics Committee of the Clementino Fraga Filho University Hospital at the Federal University of Rio de Janeiro, logged under number 103/11 and registered in ClinicalTrials.gov (NCT02086396). The study participants were fully informed regarding the procedures to which they were subjected throughout the study, and their formal consent was obtained through a free and informed consent form.

Eligibility criteria

Women, aged 20 to 59 years, with a body mass index (BMI) above 30 kg/m^2,[15] were included in this study. Women who were diabetic; those undergoing dietary or drug treatment for weight loss or using any type of supplement; pregnant or nursing women; and women with pacemakers, due to the use of bioelectrical impedance, were excluded from this study.

Sample size calculation

Sample size was calculated using the Openepi program, version 3. Our study considered a 95% confidence interval (95% CI), a power of 80%, and a 1:1 ratio for group sample size. The initial sample size was

calculated based on the pilot study (Supplementary Table 1), using body weight as a parameter, selecting 36 as the minimum number of individuals for each group. A 30% loss in subjects was predicted based on the pilot study, thus producing a plan for a total of 94 individuals .

Study groups

The eligible patients were randomized into the following two groups: hypoenergetic diet + pumpkin seed flour (PSFG) (n = 47) and hypoenergetic diet + placebo (PG) (n = 53). Randomization was performed by individuals who were not involved in the research project. The PSF and placebo were randomly coded in numerical sequence. The volunteers who met the eligibility criteria, signed the Free and Informed Consent Form, and attended the first appointment were allocated in this numerical sequence and represented by the code corresponding to PSFG or PG. Study blinding was opened only after all volunteers had completed the study.

The participants were followed for three months, totaling four consultations (one per month after baseline).

We recruited 264 individuals, 40% of whom did not participate in the study, because they rejected the experimental protocol (Supplementary Figure 1). The study began with 158 individuals, and 37% (n = 58) dropped out of the study for the following reasons: abandoning the study without explanation (36%, n = 21), failure to maintain the diet (31%, n = 18), health problems (3%, n = 2), nausea (9%, n = 5), edema (7%, n = 4), lack of time to attend consultations (12%, n = 7), and laxative use (2%, n = 1).

There was no difference in the variables studied between the participants who dropped out of the study and those who completed it, except for age (data not shown).

Study design

A randomized, double-blind, placebo-controlled clinical trial was conducted for 90 days, providing either PSF or placebo in conjunction with an individually calculated hypoenergetic diet. This study was conducted in the Clementino Fraga Filho University Hospital at the Federal University of Rio de Janeiro, Brazil, recruiting volunteers among university hospital staff and patients and university students who met the inclusion criteria.

The inclusion criteria were confirmed using socioeconomic status questionnaires, 24-hour food record recalls, and physical activity questionnaires. During the screening, the first consultation was scheduled and guidelines were given regarding 12-hour fasting for blood sampling and preparation for body composition evaluation by bioelectrical impedance.

Consultations were held monthly, defined as T_0 (baseline), T_{30} (30 days), T_{60} (60 days), and T_{90} (90 days). During the first consultation (T_0), the participants were randomized into the PG or PSFG. The following were evaluated during all of the consultations: anthropometric indices, body composition, diet, blood pressure, and biochemical analysis (blood sampling).

Dietary treatment

A balanced and individualized hypoenergetic diet plan was prescribed, calculated according to the dietary reference intake equations for women.[16] Next, 513 kcal was subtracted from each individual's diet to obtain a lowered body weight of approximately 2 kg/month by the adipose tissue energetic value method.[17] An additional 82 kcal was also subtracted, corresponding to the mean caloric value of PSF or placebo.

The diet plan was provided with the sealed and labeled packaging of the PSF or placebo in sufficient quantity for 32 days. The PSF and placebo were packed in tear-opening caplet blister foil containing 20 g. The participants were instructed to take the caplet daily at lunch. Compliance with PSF or placebo consumption was evaluated by counting the remaining caplets between monthly consultations. The analysis of the nutritional composition of the PSF and the placebo are described in the Supplementary Methodology, and the results are presented in Supplementary Tables 2 and 3.

Anthropometric attribute evaluation

Anthropometric evaluation was conducted monthly, including measurements of body mass, height, neck circumference (NC),[18] and waist circumference.[19] Body composition (fat mass [FM]) was tested by bioelectrical impedance (Biodynamics, model 450), and blood pressure was measured.[20] Waist to height ratio was determined according to Ashwell,[21] and the conicity index was determined according to Valdez.[22]

Biochemical analysis

Blood samples were taken from the women after a 12-hour fast (14-hour maximum) via gel tube. After clotting, the blood samples were centrifuged for 15 minutes at 4,000 g to obtain serum.

Concentrations of glucose, triglycerides (TG), total cholesterol, and high-density lipoprotein cholesterol (HDL-c) in serum were obtained in duplicate by an automated method (BioSystems A25 automated analyzer) using BioSystems commercial kits. LDL-c, and very LDL-c was calculated using the Friedewald formula,[23] which is only valid for TG concentrations below 400 mg/dL.

Insulin concentration was analyzed by the radioimmunoassay method.[24] Insulin resistance was estimated by the homeostasis model assessment index (HOMA-IR),[25] and insulin sensitivity was calculated using the quantitative insulin sensitivity check index (QUICKI).[26]

Dietary analysis

Dietary consumption was determined by 24-hour dietary recall during screening and, subsequently, by food records every four days, including the weekend and the evening before consultation, in order to evaluate compliance with the experimental diet and diet quality using the revised diet quality index for the Brazilian population (R-DQI).[27] A more detailed description of the dietary analysis and calculation of the R-DQI is available in the Supplementary Methodology.

Statistical analysis

Statistical analyses were performed using IBM® SPSS® Statistics software, version 21. Categorical variables were expressed as percentages and analyzed using the chi-square test. Continuous variables were presented as mean ± standard deviation. The normality of continuous variables was verified using the Kolmogorov-Smirnov test. Student's paired t-test was used for pre- and post-intervention comparisons, and Student's unpaired t-test was used for comparison between groups. Pearson´s linear correlation coefficient was applied to assess the R-DQI at baseline and at the end of the study. Analysis of variance for repeated samples was used to verify changes in variables during the study times. Results with p values < 0.05 were considered statistically significant.

Results

The study was conducted with 100 participants, whose baseline characteristics were as follows (Table 1): mean age was 43.18 ± 8.46 years old; 64% were non-white (n = 64); and 90% (n = 90) had > 12 years of educational background. There were no significant differences in the baseline characteristics between the studied groups (Table 1).

Table 1 – Baseline characteristics of the study participants

Variables	All (n = 100)	PSFG (n = 47)	PG (n = 53)	p-value
Age (years old)[a]	43.18±8.46	43.91±8.53	42.52±8.43	0.299
Skin color - non-white (% / n)	64(64)	66(31)	62(33)	0.126
Marital status – married (% / n)	66(66)	60(28)	64(34)	0.581
Educational background - more than twelve years (% / n)	90(90)	91(43)	87(46)	0.796
Smoking	3(3)	2 (1)	4(2)	0.612
Alcoholism	31(31)	34(16)	28(15)	0.397
Systemic arterial hypertension	30(30)	30(14)	28(16)	0.273
Dyslipidemia	93(93)	89(42)	96(51)	0.468

PG: placebo group; PSFG: pumpkin seed flour group.
aValues expressed as mean ± standard deviation. Unpaired Student's t-test for continuous variables. Chi-squared test for dichotomous variables. Values considered statistically significant at p < 0.05.

In classifying the women according to diet quality based on the R-DQI, 70% had healthy diets, while 30% had diets that needed improvement. After 12 weeks, the number of women classified with healthy diets increased from 70% to 87%, and the number of those classified with diets that needed improvement decreased from 30% to 12%. No participants were classified as having poor diets at any point during the study. A correlation of the RDQ-I was also observed at the beginning and end of the study (r = 0.63).

Table 2 shows the stratified dietary data according to diet quality at the end of the study. Those classified as having healthy diets, in both PSFG and PG, had a significant reduction in energy intake ($p < 0.0010$, $p < 0.001$), proteins ($p = 0.034$, $p = 0.010$), and sodium ($p = 0.007$, $p = 0.002$) after twelve weeks. Although not statistically significant, PSFG showed a reduction in intake of carbohydrates, monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), and total cholesterol, as well as a higher intake of total lipids, SFA, and fiber. By contrast, PG exhibited a reduction in intake of total lipids, MUFA, PUFA, and fiber, as well as a higher intake of carbohydrates and SFA, demonstrating differences in the consumption profile (Table 2).

Energy intake significantly differed in the PSFG and PG between baseline and the end of the study in participants classified as having diets needing improvement. PSFG also showed significantly lower sodium levels after twelve weeks (Supplementary Table 4).

Table 3 showed the analysis of the means of anthropometric indices, body composition, and blood pressure between the two groups throughout the study. Both groups had reduced BMI, waist to height ratio, conicity index, and systolic blood pressure at the end of the study; only PSFG presented lower NC and FM.

When analyzing the biochemical variables, both groups presented higher HDL-c and QUICKI, and only PSFG showed significantly lower TG, blood insulin, and HOMA-IR (Table 4).

Table 2 – Dietary data according to healthy diet classification at the end of the study

Variables[a]	PSFG (n=41)			PG (n=46)			Comparison between first and last time-points (last minus first)[c]		
	T_0	T_{90}[b]	p value	T_0	T_{90}[b]	p value	PSFG (n = 41)	PG (n = 46)	p value
Energy (kcal)	2149.8±757.2	1562.6±513.5	<0.001	2168.4±797.3	1559.1±531.6	<0.001	−587.2±877.5	−609.3±839	0.905
Protein (g/kg/weight)	1.1±0.5	0.9±0.4	0.034	1.1±0.4	0.9±0.5	0.010	−0.2±0.6	−0.2±0.6	0.803
Carbohydrates (%)	52.5±11.4	50.4±12.4	0.360	52.9±9.1	53.9±12.8	0.642	−2.1±14.8	1.0±14.2	0.319
Lipids (%)	27.6±12.1	28.6±14.1	0.713	27.9±8.8	26.1±12.7	0.395	1.0±16.8	−1.9±14.4	0.406
SFA (%)	11.4±6.8	12.5±9.2	0.466	11.2±5.4	11.6±7.9	0.729	1.1±9.9	0.4±8.5	0.726
MUFA (%)	9.7±4.7	9.0±4.8	0.513	9.3±3.6	7.9±4.1	0.094	−0.7±6.6	−1.3±5.3	0.619
PUFA (%)	3.4±2.2	2.8±1.6	0.168	3.5±2.8	2.7±2.1	0.133	−0.6±2.6	−0.8±3.4	0.759
Cholesterol (mg)	277.2±142.1	258.5±149.3	0.529	300.2±147.0	269.6±140.2	0.277	−18.6±188.2	−30.6±188.7	0.768
Total fiber (mg)	25.9±13.0	26.5±7.5	0.795	26.2±10.9	23.9±6.7	0.344	0.5±13.4	−1.7± 12.0	0.415
Sodium (mg)	2149.1±1309.7	1618.1±846.9	0.007	2262.5±1060.1	1607.8±927.7	0.002	530.9±1193.5	654.7±1356.4	0.654

MUFA: monounsaturated fatty acid; PG: placebo group; PSFG: pumpkin seed flour group; PUFA: polyunsaturated fatty acid; SFA: saturated fatty acid; [2]T_0: onset of the study; T_{90}: after 90 days.

[a]Results expressed as mean ± standard deviation. [b]Paired Student's t-test. [c]Unpaired Student's t-test between the deltas of the dietary data and R-DQI for the Brazilian population data (poor diet and diet needing improvement versus healthy diet).

*Statistically significant at $p < 0.05$.

Table 3 – Analysis of the means of the anthropometric indices, body composition, and blood pressure between both groups throughout the study

Variables[a]	T_0	T_{30}	T_{60}	T_{90}	p value[b]
BMI (kg/m2)					
PSFG	36.8±5.2	35.9±5.0	35.5±5.0	35.4±4.9	<0.001
PG	36.5±5.26	35.8±4.9	35.7±4.9	35.5±4.9	<0.001
Waist to height Ratio (cm/m)					
PSFG	68.2±6.7	66.3±6.9	65.5±7.1	65.3±7.3	<0.001
PG	66.8±7.2	65.7±7.5	65.5±7.5	65.3±7.4	<0.001
Conicity index					
PSFG	1.4±0.1	1.3±0.1	1.3±0.1	1.3±0.1	<0.001
PG	1.3±0.1	1.3±0.1	1.3±0.1	1.3±0.1	0.029
NC (cm)					
PSFG	38.2±2.6	27.7±2.5	37.5±2.4	37.7±2.6	<0.001
PG	37.8±5.3	38.1±2.7	37.9±2.9	37.9±2.8	0.365
SBP (mmHg)					
PSFG	123.8±15.6	123.5±16.4	117.0±11.2	118.5±12.6	0.005
PG	124.3±17.0	120.9±16.4	119.0±14.3	119.4±14.9	0.039
DBP (mmHg)					
PSFG	80.6±10.1	80.3±11.8	77.0±10.4	78.8±9.4	0.189
PG	81.7±12.7	79.6±11.1	76.4±10.7	75.7±10.5	0.004
FM (kg)					
PSFG	41.6±8.1 (23)	40.6±7.8	40.2±7.6	39.9±7.8	0.010
PG	40.3±10.6 (25)	39.7±9.4	39.2±9.5	38.6±9.7	0.070

BMI: body mass index; DBP: diastolic blood pressure; NC: neck circumference; PSFG: pumpkin seed flour; PG: placebo group; SBP: systolic blood pressure; FM: fat mass. T_0: onset of the study; T_{30}: after 30 days; T_{60}: after 60 days; T_{90}: after 90 days; WC: waist circumference. [a]Results expressed as mean ± standard deviation; [b]Comparison test for repeated samples.
**Statistically significant: $p < 0.05$.*

Discussion

This is the first study of its kind to investigate the effect of PSF consumption combined with a hypoenergetic diet on anthropometric indices, glucose, and lipid metabolism in obese women in Brazil. We hypothesized that pumpkin seeds can be used as an adjunct in treating obesity and its metabolic disorders, and our hypothesis was supported by the findings, as evidenced by reduction in NC, FM, TG, insulin, and HOMA-IR.

Hypoenergetic diets are used to treat obesity and help prevent CVD. A systematic review and meta-analysis evaluating 121 randomized trials with 21.942 overweight and obese adults showed that, in 6 months, low-carbohydrate and low-fat diets could reduce weight, systolic and diastolic blood pressure, and LDL.[28] Furthermore, the authors observed that, at the end of 12 months, all hypoenergetic interventions (low carbohydrate, low fat, and moderate macronutrient) were beneficial in reducing cardiovascular risk factors.[28] In our study, we prescribed a diet balanced in macronutrients, and, in both groups, the hypoenergetic intervention contributed to weight loss and was favorable for the reduction of other cardiovascular risk factors.

In addition to caloric restriction for weight loss, the adoption of a good quality diet emphasizing an increased

Table 4 – Analysis of the means of the biochemical variables between both groups throughout the study

Variables[a]	T_0	T_{30}	T_{60}	T_{90}	p-value[b]
TC (mg/dL)					
PSFG	200.7±44.3	191.3±44.9	197.1±42.6	199.9±36.9	0.159
PG	215.9±49.6	209.1±49.6	213.5±50.8	210.7±53.4	0.141
HDL-c (mg/dL)					
PSFG	46.2±11.7	46.4±12.2	48.8±13.7	49.4±13.6	0.031
PG	46.3±10.8	45.8±11.3	48.2±11.1	50.2±12.4	0.010
LDL-c (mg/dL)					
PSFG	127.4±37.5 (46)	118.5±41.1	123.4±35.9	121.7±33.2	0.098
PG	137.6±43.9 (49)	131.4±45.2	133.9±42.6	128.8±45.5	0.056
VLDL-c (mg/dL)					
PSFG	26.6±10.9 (46)	24.0±9.6	24.5±10.2	26.4±11.9	0.441
PG	27.9±11.4 (49)	26.1±10.5	25.9±10.7	25.97±9.7	0.289
TG (mg/dL)					
PSFG	139.1±68.2 (47)	122.9±50.5	121.8±52.7	136.7±66.5	0.025
PG	151.3±70.6	149.5±87.8	146.9±83.8	141.9±71.8	0.749
Glucose (mg/dL)					
PSFG	98.2±15.2	96.3±10.6	95.7±10.1	97.1±11.7	0.411
PG	98.5±11.9	96.9±9.8	96.4±14.8	97.3±13.4	0.523
Insulin (mg/dL)					
PSFG	13.7±9.1	11.0±5.9	11.7±6.7	9.8±5.9	0.003
PG	18.7±29.4	17.5±28.8	18.5±31.4	15.5±24.2	0.234
HOMA-IR					
PSFG	3.3±2.3	2.6±1.4	2.8±1.7	2.4±1.6	0.018
PG	4.7±7.5	4.1±6.6	4.3±6.8	3.8±5.9	0.183
QUICKI					
PSFG	3.0±0.3	3.1±0.3	3.1±0.6	3.3±0.7	0.054
PG	3.0±0.5	3.1±0.6	3.2±0.8	3.3±0.8	0.044

HDL-c: high-density lipoprotein cholesterol; HOMA-IR: homeostasis index resistance; LDL-c: low-density lipoprotein cholesterol; PSFG: pumpkin seed flour; PG: placebo group; QUICKI: sensibility index resistance. [2]T_0: onset of the study; T_{30}: after 30 days; T_{60}: after 60 days; T_{90}: after 90 days; TC: total cholesterol; TG: triglycerides; VLDL-c: very low-density lipoprotein cholesterol. [a]Results expressed as mean ± standard deviation. [b]Comparison test for repeated samples.
**Statistically significant: $p < 0.05$*

consumption of fruits, vegetables, whole grains, nuts, seeds, and unsaturated fats is necessary to reduce cardiovascular risk;[29] for this reason, the R-DQI was calculated to assess dietary adequacy at the beginning and end of the clinical trial. In this study, the baseline dietary analysis demonstrated that 70% of the women had healthy diets, whereas 30% had diets that needed improvement according to classification by R-DQI, and, after 90 days, the number of women classified as having diets that needed improvement decreased. Our results may be explained by educational background level, insofar as 90% of the women had > 12 years of

education, and they were in a university environment as workers, students, or patients of the university hospital. Thus, they had easy access to information about healthy eating habits. Kang et al.[30] also proved that diet quality is associated with sociodemographic and lifestyle characteristics. The average DQI score was higher in individuals with advanced age, higher education, and a greater amount of physical activity, as well as in those consuming a multivitamin supplement. Moreover, the women classified as having diets that needed improvement showed a significantly lower energy intake. The PSFG also presented a significantly lower sodium intake at the end of the study. These results corroborate those found by Al-Nimr et al.,[31] who also observed lower sodium intake after intensive nutrition counseling in obese patients. The reduced sodium intake observed in our study was most likely due to the nutritional education encouraging the women to change their dietary patterns, replacing high consumption of sodium-rich, industrialized, and processed products with diets rich in fruits, vegetables, and whole grains. This behavioral change contributed to lowering the participants' systolic blood pressure, which is an important risk factor for CVD.

Our study investigated whether PSF associated with a hypoenergetic diet could have an additional effect on the treatment of obesity. In our study, there were significantly lower NC and FM values in the PSFG after twelve weeks. The reduction in NC is considered relevant, as higher NC values (thicker neck) are associated with increased cardiometabolic risk, because they are directly associated with lipid profile and glycemia.[32] Pumpkin seeds are rich in fiber. This nutrient can help regulate body weight through some mechanisms that are already known, such as reducing gastric emptying, inducing the secretion of appetite-suppressing hormones, improving insulin sensitivity that modulates glucose and lipid oxidation, and influencing the composition of the intestinal microbiota.[33] Randomized clinical trials have shown beneficial effects of soluble fibers in reducing BMI, body fat, glucose, and insulin when compared to placebo in individuals with excess body mass, showing that supplementation improves the health of these individuals.[34]

At the end of our study, the PSFG presented significantly lower TG concentrations; thus, dietary treatment with PSF may be beneficial for cardiovascular health, since TG is associated with the pathogenesis of CVD. Similarly, pumpkin seed oil and flour significantly reduced blood TG, total cholesterol, and glucose in rats.[10,35] Gossell-Williams et al.,[13] did not report a reduction in blood TG levels in a pilot study with postmenopausal women who consumed pumpkin seed oil for twelve weeks; they did, however, demonstrate an increase in HDL-c concentrations, as well as an overall improvement in the atherogenic index, which is possibly associated with the risk of atheroma. They also suggested that supplementation with pumpkin seed oil is favorable for cardiovascular health.

Previous studies carried out in an experimental model of Wistar rats with streptozotocin-induced diabetes showed the effect of *Cucurbita maxima* seed extract on reducing blood glucose concentrations. This effect was possibly caused by stimulating pancreatic insulin secretion by the β cells of the islets of Langerhans.[9, 36] Although the potential hypoglycemic and antidiabetic effects of pumpkin seed have been described in the literature,[7-10,36] studies in humans are still scarce and inconclusive. In a randomized, simple-blind, placebo-controlled study involving 15 normoglycemic adults, the acute consumption of 65 g of pumpkin seed significantly reduced postprandial plasma glucose.[14] However, as this was an acute study, it is still necessary to prove this long-term effect in a population with a risk factor.

The present study also showed a reduction in insulin and HOMA-IR concentrations in the PSFG, supporting the hypothesis that pumpkin seed may have beneficial effects on insulin resistance and thus prevent type 2 diabetes mellitus, an important cardiovascular risk factor. As previously described, pumpkin seeds contain fibers, MUFA, and PUFA, which can improve insulin sensitivity, endothelial function, β-cell function, inflammation, and oxidative stress.[34,36-38] This could be an important benefit in Brazilian women, who have shown an increased prevalence of diabetes associated with obesity and being overweight.[2]

In this study, some side effects appeared after twelve weeks, such as nausea (15%, n = 4 in PSFG; 3%, n = 1 in PG) and abdominal distension (11%, n = 3 in PSFG; 3%, n = 1 in PG). The smell of PSF resembles that of peanuts, and this may have contributed to nausea. The high fiber concentration in PSF may contribute to abdominal distention and satiety.

There are limitations in this study, as we did not measure plasma fatty acids to verify adherence to PSF or placebo. Adherence to PSF or placebo was controlled by requesting unused caplets at the end of each monthly consultation and subsequently counting

them. The loss of participants is common in longer studies using diets with or without supplementation. However, no differences were found between those who participated and those who dropped out of the study. Notwithstanding these limitations, this was the first study to assess the consumption of PSF in obese individuals. Patients were controlled by hypoenergetic diet. A placebo was also used and a greater number of participants took part in our study than in other clinical trials that evaluated the effect of PSF on lipid metabolism and glucose. Therefore, this study can serve as a basis for further investigation.

Conclusion

PSF consumption combined with the hypoenergetic diet significantly reduced NC, FM, and HOMA-IR in obese women. We found that the prescribed experimental diet improved diet quality after twelve weeks. Our data make it possible to infer that the hypocaloric diet, and PSF consumption exhibited beneficial weight loss, FM, systolic blood pressure, and blood insulin, as well as raised HDL-c, HOMA-IR, and QUICKI levels among obese women.

Author Contributions

Conception and design of the research: Carvalho APSO, Oliveira GMM, Rosa G. Acquisition of data: Carvalho APSO, Nogueira Neto JF; analysis and interpretation of the data: Carvalho APSO, Aranha LN, Luiz RR, Nogueira Neto JF, Oliveira GMM, Rosa G; statistical analysis: Luiz RR; obtaining financing: Oliveira GMM, Rosa G; writing of the manuscript: Carvalho APSO, Aranha LN, Soares LA, Oliveira GMM, Rosa G; critical revision of the manuscript for intellectual content: Aranha LN, Luiz RR, Oliveira GMM, Rosa G.

Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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Study Association

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Ethics Approval and Consent to Participate

This study was approved by the Ethics Committee of the Hospital Universitário Clementino Fraga Filho (HUCFF/UFRJ) under the protocol number 103/11. All the procedures in this study were in accordance with the 1975 Helsinki Declaration, updated in 2013. Informed consent was obtained from all participants included in the study.

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