Chia Flour Supplementation Reduces Blood Pressure in Hypertensive Subjects

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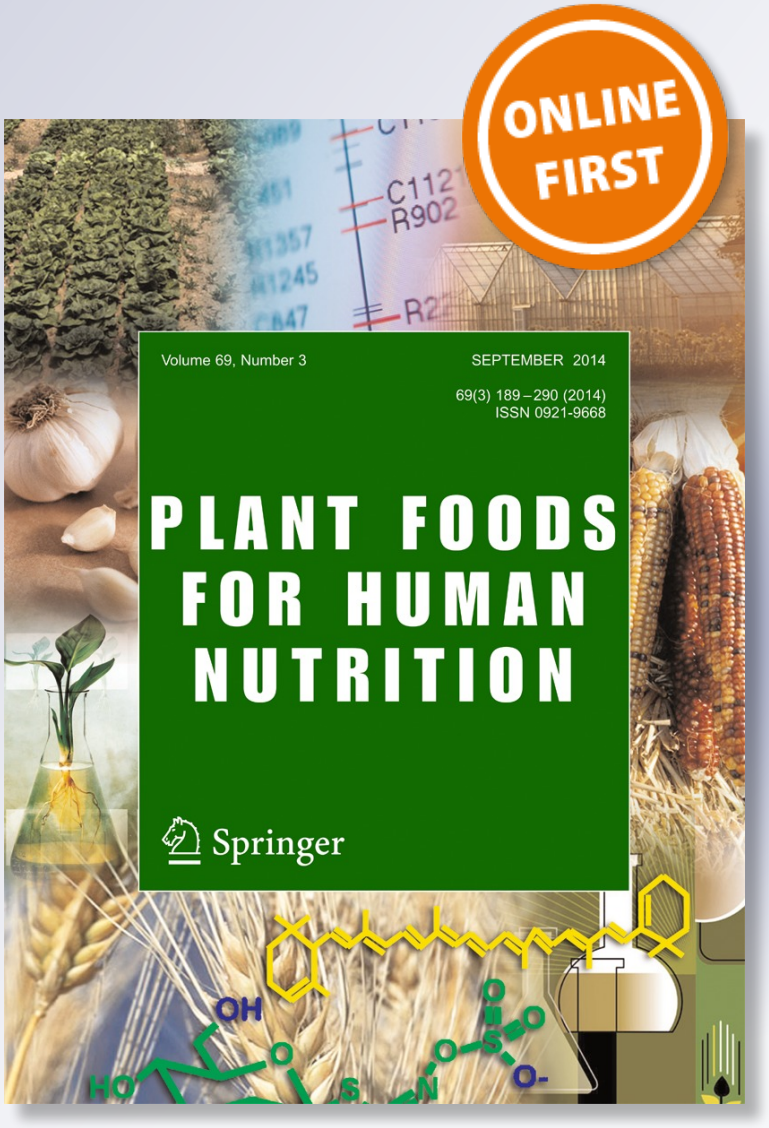

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ORIGINAL PAPER

Chia Flour Supplementation Reduces Blood Pressure in Hypertensive Subjects

Luciana Tavares Toscano · Cássia Surama Oliveira da Silva · Lydiane Tavares Toscano · Antônio Eduardo Monteiro de Almeida · Amilton da Cruz Santos · Alexandre Sérgio Silva

Abstract The aim of this study was to investigate the effect of chia supplementation (*Salvia hispanica* L.) on blood pressure (BP) and its associated cardiometabolic factors in treated and untreated hypertensive individuals. The subjects were randomly assigned to one of the following groups: the hypertensive-drug treated (CHIA-MD, n=10), hypertensive untreated (CHIA-NM, n=9) and placebo (PLA-MD, n=7) groups. The subjects consumed 35 g/day of either chia flour or a placebo for 12 weeks. The clinical and ambulatory BP, inflammation, oxidative stress and markers for nitric oxide were measured. While the PLA-MD group showed no changes in BP, there was a reduction in the mean clinical blood pressure (MBP) in the CHIA (111.5±1.9 to 102.7±1.5 mmHg, p<0.001) and CHIA-MD (111.3±2.2 to 100.1±1.8 mmHg, p<0.001) groups. The CHIA-NM group showed no reduction in the MBP but did show a decreased systolic BP (146.8±3.8 to 137.3±3.1 mmHg, p<0.05). The clinical BP reduction was demonstrated by a 24 h ambulatory systolic reduction in all of the supplemented groups. However, the mean ambulatory BP was reduced only in the CHIA (98.1±2.4 to 92.8±2.2 mmHg, p<0.05) group, and there was no change in the diastolic component in either of the CHIA groups. The lipid peroxidation was reduced in the CHIA (p=0.04) and CHIA-NM (p=0.02) groups compared with the PLA-MD group. A reduction in the plasma nitrite levels was observed only in the CHIA group (p=0.02). Chia flour has the ability to reduce ambulatory and clinical BP in both treated and untreated hypertensive individuals.

Keywords Blood pressure · Chia flour · Oxidative stress · Inflammation · Hypertension

Abbreviations

ABPM	Ambulatory blood pressure monitoring
AGP α-1	Acid glycoprotein
BP	Blood pressure
BMI	Body mass index
CHIA-MD	Chia groups previously treated with medication
CHIA-NM	Chia without medication
DBP	Diastolic blood pressure
hs-CRP	High sensitivity C-reactive protein
MBP	Mean blood pressure
MDA	Malondialdehyde
PLA-MD	Placebo with medication
SBP	Systolic blood pressure
WC	Waist circumference

Introduction

Hypertension can be difficult to control, even with drug therapy [1]. Thus, additional treatments, such as dietary interventions [2] and individual foods [3–5], can be a complementary approach to drug therapy to better control blood pressure (BP). The intake of whole grains has been reported to provide better glycemic and lipid control, increase insulin sensitivity and reduce inflammation, oxidative stress and BP [6].

Studies have shown that chia seeds (*Salvia hispanica* L.) are a whole grain of high nutritional value due to their high

L. T. Toscano · C. S. O. da Silva · L. T. Toscano
Department of Nutrition, Federal University of Paraiba, Cidade universitária, João Pessoa, Paraiba Zip Code 58051-900, Brazil

A. E. M. de Almeida · A. da Cruz Santos · A. S. Silva
Department of Physical Education, Federal University of Paraiba, Cidade universitária, João Pessoa, Paraiba Zip Code 58051-900, Brazil

A. S. Silva (✉)
Rua Silvino Lopes, 410 /apto 804, Tambaú, João Pessoa, PB Zip Code 58039-190, Brazil
e-mail: alexandresergiosilva@yahoo.com.br

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content of dietary fiber, proteins, lipids (represented by α-linolenic acid) and polyphenols such as myricetin, quercetin, kaempferol and caffeic acid [7, 8]. In humans, a dose–response reduction in postprandial glycemia was observed in healthy subjects [9] and a reduction in the C-reactive protein and Von Willebrand factor in diabetic patients [10] with dietary chia supplementation.

In the literature, only three studies have evaluated the effects of chia in regard to BP. Vuksan et al. [10] found a reduction in systolic BP (− 6.3±4 mmHg) in diabetic patients who ingested 37 g / day of chia seeds for 12 weeks. However, Nieman et al. [11] found no changes in BP in overweight or obese adults who consumed 50 g / day of chia seeds for 12 weeks. A similar result was reported by Nieman et al. [12], who also found no changes in BP in overweight women who consumed 25 g / day of chia seeds for 10 weeks. Therefore, data are still scarce and controversial regarding the effects of chia on the control of blood pressure levels.

In these previous studies, blood pressure measurements were obtained on single measures immediately before and after the study. Thus, multiple measurements and 24-h ambulatory measurements may help to clarify whether chia's efficacy for controlling BP. Furthermore, the influence of medications on the blood pressure's response to chia may also explain these previous controversial results.

In this context, the present study was conducted to evaluate the effect of 12 weeks of chia flour supplementation (*Salvia hispanica* L.) using clinical and 24-h ambulatory measurements and to determine whether oxidative stress, inflammation and endothelial functions are associated with the possible reduction of BP in pharmacologically untreated hypertensive individuals and in those previously treated with drug therapy.

Materials and Methods

Subjects Hypertensive individuals of both sexes were recruited to participate in a randomized, double-blind, experimental and placebo-controlled study. The following inclusion criteria were adopted: age between 35 and 65 years; clinically diagnosed with mild / stage 1 hypertension according to the VI Brazilian Guidelines on Hypertension [13]; a body mass index (BMI) between 25 and 35 kg / m^2; no other known diseases, no habitual chia consumption; not using medications for weight loss or to treat inflammation; be under antihypertensive drug therapy (for the group with drug treatment); and having hypertension diagnosed by a physician but not yet having initiated drug treatment (for the group without drug treatment).

Volunteers who, over the course of the study, changed their antihypertensive drug therapy (for the group with drug treatment), initiated drug treatment (for the group without drug treatment) or changed their eating and physical activity habits and those who consumed the amount of chia provided were excluded from the study. The study was submitted to the Ethics Committee of Research involving Humans of the "Lauro Wanderley" University Hospital—Federal University of Paraiba and approved under protocol n° 206.338/13. All the participants were informed about the specifications of the study and signed the informed consent form as required by resolution 196/96 of the national health council.

Sample Calculation based on blood pressure reduction for systolic BP of 6.3 mmHg, with standard deviation of 4.2 mmHg in response to chia supplementation, [10], effect size was calculated in 1.5. As a result, a minimum of seven subjects was determined in each group to compose the study sample, based on alpha error of 5 % and statistical power of 0,80. The study included 29 hypertensive patients who were randomized (www.randomizer.org) for the chia previously treated with medication (CHIA-MD), the chia without medication (CHIA-NM) and the placebo with medication (PLA-MD) groups. Three volunteers did not complete the study reporting difficulties in consuming chia or placebo; therefore, the groups were as follows: (CHIA-MD, n=10), (CHIA- NM, n=9), and (PLA-MD, n=7).

Study Design The volunteers were initially submitted to nutritional assessment, clinical BP measurement, and ambulatory blood pressure monitoring (ABPM), and blood samples were collected for the analysis of malondialdehyde (MDA), plasma nitrite, high sensitivity C-reactive protein (hs-CRP), α-1 acid glycoprotein (AGP), hepatic and renal markers. Twenty-four hours after the blood collection, the groups began the 12-week supplementation protocols. Every 4 weeks, the subjects underwent nutritional follow-up and clinical BP measurements. Forty- eight hours after the intervention period, the subjects were tested for the same baseline variables.

Nutritional Evaluation The dietary intake was assessed by a 24-h dietary recall administered three times with each individual, 2 representing the weekday diet and 1 representing the weekend diet. The average of the three values was adopted to investigate the consumption of nutrients using Avanutri Revolution software version 4.0 (Avanutri®, Brazil).

Supplementation Protocols The CHIA-MD and CHIA-NM groups consumed 35 g/day of chia flour (Cacalia®, Rio Grande do Sul, Brazil), which was added to the water, yogurt, vitamins and fruit juices normally consumed by the subjects, for 12 weeks. The PLA-MD group consumed the same portion of roasted wheat bran (Vitao®, Paraná, Brazil), following the procedure of Vuksan et al. [10] adopted in a previous study. The products were delivered every 4 weeks during the

intervention and were packaged in similar individual containers in the amounts for daily consumption. The volunteers were asked to return the monthly packages to receive new products to ensure consumption control. During the first four weeks, the researchers sent a text message via telephone three times per week. Thereafter, the frequency of the messages was once per week.

Clinical Blood Pressure Measurements (Single Measure of Blood Pressure) Twenty-four hours before the beginning of the intervention, every 4 weeks during the intervention and 48 h after the 12 weeks of supplementation, the subjects' BP was clinically assessed. Three measurements were performed with a five-minute interval between them using a properly calibrated aneroid sphygmomanometer (WelchAllyn - DS44, USA), and the mean of the last two BP measurements was considered as the BP. The BP measurements were performed as recommended by the VI Brazilian Guidelines on Hypertension [13], observing all of the recommendations on food intake, urine voiding and prior physical activity. The measurements were obtained with the subjects in a seated position with their legs uncrossed after sitting for at least 10 min.

Ambulatory Blood Pressure Monitoring (Repeated Measurements of Blood Pressure for 24 h) The BP was measured for a period of 24 h using a Dynamapa+Cardios® device (São Paulo, Brazil). The monitor was programmed to perform measurements every 15 min during the waking period and every 30 min during sleep, according to the recommendations of the V Brazilian Guidelines for Ambulatory Blood Pressure Monitoring and the III Brazilian Guidelines for Residential Blood Pressure Monitoring [14].

Biochemical Analysis The plasma oxidant activity was quantified by the reaction of the thiobarbituric acid as described by Ohkawa et al. [15]. The plasma nitrite concentration was determined by the Griess reaction according to Green, Tannernbaum and Goldman [16]. The serum hs-CRP (intra and inter assay coefficients of variations, 0.6 and 1.3 % respectively), and AGP (intra and inter assay coefficients of variations, 0.99 and 1.57 %, respectively) were determined using Labtest kits (Minas Gerais, Brazil) according to the manufacturer's instructions. The hepatic and renal markers and lipid and glycemic profiles were quantified in serum using Labtest kits (Minas Gerais, Brazil) according to the manufacturer's recommendations in a LabMax 240 premium automated analyzer (Minas Gerais, Brazil).

Statistical Analysis The data are presented as the means±the standard error mean (SEM). The normality and homogeneity were evaluated by the Shapiro-Wilk and Levene tests. The comparisons between the CHIA and PLA-MD groups were made using the independent *t*-test, and a one-way analysis of variance (ANOVA) with a *post hoc* Tukey test was used for comparisons of the CHIA - MD, CHIA - NM and PLA-MD groups. Every 4 weeks, the BP was clinically analyzed by an ANOVA for repeated measures. P values < 0.05 were considered to be statistically significant. The software used was GraphPad Instat 3.0 (San Diego, USA).

Results and Discussion

The groups were similar for all of the variables at the baseline (Table 1). The individuals were adults, who were obese and had a high waist circumference (WC), hyperlipidemia and slightly increased blood glucose levels but had not been diagnosed with diabetes. In addition, the subjects had grade I hypertension, with a clinically measured BP of at least 130/80 mmHg at baseline and serum hs-CRP and AGP in normal values. The CHIA-MD and PLA-MD groups were treated with antihypertensive drugs of the following classes: diuretics, calcium-channel blockers, ACE inhibitors and AT_1 receptor blockers. The use of other drugs, such as statins and aspirin, was not reported by any of the volunteers.

The groups did not differ in regard to dietary intake, with their diets containing normal levels of carbohydrates (45–65 %), protein (10–35 %) and lipids (20–35 %) based on the percentage parameters for total energy intake; however, their consumption of dietary fiber, vitamins A and E, and minerals (zinc, selenium and copper) was insufficient compared with values recommended by the Dietary Reference Intake [17]. The 24-h recalls that were used every four weeks during the intervention showed that the groups did not change their eating habits during the study.

The consumption of the chia or the placebo caused no gastrointestinal, hepatic or renal disorders. The CHIA group began the study with values similar to the PLA-MD group and ended without significant changes for aspartate aminotransferase (34.9±3.0 versus 37.5±4.2), alanine aminotransferase (20.4±2.6 versus 25.8±3.5), uric acid (5.1±0.3 versus 5.0±0.3), urea (16.1±1.0 versus 19.6±1.7) and creatinine (1.0±0.0 versus 0.9±0.0).

Chia treatment did not promote any significant change in BMI or WC, glycaemia and lipid profile in any of the groups. Despite this, the mean clinically measured blood pressure (MBP) was significantly reduced in the CHIA group, by 7.9 %. The CHIA-MD group also showed a significant reduction of 10 %, while the CHIA-NM group had a non-significant reduction of only 5.4 %. In the CHIA and the CHIA–MD groups, the reduction in the MBP was due to the reduction of both the systolic and diastolic BP components. In addition, despite the absence of a significant reduction in the MBP, the CHIA-NM group showed a significant reduction of 6.5 % in the systolic pressure. The lack of significance in the diastolic

Table 1 Baseline characteristics of the study subjects

	CHIA (n=19)	CHIA-MD (n=10)	CHIA-NM (n=9)	PLA-MD (n=7)	P(g)	P(s)
Age (years)	48.8±1.8	50.9±2.8	46.6±2.1	51.4±3.1	0.48	0.59
BMI (Kg/m^2)	32.1±1.0	30.9±1.2	33.4±1.5	32.5±1.5	0.83	0.45
WC (cm)	99.9±2.2	96.2±3.0	104±2.9	102±4.2	0.62	0.17
Male	103.5±2.5	101.3±4.1	104.3±3.3	104.1±5.9	0.61	0.79
Female	94.9±3.4	94.0±3.8	101.5±1.0	96.1±6.1	0.87	0.75
Cholesterol (mg/dL)	239±17.6	244.4±30.9	233.5±16.3	240.1±15.1	0.97	0.94
LDL (mg/dL)	155±18.1	169.4±28.9	139.9±21.4	134±12.8	0.50	0.53
HDL (mg/dL)	41±2.7	44.2±2.9	37.4±4.8	40.7±3.6	0.95	0.44
VLDL (mg/dL)	41±7.7	30.8±6.5	44.5±12.0	42.3±7.5	0.92	0.50
TG (mg/dL)	214±40.4	166.2±32.2	306.5±73.3	211.4±37.5	0.97	0.15
Glycaemia (mg/dL)	112±6.7	109.4±3.8	115±13.9	105.9±7.5	0.61	0.80
SBPc (mmHg)	146.2±2.0	145.8±2.2	146.8±3.8	144.0±4.3	0.60	0.85
DBPc (mmHg)	94.2±2.0	94.3±2.4	94.2±3.6	90.1±2.4	0.28	0.57
hs-CRP (mg/L)	2.1±0.5	2.8±0.8	1.4±0.4	2.3±1.0	0.87	0.44
AGP (mg/dL)	70.2±3.0	71.8±3.2	68.4±5.3	78.4±5.6	0.18	0.36
MDA (μmol/L)	4.1±0.3	3.6±0.4	4.7±0.5	4.9±0.7	0.28	0.21
Nitrite (μmol/L)	40.2±3.9	37.5±4.2	43.9±7.2	30.3±5.8	0.22	0.36

Data are mean±SEM. (g) general: comparison between groups CHIA and PLA-MD by unpaired t test. (s) stratified: comparison between groups CHIA-MD, CHIA-NM and PLA-MD through the one-way ANOVA test. *BMI* body mass index, *WC* waist circumference, *LDL* low density lipoprotein, *HDL* high density lipoprotein, *VLDL* very low density lipoprotein, *TG* triglycerides, *SBPc clinical* systolic blood pressure, *DBPc clinical* diastolic blood pressure clinic, hs-*CRP* high sensitivity c-reactive protein, *AGP* α-1 acid glycoprotein, *MDA* malondialdehyde

pressure reduction explains the lack of a significant reduction in the MBP of this group (Table 2). The SBP reduction found in the CHIA group confirmed the findings of the study by Vuksan et al. [10], which used 37 g / day of chia seeds for 12 weeks in diabetic individuals. In the present study, the MBP and the diastolic component were also reduced, but this did not occur in the CHIA-NM group, possibly because chia may have enhanced the effects of the medications in the other groups. However, studies using chia seeds found no changes in the blood pressure levels for either the systolic or diastolic components in non-hypertensive overweight or obese adults in amounts of 50 g/day and 25 g/day for 12 and 10 weeks, respectively [11, 12].

In addition to the clinical measures performed during the intervention every four weeks, ambulatory BP monitoring in the pre- and post-intervention periods was adopted. This is important because these measures not only show the time course of the chia treatment but also minimize the effects of the "white coat" syndrome and temporal fluctuations in BP. The clinical reductions observed in the BP had already occurred by the fourth week of the treatment and were confirmed by the significant reductions in the systolic BP in all of the supplemented groups in the 24-h periods during waking and sleep, unlike the PLA-MD group (Table 3).

This is the first study in which the BP's response to chia consumption was monitored by ABPM; therefore, these results cannot be compared to those of other studies. However, the various clinical measurements obtained during the process and the ABPM demonstrated consistent results, allowing us to

Table 2 Results of the clinical blood pressure measurements obtained every four weeks of the intervention

	CHIA (n=19)	CHIA-MD (n=10)	CHIA-NM (n=9)	PLA-MD (n=7)
MBP (mmHg)				
Baseline	111.5±1.9	111.3±2.2	111.7±3.4	108.0±2.9
4 weeks	103.3±2.1***	102.9±2.8**	103.7±3.5	108.7±4.0
8 weeks	104.0±1.8***	102.6±1.9**	105.6±3.3	105.5±3.2
12 weeks	102.7±1.5***	100.1±1.8***	105.6±2.1	105.7±2.9
SBP (mmHg)				
Baseline	146.2±2.0	145.8±2.2	146.8±3.8	144.0±4.3
4 weeks	136.8±3.2**	133.5±3.1*	140.6±5.7	146.0±5.0
8 weeks	140.6±3.1	139.2±3.7	142.2±5.3	141.5±5.7
12 weeks	136.3±2.6**	133.7±4.1**	137.3±3.1*	141.2±5.2
DBP (mmHg)				
Baseline	94.2±2.0	94.3±2.4	94.2±3.6	90.1±2.4
4 weeks	86.5±2.0*	87.6±3.2	85.4±2.6	90.5±3.8
8 weeks	85.8±1.4**	84.4±1.6*	87.5±2.5	87.7±3.7
12 weeks	85.5±1.2***	83.3±1.3**	88.7±1.8	87.8±2.2

All values are represented as the mean±SEM. *MBP* mean blood pressure; *DBP* diastolic blood pressure; *SBP* systolic blood pressure. $^{*}p<0.05$ $^{**}p<0.01$ $^{***}p<0.001$ difference from the baseline

Table 3 Ambulatory blood pressure monitoring at baseline and after 12 weeks of supplementation

	CHIA (n=19)	CHIA-MD (n=10)	CHIA-NM (n=9)	PLA-MD (n=7)
24 h- MBP (mmHg)				
Baseline	95.3±2.4	93.8±3.7	97.3±2.8	90.6±1.6
12 weeks	90.0±2.2*	88.8±3.5	91.5±2.5	93.6±3.7
24 h- SBP (mmHg)				
Baseline	129.6±3.6	126.7±5.4	133.4±4.6	121.9±2.0
12 weeks	119.2±3.0*	117.6±4.3*	121.4±4.3*	125.5±5.7
24 h- DBP (mmHg)				
Baseline	78.1±2.1	77.4±3.6	79.1±2.0	74.6±2.8
12 weeks	75.3±2.0	74.4±3.4	76.4±1.8	77.7±3.5
Awake- MBP (mmHg)				
Baseline	98.1±2.4	96.3±3.5	100.5±3.0	93.0±2.3
12 weeks	92.8±2.2*	91.0±3.2	93.6±2.2	97.2±3.2
Awake - SBP (mmHg)				
Baseline	132.4±3.7	128.5±4.9	137.5±5.4	123.2±1.7
12 weeks	122.5±3.0*	119.8±3.9*	126.1±4.8*	128.8±5.1
Awake - DBP (mmHg)				
Baseline	81.0±2.2	79.9±3.5	82.4±2.2	78.0±3.8
12 weeks	78.0±2.1	76.5±3.2	77.6±1.9	81.5±3.4
Asleep- MBP (mmHg)				
Baseline	86.9±2.6	87.7±4.4	85.8±2.5	86.0±1.5
12 weeks	82.0±2.2	82.6±4.0	81.1±0.9	87.4±5.1
Asleep - SBP (mmHg)				
Baseline	121.6±3.9	122.3±6.6	120.6±3.1	119.0±2.7
12 weeks	110.9±3.1*	111.8±5.1*	109.7±2.8*	118.1±7.2
Asleep - DBP (mmHg)				
Baseline	69.6±2.4	70.6±3.8	68.2±2.6	69.4±2.7
12 weeks	67.6±2.3	68.0±4.1	67.0±1.2	71.8±4.9

All values are represented as the mean±SEM. *MBP* mean blood pressure; *DBP* diastolic blood pressure; *SBP* systolic blood pressure. * Difference from the baseline, $p<0.05$

conclude that chia exerts a significant hypotensive effect on hypertensive patients.

In this study, we distinguished between the hypertensive patients using medications and those not using medications. This methodological procedure proved to be useful, as we observed that although the overall effect of chia was a reduction in BP, significant differences between the groups existed. Therefore, the results of this study suggests that the distinction

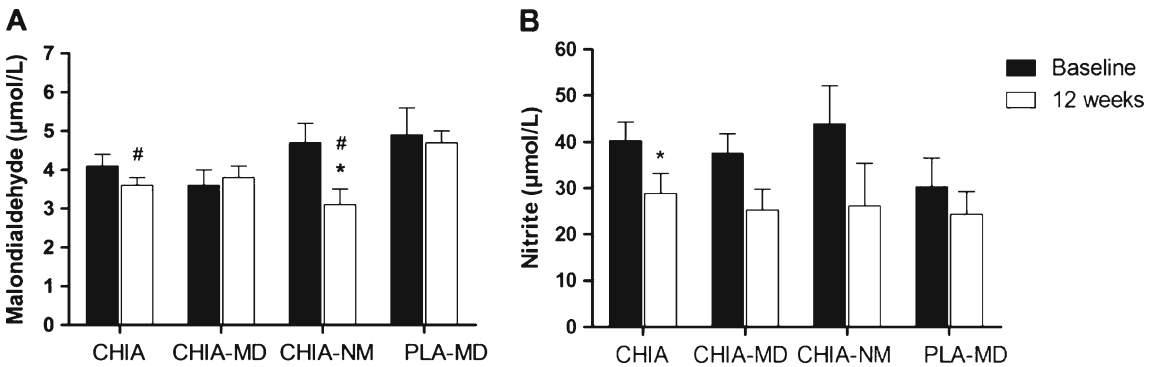

Fig. 1 Serum concentrations of malondialdehyde and nitrite at baseline and after 12 weeks of intervention. The data are described as the mean± SEM. * difference from the baseline; # difference compared with the PLA-MD group, $p<0.05$

of the subjects in terms of their medications needs to be considered in future studies, as it does not appear to have been considered in the previous studies on intervention with chia or other nutrients [10, 18–20].

Interestingly, the CHIA-MD and PLA-MD groups showed baseline BPs very similar to the CHIA-NM group, which may indicate the ineffectiveness of their drug therapy. In fact, studies have shown that despite the adherence of some hypertensive patients to drug therapy, the BP control levels are still unsatisfactory [1, 2]. One explanation for this lack response to treatment may be that the hypertensive mechanisms are pleiotropic, whereas the drugs typically act on only one of these mechanisms. Despite these difficulties, the present study showed that the adoption of a nutritional approach was able to reduce the BP even in individuals who were resistant to drug treatment. These data not only give credibility to the antihypertensive power of chia but also reinforce the recommendations that antihypertensive treatment is much more when drug therapy is used in combination with other interventions [21].

Chia seeds contain a higher proportion of α-linolenic acid (~60 %) than any other known plant source [22], and this essential fatty acid is beneficial to cardiovascular health, exerting both antioxidant and anti-inflammatory effects [23–25]. In consideration of this data, we propose investigating the antioxidant and anti-inflammatory effects of chia. The serum concentrations of the inflammatory markers hs-CRP and AGP remained unchanged when comparing the baseline and post-intervention periods of the chia groups in relation to the placebo in this study. However, the serum MDA of the CHIA-NM group decreased (p=0.04) after 12 weeks without significant changes in the other groups. Nevertheless, the comparison between the groups showed that the CHIA and CHIA - NM groups completed the study with lower MDA values (p= 0.04 and p=0.02, respectively) compared with the PLA - MD group (Fig. 1, panel A). As for the serum nitrite concentration, a reduction (p=0.02) was observed only in the CHIA group after 12 weeks of supplementation (Fig. 1, panel B).

While a reduction of this variable by the lower activity of the MDA was found in this study, Nieman et al. [11] reported no increase in the total antioxidant capacity. One possible explanation for these controversial results may be that different variables were adopted for the oxidative stress. Therefore, studies aimed at measuring oxidative stress may need to adopt more variables.

Unlike our study, Vuksan et al. [10] found a reduced inflammatory status using hs-CRP. However, it is noteworthy that this difference was due to an increase in the serum concentrations hs-CRP in the control group compared with the experimental group at the end of the intervention. Thus, the lack of the chia's capacity to decrease the hs-CRP in our study may corroborate this previous study.

Although this was the first study in which reducing blood pressure by chia are differentiated according to the presence or absence of drug therapy, this aspect needs to be better explained because the groups were reduced in size after stratified by medication use. However, the minimum sample size was calculated for seven subjects, which was the exact size of the smaller subgroup. Although the observed reduction in lipid peroxidation as effect of chia, there was no verified whether this effect would be accompanied by increased antioxidant capacity, thus this is a suggestion for future studies to minimize this limitation in this study.

Taken together, the results of this study show that the consumption of chia flour is consistently able to decrease the BP in hypertensive individuals, even in patients previously treated with medications in a manner similar to the patients not using medications. This phenomenon was accompanied by lipid peroxidation reduction but not by changes in the inflammatory markers.

Conflict of Interest The authors declare that they have no conflicts of interest.

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