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Orange juice associated with a balanced diet mitigated risk factors of metabolic syndrome: A randomized controlled trial

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HIGHLIGHTS

- The daily consumption of orange juice improved the diet quality of patients with MetS.
- Orange juice, along with a balanced diet, improved glycemic control in patients with MetS.
- Orange juice, along with a balanced diet, improved the lipid profile of patients with MetS.
- Orange juice, along with a balanced diet, reduces systemic inflammation in MetS patients.
- Dietary intervention associated with daily consumption of orange juice reversed MetS in 35% of patients.

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ABSTRACT

In this 12 weeks randomized parallel controlled trial, we investigated whether the daily intake of orange juice (OJ) associated with a balanced diet attenuates risk factors in individuals with metabolic syndrome (MetS) and reverses this condition. Patients were divided into two groups: control (n = 36) and OJ (n = 36), which adopted a balanced diet according to the MetS guidelines. In addition, the OJ group consumed 500 mL/d OJ, maintaining the recommended dietary energy intake but adding more vitamin C (133%) and folic acid (43%) than controls. After the intervention, both groups showed a mean reduction of glucose (−3%), cholesterol (−7.5%), HDLC (−8%), BMI (−2%), waist circumference (−5.5%), and systolic and diastolic blood pressure (−8% and −9.5%, respectively). However, only the OJ group decreased insulin (−9%), insulin resistance (- 8%), LDL-C (−4%), CRP (−28%) and higher hsCRP levels (−61%), while the control group reduced exclusively triglycerides (−8.4%). Both groups showed a slight increase in antioxidant capacity (1%). The reversion of MetS to normality was similar in both groups: 12 out of 36 controls (33%) and 13 out of 36 subjects supplemented with OJ (36%). MetS reversal was due to a decrease in the risk factors, such as systolic pressure in the controls, and high glucose, insulin resistance, systemic inflammation and LDL-C, without altering HDL-C, in the OJ group. In conclusion, both treatments reduced risk factors and together reversed more than 30% MetS to normal, but the addition of OJ mitigated more risk factors than the balanced diet alone. (NCT 03301675).

1. Introduction

Currently, there is great interest in studying the contribution of diet composition to the development, reversal and prevention of obesity and metabolic disorders associated with metabolic syndrome (MetS). This clinical condition occurs most often in overweight and obese individuals, and it is characterized by a set of metabolic risks, including atherogenic dyslipidemia (low HDL and hypertriglyceridemia), hyperglycemia, high blood pressure, and abdominal obesity. The combination of these factors may increase the risk of cardiovascular disease and diabetes [1]. Underneath this condition, an unhealthy eating pattern, with low consumption of fruits and vegetables, and a high intake of salt, sugar and saturated fat, associated with a sedentary lifestyle, is implicated in the development of MetS [2–4]. As stated by the

Abbreviations: MetS, Metabolic Syndrome; OJ, Orange Juice; BMI, Body Mass Index; HDL-C, High-density lipoprotein; DRI, Dietary Reference Intake; HOMA-IR, Evaluation by the Homeostasis Model; TC, Total Cholesterol; LDL-C, low-density lipoprotein; AST, Aspartate Aminotransferase; ALT, Alanine Aminotransferase; ALP, Alkaline Phosphatase; Gama-GT, Gamma Glutamyl Transferase; CRP, C-reactive protein; hsCRP, high-sensitive C-reactive protein; SFA, Saturated Fatty Acid; ABTS, 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid); AMPK, AMP-activated protein kinase; GLUT4, Glucose transporter type 4

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International Diabetes Federation (IDF), the primary intervention for MetS is the promotion of a healthy lifestyle, including a moderate calorie restriction to achieve at least 5% body weight loss, moderate increase in physical activity and change in diet composition [5].

According to the Brazilian guidelines for the treatment of MetS patients, a personalized diet plan should be adopted, focused on a balanced diet, with a higher consumption of fruits, vegetables, legumes, low-fat dairy products, fish, lean meats, liquid oils, whole grains, nuts and seeds [6]. This pattern seems to offer benefits for reducing the risk parameters of MetS [7]. Thus, improvement in diet quality and the adoption of a balanced eating plan, with implementation of physical activity, are key points for the MetS treatment [8]. In this context, the consumption of orange juice (OJ) provides many nutrients and bioactive compounds, such as vitamin C, potassium, folate and citrus flavonoids, and is associated with better diet quality, providing significant health benefits to consumers [9].

However, there is a misperception among health professionals that consuming fruit juices, such as orange juice, leads to weight gain and obesity, based on high in free sugars and low fiber contents. However, much of this evidences do not separate sugar-sweetened fruit beverages from 100% fruit juices [10]. On the other hand, careful epidemiological and clinical studies suggest that moderate consumption of 100% fruit juice is associated with lower BMI and reduced indices of blood cholesterol and MetS [11], but not with a lower risk of hypertension or type 2 diabetes [12–15]. In fact, a hundred percent pure OJ contains only intrinsic sugars such as sucrose, fructose and glucose, totaling approximately 20 g in 8 oz [9]. In addition, 100% fruit juice is a complex food matrix with valuable nutrients, such as vitamins, minerals and phytochemicals that counteract possible harmful effects of simple sugars. In contrast, fruit drinks containing added sugar, such as sucrose or fructose-rich corn syrup, have been associated with adverse health effects that can lead to obesity and type 2 diabetes [11,13,16].

Randomized clinical trials (RCTs) have shown the potential of 100% fruit juices in health promotion because they improved glycemic control and cardiovascular risk factors [14,17–19]. In addition, a recent review pointed out that polyphenol-rich foods , such as 100% fruit juices, are effective in improving the clinical condition of MetS patients [20]. Based on these findings and previous evidence, the present study tested the hypothesis that 100% OJ consumption as a source of micronutrients, antioxidants and bioactive compounds may prevent the aggravation of risk factors in individuals with MetS. The study aimed to associate daily consumption of 100% orange juice with balanced diet as a nutritional strategy for the treatment of MetS with reduction of glycemia as a primary outcome.

2. Material and methods

2.1. Trial design

This was a 12-week, parallel group, randomized clinical trial of MetS individuals (n = 84), conducted at the Laboratory of Nutrition, School of Pharmaceutical Sciences, UNESP, Araraquara, SP, Brazil. Participants were allocated into two groups: control group (n = 42), subjects that received nutritional guidance during the experimental period; and OJ group (n = 42), with nutritional guidance associated with daily consumption of 100% OJ (500 ml). This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by the Ethics Board of Pharmaceutical School, UNESP (# 1.657.604), and the study was registered in the Clinical Trial Protocol Registration and Results System (# NCT03301675, URL: https://clinicaltrials.gov/ct2/show/NCT03301675). All participants signed a written Informed Consent Form in two copies, one for the patient and another for the Ethics Board, before start the study.

2.2. Participants

The recruitment process took place over three months (June–September 2016) and was publicized on local radio, social networks and posters spread throughout the UNESP units in Araraquara and in commercial establishments. Eligibility criteria were men and women between 40 and 60 years with MetS [1]: waist circumference: man ≥ 90 cm and woman ≥ 80 cm [2]; triglycerides ≥ 150 mg/dL [3]; HDL-C man ≤ 40 mg/dL and woman ≤ 50 mg/dL [4]; blood pressure ≥ 130/≥ 85 mm Hg and [5] fasting glucose ≥ 100 mg/dL (considering the use of medication for hypertriglyceridemia, type 2 diabetes, arterial hypertension and low high-density lipoprotein (HDL-C) [1,4]; 25 ≥ BMI ≤ 39,9 kg/m [21]; like to consume OJ; use of digital communication (e-mail, messaging application, telephone messages). Individuals not included were pregnant/nursing mothers; use of vitamin supplements in the last three months, and individuals with diseases that require dietary recommendations, such as: type 2 diabetes with insulin therapy and carbohydrate counts, cancer, chronic liver and kidney disease.

During the recruitment, interviews were conducted by nutritionists and they collected clinical, dietary history, practice of physical activity (sedentary, low active, active and very active) according to Dietary Reference Intake (DRI) [22]. Glucose, triglycerides and HDL-C were measured simultaneously using portable equipment CardioCheck® PA on 12-h fasting blood of the volunteers; Waist circumference was measured at the smallest circumference between the lower extremity of the last rib and the anterior superior iliac spine [23], and blood pressure was measured using ReliOn automatic and digital equipment, HEM-741CRELN, USA, on the same day of the interviews. At the end of this period, of the 187 eligible individuals 103 were excluded: 96 did not meet the inclusion criteria and 7 gave up prior to randomization. Therefore, 84 individuals have started the study (Fig. 1).

2.3. Interventions

Over the 12 weeks, the volunteers had biweekly consultations with two trained and certified nutritionists, at baseline, 2nd, 4th, 6th, 8th, 10th, and 12th week. The prescribed diet plans was individualized, based on the 3-day food record previously filled by each participant, according to recommendations from the I Brazilian Guideline for Metabolic Syndrome [5]. Diet plans for both groups presented a similar nutritional composition, with a few differences for the OJ group due to the contribution of this juice in their diet (Table 1).

The 3-day food record was performed on three non-consecutive days, two weekdays and one weekend day, and was filled out by all the participants at the baseline, 6th and 12th weeks. Data included all food and beverage consumed, based on portion size of local standard kitchen utensils, and also location and time schedule for meals and snacks. Dietitians instructed volunteers to fill out the food-record immediately after eating, in order to minimize memory-related errors and to avoid possible underreporting.

The diet plan was elaborated taking into account the Brazilian Food Guide [24], prioritizing *in natura* and minimally processed foods, and meals prepared according to healthy recipes, respecting each person's routine. The energy requirements were evaluated according to the DRI equations and took into account the basal weight [25]. The dietary plan included six meals a day, divided as follows: Breakfast: milk/dairy, coffee, whole cereals, whole wheat bread, fruits. Morning snack: milk/dairy, oatmeal, fruit yogurt, nuts ***or* 250 ml *of OJ***. Lunch: rice/brown rice, beans/legumes, raw leafy salad, roasted/baked vegetable, grilled meat or egg, and fruit. Afternoon snack: milk/dairy, oatmeal, fruit yogurt, whole grain cereal, mixed nuts ***or* 250 ml *OJ***. Dinner: rice/brown rice, beans/legumes, raw leafy salad, roasted/baked vegetable, grilled meat or egg, fruit or natural snack made with wheat bread, chicken/tuna/ricotta sausage, leaf and vegetable salad, and fruit. Supper: tea, milk/dairy products, and toast.

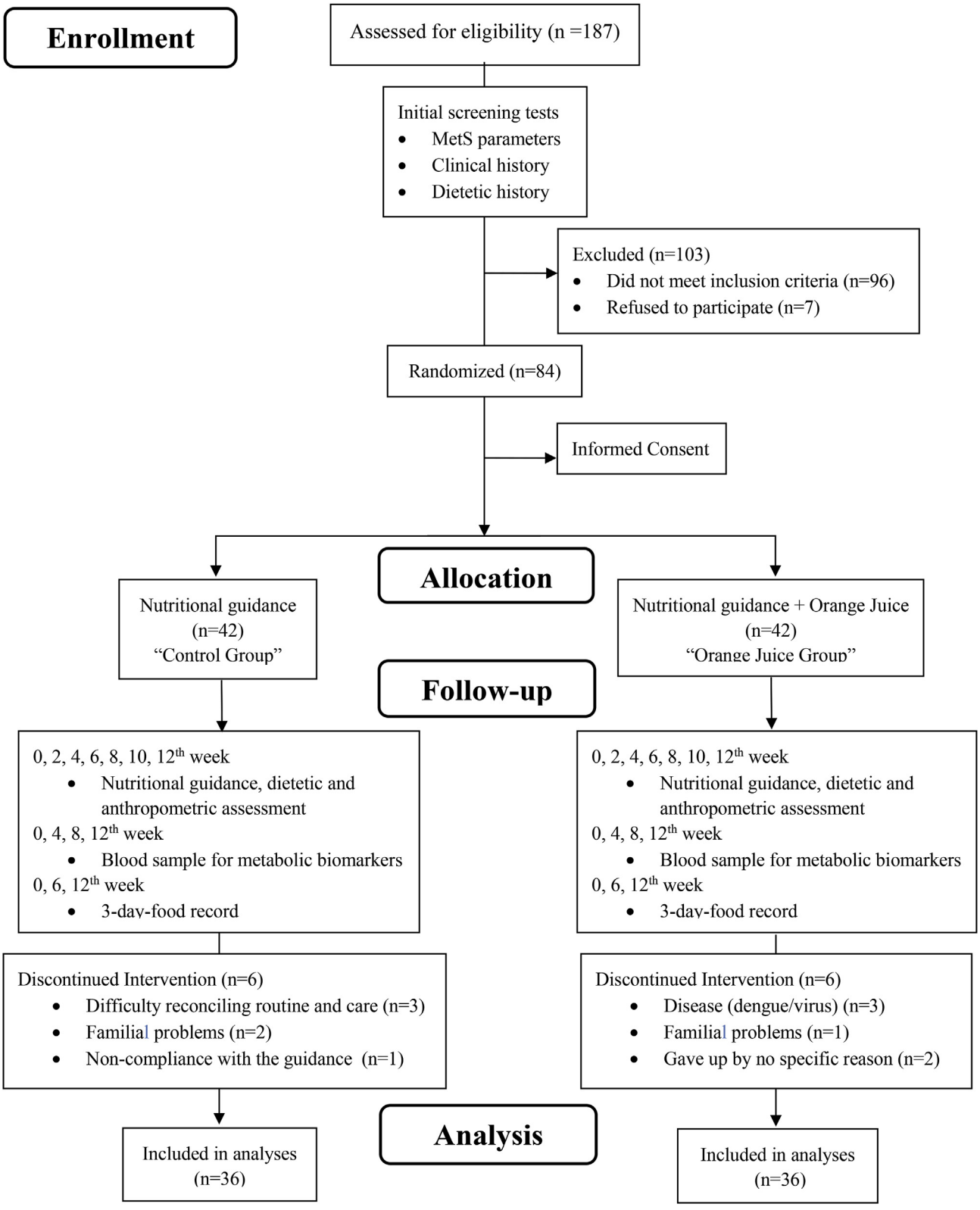

Fig. 1. Trial design diagram showing flow of participants (CONSORT-2010).

The OJ group received 4 L of juice weekly, and they were instructed to drink 250 ml as a morning snack, and plus 250 ml as an afternoon snack. The Control group was advised to consume few citrus fruits or citrus juices, without exceed one serving per week. Other types of non-citrus fruits and juices were allowed according to each individual plan. Morning and afternoon snacks of the control group were designed to have a similar amount of energy as the OJ group. A 24-h food recall was performed individually and adherence to the proposed diet was verified by the nutritionist biweekly.

All participants were contacted by telephone during the experiment as many times as necessary. Online messages were used to remind patients about blood collection, clinical examination, consultation and

Table 1
Nutritional composition of the meal planning for MetS subjects.

Nutrients	Control (n = 36)	Orange juice (n = 36)
Energy, kcal	1876 ± 318	1971 ± 321
Carbohydrate, g	223 ± 37	240 ± 43
Protein, g	111 ± 21	117 ± 23
Total fat, g	59 ± 09	60 ± 11
Fiber, g	25 ± 05	27 ± 06
Vitamin C, mg	147 ± 42	328 ± 35 *
Folate, mcg	339 ± 87	486 ± 78 *
Sodium, mg	1588 ± 245	1673 ± 274
Potassium, mg	4457 ± 838	4658 ± 816
Cholesterol, mg	252 ± 37	245 ± 32
Saturated fatty acid, g	21 ± 04	22 ± 03

Data are shown as mean ± SD.
*Differences between groups detected by independent Test t, significance $p < 0.05$.

distribution of OJ, among other relevant assignments. Regular messages with and between participants by the mobile application ("WhatsApp") allowed good adherence to the experiment, as well as promoting integration and generating incentives to test new recipes and answer questions about diet and orange juice, as well as post positive messages of how to achieve and maintain good health.

100% OJ consumed in this study was obtained by commercial process (Not From Concentrate – NFC), from Pera Rio oranges, supplied by Citrosuco (Matao, SP, Brazil). The juice was filled in 2-L clear PET bottles that were kept frozen until delivery. Participants were advised to thaw the juice under refrigeration before consuming it in one week. The characteristics of the OJ were 0.7% total titratable acidity, 11° Brix total soluble solids, pH 3.95, 189 mg ascorbic acid, 34 mg gallic acid equivalents of phenolic compounds, 950 TEAC μmol antioxidant capacity, 240 kcal, 44 g total sugar, 121.6 mg hesperidin and 26.6 mg naringin (500 ml) taken in two 250 ml doses. OJ analysis was performed in our laboratory and are described in detail elsewhere [15,26].

2.4. Outcomes

Fasting glycemia was defined as the primary endpoint; and the secondary outcomes are listed below [1]: MetS components: waist circumference, triglycerides, HDL-C, systolic blood pressure, and fasting glucose [2]. Other biochemical parameters are: insulin, Homeostasis Model Assessment (HOMA-IR), total cholesterol (TC), low-density lipoprotein (LDL-C), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma-glutamyl transferase (gamma-GT), C-reactive protein (CRP), high-sensitive C-reactive protein (hs-CRP) and antioxidant capacity [3]; Body composition: weight, BMI, muscle mass, fat mass, visceral fat area [4]. Dietary parameters: consumption of energy, protein, carbohydrates, fats, cholesterol, saturated fatty acids, vitamin C, folate, potassium, and sodium.

2.5. Biochemical assessment

Overnight fasting blood samples were collected at baseline, 4th, 8th and 12th week from each participant by trained and qualified technicians from São Lucas Clinical Analyzes Laboratory, Araraquara, SP, Brazil. Total blood was centrifuged and serum was aliquoted, properly identified and stored at −80 °C until the analytical procedures were performed. Fasting glycemia and the secondary measurements, such as triglycerides, TC, LDL-C, HDL-C, AST, ALT, ALP, GGT and CRP were evaluated using VITROS 250 Chemistry System, Johnson & Johnson® Ortho Clinical Diagnostics. Insulin was quantified by electrochemiluminescence immunoassay. Insulin resistance was calculated by the HOMA1-IR equation [glycemia (mmol/L) x 0.0555 × insulinemia (μU/mL)]/22.5 [27] with cutoff point ≥ 2.71 [28]. The analysis of hs-CRP′ was performed using the automated immunoturbidimetry method, and the total antioxidant capacity was evaluated by the 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) radical method [29].

2.6. Anthropometrics, hemodynamics and dietetic assessments

Height was measured by stadiometer at the first consultation with the nutritionist. Body weight, muscle mass, fat mass, fat percentage and visceral fat area were performed with high frequency tetrapolar bioimpedance equipment (Inbody 720®). Volunteers were instructed to fast for 2 h, not to drink alcohol in the last 24 h, to urinate at least 20 min before the test, to wear light clothes and to remove any type of metallic ornament and not to practice intense physical activity in the last 24 h. Waist circumference was measured at the smallest circumference between the lower extremity of the last rib and the superior iliac spine [23]. Blood pressure was measured with automatic digital equipment (ReliOn®). All measurements were taken at baseline, 2nd, 4th, 6th, 8th, 10th and 12th weeks. The quantitative analysis of energy and nutrients was performed with Dietbox software, using the Brazilian Food Composition Table [30,31], based on a non-consecutive 3-day food record.

2.7. Sample size

Sample size was established using BioEstat 5.0, type I error $\alpha = 0.05$, and a type II error $\beta = 0.2$ (80% power), based on a similar clinical study[30], where the reduction of fasting glycemia was observed after hesperidin treatment. Thus, the minimum sample size estimated was 36 individuals per group. The final sample size, considering a dropout rate of 15% (n = 42), was 84 individuals.

2.8. Randomization

The selected participants were stratified by sex, and distributed according to computer-generated random numbers, followed by a randomized block. An independent researcher allocated the subjects in two groups: control group and OJ group. The two nutritionists responsible for the intervention performed all clinical tasks and the OJ delivery schedule during the study period.

2.9. Statistical analysis

Results were expressed as mean ± SD. The normality of the data was evaluated by asymmetry and kurtosis. Student's t-test for independent samples was applied between groups to compare the food plan and the baseline data. Two-way ANOVA followed by post-hoc analysis (Sidak) was used to verify changes in primary and secondary outcomes over time and between groups. Statistical software SPSS v.22 was used to verify the statistical differences. The evaluation of the risk factors of MetS was made by comparing the frequencies before and after the intervention.

3. Results

3.1. Population

Seventy-two participants with an age of 48 ± 9 years, 68% female, completed the study in 12 weeks, from September to December 2016. All participants were obese (BMI ≥ 30 kg/m^2). For educational level, the majority had completed high school education (51%), 31% had college education, and a small portion were postgraduate (6%); 76% reported having income up to 4 times minimum wage, 14% between 4 and 8 times minimum wage and 10% between 8 and 10 times minimum wage. Regarding the practice of physical activity, 35% reported regularly practicing light walks, 65% did not practice any type of activity, and there was no change in this profile throughout the study. No participant used dietary supplements and only two individuals declared themselves smokers. All participants started the interventions under the

Table 2
Baseline characteristics of MetS subjects treated with orange juice over 12 weeks.

Groups	Control (n = 36)	Orange Juice (n = 36)		All subjects (n = 72)
Demographics				
Sex, woman [n (%)]	25 (69%)	24 (67%)		49 (68%)
Age, years	46 ± 9	49 ± 9		48 ± 9
Education level [n (%)]				
Elementary school	3 (9%)	6 (16%)		9 (13%)
High school	18 (50%)	19 (53%)		37 (51%)
College	12 (33%)	10 (28%)		22 (31%)
Post-graduation	3 (8%)	1 (3%)		4 (6%)
Biophysical parameters			***(P §)***	
Weight, kg	95 ± 15	96 ± 16	*(0.91)*	95 ± 16
Height, m	1.65 ± 0.1	1.67 ± 0.1	*(0.15)*	1.66 ± 0.1
Body mass index, kg/m^2	35.1 ± 4.1	34.0 ± 4.2	*(0.30)*	34.6 ± 4.1
Waist circumference, cm	106 ± 11	104 ± 10	*(0.30)*	105 ± 11
Lean mass, kg	31 ± 7	32 ± 7	*(0.52)*	31 ± 7
Fat mass, kg	40 ± 9	39 ± 9	*(0.49)*	40 ± 9
Visceral Fat Area, cm^3	155 ± 30	154 ± 21	*(0.81)*	155 ± 26
Systolic Blood Pressure, mmHg	139 ± 17	136 ± 16	*(0.48)*	137 ± 16
Diastolic Blood Pressure, mmHg	88 ± 10	85 ± 11	*(0.40)*	87 ± 11
Metabolic biomarkers				
Glycaemia, mg/dL	105 ± 15	101 ± 11	*(0.13)*	103 ± 13
Insulin, μU/dL	18.5 ± 9.0	18.6 ± 7.7	*(0.95)*	18.6 ± 8.3
HOMA-IR	4.50 ± 2.05	4.47 ± 1.63	*(0.94)*	4.48 ± 1.84
Triglycerides, mg/dL	201 ± 72	196 ± 72	*(0.78)*	199 ± 72
Total cholesterol, mg/mL	185 ± 33	190 ± 41	*(0.59)*	188 ± 37
HDL-C, mg/dL	55 ± 12	55 ± 7	*(0.83)*	55 ± 10
LDL-C, mg/mL	84 ± 39	87 ± 28	*(0.70)*	86 ± 34
ALT, U/L	34 ± 12	34 ± 12	*(0.84)*	34 ± 12
AST, U/L	22 ± 9	22 ± 7	*(0.77)*	22 ± 8
Alanine phosphatase, U/L	76 ± 22	67 ± 28	*(0.11)*	71 ± 25
Gamma-GT, U/L	37 ± 20	34 ± 15	*(0.48)*	36 ± 18
Inflammatory biomarkers				
c-Reactive Protein, mg/dL	0.82 ± 0.48	0.79 ± 0.76	*(0.16)*	0.81 ± 0.63
hs-C Reactive Protein, mg/dL	0.54 ± 0.41	0.54 ± 0.52	*(0.10)*	0.54 ± 0.47
Oxidative stress				
Antioxidant capacity, μM	1.63 ± 0.01	1.63 ± 0.04	*(0.16)*	1.63 ± 0.02

Data showed as mean ± SD.
§ Independent T-test for all variables tested P > 0.05.

same conditions (Table 2). Most of participants (68%) used drugs to treat hypertension (51%), hyperglycemia (35%), dyslipidemias (17%) and hypothyroidism (18%), and this use did not change throughout the study. The experimental group, which consumed 500 ml/day of 100% OJ, reported no adverse effects. The withdrawal of the participants from the OJ group had no relation to juice intake, and the reasons for their withdrawal were similar to those who gave up in the control group (Fig. 1, item Discontinued Intervention).

3.2. Biochemical markers

Both treatment promoted a significant reduction in fasting blood glucose, 4% in the OJ group and 2.6% in control group, but only the OJ group had a total reduction of 9% in fasting insulin, while control group decreased by just 3.8% at the end of the study. At the baseline period, 81% of participants in the control group and 86% in the OJ group were insulin resistant, but after 12 week the OJ group decreased HOMA-IR by 8%, while the control group reduced only 4% (Table 3). When we evaluated individuals with upper threshold values for HOMA-IR (≥2.4), only the OJ group significantly decreased the number of subjects with insulin resistance after 12 weeks . The OJ group showed a significant reduction of total cholesterol (−8.5%), LDL-C (−4%), HDL-C (−6.1%), and triglycerides (−6.7%) ($p \leq 0.05$), while the Control group showed a respective reduction of total cholesterol (−6.4%), HDL-C (−9.6%), triglycerides (−8.4%), and no reduction for LDL-C (Table 3). No differences were detected between groups (intergroup), such as Control versus OJ ($p > 0.05$).

3.3. Liver enzymes, CRP and antioxidant capacity

There was no change in AST and ALT levels throughout the treatment for either group. However, we observed a reduction of serum Gama-GT and ALP levels by 21% and 26%, respectively, for the Control group, and by 13% and 14% for the OJ group (Table 4). Only the OJ group showed a significant decrease of 28% in serum CRP ($p \leq 0.05$). Considering the individuals with upper threshold values for CRP (≥0.8 mg/dL), and hs-CRP (≥1 mg/dL), we detected a decrease of 36% and 61%, respectively for CRP and hsCRP in OJ group after 12 weeks. On the other hand, the antioxidant capacity significantly increased in both groups: by 1.2% in the control group and 0.9% in the OJ group. No differences were detected between groups (intergroup), such as Control versus OJ ($p > 0.05$).

3.4. Anthropometric and blood pressure assessment

Both treatments promoted a reduction of 2% in body weight and 2% in BMI ($p \leq 0.05$). In addition, all participants decreased waist circumference by 6%, fat mass by 5%, and visceral fat area by 4.5% ($p \leq 0.05$), but maintained lean mass ($p \leq 0, 05$). Systolic blood pressure decreased by 8.6% in the control group, while the OJ group decreased by 6.6% ($p \leq 0.05$). Diastolic blood pressure decreased by 11% in the control group and 8% in the OJ group ($p \leq 0.05$). There was no significant difference between the anthropometric and hemodynamic parameters between the groups (Table 5). No differences were detected between groups (intergroup), such as Control versus OJ ($p > 0.05$).

3.5. Dietetic assessment

The OJ group showed an increase of 157% ($p \leq 0.01$) in vitamin C intake, while the control group had an increase of 21% ($p > 0.05$). The OJ group increased the consumption of folic acid by 132% ($p \leq 0.05$), while the Control group had an increase of 55% ($p \leq 0.05$). The intake of vitamin C and folic acid was 133% and 43%, respectively, higher in the OJ group than in the control group at the end of the 12 weeks of intervention (Table 6)

The effectiveness of the nutritional guidance was observed by the significant reduction in the energy intake in the control group (23%) and the OJ group (18%). This reduction was due to the lower carbohydrate intake in the control group (−24%) and OJ group (−21%); and lower lipids consumption in the control group (−27%) and OJ group (−24%), with no changes in protein intake, which was maintained at 1 g/kg body weight in both groups. In addition, participants consumed 46% more sodium at the beginning of the experiment than the recommended daily dose, but they reduced their intake by 26% at the end of the study. Both groups had a significant increase in dietary fiber by 24% in the OJ group and 29% in the control group. The OJ group showed a significant reduction by 16% in cholesterol intake and 29% in SFA, whereas the control group decreased cholesterol by 9% and SFA by 36%, with no statistical differences between groups (Table 6). No differences were detected between groups (intergroup), such as Control versus OJ ($p > 0.05$).

Table 3
Metabolic biomarkers of MetS subjects submitted to orange juice allied to a dietetic intervention over 12 weeks.

Variables	Weeks					
	0	4	8	12	$\Delta_{(12-0\,wk)}$	*P*
Glycemia, mg/mL						
Control (n = 36)	106 ± 15[a]	106 ± 17[a]	101 ± 15[b]	103 ± 15[b]	−2.6%	*0.05*
Orange juice (n = 36)	101 ± 11[a]	102 ± 14[a]	99 ± 11[a,b]	97 ± 10[b]	−4.0%	*0.02*
Insulin, μU/mL						
Control (n = 36)	18.5 ± 9.0[a]	19.0 ± 8.2[a]	17.5 ± 8.1[a]	17.8 ± 7.9[a]	−3.8%	*0.98*
Orange juice (n = 36)	18.6 ± 7.7[a]	19.2 ± 8.4[a]	17.9 ± 8.9[a]	16.9 ± 7.1[b]	−9.0%	*0.17*
HOMA-IR						
Control (n = 36)	4.50 ± 2.05[a,b]	4.72 ± 2.01[b]	4.18 ± 1.80[a]	4.31 ± 1.72[a]	−4.2%	*0.05*
Orange juice (n = 36)	4.47 ± 1.63[a]	4.54 ± 1.79[a]	4.22 ± 1.95[b]	4.11 ± 1.88[b]	−8.0%	*0.02*
Total Cholesterol, mg/mL						
Control (n = 36)	185 ± 33[a]	174 ± 33[b]	179 ± 33[a,b]	174 ± 30[b]	−6.4%	*0.04*
Orange juice (n = 36)	190 ± 41[a]	183 ± 40[a,b]	180 ± 35[b,c]	174 ± 34[c]	−8.5%	*< 0.001*
LDL-C, mg/mL						
Control (n = 36)	84.2 ± 38.8[a]	81.2 ± 32.9[a]	83.0 ± 27.1[a]	83.6 ± 31.0[a]	−0.8%	*0.55*
Orange juice (n = 36)	87.3 ± 27.8[a]	91.3 ± 33.9[a]	87.3 ± 29.7[a]	83.9 ± 29.0[a]	−3.9%	*0.49*
HDL-C, mg/dL						
Control (n = 36)	55.0 ± 12.3[a]	50.0 ± 12.0[b]	49.3 ± 11.5[b]	49.7 ± 10.5[b]	−9.6%	*< 0.001*
Orange juice (n = 36)	55.5 ± 7.4[a]	52.1 ± 10.7[b]	53.2 ± 8.8[a,b]	52.1 ± 8.1[b]	−6.1%	*0.05*
Triglycerides, mg/mL						
Control (n = 36)	201 ± 72[a]	182 ± 67[b]	185 ± 59[b]	184 ± 61[b]	−8.4%	*0.01*
Orange juice (n = 36)	196 ± 72[a]	182 ± 87[a]	186 ± 68[a]	183 ± 70[a]	−6.7%	*0.79*

Two-way repeated measures ANOVA followed by Sidak post hoc: "orange juice" versus "control group" for 12 wks, $p < 0.05$.
a,b Different letters mean difference between weeks within the same group.
$\Delta_{(12-0)}$ indicates difference between 12th and 0 week.

Table 4
Metabolic biomarkers of liver enzymes, CRP and antioxidant capacity of MetS subjects treated with orange juice over 12 weeks.

Variables	0 week	12 week	$\Delta_{(12-0\,wk)}$	*P*
Liver enzymes				
AST, U/L				
Control (n = 36)	22 ± 9	22 ± 10	0%	*1.00*
Orange juice (n = 36)	22 ± 7	23 ± 7	4,5%	*0.71*
ALT, U/L				
Control (n = 36)	34 ± 12	30 ± 10[a]	−9.5%	*0.03*
Orange juice (n = 36)	34 ± 12	32 ± 9	−6.4%	*0.14*
Alanine phosphatase, U/L				
Control (n = 36)	76 ± 22	57 ± 18[a]	−26%	*< 0.001*
Orange juice (n = 36)	67 ± 29	58 ± 19[a]	−14%	*0.002*
Gamma-GT, U/L				
Control (n = 36)	37 ± 20	29 ± 15[a]	−21%	*0.001*
Orange juice (n = 36)	34 ± 15	30 ± 14	−13%	*0.004*
Inflammatory markers				
CRP, mg/dL				
Control (n = 36)	0.82 ± 0.48	0.78 ± 0.55	−5%	*0.64*
Orange juice (n = 36)	0.79 ± 0.76	0.57 ± 0.45[a]	−28%	*0.05*
CRP ≥ 0.8 mg/dL				
Control (n = 19)	1.18 ± 0.31	0.99 ± 0.62	−16%	*0.29*
Orange juice (n = 15)	1.46 ± 0.77	0.93 ± 0.48[a]	−37%	*0.01*
hs-CRP, mg/dL				
Control (n = 36)	0.54 ± 0.41	0.45 ± 0.35	−17%	*0.17*
Orange juice (n = 36)	0.54 ± 0.52	0.44 ± 0.31	−17%	*0.19*
hs-CRP ≥ 1.0 mg/dL				
Control (n = 6)	1.18 ± 0.13	0.84 ± 0.38	−29%	*0.06*
Orange juice (n = 6)	1.48 ± 0.35	0.57 ± 0.27[a]	−61%	*< 0.001*
Antioxidant capacity, μM				
Control (n = 36)	1.63 ± 0.01	1.65 ± 0.02[a]	1.2%	*< 0.001*
Orange juice (n = 36)	1.63 ± 0.03	1.64 ± 0.02[a]	0.9%	*0.01*

Two-way repeated measures ANOVA followed by Sidak post hoc to compare "orange juice" and "control group" over 12 wk $p < 0.05$.
$\Delta_{(12-0)}$ indicates difference between the 12th and - 0 week.
[a] Means difference between weeks within the same group.

3.6. Evaluation of MetS factors

At baseline, 97% of subjects with MetS had increased waist circumference, 72% elevated triglyceride levels, 68% hypertension, 57% fasting glucose ≥100 mg/dL, and 30% had low HDL-C (Fig. 2). After 12 weeks of treatment, 36% of the control group and 39% of the OJ group has less than three risk factors, reverting their MetS condition (Fig. 2). Among individuals who remained with MetS, about 36% of the control group and 25% of the OJ group decreased one or two parameters of MetS (see Fig. 3).

4. Discussion

Daily consumption of OJ for 12 weeks improved the diet quality of MetS patients, and helped decrease glycemia and insulin resistance, demonstrating that 100% OJ associated with a balanced diet ameliorates glycemic control. Previous clinical trials have shown that regular consumption of OJ has alleviated cardiometabolic risk parameters, such as glycemic control, insulin resistance, lipid profile, arterial hypertension among others variables in individuals with MetS [14], obesity [18] and hypercholesterolemia [33]. In addition, experimental studies have shown that 100% fruit juices positively favor glycemic control, probably due to polyphenols and/or soluble fibers. These results are supported by epidemiological evidence in populations exposed to diets rich in polyphenols [34] and some clinical studies have shown benefits of citrus flavonoids in cardiometabolic risk factors related to the MetS [20,32].

Citrus flavanones present in OJ, hesperidin and naringin, appear to contribute to the reduction of glycemia and improvement insulin resistance in type 2 diabetes, obesity and in MetS models [35–38]. Both flavanones increase hepatic expression of glucokinase-mRNA, while naringin promotes downregulation of gluconeogenic enzymes involved in the synthesis of hepatic glucose [35–38]. An important mechanism that explains the specific action of naringin is the positive regulation of AMP-activated protein kinase (AMPK) related signaling pathway. Activated AMPK also favors glucose transporter type 4 (GLUT4) translocation, enhancing glucose uptake, and improves insulin resistance by IRS-1 receptor phosphorylation and by attenuating phosphorylation of transcription factor-signaling pathways linked to inflammation and IR [37,39]. Our study showed an improvement in insulin resistance, measured by the HOMA-IR index on MetS individuals treated with OJ. This result reflected a significant decrease in glycemia in the OJ group

Table 5
Biophysical parameters of MetS subjects treated with orange juice over 12 weeks.

Parameters	Weeks								$P_{(0\text{-}12\,wk)}$
	0	2	4	6	8	10	12	$\Delta_{(12-0\,wk)}$	
Weight, kg									
Control (n = 36)	95.3 ± 15.3[a]	94.8 ± 15.0[a,b]	94.1 ± 14.9[b,c]	93.8 ± 13.4[b,c]	93.5 ± 14.8[c,d]	93.2 ± 14.7[c,d]	93.1 ± 14.9[d]	−2%	*< 0.001*
Orange juice (n = 36)	95.7 ± 15.8[a]	94.7 ± 16.1[a,b]	94.7 ± 15.5[b]	94.6 ± 15.4[b]	93.9 ± 15.3[b]	94.1 ± 15.3[b]	93.9 ± 15.4[b]	−2%	*0.001*
BMI, kg/m^2									
Control (n = 36)	35.1 ± 4.0[a]	34.9 ± 4.0[a,b]	34.6 ± 4.0[a,b]	34.5 ± 3.9[a,b]	34.4 ± 3.9[b]	34.3 ± 3.9[b]	34.2 ± 4.0[b]	−2%	*< 0.001*
Orange juice (n = 36)	34.0 ± 4.2[a]	33.7 ± 4.4[a]	33.7 ± 4.2[a]	33.6 ± 4.2[a]	33.4 ± 4.4[b]	33.5 ± 4.1[b]	33.4 ± 4.1[b]	−2%	*0.004*
Lean mass, kg									
Control (n = 36)	30.6 ± 6.8[a]	30.3 ± 6.1[a]	30.5 ± 6.5[a]	30.5 ± 6.4[a]	30.5 ± 6.5[a]	30.4 ± 6.4[a]	30.4 ± 6.4[a]	0%	*0.77*
Orange juice (n = 36)	31.6 ± 6.7[a]	31.8 ± 6.7[a]	31.9 ± 6.7[a]	31.8 ± 6.6[a]	31.2 ± 6.5[a]	31.7 ± 6.5[a]	31.8 ± 6.5[a]	0%	*0.81*
Fat mass, kg									
Control (n = 36)	40.5 ± 9.2[a]	40.4 ± 9.1[a]	39.4 ± 9.3[b]	39.2 ± 9.3[b,c]	39.0 ± 9.2[b,c,d]	38.8 ± 9.1[c,d]	38.5 ± 9.2[d]	−5%	*< 0.001*
Orange juice (n = 36)	39.1 ± 8.8[a]	38.6 ± 8.8[a]	37.6 ± 9.2[b,c]	37.6 ± 9.9[b,c]	37.9 ± 8.8[b]	37.3 ± 9.0[c]	37.1 ± 9.1[c]	−5%	*< 0.001*
Waist circumference, cm									
Control (n = 36)	106 ± 11[a]	104 ± 10[b]	103 ± 11[b]	101 ± 9[c]	100 ± 9[c,d]	100 ± 9[c,d]	99 ± 8[d]	−7%	*< 0.001*
Orange juice (n = 36)	104 ± 10[a]	102 ± 11[a,b]	102 ± 11[b]	100 ± 8[c]	100 ± 9[c]	99 ± 9[c]	99 ± 9[c]	−4%	*< 0.001*
Visceral Fat Area (VFA), cm^3									
Control (n = 36)	155 ± 30[a]	155 ± 28[a,b]	152 ± 30[b,c]	150 ± 29[c,d]	150 ± 30[c,d]	149 ± 29[d]	148 ± 30[d]	−5%	*< 0.001*
Orange juice (n = 36)	154 ± 21[a]	153 ± 20[a]	149 ± 20[b]	149 ± 21[b]	150 ± 21[b]	149 ± 22[b]	148 ± 23[b]	−4%	*< 0.001*
Systolic Blood Pressure, mmHg									
Control (n = 36)	139 ± 17[a]	138 ± 17[a]	131 ± 16[b]	129 ± 13[b,c]	128 ± 17[b,c]	127 ± 14[c]	127 ± 16[c]	−9%*	*< 0.001*
Orange juice (n = 36)	136 ± 16[a]	136 ± 16[a]	128 ± 12[b]	130 ± 13[b]	129 ± 12[b]	129 ± 14[b]	127 ± 15[b]	−7%	*< 0.001*
Diastolic BloodPressure, mmHg									
Control (n = 36)	87.5 ± 10.2[a]	86.5 ± 10.1[a]	80.0 ± 8.3[b]	80.3 ± 7.2[b]	79.1 ± 11.4[b]	81.5 ± 10.7[b]	77.6 ± 11.3[b]	−11%	*< 0.001*
Orange juice (n = 36)	85.4 ± 10.8[a]	85.4 ± 10.8[a]	79.7 ± 11[b]	80.9 ± 12[b]	79.6 ± 11.7[b]	79.9 ± 10.9[b]	78.3 ± 16.0[b]	−8%	*0.01*

Two-way repeated measures ANOVA followed by Sidak post hoc to compare "orange juice" and "control group" over 12 wk $p < 0.05$.
[a, b] Different letters mean difference between time (week) within the same group. It was not detected differences between groups for any parameter ($p > 0.05$).
$\Delta_{(12\text{-}0)}$ indicates difference between the 12th and 0 week.

associated with a non-significant reduction of 9% in the basal insulin of these patients. There was also a trend towards improved insulin sensitivity after supplementation with isolated hesperidin for three weeks in MetS adults [40].

The oral administration of hesperidin and naringin in an experimental study significantly decreased fasting glucose and HOMA-IR levels [41]. These results are explained by the authors to be due to antioxidant properties of these compounds, since they promoted an increase in the activity of antioxidant defense system of treated animals [41]. In this context, in addition to flavanones, vitamin C appears to exert positive actions on glycemic and insulinemic control [42]. Because it is known for its antioxidant activities, vitamin C is able to eliminate reactive oxygen species and regenerate antioxidant molecules such as α-tocopherol, glutathione and β-carotene. A higher antioxidant capacity, in turn, is related to the reduction of insulin resistance and better control of glucose [42].

Although the mechanisms associated with vitamin C potential to reducing glucose levels are not completely understand, it is suggested that their antioxidant action may improve endothelial function and reduce oxidative stress, which contributes to the reduction of insulin resistance [43]. In our study, although vitamin C intake was higher in the OJ group, an increase in serum antioxidant capacity was also observed for control subjects. This can be explained by the improvement in overall diet quality in both groups; the intake of vitamin C in the control group remained adequate from the beginning to the end of the experiment, even though it came from sources other than OJ. Thus, we suggest that vitamin C may play an important role in improving insulin resistance observed in subjects who consumed OJ. Our study, therefore, is in line with recent meta-analyzes that show consumption of 100% fruit juice rich in polyphenols does not present any impairment in glucose and insulin homeostasis, and it is not associated with the risk of developing type 2 diabetes or damage in individuals with type 2 diabetes already diagnosed [17,44–46].

Our study showed a reduction of 28% in CRP only in the OJ group ($p < 0.05$). Regarding the reference blood levels of CRP (0.8 mg/dL) or hs-CRP (1 mg/dL) [47], only those individuals with consumption of OJ reduced higher values of CPR (≥0.8 mg/dL) and hs-CRP (≥1 mg/dL). This result has relevance in the clinical practice and suggests the anti-inflammatory effect from OJ, as sustained in previous studies [14,18,48]. In agreement, MetS individuals who consumed 300 ml of natural citrus juice (95% mixture of citrus juices and 5% *A. melanocarpa extract*) daily for six months had a reduction of CRP, concluding that natural fruit juice, especially citrus fruits, may have a beneficial effect on predictive parameters of cardiovascular disease in these patients [49]. High-sensitive CRP has been associated with abdominal obesity and associated metabolic disorders and highlighted as a possible predictive marker for the risk of MetS, cardiovascular disease and type 2 diabetes [47,50]. It is known that insulin resistance is associated with low-grade inflammation induced by several pro-inflammatory mediators in obesity and MetS [3,51]. Thus, the anti-inflammatory effects found in this study may also be related to the reduction of HOMA-IR in participants with insulin resistance who took OJ. In addition, OJ consumption did not induce hepatic toxicity in general, since no change was observed in AST and ALT levels; however there was a reduction in ALP and Gamma-GT concentrations. These results are in agreement with other studies that showed no toxicity or impairment of liver function after daily consumption of OJ or grapefruit juice [18,52].

Regarding the lipid profile, studies have shown that supplementation with hesperidin or OJ leads to significant reductions in the circulating concentration of total cholesterol and LDL-C [18,40,53–56]. Flavonoids can promote a hypolipidemic effect by the reduction of very-low density lipoprotein secretion associated with an increased activity of hepatic LDL receptors, accelerating the clearance of circulating LDL-C particles [14,57]. Even with no difference between groups, we observed a greater trend of reduction in total cholesterol and LDL-C in the OJ group (8.5% and 6.4% versus 6.4% and 0.8% in the control group, respectively). The initial values of LDL-C were not higher than normal and it could not explain significant changes in this variable for this study.

Although *in vitro* and animal studies indicate that flavanones may exert antihypertensive effects, and some clinical studies also point to these benefits [14,19,59] some intervention studies with flavonoids

Table 6
Dietary consumption of energy, macro and micronutrients in MetS subjects treated with orange juice for 12 weeks.

Nutrients	Weeks				*P*
	0	6	12	Δ (12–0 wk)	
Energy, kcal					
Control	2149 ± 816[a]	1682 ± 350[b]	1649 ± 349[b]	−23%	*< 0.001*
Orange juice	2045 ± 669[a]	1758 ± 412[b]	1673 ± 463[b]	−18%	*0.01*
Protein, g					
Control	93.1 ± 34.5[a]	91.7 ± 32.7[a]	93.3 ± 31.9[a]	0%	*0.92*
Orange juice	86.7 ± 32.9[a]	95.9 ± 29.3[a]	91.3 ± 26.2[a]	0%	*0.46*
Carbohydrate, g					
Control	266 ± 135[a]	208 ± 66[b]	202 ± 67[b]	−24%	*0.001*
Orange juice	258 ± 99[a]	209 ± 49[b]	205 ± 60[b]	−21%	*0.002*
Fiber, g					
Control	19.8 ± 7.2[a]	21.2 ± 6.6[a]	25.6 ± 8.3[b]	29%	*< 0.001*
Orange juice	21.3 ± 5.2[a]	23.7 ± 5.1[a]	26.4 ± 7.5[b]	24%	*< 0.001*
Total fat, g					
Control	75.2 ± 29.0[a]	54.3 ± 20.5[b]	55.3 ± 21.0[b]	−26%	*< 0.001*
Orange juice	69.3 ± 36.4[a]	52.1 ± 19.0[b]	52.4 ± 17.4[b]	−24%	*0.001*
Cholesterol, mg					
Control	322 ± 127[a]	270 ± 108[a]	292 ± 108[a]	−9%	*0.27*
Orange juice	315 ± 146[a]	292 ± 126[a]	266 ± 103[a]	−15%	*0.07*
Saturated FA, g					
Control	25.5 ± 11.6[a]	14.6 ± 8.2[b]	16.2 ± 9.5[b]	−36%	*< 0.001*
Orange juice	23.0 ± 10.9[a]	13.0 ± 5.2[b]	15.9 ± 8.7[b]	−31%	*0.001*
Vitamin C, mg					
Control	132 ± 45[A,a]	166 ± 51[A,a]	160 ± 35[A,a]	22%	*0.10*
Orange juice	145 ± 36[A,a]	377 ± 134[B,b]	373 ± 111[B,b]	157%	*< 0.001*
Folate, mcg					
Control	143 ± 22[A,a]	219 ± 29[A,b]	222 ± 36[A,b]	55%	*< 0.001*
Orange juice	136 ± 27[A,a]	311 ± 94[B,b]	318 ± 98[B,b]	133%	*< 0.001*
Potassium, mg					
Control	2469 ± 939[a]	2642 ± 900[b]	3281 ± 1040[b]	33%	*< 0.001*
Orange juice	2425 ± 919[a]	2978 ± 634[b]	3439 ± 852[c]	42%	*< 0.001*
Sodium, mg					
Control	2269 ± 1036[a]	1606 ± 510[b]	1666 ± 571[b]	−27%	*< 0.001*
Orange juice	2107 ± 955[a]	1602 ± 241[b]	1558 ± 319[b]	−26%	*0.002*

Two-way repeated measures ANOVA followed by Sidak post hoc to compare "orange juice" and "control group" over 12 wk $p < 0.05$.
[a, b] Different lowercase letters mean difference between times within the same group.
[A, B] Different upper case letters mean difference between groups.

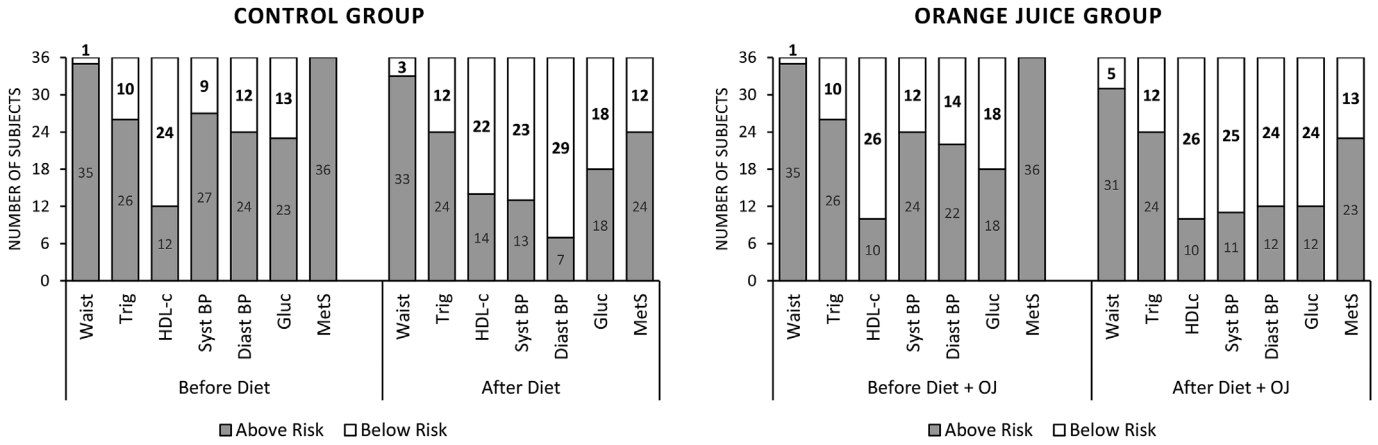

Fig. 2. Number of subjects with risk factors above or below MetS threshold (last column), at baseline and after 12 weeks of intervention with balanced diet (Control Group, n = 36) versus balanced diet with orange juice (OJ Group, n = 36).

have not detected changes in blood pressure, regardless of the improvement in other parameters of endothelial function [52]. Other hemodynamic parameters were not evaluated in this study; however, we observed a significant reduction in systolic and diastolic pressure in both interventions. In this case, we can suggest that dietary modification, which led to a significant reduction in sodium intake and weight loss, may have had a priority influence on the improvement in participants' blood pressure.

Another concern regarding the consumption of 100% fruit juice refers to weight gain, justified by the natural sugar of the fruit and its energy contribution. In agreement with our study, others did not observe any increase to body weight after 12 weeks of daily consumption of OJ in overweight and obese men, without considering other health conditions. In fact, the participants had a balanced energy consumption since there was no change in weight in both interventions, OJ or control drink [11]. Other studies did not observe weight gain with the inclusion of citrus juices in the diet [14,18,19,52], and no association was found between OJ intake and weight gain in children with controlled energy

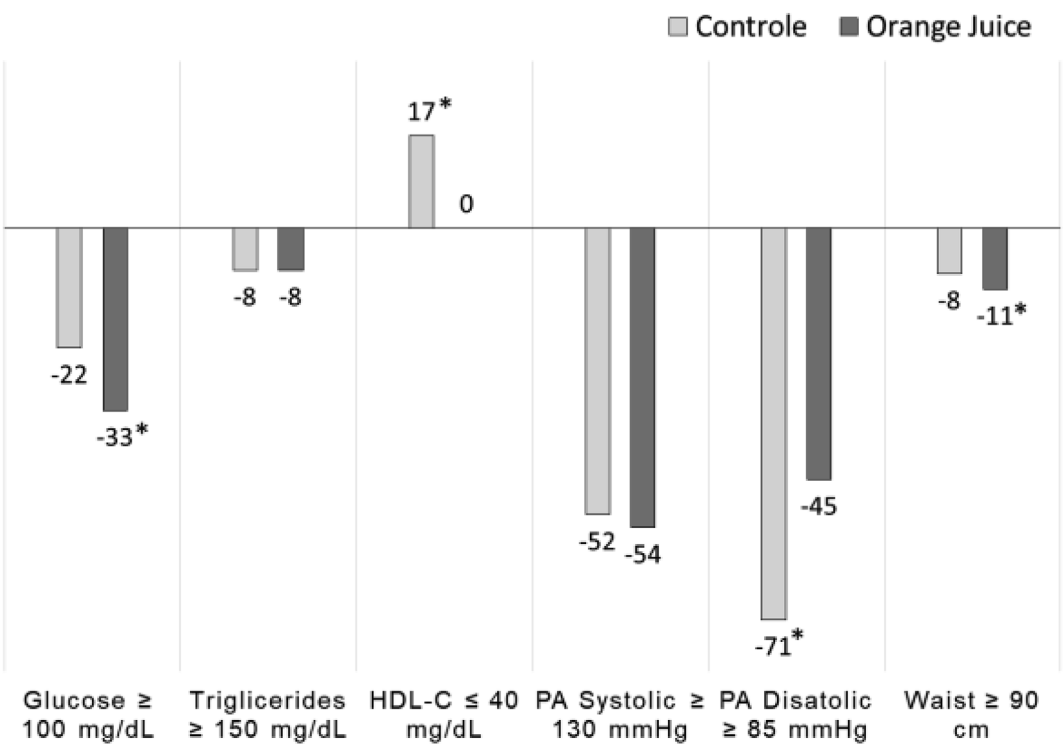

Fig. 3. Changes in MetS risk factors (% of subjects) after 12 weeks of intervention with balanced diet (Control Group, n = 36), or balanced diet plus orange juice (OJ Group, n = 36) (*p < 0.05).

intake, and in adults consumption of 100% fruit juice is inversely associated with BMI [57–60].

Participants from both intervention groups lost body weight and fat mass, with emphasis on visceral fat that decreased 4.5% VFA and 6% waist circumference, but maintained lean mass. A similar result was found previously with obese subjects on a caloric restriction diet plus 500 ml of 100% OJ for 12 weeks. They had an average body weight loss of 6 kg and 8% of body fat loss, with none muscle mass loss [18]. Our results suggested that a balanced diet with the right amounts of energy and macronutrients was able to promote fat loss, without impairing muscle mass.

Nutritional guidance in this study did not prioritize the restriction of energy intake, but rather the adequacy of the routine dietary choices of participants, providing a modification in the profile of food consumed. As observed through dietary records, there was an increase in consumption of high nutritional value foods such as foods *in natura* and/or minimally processed, with the inclusion of fruits, juice and vegetables, and reduction in intake of high energetic density foods, rich in sodium and added sugar, as ultra-processed food. The change in dietary pattern resulted in less energy intake in both groups, with less carbohydrates, total fat, cholesterol and saturated fatty acid, but with more fiber and micronutrients. In the OJ group, as expected, there was a higher intake of vitamin C and folate compared to the control group. The I Brazilian Guideline of Diagnosis and Treatment of the Metabolic Syndrome [7] predicts that the food changes must promote a reduction of 5–10% of body weight. In our study, the weight loss was below this level, but improved MetS parameters after 12 weeks of intervention. Weight loss, especially visceral fat reduction, promotes potential effects on the reduction and reversal of cardiometabolic markers [60].

These results agree with epidemiological studies showing that the consumption of 100% fruit juice was associated with increase of important nutrients, including vitamins C, B-complex , such as B-6, folate and thiamine, magnesium and potassium, promoting better diet quality besides a higher consumption of fruits in children and adults [9,59].

This study investigated the combined effects of nutrients (sugars, vitamis, minerals) and bioactive components (antioxidants and flavonoids) contained in OJ from clear oranges (Pera Rio variety). Therefore, it was sugested that hesperidin, the main flavonoid of OJ, plays a relevant role in the observed results. Similar studies with “red OJ”, made from red-flashed oranges, or “blood OJ” from blood oranges, showed distinct effects on the cardiometabolic risks based on their predominant bioactive component, as lycopene (red OJ) [14], or anthocyanin (blood OJ) [61]. Both compounds enhance the antioxidant capacity and anti-inflammatory outcomes in the blood and organs. In fact, the synergy or the interaction among citrus flavonoids and other bioactive compounds are in consideration in nutrigenomics field nowadays.

The molecular mechanisms underlying to bioactive citrus compounds are still under study, but some of them were recognized for lowering levels of inflammatory mediators, such as IL-6 and hsCRP. Indeed they inhibit IL-6 and TNF-α mRNA expression, activate PPARγ expression and inhibit nuclear factor kappa B (NFκB), with consequent reduction of inflammatory cytokine secretion, increasing adiponectin and sensitivity to insulin [19,20,37,38]. All of these effects may improve the metabolic condition of MetS subjects who intake sources of bioactive compounds, as OJ, or citrus flavonoid supplements. In conclusion, we are able to show that OJ behaved as a functional food, increasing the density of bioactive compounds in a balanced diet, and improving or reversing cardiometabolic risks in individuals with MetS.

CRediT authorship contribution statement

Olivia Ponce: Data curation, Formal analysis, Writing - original draft, Writing - review & editing. **Renata Benassi:** Data curation, Formal analysis. **Thais Cesar:** Conceptualization, Funding acquisition, Supervision, Writing - review & editing.

Declaration of competing interest

The authors do not have any conflicts of interest and the source of funding is independent of the objectives and results, and all authors

have read and understood the guidelines about copyright, ethical guidelines, following legal requirements of the study country.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.jnim.2019.100101.

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