



Applied nutritional investigation

Orange juice allied to a reduced-calorie diet results in weight loss and ameliorates obesity-related biomarkers: A randomized controlled trial



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ABSTRACT

Objective: Assumptions have linked orange juice (OJ) consumption with weight gain and adverse effects on health due to its sugar content; however, epidemiologic studies have not shown increased risk for overweight or obesity with the consumption of 100% OJ. The aim of this study was to verify whether the combination of a reduced-calorie diet (RCD) and 100% OJ contribute to weight loss, promote changes in glucose and lipid metabolism, and improve diet quality in obese individuals.

Methods: A randomized controlled trial with 78 obese patients (age 36 ± 1 y, body mass index [BMI] 33 ± 3 kg/m²) were enrolled in two groups: Individuals in the OJ group submitted to an RCD that included OJ (500 mL/d), and individuals in the control group submitted to an RCD without OJ. Body composition, biochemical biomarkers, and dietary intake were analyzed over a 12-wk period.

Results: Both treatments had similar outcomes regarding body weight (-6.5 kg; $P = 0.363$), BMI (-2.5 kg/m²; $P = 0.34$), lean mass (-1 kg; $P = 0.29$), fat mass (-5 kg; $P = 0.58$), body fat (-3% ; $P = 0.15$), and waist-to-hip ratio (-0.1 ; $P = 0.79$). Insulin levels in the OJ group decreased by 18% ($P = 0.05$), homeostasis model assessment–insulin resistance by 33% ($P = 0.04$), total cholesterol by 24% ($P = 0.004$), low-density lipoprotein cholesterol by 24% ($P \leq 0.001$), and high-sensitivity C-reactive protein levels by 33% ($P = 0.001$) compared with the control group. Consumption of energy and nutrients was similar between the two groups, but vitamin C and folate increased by 62% ($P \leq 0.015$) and 39% ($P = 0.033$), respectively, after OJ intervention.

Conclusion: When consumed concomitantly with an RCD, OJ does not inhibit weight loss; ameliorate the insulin sensitivity, lipid profile, or inflammatory status, or contribute nutritionally to the quality of the diet.

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Introduction

Obesity is associated with a cluster of complications, including impaired glucose tolerance, dyslipidemia, hypertension, and systemic inflammation, which are independent risk

factors for cardiovascular disease and diabetes [1]. Currently, it is well recognized that diets rich in fruits and vegetables contribute to weight control and regulation of metabolic parameters, preventing or reversing the aforementioned conditions [2]. Such foods, rich in water and fiber, reduce the energy density of the diet, promote satiety, and decrease calorie intake [3]. Furthermore, fruits and their juices are classified as nutrient-dense foods, providing vitamins, minerals, and bioactive compounds with relatively few calories [4]. In the context of nonpharmacologic therapies for obesity, lifestyle modifications, including a reduced-calorie diet (RCD) [5] combined or not with physical activity, along with behavioral techniques, have been recommended as fundamental strategies for successful loss and maintenance of weight [6].

CitrusBr provided funding for this study. CR enrolled participants, collected data, and wrote the first version of the paper. GD generated the random-allocation sequence, assigned participants to interventions, and edited the first version of the paper. TC conceived and designed the study. All authors contributed significantly to analysis and interpretation of data, discussion, editing, and approval of the final version of this paper. The authors have no conflicts of interest to declare.

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Assumptions recently have emerged about the role of the consumption of fruit juices in the obesity epidemic, contributing to weight gain in children [7] and adults [8]. Nevertheless, those studies have not distinguished between “fruit juice-containing beverages” and “100% fruit juices,” whose compositions are different in terms of energy and nutrients, sugars, and bioactive compounds [9,10]. Recent evidence has shown that the daily consumption of orange juice (OJ) does not contribute to adiposity or weight gain [11], insulin resistance and inflammation [12,13], or dyslipidemia [14], contradicting the negative reports about consumption of OJ.

Although there are still controversies linking the consumption of fruit juice to weight gain, to our knowledge there are no studies regarding the possible role of OJ consumption on weight loss induced by an RCD. Thus, the aim of the present study was to assess the combination of an RCD and 100% OJ on body composition, food intake, and metabolic biomarkers of obesity.

Material and methods

Trial design

A 12-wk, parallel group, randomized (block size) controlled trial was conducted in the Pharmacy School, Sao Paulo State University, Brazil. The recruitment process began in September 2015, and the intervention was carried out in October 2015. The sample number took into account variations in body weight with a type I error $\alpha = 0.05$ and a type II error $\beta = 0.2$ (80% power). Participants were assigned to one of the two groups, “orange juice (OJ)” or “control” by a random-number generator program. The OJ group ($n = 39$) consumed an RCD that included OJ (500 mL/d); the control group ($n = 39$) consumed only an RCD. Body composition measurements were collected monthly; blood samples and dietary questionnaires were collected every 2 wk (Fig. 1). Primary and secondary endpoints were the reduction of weight considering a weight loss by 5% between initial and final body weight and beneficial modification of the levels of obesity-related metabolic biomarkers considered, respectively.

Participants

Of the 89 volunteers who were screened, 5 were excluded because they did not meet the criteria for inclusion; 84 (ages 18–50 y) were eligible and enrolled in the study. Six individuals dropped out for the following reasons: personal ($n = 4$), pregnancy ($n = 1$), and moved away ($n = 1$). Thus, 78 volunteers completed the intervention and were included in the final analysis. Eligibility criteria were body mass index (BMI) ≥ 30 or ≤ 40 kg/m². Exclusion criteria were dieting over the past year; use of drugs, vitamins, and dietary supplements; alcohol consumption (>20 g alcohol/d); and intense physical activity (>5 h/wk). All participants declared to be sedentary and denied the practice of physical activity during the trial period. The Ethics Board of Pharmacy School, UNESP, approved the study. All participants provided written informed consent. This clinical study has been declared on the website ClinicalTrials.com.

Interventions

Food intake was estimated using 3-d food records, applied six times for each patient: 0-, 2-, 4-, 6-, 8-, and 12-wk. Energy, macronutrient, total lipids, cholesterol, saturated fatty acids, vitamin C, and folate were calculated by Avanutri Software based on Brazilian Food Composition Table [15]. To design the RCD plan, we used the information of first 3-d food records with the objective of respecting the personal food tastes of the participants. The individualized diet plan was prescribed in accordance to the total energy expenditure (TEE) for each individual, minus 500 kcal/d (30% TEE) [16] and macronutrient distribution was adjusted in accordance with the Acceptable Macronutrient Distribution Range [17]. Participants were advised to substitute equivalent foods using a food replacement list (FRL) based on MyPlate's food groups, as fruits, vegetables, grains, protein, dairy, and others (oil, sugar, salt) [18]. Food portion size was based on Brazilian standard kitchen utensils, and all participants were advised by the dietitian to use them to measure their own consumption. The dietary plan was composed of six meals daily: breakfast (e.g., fat-free milk and coffee, whole-grain bread with margarine, and an apple); snack 1 (250 mL OJ or energy-equivalent item from food groups); lunch (e.g., brown rice, beans, grilled lean meat, salad, cooked vegetables); snack 2 (250 mL OJ or energy-equivalent item from food groups); dinner (e.g., brown rice, beans, grilled lean meat, cooked vegetables, and salad); and snack 3 (e.g., unsweetened tea plus 120 kcal from FRL). Nutritionists assessed compliance of diet plan and OJ consumption every 2 wk, closely monitoring each patient during the procedure.

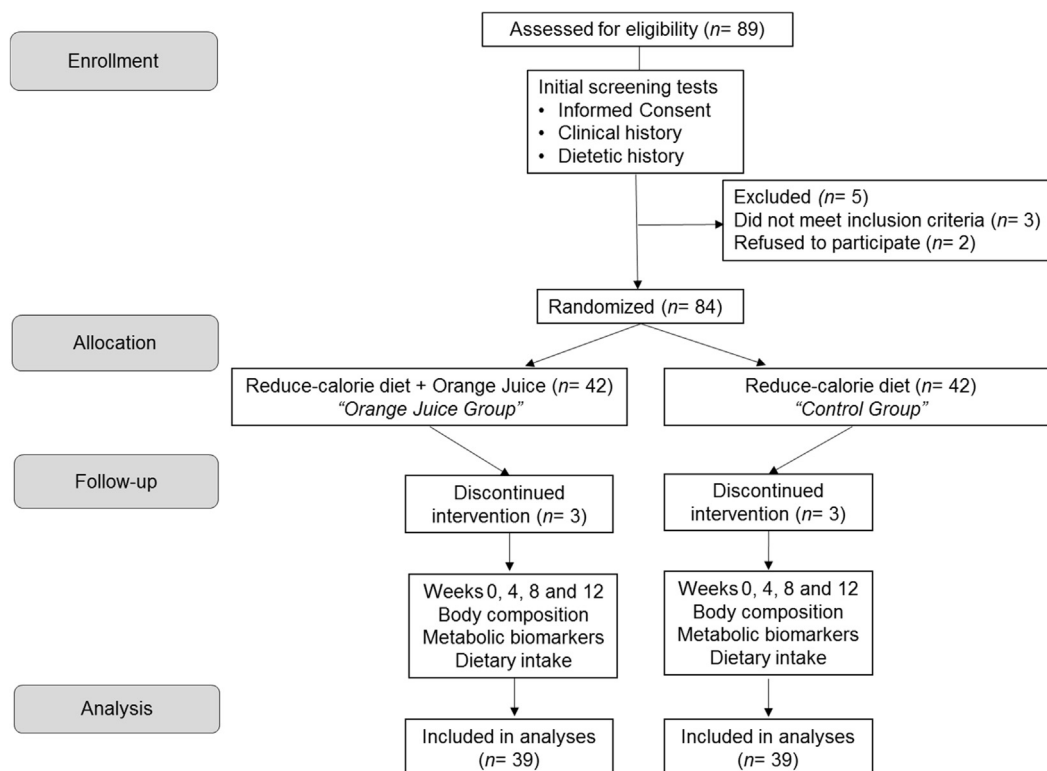


Fig. 1. Trial design.

Body composition measurements were performed using standardized procedures [19]. Bioelectrical impedance (InBody 720, Biospace, Tokyo, Japan) was used to determine body weight (kg) and composition, including fat mass (kg), lean mass (kg), and percentage of body fat with the participants barefoot and wearing light clothes. Overnight fasting blood samples were obtained at the inception of the trial and after weeks 4, 8, and 12 of intervention, and serum was stored at -80°C . Metabolic biomarkers were performed using commercial kits and included triacylglycerol (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), glucose, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and γ -glutamyl transferase (GGT; Labtest, Brazil); low-density lipoprotein cholesterol (LDL-C) [20]; and high-sensitivity C-reactive protein (hs-CRP; Dade Behring, Deerfield, IL, USA). Homeostasis model assessment–insulin resistance (HOMA-IR) was calculated, and the cutoff was set at ≥ 2.71 [21]. Lipid peroxidation was assessed by thiobarbituric acid reactive substances assay [22] and total antioxidant capacity (TAC) by radical 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) assay [23].

Citrosuco (Matao, SP, Brazil) provided 100% OJ, which was obtained from Pera Rio oranges. The characteristics of the OJ were 0.7% total titratable acidity and 15°Brix total soluble solids, 204 mg ascorbic acid, 34 mg phenolic compounds of gallic acid equivalent, 950 trolox equivalent antioxidant capacity μmol antioxidant capacity, 240 kcal, 44 g total sugar, 162 mg hesperitin, and 7.7 mg naringenin [24] in two doses of OJ (500 mL). The analysis of OJ was performed in our laboratory and is described in detail elsewhere [11].

Statistical analysis

Kolmogorov–Smirnov and Levene's tests were used to assess normality and homogeneity, respectively. We conducted *t* test to identify possible differences between OJ and control groups at baseline. Two-way repeated measures analysis of variance followed by Sidak post hoc test were applied to compare changes within and between OJ and control groups over the 12-wk time period. Significance was set at $P \leq 0.05$.

Results

Participants

Seventy-eight individuals (24 men; 54 women) ages 36 ± 1 y with a BMI 33 ± 3 kg/m² were included in the data analyses. Participants from both groups started the intervention under the same conditions (Table 1). Fasting serum levels of glucose, insulin, TC, LDL-C, non-HDL-C, TG, ALP, AST, ALT, and GGT were within the reference values for all participants. However, 53 individuals were above the HOMA-IR cutoff point (2.71), indicating that 68% of the population was insulin resistant. Additionally, 66% of the individuals showed elevated basal levels of hs-CRP in relation to reference values, whereas HDL-C levels were $\sim 25\%$ below optimal recommended value [25] (Table 1).

Body composition

In the OJ group, body weight gradually declined at each time point: 3 kg at 4 wk, 2 kg at 8 wk, and 2 kg at 12 wk, for a loss of 7 kg at the end of the trial, and BMI was reduced from 33 to 31 kg/m². A loss of 1 kg of lean mass occurred by week 4, and it remained until week 12. Average fat mass was reduced by 2 kg at 4 wk, followed by 2 kg at 8 wk and 1 kg at 12 wk, for a loss of 5 kg and a reduction of 3% of body fat. Also, a loss of 8 and 5 cm of waist and hip circumference, respectively, contributed to reducing the waist-to-hip ratio (WHR) from 1 to 0.9 (Table 2). Control group participants also exhibited reduced body weight by 3 kg at 4 wk, followed by 2 kg at 8 wk and 1 kg at 12 wk, for a loss of 6 kg, and BMI declined from 34 to 31 kg/m². A loss of 1 kg of lean mass by 4 wk was observed and remained the same until week 12. A decrease of fat mass was verified of 2 kg at 4 wk, 2 kg at 8 wk, and 1 kg at 12 wk, for a loss of 5 kg of fat mass and a reduction of 3% of body fat. Moreover, a loss of 9 and 5 cm on waist and hip circumference, respectively, and a change in the WHR of 1 to 0.9 (Table 2) was observed. Thus,

there was no difference in body composition between the OJ and control groups.

Metabolic biomarkers

Over time, the OJ group showed a reduction of blood glucose by 8%, insulin by 27%, HOMA-IR index by 34%, TC by 16%, LDL-C by 29%, non-HDL-C by 20%, TG by 22%, hs-CRP by 40%, ALP by 7%, AST and ALT by 14%, GGT by 16%, and lipid peroxidation by 47%. However, no change was observed in TAC (Table 2). The control group showed a reduction by 5% in blood glucose, 13% in insulin, 10% in HOMA-IR index, 9% in total LDL and non-HDL-C, 21% in TGs, 20% in hs-CRP, 7% in ALP, 10% in AST, 4% in GGT, and 47% in lipid peroxidation, with no change in ALT or TAC (Table 2). The OJ group had a higher reduction in insulin and HOMA-IR levels, respectively, by 27% and 41% at 8 wk and by 18% and 33% at 12 wk. Additionally, the OJ group showed a decrease in TC and hs-CRP levels of 24% and 33%, respectively, after 12 wk of intervention compared with the control group (Table 2).

Diet

There was a reduction in the quantity of energy and the intake of most nutrients as well as an increase in vitamin C and folate in both intervention groups (Fig. 2). In the OJ group, the average intake of total energy decreased by 630 kcal compared with the initial levels (0 wk); carbohydrates were lower by 46% kcal, protein by 4% kcal, lipids by 30% kcal, cholesterol by 24%, and saturated fatty acids (SFAs) by 25%. Vitamin C and folate intake increased by 184 mg and 232 μg , respectively, by the end of the study. At the end of the trial, the control group showed a reduction in the intake of energy (491 kcal), carbohydrates (46% kcal), protein (7% kcal), lipids (32% kcal), cholesterol (21%), and SFAs (21%) and an increase in vitamin C and folate intake (43 mg and 70 μg , respectively; Fig. 2).

Table 1

Baseline characteristics of obese participants submitted to orange juice allied to a reduced-calorie diet intervention over 12 wk

Variables	Orange juice group (n = 39)	Control group (n = 39)	All participants (N = 78)
Age, y	37 $\pm$ 1	35 $\pm$ 1	36 $\pm$ 1
BMI, kg/m ²	33 $\pm$ 3	34 $\pm$ 4	33 $\pm$ 3
Glucose, mg/dL	87 $\pm$ 7	85 $\pm$ 8	86 $\pm$ 7
Insulin, $\mu\text{U/mL}$	15 $\pm$ 6	15 $\pm$ 6	15 $\pm$ 6
HOMA-IR	3.2 $\pm$ 1.5	3.1 $\pm$ 1.3	3.1 $\pm$ 1.4
Total cholesterol, mg/dL	185 $\pm$ 21	181 $\pm$ 31	183 $\pm$ 27
LDL-C, mg/dL	119 $\pm$ 27	115 $\pm$ 27	118 $\pm$ 27
HDL-C, mg/dL	44 $\pm$ 8	47 $\pm$ 11	45 $\pm$ 10
Non-HDL-C, mg/dL	145 $\pm$ 27	133 $\pm$ 29	139 $\pm$ 28
Triacylglycerols, mg/dL	140 $\pm$ 40	141 $\pm$ 43	140 $\pm$ 41
hs-CRP, mg/dL	0.5 $\pm$ 0.1	0.5 $\pm$ 0.1	0.5 $\pm$ 0.1
ALP, U/L	73 $\pm$ 18	72 $\pm$ 16	73 $\pm$ 17
AST, U/L	21 $\pm$ 9	21 $\pm$ 7	21 $\pm$ 8
ALT, U/L	23 $\pm$ 8	20 $\pm$ 9	21 $\pm$ 14
GGT, U/L	25 $\pm$ 7	24 $\pm$ 6	25 $\pm$ 7

ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; GGT, γ -glutamyl transferase; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment–insulin resistance; hs-CRP, high-sensitivity C-reactive protein; LDL-C, low-density lipoprotein cholesterol

Data are presented as mean $\pm$ SE

Independent *t* test, $P < 0.05$

Table 2

Body composition and metabolic markers of obese participants submitted to orange juice allied to a reduced-calorie diet intervention over 12 wk

Variables	Orange juice (n = 39)				Control (n = 39)			
	0 wk	4 wk	8 wk	12 wk	0 wk	4 wk	8 wk	12 wk
Body composition								
Body weight, kg	97 ± 12 ^a	94 ± 12 ^b	92 ± 12 ^c	90 ± 11 ^d	98 ± 12 ^a	95 ± 12 ^b	93 ± 12 ^c	92 ± 11 ^d
BMI, kg/m ²	33 ± 3 ^a	32 ± 3 ^b	31 ± 3 ^c	31 ± 3 ^c	34 ± 4 ^a	33 ± 3 ^b	32 ± 3 ^c	31 ± 3 ^d
Lean mass, kg	31 ± 6 ^a	30 ± 6 ^b	30 ± 6 ^b	30 ± 6 ^b	30 ± 5 ^a	29 ± 5 ^b	29 ± 5 ^b	29 ± 5 ^{b,c}
Fat mass, kg	36 ± 10 ^a	34 ± 10 ^b	32 ± 10 ^c	31 ± 10 ^d	38 ± 10 ^a	36 ± 10 ^b	34 ± 10 ^c	33 ± 9 ^d
Body fat, %	37 ± 9 ^a	36 ± 9 ^b	34 ± 9 ^c	34 ± 9 ^c	40 ± 8 ^a	39 ± 9 ^b	38 ± 9 ^c	37 ± 8 ^d
Waist circumference, cm	104 ± 10 ^a	100 ± 10 ^b	97 ± 10 ^c	96 ± 9 ^c	102 ± 10 ^a	98 ± 10 ^b	95 ± 10 ^c	93 ± 9 ^d
Hip circumference, cm	113 ± 9 ^a	110 ± 9 ^b	109 ± 10 ^c	108 ± 8 ^d	114 ± 9 ^a	113 ± 9 ^b	110 ± 8 ^c	109 ± 8 ^d
WHR	1.0 ± 0.1 ^a	1.0 ± 0.1 ^a	0.9 ± 0.1 ^b	0.9 ± 0.1 ^b	1.0 ± 0.1 ^a	1.0 ± 0.1 ^a	0.9 ± 0.1 ^b	0.9 ± 0.1 ^b
Metabolic biomarkers								
Glucose, mg/dL	87 ± 10 ^a	83 ± 8 ^b	82 ± 7 ^{bc}	80 ± 7 ^c	85 ± 6 ^a	81 ± 6 ^b	81 ± 7 ^b	81 ± 6 ^b
Insulin, μU/mL	15 ± 6 ^{A,a}	13 ± 5 ^{A,b}	11 ± 4 ^{A,c}	11 ± 4 ^{A,c}	15 ± 6 ^{A,a}	15 ± 7 ^{A,a}	14 ± 5 ^{B,b}	13 ± 6 ^{B,c}
HOMA-IR	3.2 ± 1.5 ^{A,a}	2.8 ± 1.2 ^{A,b}	2.2 ± 0.8 ^{A,c}	2.1 ± 0.8 ^{A,c}	3.1 ± 1.3 ^{A,a}	3.1 ± 1.4 ^{A,a}	2.9 ± 1.4 ^{B,b}	2.7 ± 1.1 ^{B,c}
Total cholesterol, mg/dL	185 ± 21 ^{A,a}	173 ± 21 ^{A,b}	170 ± 28 ^{A,c}	155 ± 20 ^{A,d}	181 ± 30 ^{A,a}	172 ± 30 ^{A,b}	171 ± 28 ^{A,b}	165 ± 28 ^{B,c}
LDL-C, mg/dL	119 ± 27 ^{A,a}	111 ± 24 ^{A,b}	107 ± 26 ^{A,b}	85 ± 18 ^{A,c}	115 ± 27 ^{A,a}	109 ± 23 ^{A,b}	108 ± 26 ^{A,b}	105 ± 27 ^{B,b}
HDL-C, mg/dL	44 ± 8 ^a	42 ± 8 ^b	43 ± 9 ^{a,b}	44 ± 10 ^a	47 ± 11 ^a	44 ± 10 ^b	44 ± 10 ^b	44 ± 10 ^b
Non-HDL-C, mg/dL	144 ± 27 ^a	135 ± 24 ^b	130 ± 25 ^c	115 ± 24 ^d	133 ± 30 ^a	130 ± 30 ^b	127 ± 29 ^c	121 ± 27 ^d
Triacylglycerols, mg/dL	140 ± 40 ^a	127 ± 41 ^b	117 ± 40 ^c	109 ± 34 ^d	141 ± 43 ^a	133 ± 40 ^b	119 ± 40 ^c	112 ± 38 ^c
hs-CRP, mg/dL	0.5 ± 0.1 ^{A,a}	0.4 ± 0.1 ^{A,b}	0.4 ± 0.1 ^{A,b}	0.3 ± 0.1 ^{A,c}	0.5 ± 0.1 ^{A,a}	0.5 ± 0.1 ^{A,a}	0.4 ± 0.1 ^{A,b}	0.4 ± 0.1 ^{B,b}
ALP	73 ± 18 ^a	71 ± 15 ^b	70 ± 16 ^b	68 ± 18 ^c	72 ± 18 ^a	69 ± 16 ^b	68 ± 15 ^b	67 ± 15 ^b
AST, U/L	21 ± 9 ^a	21 ± 6 ^a	19 ± 5 ^b	18 ± 5 ^b	21 ± 7 ^a	20 ± 6 ^{a,b}	19 ± 6 ^b	19 ± 6 ^b
ALT, U/L	22 ± 8 ^a	22 ± 8 ^a	19 ± 8 ^b	19 ± 9 ^b	20 ± 9 ^a	23 ± 10 ^b	21 ± 9 ^{a,c}	20 ± 7 ^{a,c}
GGT, U/L	25 ± 7 ^a	22 ± 9 ^b	22 ± 9 ^b	21 ± 8 ^c	24 ± 6 ^a	23 ± 7 ^b	23 ± 8 ^b	23 ± 8 ^b
Antioxidant capacity, μM	1.8 ± 0.03 ^a	1.8 ± 0.04 ^a	1.9 ± 0.04 ^a	1.9 ± 0.04 ^a	1.8 ± 0.08 ^a	1.8 ± 0.08 ^a	1.8 ± 0.05 ^a	1.8 ± 0.04 ^a
Lipid peroxidation, (MDA) mM	1.5 ± 0.9 ^a	1.2 ± 0.6 ^b	1.2 ± 0.6 ^b	0.8 ± 0.4 ^c	1.7 ± 0.8 ^a	1.5 ± 0.7 ^b	1.5 ± 0.7 ^b	0.9 ± 0.5 ^c

ALP, alanine phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, γ-glutamyl transferase; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment–insulin resistance; hs-CRP, high-sensitivity C-reactive protein; LDL-C, low-density lipoprotein cholesterol; MDA, malondialdehyde; WHR, waist-to-hip ratio

Two-way repeated measures analysis of variance followed by Sidak post hoc test to compare changes within and between orange juice and control groups over 12-wk intervention period; $P < 0.05$

Different lowercase letters (a, b, c, d) indicate difference between the times within the group, and different uppercase letters (A, B) indicate difference, between groups

Discussion

This study brought to light new findings in the debate concerning the possible contribution of 100% fruit juice consumption to body weight and obesity-related diseases. The association of RCD with OJ induced a similar body weight loss as that of RCD alone, but also promoted a greater reduction in the levels of blood serum cholesterol, LDL-C, HOMA-IR, and hs-CRP and supplied vitamin C and folate. To our knowledge, this is the first study to evaluate the combination of RCD and 100% OJ in obese patients. Moreover, the dietary follow-ups helped participants adopt the prescribed diet plan more easily, promoting a loss of 6 kg of body weight, a reduction of 8% of fat mass, and a drop of 3% in lean mass over a 12-wk period. There also was a reduction of BMI, waist and hip circumferences, and the WHR. The adaptation to the diet over time seems to have been a key factor in sparing the loss of lean mass, suggesting that the consumption of OJ does not hinder the weight-reducing effect of an RCD.

A systematic review examined the associations between the consumption of 100% fruit juice and weight status, nutrient intake, and nutrient adequacy, showing no association between 100% fruit juice consumption (apple and OJ) and weight or adiposity gain in children after controlling energy intake [26]. Epidemiologic surveys with adults have revealed that consumption of 100% fruit juice is associated with lower BMI and reduction of some risk factors for metabolic syndrome [27]. Clinical studies have reported the beneficial effects of regular consumption of OJ on lipid profile by the reduction of TC and LDL-C in overweight and obese individuals [11], metabolic syndrome [12,13], and hypercholesterolemia [14].

Recently, some scientists and clinicians have been indiscriminately mentioned the consumption of beverages containing

natural or added sugar as detrimental, thus affecting their consumption by the public [12]. However, the differences among types of juices have not been disclosed clearly. For instance, 100% citrus juice does not contain extrinsic sugars and therefore are not a sugar-sweetened beverage [28]. Thus, the attention should be focus on the food quality, dietary total energy, and lifestyle, more than discouraging the consumption of fruit juices, which have an important nutritional contribution for the diet [27,29].

In both groups of individuals, a mild and similar reduction in fasting glucose levels was observed, showing that OJ as part of an RCD did not contribute to increments of glucose over time. There was a similar reduction of insulin levels in both groups until the 7-wk time point, but from weeks 8 to 12, only the OJ group had a continued decrease in insulin. In the present study, a reduction of HOMA-IR was observed in both users and non-users of OJ, but this change was greater after 8 wk in the OJ group. Although this study was not designed to test the influence of OJ or its components on insulin levels, others have shown that hesperidin indirectly affects insulin resistance status by two pathways. First, it was shown that OJ [30] and hesperidin [31] can stimulate the growth of gut microorganisms, which increases the production of short-chain fatty acids that modulate adipose tissue, skeletal muscle, and liver tissue function, improving glucose homeostasis and insulin sensitivity [32]. Second, metabolites of hesperidin, 7-O-glucuronide and 3'-O-glucuronide, activate peroxisome proliferator-activated receptor-γ that alters the transcription of genes that encode the expression of adiponectin and glucose transporter 4, improving insulin sensitivity, inflammation, plasma levels of free fatty acids, and blood pressure [33].

The OJ group had lower TC (17%) and LDL-C (30%) levels than the control group after 12 wk of RCD. Indeed, lowered blood cholesterol was expected due to the effects of hesperidin and

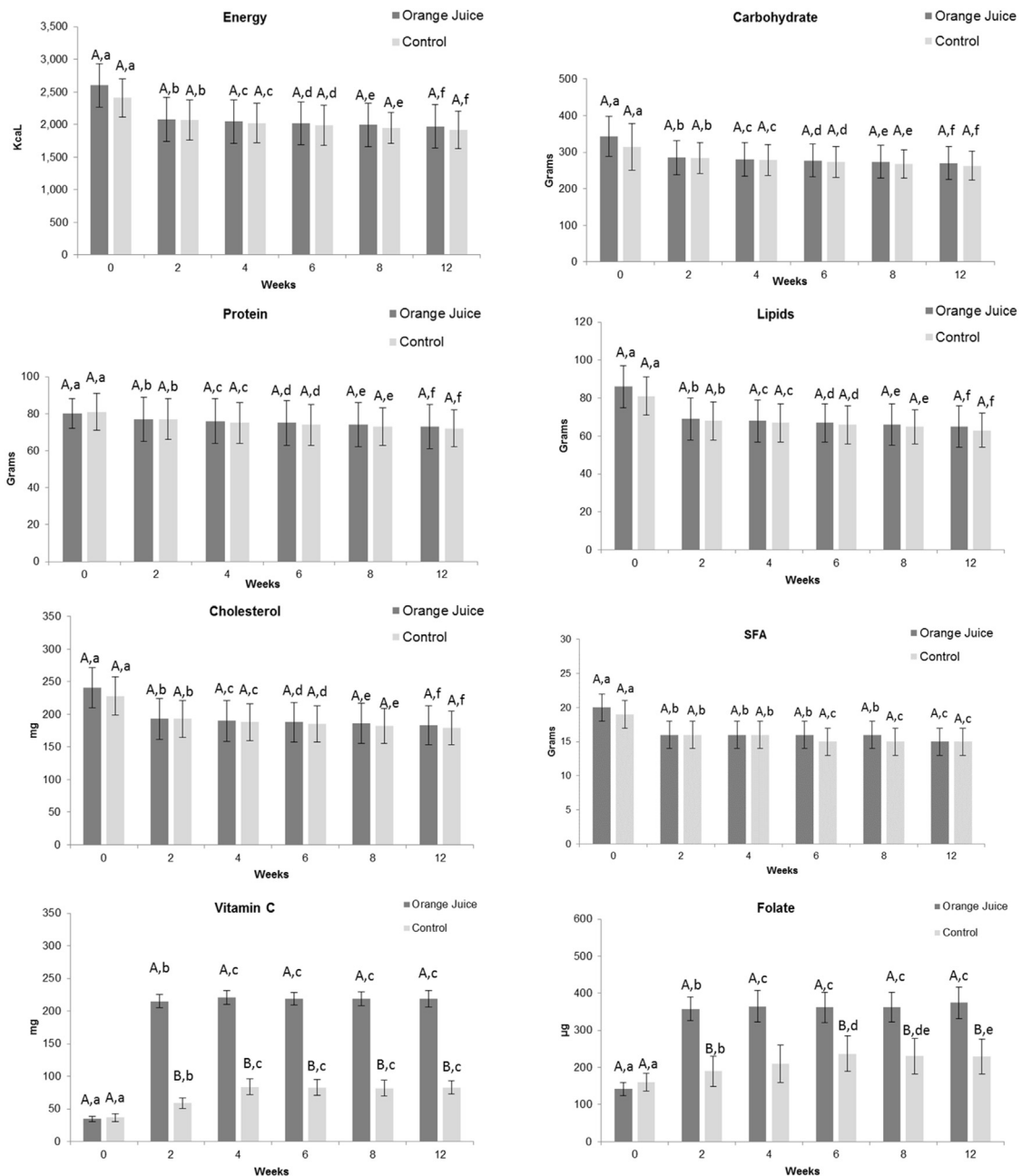


Fig. 2. Intake of energy and macronutrients of obese participants submitted to orange juice allied reduced-calorie diet intervention over 12 wk. Values are means $\pm$ SD. Two-way repeated measures analysis of variance followed by Sidak post hoc test to compare changes within and between orange juice and control groups over the 12-wk intervention ($P < 0.05$). Different lowercase letters mean difference between the times within the group; different uppercase letters mean difference between groups.

naringin in reducing the hepatic secretion of very low-density lipoprotein and, consequently, LDL-C into the bloodstream. These changes lead to increased hepatic receptors that speed the clearance of circulating LDL-C particles [34].

The antiinflammatory role of OJ associated with an RCD was evidenced by the decrease of hs-CRP (33%). Analysis over the 12-wk period showed a reduction from 0.5 to 0.3 mg/dL in hs-CRP levels in the OJ group, suggesting a change of 40% in inflammatory status by the end of the trial, compared with a reduction of 20% in the controls. High hs-CRP levels in blood serum (>0.3 mg/dL) have been associated with an increased risk for coronary events and, more recently, with low-grade

inflammation induced by visceral adiposity [35]. The accumulation of visceral adipose tissue induces increased expression of interleukin-6 and, consequently, the increase of CRP expression [36]. Therefore, we suggest that 100% OJ could be considered a beverage with antiinflammatory proprieties, which is supported by previous studies [36–38].

Concerning dietary intake, we observed a gradual reduction of macronutrients, cholesterol, and SFA in both groups. These changes can be attributed to adaptation to the prescribed diet, which was quite different from the previous dietary pattern of all individuals. As expected, OJ consumption over time improved the folate intake from 142 to 374 μ g, adjusting to $\sim 95\%$ of the

recommended daily requirements. With respect to vitamin C, there was a 500% increase in its intake, exceeding three times the recommended daily requirement (75–90 mg), without exceeding the tolerable upper limit intake. Considering only the effect of interventions, the OJ group had a significant increase in vitamin C and folate intake by 163% and 62%, respectively, compared with the control group.

Despite the Dietary Guidelines for Americans [4] recommendations of 8 ounces of 100% fruit juice being equivalent to one serving of whole fruit, the consumption of 16 ounces of OJ does not seem detrimental based on the parameters analyzed during the 12 wk of intervention. Furthermore, the amount offered to the participants was supported by previous studies [11,13,14]. A systematic review shows evidence that supports a positive relationship between 100% fruit juice consumption and disease prevention [26]. In general, consuming 100% fruit juices may help individuals meet their nutritional needs, especially in terms of micronutrients, bioactive compounds, and antioxidants, similar to the whole fruit [39].

Limitations of the present study included the midterm follow-up of the RCD combined with OJ, and the lack of blindness of participants.

Conclusion

The present study concluded that the addition of OJ does not affect the weight loss induced by RCD. Additionally, this combination of RCD and OJ does not increase serum glucose, but it does improve insulin sensitivity, antiinflammatory status, and the nutritional quality of the diet. Thus, moderate consumption of OJ provides benefits allied to a weight loss intervention and has no adverse effect on body weight and metabolic parameters in obese patients.

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References

- [1] Stoner L, Cornwall J. Did the American Medical Association make the correct decision classifying obesity as a disease? *Australas Med J* 2014;7:462–4.
- [2] Giugliano D, Ceriello A, Esposito K. The effects of diet on inflammation: emphasis on the metabolic syndrome. *J Am Coll Cardiol* 2006;48:677–85.
- [3] Tohill BC, Seymour J, Sordula M, Kettel-Khan L, Rolls BJ. What epidemiologic studies tell us about the relationship between fruit and vegetable consumption and body weight. *Nutr Rev* 2004;62:365–74.
- [4] U.S. Department of Agriculture and U.S. Dietary Guidelines for Americans 2010. 7th ed. Washington, DC: U.S. Government Printing Office.
- [5] Soare A, Weiss EP, Pozzilli P. Benefits of caloric restriction for cardiometabolic health, including type 2 diabetes mellitus risk. *Diabetes Metab Res Rev* 2014;30:41–7.
- [6] Verheggen RJ, Maessen MF, Green DJ, Hermus AR, Hopman MT, Thijssen DH. A systematic review and meta-analysis on the effects of exercise training versus hypocaloric diet: distinct effects on body weight and visceral adipose tissue. *Obes Rev* 2016;17:664–90.
- [7] Shefferly A, Scharf RJ, DeBoer MD. Longitudinal evaluation of 100% fruit juice consumption on BMI status in 2–5-year-old children. *Pediatr Obes* 2016;11:221–7.
- [8] Pan A, Malik VS, Hao T, Willett WC, Mozaffarian D, Hu FB. Changes in water and beverage intake and long-term weight changes: results from three prospective cohort studies. *Int J Obes* 2013;37:1378–85.
- [9] Hyson DA. A review and critical analysis of the scientific literature related to 100% fruit juice and human health. *Adv Nutr* 2015;6:37–51.
- [10] Rampersaud GC, Valim MF. 100% citrus juice: nutritional contribution, dietary benefits, and association with anthropometric measures. *Crit Rev Food Sci Nutr* 2015;57:129–40.
- [11] Dourado GK, Cesar TB. Investigation of cytokines, oxidative stress, metabolic, and inflammatory biomarkers after orange juice consumption by normal and overweight subjects. *Food Nutr Res* 2015;59:28147.
- [12] Simpson EJ, Mendis B, Macdonald IA. Orange juice consumption and its effect on blood lipid profile and indices of the metabolic syndrome: a randomised, controlled trial in an at-risk population. *Food Funct* 2016;7:1884–91.
- [13] Silveira JQ, Dourado GK, Cesar TB. Red-fleshed sweet orange juice improves the risk factors for metabolic syndrome. *Int J Food Sci Nutr* 2015;66:830–6.
- [14] Aptekmann NP, Cesar TB. Long-term orange juice consumption is associated with low LDL-cholesterol and apolipoprotein B in normal and moderately hypercholesterolemic subjects. *Lipids Health Dis* 2013;12:119.
- [15] Unicamp. Tabela Brasileira de Composição de Alimentos. TACO. 2^a ed. São Paulo: Brazil: Campinas; 2006.
- [16] Institute of Medicine (IOM). Dietary reference intakes: the essential guide to nutrient requirements. Available at: <http://www.nap.edu/catalog/11537.html/>. Accessed April 20, 2015.
- [17] Institute of Medicine (IOM). Dietary references intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein and aminoacids (macronutrients). Available at: <https://www.nap.edu/read/10490/chapter/1>. Accessed April 20, 2015.
- [18] United States Department of Agriculture: Center for Nutrition Policy and Promotion. MyPlate background. Available at: <http://www.cnpp.usda.gov/Publications/MyPlate/Background.pdf>. Accessed December 5, 2016.
- [19] World Health Organization. Obesity: prevention and managing the global epidemic: report of a WHO consultation on obesity. Geneva, Switzerland: WHO; 1998.
- [20] Friedwald WT, Levy RI, Friedrickson DS. Estimation of concentration of LDL cholesterol in plasma without preparation or ultracentrifugation. *Clin Chem* 1972;18:499–502.
- [21] Matthews D, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and β -cell function from fasting plasma glucose and insulin concentration man. *Diabetologia* 1985;28:412–9.
- [22] Yagi K. Simple assay for the level of total lipid peroxides in serum or plasma. *Methods Mol Biol* 1998;108:101–6.
- [23] Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic Biol Med* 1999;26:1231–7.
- [24] Silveira JQ, Cesar TB, Manthey JA, Baldwin EA, Bai J, Raitore S. Pharmacokinetics of flavanone glycosides after ingestion of single doses of fresh-squeezed orange juice versus commercially processed orange juice in healthy humans. *J Agric Food Chem* 2014;62:12576–84.
- [25] Ridker P, Buring J, Rifai N. Development and validation of improved algorithms for the assessment of global cardiovascular risk in women: the Reynolds risk score. *JAMA* 2007;297:611–9.
- [26] Crowe-White K, O’Neil CE, Parrott JS, Benson-Davies S, Droke E, Gutschall M, et al. Impact of 100% fruit juice consumption on diet and weight status of children: An evidence-based review. *Crit Rev Food Sci Nutr* 2016;56:871–84.
- [27] O’Neil E, Nicklas TA, Rampersaud GC, Fulgoni VL. 100% orange juice consumption is associated with better diet quality, improved nutrient adequacy, decreased risk for obesity, and improved biomarkers of health in adults: National Health and Nutrition Examination Survey, 2003–2006. *Nutr J* 2012;11:107.
- [28] US FDA. U.S. Food and Drug Administration Agency Information Collection Activities. Available at: <http://www.gpo.gov/fdsys/pkg/FR-2011-12-29/html/2011-33303.htm>. Accessed August 17, 2016.
- [29] Bolling BW, Chen YY, Chen CYO. Contributions of phenolics and added vitamin C to the antioxidant capacity of pomegranate and grape juices: synergism and antagonism among constituents. *Int J Food Sci Technol* 2013;48. <http://dx.doi.org/10.1111/ijfs.12261>.
- [30] Duque AL, Monteiro M, Adorno MA, Sakamoto IK, Sivieri K. An exploratory study on the influence of orange juice on gut microbiota using a dynamic colonic model. *Food Res Int* 2016;84:160–9.
- [31] Unno T, Hisada T, Takahashi S. Hesperetin modifies the composition of fecal microbiota and increases cecal levels of short-chain fatty acids in rats. *J Agric Food Chem* 2015;63:7952–7.
- [32] Canfora EE, Jocken JW, Blaak EE. Short-chain fatty acids in control of bodyweight and insulin sensitivity. *Nat Rev Endocrinol* 2015;11:577–91.
- [33] Gamo K, Miyachi H, Nakamura K, Matsuura N. Hesperetin glucuronides induce adipocyte differentiation via activation and expression of peroxisome proliferator-activated receptor- γ . *Biosci Biotechnol Biochem* 2014;78:1052–9.
- [34] Assini JM, Mulvihill EE, Huff MW. Citrus flavonoids and lipid. *Curr Opin Lipidol* 2013;24:34–40.
- [35] Stepień A, Stepień M, Wlazel RN, Rysz MP. New indices of visceral adiposity and its correlation with hs-CRP in patients with obesity. *Med Sci Tech* 2011;52:91–5.

- [36] Devaraj S, Autret C, Jialal I. Reduced-calorie orange juice beverage with plant sterols lowers C-reactive protein concentrations and improves the lipid profile in human volunteers. *Am J Clin Nutr* 2006;84:756–61.
- [37] Buscemi S, Rosafio G, Arcoleo G, Mattina A, Canino B, Montana M, et al. Effects of red orange juice intake on endothelial function and inflammatory markers in adult subjects with increased cardiovascular risk. *Am J Clin Nutr* 2012;95:1089–95.
- [38] Deopurkar R, Ghanim H, Friedman J, Abuaysheh S, Sia CL, Mohanty P, et al. Differential effects of cream, glucose, and orange juice on inflammation, endotoxin, and the expression of Toll-like receptor-4 and suppressor of cytokine signaling-3. *Diabetes Care* 2010;33:991–7.
- [39] United States Department of Agriculture. USDA branded food product database. Available at: <https://ndb.nal.usda.gov/ndb/foods/>. Accessed May 20, 2016.