



Green Juice in Human Metabolism: A Randomized Trial

Marina Chiochetta, Eduarda Jardim Ferreira, Isabel Taís da Silva Moreira, Richard Chuquel Silveira de Avila, Alcyr Alves de Oliveira, Fernanda Michielin Busnello, Elizandra Braganhol & Alethéa Gatto Barschak

To cite this article: Marina Chiochetta, Eduarda Jardim Ferreira, Isabel Taís da Silva Moreira, Richard Chuquel Silveira de Avila, Alcyr Alves de Oliveira, Fernanda Michielin Busnello, Elizandra Braganhol & Alethéa Gatto Barschak (2018): Green Juice in Human Metabolism: A Randomized Trial, Journal of the American College of Nutrition, DOI: [10.1080/07315724.2018.1457458](https://doi.org/10.1080/07315724.2018.1457458)

To link to this article: <https://doi.org/10.1080/07315724.2018.1457458>



Published online: 27 Apr 2018.



Submit your article to this journal [↗](#)



View related articles [↗](#)



View Crossmark data [↗](#)



Green Juice in Human Metabolism: A Randomized Trial

Marina Chiochetta^a, Eduarda Jardim Ferreira^b, Isabel Taís da Silva Moreira^b, Richard Chuquel Silveira de Avila^b, Alcyr Alves de Oliveira^c, Fernanda Michielin Busnello^d, Elizandra Braganhol^e, and Alethéa Gatto Barschak^a

^aDepartamento de Ciências Básicas da Saúde, Programa de Pós-Graduação em Patologia, Universidade Federal de Ciências da Saúde de Porto Alegre, Porto Alegre, Rio Grande do Sul, Brazil; ^bUniversidade Federal de Ciências da Saúde de Porto Alegre, Porto Alegre, Rio Grande do Sul, Brazil; ^cPrograma de Pós-Graduação em Psicologia, Universidade Federal de Ciências da Saúde de Porto Alegre, Porto Alegre, Rio Grande do Sul, Brazil; ^dPrograma de Pós-Graduação em Ciências da Nutrição, Universidade Federal de Ciências da Saúde de Porto Alegre, Porto Alegre, Rio Grande do Sul, Brazil; ^ePrograma de Pós-Graduação em Biociências, Universidade Federal de Ciências da Saúde de Porto Alegre, Porto Alegre, Rio Grande do Sul, Brazil

ABSTRACT

Objective: Fruits and vegetables contain many compounds presenting potential antioxidant activity. The objective of this study was to evaluate the effect of a green juice recipe in adult metabolism in order to identify new preventive dietary sources.

Method: This was a single-blind randomized controlled clinical trial. Recruitment and data were, respectively, made and collected at the Universidade Federal de Ciências da Saúde de Porto Alegre. Individuals who met all the inclusion criteria during the period of recruitment were included. Green juice (experimental group) or placebo (control group) were consumed from Monday to Friday between 8 and 9 am, in the amount of 300 mL for 60 days (except Saturdays and Sundays). To verify the effect of green juice on metabolism, the following were evaluated: (a) glycemia, plasma lipid profile, renal and liver functions, redox profile, and antioxidant enzymes; (b) anthropometry; and (c) well-being and anxiety.

Results: This study included 14 participants in the test group (juice group) and 13 controls (placebo group), with mean ages of 31.07 and 30.15 years, respectively. We did not observe a significant difference between the treatments. Dietary properties of vegetable and fruit juices are an area of significant interest.

Conclusions: Together with an analysis of previous works, we suggest that green juice did not cause an improvement in metabolic function and there is a need for further research on this issue, mainly through different interventions and other samples.

ARTICLE HISTORY

Received 14 January 2018
Accepted 20 March 2018

KEYWORDS

Bioactive compounds; diets; general nutrition; supplements and functional foods; metabolism

Introduction

In the last decade, preventive medicine has undergone a great advance, especially in developed countries. Research has demonstrated that nutrition plays a crucial role in chronic diseases prevention, as most of them can be related to diet. Functional food proposes the concept that food is not only necessary for staying alive but also a source of mental and physical well-being. Therefore, functional food contributes to prevention and reduction of risk factors for several diseases or enhances certain physiological functions (1). A food can be regarded as functional if it exhibits an extra function, which is often related to health improvement.

The main antioxidants in vegetables are vitamins C and E, carotenoids, and phenolic compounds, especially flavonoids. These antioxidants absorb free radicals and inhibit the initiation or interrupt the chain of propagation of oxidative reactions promoted by free radicals (2,3). Epidemiological studies have shown a close correlation between fruit consumption and a reduction of chronic disease risk. It is believed that the combination of vitamins, minerals, phenolic antioxidant compounds, and fiber is responsible for the desired effect (4).

Antioxidant phytochemicals can be found in many foods and medicinal plants and play an important role in prevention and treatment of chronic diseases caused by oxidative stress. They often possess strong antioxidant and free radical-scavenging abilities as well as anti-inflammatory action. Besides, anticancer, anti-aging, and protective action for cardiovascular diseases, diabetes mellitus, obesity and neurodegenerative diseases are also phytochemicals health benefits (5).

The production of reactive oxygen species (ROS) and reactive nitrogen species (RNS), among other reactive species, is an integral part of human metabolism, and it is observed in several physiological conditions, including aging and immune/inflammatory responses (6). ROS and RNS have important biological functions, as in phagocytosis, a phenomenon in which reactive species are produced to eliminate the attacking agent. The organism has an efficient antioxidant system that is able to control and maintain the balance. However, oxidative stress results from imbalance between pro-oxidant and antioxidant system (7,8), with a predominance of oxidants, presenting consequent damage.

To minimize damage to macromolecules and tissues, living organisms use antioxidant molecules that limit oxidative stress

promoted by ROS when oxidation levels exceed the acceptable limit. This defense mechanism can detoxify the microenvironment of oxidative agents even before they can cause injury or block ROS production. Molecules with antioxidant activity allow erythrocytes to resist oxidative attacks, preventing accelerated lipid peroxidation and other cellular damage such as protein inactivation (9).

The antioxidant system is classified as enzymatic and non-enzymatic. The enzymatic is represented mainly by antioxidant enzymes: superoxide dismutase (SOD), which catalyzes the dismutation of superoxide radical anion ($O_2^{\bullet-}$) to hydrogen peroxide (H_2O_2) and O_2 ; catalase (CAT), whose active site contains a heme group that acts on decomposition of H_2O_2 to O_2 and H_2O ; and glutathione peroxidase, which acts on peroxides in general by using glutathione as a cofactor (10). The non-enzymatic antioxidant system consists of several compounds, with emphasis on glutathione, the main intracellular antioxidant substance; on tocopherols (vitamin E), molecules that qualitatively exhibit biological activity of α -tocopherol (the most potent compound and usually the predominant form) that works by blocking the propagation stage of polyunsaturated fatty acids' (from membranes and lipoproteins) lipid peroxidation; and on ascorbate (vitamin C), a molecule that acts directly as an antioxidant on ROS in an aqueous biological environment, preventing initiation of lipid peroxidation and resulting in the formation of ascorbyl radical anions or indirectly by regenerating vitamin E, which acts as an antioxidant in the lipophilic phase of the membrane (10,11).

There are also other antioxidant molecules such as uric acid, tannic acid, and β -carotene as well as transition metal ion transport proteins such as transferrin (iron transport) and ceruloplasmin (copper transport and oxidation of iron to be captured by transferrin) (11).

The intake of green juices is widespread in the population; however, there is no consensus about its composition. Green juices have aroused great interest among researchers because their components present different functional properties assisting the immune system and reducing the action of ROS/RNS, which are involved in pathological conditions (12). It has been demonstrated that green juice decreased lipid peroxidation and the activity of the antioxidant enzyme CAT in liver of rats, suggesting a beneficial effect of the juice, by modulating cell redox state. Furthermore, it was found that green juice reduced weight gain in these animals by approximately 20%, indicating that this supplementation could have a beneficial effect on protection against obesity (13). The main hypothesis of this study is that the human population can benefit from the effects of green juice consumption. Therefore, the aim of this study was to verify the effect of green juice on human metabolism through the evaluation of biochemical parameters, redox profile, body mass index (BMI), and well-being.

Methods

Participants

The investigated sample was composed of individuals from the academic community of Universidade Federal de Ciências da Saúde de Porto Alegre (UFCSPA), Rio Grande do Sul, Brazil.

Individuals who met all the inclusion criteria during the period of recruitment (July to August 2015) were included. Written informed consent was obtained from all the volunteers. The present study was approved by the Ethics Committee of the UFCSPA (protocol number 1.074.266).

Inclusion and exclusion criteria

The target population consisted of healthy adults of both sexes aged between 18 and 59 years. Smokers, individuals with a diagnosed pathology, individuals using any dietary or vitamin and/or mineral supplement, and individuals who had any kind of allergy or intolerance to juice components and/or placebo were excluded.

Study design

This study was characterized as a single-blind randomized controlled trial.

Procedures

Individuals received supplementation with a recipe of fruit-and-vegetable juice (supplemented group) or an artificial green beverage of powdered gelatin and powdered drink mixes (control group, placebo).

The selected individuals were divided into two groups: (a) control group (placebo): individuals who received gelatin juice (300 mL/d) and (b) supplemented group: individuals who received green juice (300 mL/d). The administration of gelatin juice (control group) or green juice (supplemented group) was held for 9 weeks from Monday to Friday, from 8 to 9 AM. Individuals underwent measurement and assessment at three time points: before the day of starting supplementation, on the 30th day, and on the 60th day.

Juice preparation

The green juice was prepared using the following components: Gala apple (*Malus domestica Borkhausen*), orange (*Citrus sinensis* (L.) Osbeck), and green vegetables such as lettuce (*Lactuca sativa*), green cabbage (*Brassica oleracea*, cultivar Acephala), head cabbage (*Brassica oleracea*, cultivar Capitata), and cucumber (*Cucumis sativus*). All components were commercially obtained in Porto Alegre, Rio Grande do Sul, Brazil. The preparation of the green juice consisted of processing one apple, one lettuce leaf, one green cabbage leaf, one head cabbage leaf, and one-third part of one cucumber with 500 mL of water and the strained juice of one orange and then separating the solid parts. Thereafter, 300 mL were withdrawn and poured in a Styrofoam cup to be offered to the volunteers. Green juice was prepared daily in the Dietary Technique and Gastronomy Laboratory of UFCSPA daily.

Placebo

Volunteers from the placebo group received a beverage composed by 1 package of pineapple and mint flavor juice, 1 package of pineapple and ginger flavor juice (Clight[®], Mondelez

International, Paraná, Brazil, 2016), and 1 package of lemon gelatin powder (Magro[®], Paraná, Brazil, 2016) at a final volume of 2 L of water. Then, 300 mL was offered to volunteers. The juice was prepared in the Dietary Laboratory of UFCSPA daily.

Blood collection

Volunteers had to fast for 12 hours before blood collection. The blood was collected three times throughout the study period—before beginning supplementation, in the fifth week, and at the end—always on the same weekday.

Blood was collected through venous puncture in ethylenediaminetetraacetic acid tubes or tubes without anticoagulant. Biological material was centrifuged for 10 minutes at 3000 rpm; plasma or serum were separated and frozen at -80°C . Erythrocytes were washed three times in a double-volume NaCl 0.9% solution and centrifuged for 10 minutes at 3000 rpm after each washing. Obtained erythrocytes were diluted in water at the proportion of 1:10 and stored in a -80°C freezer.

Biochemical determinations

Biochemical profile evaluation

Plasmatic levels of total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, very-low-density lipoprotein cholesterol, triacylglycerols, glucose, urea, creatinine, proteins, aspartate aminotransferase, alanine aminotransferase, and lactate dehydrogenase were measured in blood serum according to fabricant instructions (Bioclin/Quibasa, Minas Gerais, Brazil, ©2012).

Redox profile determination

Thiobarbituric acid reactive substances (TBARS)

TBARS, a measure of lipid peroxidation, were determined in plasma. Samples were mixed with 20% trichloroacetic acid and 0.8% thiobarbituric acid and heated in a boiling water bath for 60 minutes. TBARS were determined by the absorbance at 535 nm and reported as nmol TBARS/mg of protein (14).

Carbonyls

Carbonyl content was determined spectrophotometrically (15). The damage measure was taken from an absorbance reading at 370 nm. Results were reported as nmol of carbonyl groups/mg of protein.

Sulfhydryls (SH)

This method is based on the 5,5'-dithiobis (2-nitrobenzoic acid) reduction by thiols that produces a yellow solution, thionitrobenzoate (TNB), whose absorption is measured spectrophotometrically at 412 nm (16). Results were reported as nmol TNB/mg of protein.

Antioxidant enzymes

CAT assay

Activity was assayed in erythrocytes according to Aebi (17), based on the decomposition of H_2O_2 monitored spectrophotometrically at 240 nm, at ambient temperature. One CAT unity

is defined as 1 μmol of hydrogen peroxide consumed per minute, and the specific activity was reported as units/mg of protein.

SOD assay

SOD activity was measured spectrophotometrically at 480 nm (18). This method is based on the inhibition of adrenaline autoxidation in alkaline medium by the enzyme. Results were reported as units/mg of protein.

Protein determination

Protein was determined by the method of Lowry et al. (19) using bovine serum albumin as standard.

Body composition evaluation

The nutritional status of individuals was assessed by measuring weight (kg), height (m), waist circumference (WC), and BMI. Weight was measured by a Welmy calibrated digital scale (Welmy, Brazil), which has a range of up to 200 kg. Individuals were standing erect and still at the center of the weight scale, without shoes, wearing light clothes, head up, and staring at a fixed point at eye level, to have their weight measured. Weight was measured in a reserved room. Stadiometer range was up to 2.0 m. BMI was calculated by the ratio between total body mass (kg) and squared height (m^2) (20). WC was measured by inelastic and inextensible tape measure with individuals in the standing position. Measurements were taken at minimal inspiration, surrounding the abdominal region, at the midpoint between the iliac crest and the last rib (21).

Food consumption

Food consumption was assessed through a food survey. The 24-hour dietary recall (22) was used throughout the study period, which was 9 weeks. Energy, carbohydrate, protein, fat, and fiber were measured as nutritional parameters. These recalls were held three times throughout the study period: in the first, fifth, and ninth weeks, always on the same weekday.

Indicators of well-being and anxiety

The aim of this survey was to evaluate indicators of anxiety, life satisfaction, and self-efficacy before and after a period of green juice use as dietary supplementation.

The two groups received treatment for 9 weeks and underwent two assessments with the following tests: the Beck Anxiety Inventory (23) and the General Self-Efficacy Scale (24). Tests were adapted and validated for Brazilians (25,26). The tests were completed by the participants before beginning supplementation period and at the end.

Statistical analysis

Statistical analysis was performed using SPSS v. 20-IBM software (Statistical Product and Service Solutions, New York, USA). The normality of data was analyzed through the Shapiro-Wilk test. The analysis of quantitative variables that

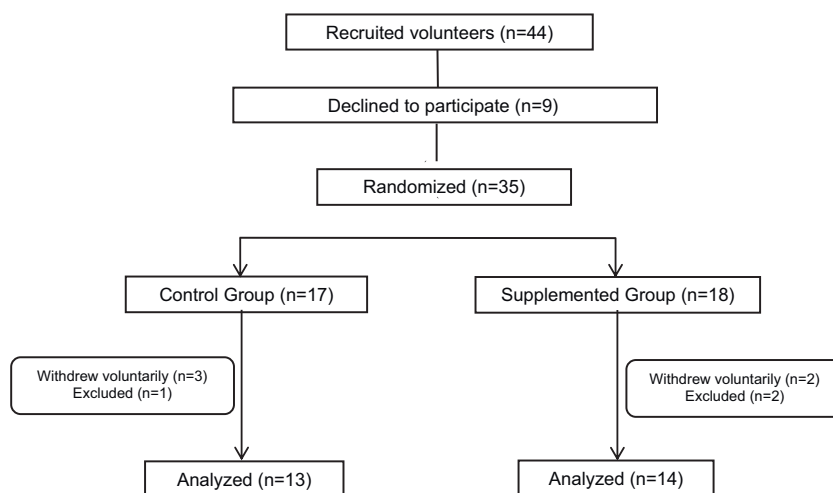


Figure 1. Participant flowchart during the study, from enrollment to completion.

Table 1. Sample Characterization.

	Juice Group (n = 14)	Control Group (n = 13)	p
Female, n (%) ^a	11/14 (78.6)	11/13 (84.6)	1.00
Age, years	31.07 ± 11.09	30.15 ± 8.52	0.81
BMI, kg/m ²	25.41 ± 4.11	23.5 ± 2.85	0.17

^aData are expressed as mean ± standard deviation.

BMI = body mass index.

*Chi-square tests.

**Independent t test.

presented normal distribution was performed through repeated measures analysis of variance. The *post hoc* Bonferroni test was used as necessary. Values of $p < 0.05$ were considered significantly different from control.

Results

Forty-four volunteers were recruited. Nine of them (20.45%) declined to participate. A total of 35 agreed to participate in the study: 18 individuals in the supplemented group (2 of them withdrew voluntarily and 2 were excluded as outliers) and 17 in the control group (3 of them withdrew voluntarily and 1 was excluded for changing diet during the study). Fourteen participants remained in the juice group and thirteen participants remained in the placebo one (see [Figure 1](#) for the participant flow chart). Mean ages were 31.07 and 30.15 years in the juice and control groups, respectively. Demographic details of the individuals by randomized group are shown in [Table 1](#). No significant differences were found between groups.

There was no difference in diet composition intake (carbohydrates, lipids, proteins, and fiber) of participants during the

development of this experimental protocol, as analyzed by 24-hour dietary recall (data not shown). In addition, green juice consumed for 9 weeks did not alter anthropometric measurements of supplemented group when compared to the control group ([Table 2](#)). Markers of biochemical functions are shown in [Table 3](#). We found no significant differences in biochemical markers between the groups.

Oxidative stress markers (carbonyl, SH, and TBARS) and antioxidant enzymes (SOD and CAT) are shown in [Table 4](#). No significant differences were found between the groups. No significant differences were found in quality of life or well-being between the groups ([Table 5](#)).

Discussion

Fruits and vegetables, apart from being good sources of vitamins, minerals, and fiber, are rich sources of potentially bioactive compounds known as phytochemicals. These compounds are not considered nutrients, but they are thought to provide much of the disease prevention potential of fruits and vegetables in human health (27–29). However, there are many unanswered questions related to fruit juice and human health. In this context, the present study aimed to investigate the effect of green juice (which combines fruits and vegetables) on human metabolism, considering biochemical and redox profile, as well as on well-being.

In the volunteers in our study, green juice supplementation did not modify plasmatic biochemical parameters such as glucose and lipid profile. In a recent study, increasing fruit and vegetable intake did not alter plasma glucose or lipid status in participants, despite significantly increasing circulating folate

Table 2. Anthropometric Outcomes.

	Juice Group			Control Group			p
	1st	2nd	3rd	1st	2nd	3rd	
Weight, kg	71.54 ± 14.18	71.81 ± 13.91	71.00 ± 14.36	63.60 ± 10.91	63.06 ± 9.83	63.34 ± 10.44	0.402
BMI, kg/m ²	25.23 ± 3.99	25.00 ± 3.94	24.88 ± 4.14	23.20 ± 2.83	22.97 ± 2.68	22.72 ± 2.60	0.596
WC, cm	82.77 ± 12.03	84.71 ± 11.17	83.28 ± 12.02	80.32 ± 9.64	80.78 ± 8.07	78.35 ± 8.13	0.357

Data are expressed as mean ± standard deviation.

BMI = body mass index; WC = waist circumference.

Table 3. Biochemical Outcomes.

	Juice Group			Control Group			p
	1st	2nd	3rd	1st	2nd	3rd	
Glucose, mg/mL	92.3 ± 10.3	91.6 ± 9.9	89.5 ± 11.1	91.6 ± 6.1	93.3 ± 6.5	90.9 ± 7.5	0.630
Triglycerides, mg/dL	119.7 ± 45.5	106.3 ± 36.8	108.7 ± 47.8	119.8 ± 68.4	103.0 ± 63.7	111.3 ± 47.8	0.891
Cholesterol, mg/dL	192.9 ± 31.9	196.7 ± 37.0	189.0 ± 36.3	181.5 ± 25.5	189.9 ± 28.4	188.5 ± 28.6	0.256
HDL-C, mg/dL	64.7 ± 14.8	67.1 ± 13.8	64.6 ± 12.9	64.0 ± 12.8	68.8 ± 16.5	67.6 ± 17.1	0.396
LDL-C, mg/dL	104.3 ± 34.2	108.3 ± 33.9	102.7 ± 35.9	93.6 ± 19.8	100.4 ± 20.2	98.7 ± 17.7	0.463
Urea, mg/dL	33.3 ± 11.8	30.1 ± 8.4	30.6 ± 4.8	29.0 ± 8.4	27.1 ± 5.9	26.6 ± 12.1	0.915
Creatinine, mg/dL	0.8 ± 0.1	0.9 ± 0.2	0.9 ± 0.2	0.8 ± 0.1	0.9 ± 0.2	0.9 ± 0.1	0.843
Uric acid, mg/dL	4.8 ± 0.9	4.5 ± 1.0	4.2 ± 0.8	4.7 ± 1.1	4.4 ± 1.2	4.3 ± 1.2	0.613
AST, U/L	19.2 ± 4.4	20.6 ± 4.7	22.9 ± 5.9	20.1 ± 5.3	20.2 ± 4.0	20.9 ± 3.8	0.262
ALT, U/L	7.4 ± 3.4	9.5 ± 4.6	12.3 ± 5.7	8.8 ± 8.2	10.8 ± 8.1	11.8 ± 9.6	0.351
LDH, U/L	195.9 ± 47.5	219.9 ± 34.4	230.4 ± 37.4	224.2 ± 32.7	221.9 ± 52.7	234.3 ± 34.1	0.094
Iron, mcg/dL	68.7 ± 29.9	69.6 ± 28.2	70.9 ± 23.8	58.7 ± 20.8	94.9 ± 54.4	73.4 ± 23.9	0.151
Total protein, g/dL	6.8 ± 0.5	6.7 ± 0.7	6.7 ± 0.3	6.7 ± 0.4	6.9 ± 0.4	6.6 ± 0.3	0.075

Data are expressed as mean ± standard deviation.

HDL-C = high-density lipoprotein cholesterol; AST = aspartate aminotransferase; ALT = alanine aminotransferase; LDH = lactate aminotransferase; LDL-C = low-density lipoprotein cholesterol.

Table 4. Oxidative Stress Outcomes.

	Juice Group			Control Group			p
	1st	2nd	3rd	1st	2nd	3rd	
Carbonyl, mmol/mg protein	0.47 ± 0.09	0.43 ± 0.09	0.43 ± 0.08	0.47 ± 0.08	0.44 ± 0.09	0.41 ± 0.08	0.781
SH, nmol TNB/mg protein	0.66 ± 0.50	0.62 ± 0.50	1.01 ± 1.04	1.27 ± 0.70	1.21 ± 0.77	1.25 ± 0.65	0.275
SOD, units/mg protein	3221 ± 532	3230 ± 572	3121 ± 635	3055 ± 926	3028 ± 780	2780 ± 640	0.699
TBARS, nmol TBARS/mg protein	0.05 ± 0.02	0.07 ± 0.04	0.06 ± 0.02	0.05 ± 0.02	0.07 ± 0.03	0.09 ± 0.06	0.406
CAT, U/mg protein	296.1 ± 70.1	286.1 ± 55.6	296.8 ± 59.8	308.5 ± 90.4	290.0 ± 109.7	280.4 ± 81.1	0.598

Data are expressed mean ± standard deviation.

SH = sulfhydryls; TNB = thionitrobenzoate; SOD = superoxide dismutase; TBARS = thiobarbituric acid reactive substances; CAT = catalase.

levels (30). It is notable that many studies did not find an association between fruit juice and adverse outcomes related to body weight, plasma lipids, or blood glucose in adults or children (31).

Our results also did not show any significant changes in redox profile (lipid peroxidation and protein oxidation). On the other hand, it is possible that we did not observe significant results due to a supplementation period of only 9 weeks. Of interest, an improvement in redox balance was not accompanied by a decrease in cardiovascular risk markers, which demonstrates that high consumption of fresh carrot juice without changes in lifestyle is not sufficient to improve lipid profiles (32). On the other hand, a previous study of the effect of the same green juice on rats' metabolism showed a modulation of redox state and a reduction of weight gain (13). In this published research with rats, both intervention and control groups received the same diet. However, both groups were not asked to follow any further dietary counseling in our study.

Most studies evaluating the effect of dietary supplementation had limited sample sizes of varied gender and ethnicity. In addition, these studies presented short treatment periods and a focus on early biomarkers rather than on functional end points, which are costly but more meaningful. Investigations included single-dose tests, 5-day studies, 4- to 8-week studies (most common) and, only very rarely, studies of more than a few months' duration. There are few studies controlled for variability in background diet and other confounding variables (30,31).

Additional challenges to dietary supplementation research include determining accurate dietary intake and compliance with the study protocol. In our study, we did not interfere with nutrient intake. Therefore, we recommended that volunteers keep their regular diets throughout the study. Thus, it was necessary to exclude a participant who modified his/her regular diet during the period of supplementation. Besides, differing methods of preparing juices, the variable nutrient and phytochemical content among varieties, regions, and storage conditions are inherent issues

Table 5. Psychological Outcomes.

	Juice Group		Control Group		p
	Pre-intervention	Post-intervention	Pre-intervention	Post-intervention	
BAI	7.86 ± 4.22	9.07 ± 6.33	4.64 ± 2.73	5.27 ± 4.29	0.730
GSE	54.29 ± 22.76	53.21 ± 33.09	65.45 ± 17.81	64.55 ± 20.55	0.985

Data are expressed as mean ± standard deviation.

BAI = Beck Anxiety Inventory; GSE = General Self-Efficacy Scale.

associated with the field. Furthermore, determining an appropriate dosage of juice to study is a challenge; volumes ranged from 30 mL/d to 1 L in the studies reviewed. Many investigations raised important questions regarding the bioavailability and *in vivo* metabolism of phytochemicals in juice as well as the required exposure time and concentrations needed to be effective (31).

In the present study, the sample was composed of healthy volunteers, and this can be one factor contributing to the lack of differences observed. The implications of baseline health status are uncertain. Some authors (30,33) observed a greater response in populations deemed less healthy but apparently more likely to benefit from dietary treatment. Recent investigations have involved “at-risk” persons, but traditionally most work has focused on healthy young adults who might be more “resistant” to treatment effects. The incorporation of participants with existing disease or risk factors introduces variability into a study, but it is important given the increasing risk profile of the population (31).

It is extremely important to say that there may be evidence of actual benefits on human metabolism in different future studies. There is a clear need for larger, well-controlled studies of longer duration with well-defined outcomes. We strongly recommend future work among different samples, such as adults with chronic medical conditions. Not all healthy individuals are on a fruit-and-vegetable juice-based diet. However, healthy people who consume fruits and vegetables as a dietary drink are expected to be protecting themselves from chronic diseases, due to an increase in fiber and antioxidant substance intake. Our sample was characterized by healthy people. Future studies with healthy people, but different fruit-and-vegetable juices are also recommended.

The bioaccessibility and bioavailability of each antioxidant differs greatly, and the most abundant antioxidants in ingested fruit are not necessarily those leading to the highest concentrations of active metabolites in target tissues (34). Several factors interfere with the bioavailability of antioxidants, such as food source and chemical interactions with other phytochemicals and biomolecules present in the food (35). The absence of significance may be key to clarifying some doubts concerning bioaccessibility and bioavailability of antioxidants in fruit-and-vegetable drinks.

Although our green juice recipe has not achieved significant results as a preventive diet, green juice consumption should be promoted for the maintenance of good health, because it stimulates the intake of fruits and vegetables.

Conflict of interest

The authors do not have any conflicts of interest.

Acknowledgement

We would like to thank the volunteers who participated in the study and those who helped in the execution of the project.

References

- López-Varela S, González-Gross M, Marcos A. Functional foods and the immune system: a review. *Eur J Clin Nutr.* 2002;56:S29–33. doi:10.1038/sj.ejcn.1601481. PMID:12142958.
- Podsedek A. Natural antioxidants and antioxidant capacity of Brassica vegetables: a review. *Lebensm Wiss Technol.* 2007;40:1–11. doi:10.1016/j.lwt.2005.07.023.
- Nunez-Córdoba JM, Martínez-González MA. Antioxidant vitamins and cardiovascular disease. *Curr Top Med Chem.* 2011;11:1861–69. doi:10.2174/156802611796235143. PMID:21506930.
- Silva MLC, Costa RS, Santana AS, Koblitz MGB. Compostos fenólicos. Carotenoides e atividade antioxidante em vegetais: Semina. *Ciências Agrárias.* 2010;31(3):669–82. doi:10.5433/1679-0359.2010v31n3p669.
- Zhang YJ, Gan RY, Li S, Zhou Y, Li AN, Xu DP, Li HB. Antioxidant phytochemicals for the prevention and treatment of chronic disease. *Molecules.* 2015;20:21138–56. doi:10.3390/molecules201219753. PMID:26633317.
- Halliwell B, Gutteridge JMC. *Free radicals in biology and medicine.* New York: Oxford University Press Inc.; 2007.
- Finkel T, Holbrook NJ. Oxidants, oxidative stress and the biology of ageing. *Nature.* 2000;408:239–47. doi:10.1038/35041687. PMID:11089981.
- Schafer FQ, Buettner GR. Redox environment of the cell as viewed through the redox state of the glutathione disulfide/glutathione couple. *Free Radical Biol Med.* 2001;30:1191–212. doi:10.1016/S0891-5849(01)00480-4.
- Tedesco I, Russo M, Russo P, Iacomino G, Russo GL, Carraturo A, Faruolo C, Moio L, Palumbo R. Antioxidant effect of red wine polyphenols on red blood cells. *J Nutr Biochem.* 2000;11:114–9. doi:10.1016/S0955-2863(99)00080-7. PMID:10715597.
- Cimen MYB. Free radical metabolism in human erythrocytes. *Clin Clim Acta.* 2008;390:1–11. doi:10.1016/j.cca.2007.12.025.
- Vasconcelos SML, Goulart MOF, Moura JBF, Manfredini V, Benfato MS, Kubota LT. Espécies reativas de oxigênio e de nitrogênio, antioxidantes e marcadores de danos oxidativo em sangue humano. Principais métodos analíticos para sua determinação. *Quim Nova.* 2007;30:1323–38. doi:10.1590/S0100-40422007000500046.
- Pastoriza S, Mesías M, Cabrera C, Rufián-Henares JÁ. Healthy properties of green and white teas: An up date. *Food Funct.* 2017;8:2650–62. doi:10.1039/C7FO00611J. PMID:28640307.
- Oliveira PS, Saccon TD, Da Silva TM, Costa MZ, Dutra FS, De Vasconcelos A, Lencina CL, Stefanello FM, Barschak AG. Green juice as a protector against reactive species in rats. *Nutr Hosp.* 2013;28:1407–12. PMID:24160193.
- Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem.* 1979;95:351–8. doi:10.1016/0003-2697(79)90738-3. PMID:36810.
- Reznick AZ, Packer L. Oxidative damage to proteins: spectrophotometric method for carbonyl assay. *Methods Enzymol.* 1994;233:357–63. doi:10.1016/S0076-6879(94)33041-7. PMID:8015470.
- Aksenov MY, Markesbery WR. Changes in thiol content and expression of glutathione redox system genes in the hippocampus and cerebellum in Alzheimer’s disease. *Neurosci Lett.* 2001;302:141–5. doi:10.1016/S0304-3940(01)01636-6. PMID:11290407.
- Aebi H. Catalase in vitro. *Methods Enzymol.* 1984;105:121–6. doi:10.1016/S0076-6879(84)05016-3. PMID:6727660.
- Misra HP, Fridovich I. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. *J Biol Chem.* 1972;247:3170–75. PMID:4623845.
- Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. *J Biol Chem.* 1951;193:265–75. PMID:14907713.
- Borges CF, Busnello FM, Pellanda LC. Identificação de fatores de risco cardiovascular em pais/cuidadores de crianças cardiopatas. *Arq Bras Cardiol.* 2012;99:936–43. doi:10.1590/S0066-782X2012005000085. PMID:22948242.
- Jansen I, Katzmarzyk PT, Ross R. Body mass index, waist circumference, and health risk: Evidence in support of current national institutes of health guidelines. *Arch Intern Med.* 2002;162:2074–9. doi:10.1001/archinte.162.18.2074. PMID:12374515.
- Fisberg RM, Slater B, Marchioni DML, Martini LA. *Inquéritos alimentares – métodos e bases científicas.* São Paulo (Brazil): Manole; 2005.

23. Beck AT, Epstein N, Brown G, Steer RA. An inventory for measuring clinical anxiety: psychometric properties. *J Consult Clin Psychol.* 1988;56:893–7. doi:10.1037/0022-006X.56.6.893. PMID:3204199.
24. Schwarzer R. General self efficacy scale (GSE). [cited 2012 March 5]. Available from: <http://userpage.fu-berlin.de/~health/selfscal.htm>.
25. Cunha JA. Manual da versão em português das Escalas Beck. São Paulo (Brazil): Casa do Psicólogo; 2001.
26. Hutz CS. Avaliação em psicologia positiva. Porto Alegre, Brazil: Artmed; 2014.
28. Denny A, Buttriss J. Plant foods and health: Focus on plant bioactives. European Food Information Resource (EuroFIR) Consortium. Funded under the EU 6th Framework Food Quality and Safety Thematic Priority; Contract FOOD-CT-2005-513944, 2007.
29. Del Rio D, Costa LG, Lean MEJ, Crozier A. Polyphenols and health: what compounds are involved? *Nutr Metab Cardiovasc Dis.* 2009;20:1–6. doi:10.1016/j.numecd.2009.05.015.
30. Gonzalez-Aguilar GA, Ayala-Zavala JF, De la Rosa LA, Alvarez-Parrilla E. Phytochemical changes in the postharvest and minimal processing of fresh fruits and vegetables. In: De la Rosa LA, Alvarez-Parrilla E, Gonzalez-Aguilar GA, editors. *Fruit and vegetable phytochemicals: chemistry, nutritional value and stability*. 1st ed. New York (NY): Wiley; 2010. p. 309–11.
31. Duthie SJ, Duthie GG, Russell WR, Kyle JAM, Macdiarmid JL, Rungapamestry V, Stephen S, Megias-Baeza C, Kanieswska JJ, Shaw L, et al. Effect of increasing fruit and vegetable intake by dietary intervention on nutritional biomarkers and attitudes to dietary change: a randomised trial. *Eur J Nutr.* 2017 May 30. doi:10.1007/s00394-017-1469-0. PMID:28560503.
32. 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. doi:10.3945/an.114.005728. PMID:25593142.
33. Potter AS, Foroudi S, Stamatikos A, Patil BS, Deyhim F. Drinking carrot juice increases total antioxidant status and decreases lipid peroxidation in adults. *Nutr J.* 2011;10:96. doi:10.1186/1475-2891-10-96. PMID:21943297.
34. Durga J, Bots ML, Schouten EG, Kok FJ, Verhoef P. Low concentrations of folate not hyperhomocysteinemia are associated with carotid intima-media thickness. *Atherosclerosis.* 2005;179:285–92. doi:10.1016/j.atherosclerosis.2004.10.026. PMID:15777543.
35. Manach C, Williamson G, Morand C, Scalbert A, Remesy C. Bioavailability and bioefficacy of polyphenols in humans. Review of 97 bioavailability studies. *Am J Clin Nutr.* 2005;81:230s–42s. doi:10.1093/ajcn/81.1.230S. PMID:15640486.
36. Parada J, Aguilera JM. Food microstructure affects the bioavailability of several nutrients. *J Food Sci.* 2007;72:R21–32. doi:10.1111/j.1750-3841.2007.00274.x. PMID:17995848.