Daily Intake of Pasteurized Orange Juice Decreases Serum Cholesterol, Fasting Glucose, and Diastolic Blood Pressure in Adults

LÍVIA G. BASILE¹, CLÁUDIA G. LIMA², AND THAIS B. CÉSAR*²

¹Paulista University, UNIP, Av. Juscelino K. de Oliveira, s/n – Tarraf II, São José do Rio Preto, SP, Brazil
²São Paulo State University, UNESP, Faculty of Pharmaceutical Sciences, Rod. Araraquara-Jaú km 1, Araraquara, SP, Brazil, 14801-902

**Additional index words.** orange juice, clinical trial, blood serum, lipids, glucose, hemodynamic profile

Previous studies have shown that fresh squeezed orange juice or juice from reconstituted concentrate can help prevent the development of atherosclerosis. Pasteurized orange juice presently represents the major orange juice available in the market, and because of this, it becomes necessary to determine the healthy benefits associated with this product. In this study we investigated the effect of regular consumption of pasteurized orange juice on the nutritional status, biochemical profile, and arterial blood pressure in healthy men and women. Men and women volunteered to consume pasteurized orange juice (500 mL·d⁻¹ and 750 mL·d⁻¹, respectively), for 8 weeks. Anthropometric, biochemical, hemodynamic, and dietary assessments were evaluated at baseline and at the end of the experimental period. Total cholesterol and LDL-C significantly decreased in both men and women after the consumption of orange juice, and an increase in HDL-C level was detected exclusively in women. Fasting glucose, diastolic blood pressure, and triglyceride levels dropped in men after the consumption of orange juice. Anthropometric variables did not change with orange juice consumption, only waist circumference decreased significantly in women. Consumption of orange juice increased the energy and carbohydrate intake for women; however, vitamin C and folate increased after the orange juice period for both men and women. Regular consumption of pasteurized orange juice by men (750 mL·d⁻¹) and women (500 mL·d⁻¹) reduced the risk of developing atherosclerosis, and increased the nutritional quality of their diets.

Considering that pasteurized orange juice contains the same constituents as fresh squeezed orange juice, despite slightly lower vitamin C content and greater hesperidin and apigenin contents, the hypothesis of this study is that the health benefits of consuming pasteurized orange juice are similar to those of consuming fresh orange juice or frozen orange juice concentrate. The objective of this study was to investigate how daily consumption of pasteurized orange juice affects the nutritional status, lipid, glycemic, and hemodynamic profiles of adult men and women.

Nutritional research of fresh and processed foods with functional properties have been the focus of many experimental, clinical, and epidemiological studies in an attempt to understand how the compounds present in these foods can prevent and treat chronic degenerative diseases. Previous studies have shown that a diet rich in fruits and vegetables is associated with reduced risk of cancer and cardiovascular diseases, which are the main causes of death in western countries, including Brazil (Chun et al., 2007; Kurowska et al., 2000a; Liu, 2004; Riso et al., 2005; Silalahi, 2002).

Among the substances present in fruit and vegetables, it has been suggested that vitamins C and E, β-carotene, and phenolic acids, especially flavonoids, are the components responsible for preventing chronic degenerative diseases (Chun et al., 2007; Liu, 2004). Flavonoids inhibit LDL cholesterol oxidation and platelet aggregation in vitro, and may have antioxidant activity in vivo (Kurowska et al., 2000b; Leake, 2001; Silalahi, 2002; Tapiero et al., 2002; Whitman et al., 2005). Other health benefits of flavonoids are related to their anti-inflammatory, anticancer, and anti-allergic properties; they also fight ulcers and infections and detoxify the liver (Arabbi, 2001; Erlund et al., 2001).

Flavonoids are found mainly in fruits, vegetables, grains, and beverages made from fruits or vegetables (Spencer et al., 1999). Citrus juices, especially orange and grapefruit juices, are receiving special attention because of their unique flavanone and vitamin C contents. The main flavonoids present in orange and grapefruit juices are hesperidin and naringin, respectively (Kurowska et al., 2000b). However, the clinical studies were made with concentrated or fresh orange juice, not with pasteurized orange juice. Pasteurized orange juice is available in the marketplace, but there are few nutritional studies with this juice (Deopurkar et al., 2010), although consumers prefer pasteurized orange juice because of its convenience, preservation of sensory properties, and nutritional quality.

Since chronic diseases constitute a global public health problem, clinical and nutritional studies that investigate orange juice consumption and its nutritional properties, which have been associated with the prevention of chronic diseases, are fully justified. Furthermore, knowledge of the mechanisms involved in orange juice flavonoid activity can give rise to important advances in the development of new foods with functional capacity or products with nutraceutical potential.

*Corresponding author; phone: 55 16 3301-6927; email: tcesar@fcfar.unesp.br
Materials and Methods

A total of 21 healthy women aged 20 to 53 years and 20 healthy men aged 21 to 44 years, recruited from the Paulista University (UNIP) of São José do Rio Preto, São Paulo, participated in this study. The recruitment questionnaire was distributed to all professors and employees of the university, and selection was based on certain inclusion and exclusion criteria and amount of pasteurized orange juice available. The objectives and procedures of the study were explained to the selected volunteers, who subsequently underwent anthropometric, biochemical, and dietary assessments. The project was approved by UNIP’s Research Ethics Committee, protocol number 263/08.

The inclusion criteria were: no regular consumption of orange juice, willingness to drink pasteurized orange juice daily for 8 weeks (750 mL for men and 500 mL for women), and age above 18 years. The exclusion criteria were: having thyroid, kidney and/or heart diseases; having hypertension and/or diabetes; consuming more than 30 mL of distilled beverages or one can of beer (350 mL) or 1 cup of wine (120 mL) per day; using dietary supplements; taking medications, especially lipid-lowering drugs; smoking; and women undergoing hormone replacement therapy.

The orange juice used in this study was non-fermented, non-alcoholic, non-diluted, additive-free, no-sugar-added, pasteurized, brand name Salute, supplied by the sponsor company Salute, located in São Carlos, SP, Brazil.

Nutritional assessments were done at baseline and end of the study period of 8 weeks at the nutrition clinic of UNIP. They consisted of anthropometric, biochemical and dietary assessments, and investigation of physical activity habits. The anthropometric data collected were: weight (kg), height (m), triceps skin-fold thickness (mm), mid-arm circumference (cm), and waist circumference (cm). Weight and height of the participants were determined with a leveled and calibrated mechanical scale (model R110, Welmy; 150-kg capacity), and a stadiometer with a 200-cm ruler. Body mass index (BMI) was classified according to World Health Organization criteria (2002, 2003). Mid-arm circumference and waist circumference were measured using a flexible and inelastic metric tape measure. The adipometer used to measure the triceps skin-fold thickness was the Lange Skinfold Caliper.

A validated 24-h recall (24hR) and food frequency questionnaire (FFQ) were used for dietary assessment (Aptekmann and Cesar, 2010). The participants were advised not to modify their food habits during the study. Energy, macronutrient, and micronutrient intakes were determined by the software Nut Win, version 1.5, and assessed according to the Dietary Reference Intakes (DRI) (Nutritional Research Council, 2005 and 2006).

Collection of blood and blood pressure data were done at UNIP’s nursing clinic. Blood was collected in the morning after a 12-h fast, at baseline and end of the study period (8 weeks). Blood from each participant was transferred to two test tubes and transported in ice-filled Styrofoam boxes to Sena Laboratory of Clinical Analysis, Catanduva, SP, which centrifuged the blood and performed the biochemical analyses. Commercial, enzyme-based, spectrophotometric test kits were used to determine serum TC, HDL-C, TG, and glucose. TC, TG, and HDL-C were used to estimate LDL-C and VLDL-C according to Friedwald’s formula (Friedwald et al., 1972). BP was measured with a premium aneroid sphygmomanometer and premium Rappaport stethoscope, both from Glicomed.

Statistical analyses of the data were performed with SigmaStat®, version 2.03. All variables were recorded in tables and expressed as means and standard deviations. The data were tested for normality before the statistical analyses. The paired Student’s t-test was used to compare normally distributed data and the Wilcoxon test was used to compare non-normally distributed data. The significance level was set at 5%.

Results and Discussion

The mean ages of the men and women in this study were 31.5 ± 6.6 and 35.4 ± 9.5 years, respectively. All participants were either undergraduate students or already had an undergraduate degree. Most of the men (65%) and women (81%) were inactive, i.e., they did not practice any kind of physical activity.

According to WHO BMI classification, 4.8% of the women presented grade 1 thinness, 61.9% were normal weight, 19% were overweight, 9.5% were class 1 obese, and 4.8% were class 2 obese, whereas 30% of the men were normal weight, 60% were overweight, and 10% were class 1 obese. The mean BMI of the entire sample was above the desirable upper limit of 24.9 kg·m⁻² (Table 1), a finding also reported by others (Hunt et al., 2004; Marcovina et al., 2007). Excess weight was found in 70% of the men and 33.3% of the women, confirming the trend observed in developed and developing countries, including Brazil (American Heart Association, 2005; World Health Organization, 2003). Waist circumferences greater than the maximum recommended were found in 30% of the men and 24% of the women, a risk factor for cardiovascular diseases.

BMI and waist circumference values exceeding the recommended upper limits are known to be positively associated with risk factors that predispose individuals to cardiovascular diseases (Mooradian et al., 2008). The women in this study presented a significant reduction in waist circumference after the study period, but not the men. The other studied variables (weight, BMI, mid-arm circumference, and triceps skin-fold thickness) remained unchanged with daily orange juice consumption (Table 1).

Similarly, previous studies did not find significant anthropometric changes after regular orange juice consumption (Bonifácio and Cesar, 2009; Cesar et al., 2010b; Garcia et al., 2008). However, one study found that the participants lost a significant amount of weight after consuming grapefruit or its juice for 12 weeks. A possible mechanism for this weight loss is delayed gastric emptying secondary to grapefruit and its acidity, since lowering gastric pH can delay gastric emptying (Fujioka et al., 2006). The increase in abdominal fat that accompanies obesity is a risk factor for the development of glucose intolerance and diabetes (Boyko et al., 2000; Hayashi et al., 2003). The number of insulin-resistant and type II diabetic individuals has also been increasing (Zanella et al., 2001). Energy intake by the participants of the study was not different from the recommended energy intakes for both genders. Orange juice consumption led to reduced protein and lipid intakes among the men and increased energy, carbohydrate and lipid intakes among the women (Table 2). According to the dietary recall, those who consumed orange juice during the main meals stopped consuming some other item, mainly sodas and powder juices, ignoring the recommendation given at the beginning of the study, but justifying the fact that there were no increases in the anthropometric characteristics of the participants after the study period. However, a previous study showed that there was a significant increase in the percentage of dietary energy derived from pasteurized orange juice.
According to the American Heart Association (2005) and Society of the total energy value, saturated fat intake not greater than 30% of the total energy intake for both genders. When fat intake was analyzed according to type of fat, men and women were found to consume elevated amounts of saturated fats before and at the end of the study period. Men also ingested more cholesterol than women, which could increase their lipid profile that can help prevent cardiovascular diseases. Despite the higher fat intake among women, which could increase the intake. On the other hand, iron intake was adequate given that the volunteers consumed beef and beans daily. Calcium and iron intakes remained roughly the same over the study period (Table 2). The consumption of vitamin C and folate were increased with orange juice intake, however, the intake of folate was inadequate in comparison to the nutritional recommendation of 320 µg·d⁻¹, while vitamin C was three times higher among women than the recommended (Table 2). Increased intake of these nutrients can be attributed to the orange juice since there were no other detectable dietary changes. Increased intake of these nutrients can be attributed to the orange juice since there were no other detectable dietary changes. Increased intake of these nutrients can be attributed to the orange juice since there were no other detectable dietary changes.

Table 1. Anthropometric, biochemical, and hemodynamic characteristics of men and women with daily consumption of 750 and 500 mL of orange juice, respectively, for the 8 weeks of the experiment.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Men, n = 20</th>
<th>Women, n = 21</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>After 8 wk</td>
</tr>
<tr>
<td></td>
<td>Baseline</td>
<td>After 8 wk</td>
</tr>
<tr>
<td><strong>Anthropometric</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wt (kg)</td>
<td>78 ± 12</td>
<td>79 ± 12</td>
</tr>
<tr>
<td>BMI (kg·m⁻²)</td>
<td>26 ± 3</td>
<td>26 ± 3</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>90 ± 9</td>
<td>90 ± 9</td>
</tr>
<tr>
<td>Mid-arm circumference (cm)</td>
<td>31 ± 3</td>
<td>32 ± 3</td>
</tr>
<tr>
<td>Triceps skin-fold thickness (mm)</td>
<td>17 ± 8</td>
<td>17 ± 8</td>
</tr>
<tr>
<td><strong>Biochemical</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cholesterol (mg·dL⁻¹)</td>
<td>186 ± 45</td>
<td>170 ± 36</td>
</tr>
<tr>
<td>HDL-C (mg·dL⁻¹)</td>
<td>52 ± 3</td>
<td>53 ± 3</td>
</tr>
<tr>
<td>Triglycerides (mg·dL⁻¹)</td>
<td>139 ± 55</td>
<td>126 ± 41</td>
</tr>
<tr>
<td>LDL-C (mg·dL⁻¹)</td>
<td>114 ± 22</td>
<td>104 ± 20</td>
</tr>
<tr>
<td>Glucose (mg·dL⁻¹)</td>
<td>85 ± 8</td>
<td>81 ± 6</td>
</tr>
<tr>
<td><strong>Hemodynamic</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>115 ± 9</td>
<td>112 ± 10</td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg)</td>
<td>76 ± 9</td>
<td>70 ± 7</td>
</tr>
</tbody>
</table>

*Measurements were taken at the first day in the beginning of the experiment; values are means ± SD.
*Measurements were taken at the last day in the 8th week at the end of the experiment; values are means ± SD.
*P < 0.05, difference before (baseline) and after drinking orange juice for 8 weeks, tested by the paired t-test for men’s and women’s group.

Table 2. Energy and nutrient intakes of men and women with daily consumption of 750 and 500 mL of orange juice, respectively, for the 8 weeks of the experiment.

<table>
<thead>
<tr>
<th>Dietary intakes</th>
<th>Men, n = 20</th>
<th>Women, n = 21</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>After 8 wk</td>
</tr>
<tr>
<td></td>
<td>Baseline</td>
<td>After 8 wk</td>
</tr>
<tr>
<td>Energy (kcal·d⁻¹)</td>
<td>2540 ± 853</td>
<td>2500 ± 746</td>
</tr>
<tr>
<td>Proteins (g·d⁻¹)</td>
<td>104 ± 39</td>
<td>96 ± 33*</td>
</tr>
<tr>
<td>Fats (g·d⁻¹)</td>
<td>84 ± 38</td>
<td>71 ± 39*</td>
</tr>
<tr>
<td>Carbohydrates (g·d⁻¹)</td>
<td>345 ± 137</td>
<td>368 ± 11</td>
</tr>
<tr>
<td>Cholesterol (mg·d⁻¹)</td>
<td>246 ± 140</td>
<td>213 ± 106</td>
</tr>
<tr>
<td>Saturated fatty acid (g·d⁻¹)</td>
<td>25 ± 13</td>
<td>21 ± 11</td>
</tr>
<tr>
<td>Calcium (mg·d⁻¹)</td>
<td>948 ± 547</td>
<td>823 ± 420</td>
</tr>
<tr>
<td>Iron (mg·d⁻¹)</td>
<td>19 ± 9</td>
<td>19 ± 9</td>
</tr>
<tr>
<td>Vitamin C (mg·d⁻¹)</td>
<td>105 ± 74</td>
<td>380 ± 88*</td>
</tr>
<tr>
<td>Folate (mg·d⁻¹)</td>
<td>327 ± 235</td>
<td>501 ± 196*</td>
</tr>
</tbody>
</table>

*Measurements were taken at the first day in the beginning of the experiment; values are means ± SD.
*Measurements were taken at the last day in the 8th week at the end of the experiment; values are means ± SD.
*P < 0.05, difference before (baseline) and after drinking orange juice for 8 weeks, tested by the paired t-test for men’s and women’s group.

from orange juice during the study period (Kurowska et al., 2000b). Macronutrient intakes were within the recommended percentages of the total energy intake for both genders. When fat intake was analyzed according to type of fat, men and women were found to consume elevated amounts of saturated fats before and at the end of the study period. Men also ingested more cholesterol than the recommended 200 mg·d⁻¹, both before and at the end of the study period. The consumption of fats at baseline and end of the study period decreased among men and increased among women, and the consumption of saturated fats increased among women. Despite the higher fat intake among women, which could increase total cholesterol and LDL-C, there were important changes in their lipid profile that can help prevent cardiovascular diseases. According to the dietary recommendations to prevent coronary artery disease (CAD), fat intake should not be greater than 30% of the total energy value, saturated fat intake not greater than 7%, and cholesterol intake not greater than 200 mg/day. Thus, according to the American Heart Association (2005) and Society Brasileira de Cardiologia (2007), the studied population is at increased risk of CAD.

All subjects form this study presented an inadequate calcium intake. On the other hand, iron intake was adequate given that the volunteers consumed beef and beans daily. Calcium and iron intakes remained roughly the same over the study period (Table 2). The consumption of vitamin C and folate were increased with orange juice intake, however, the intake of folate was inadequate in comparison to the nutritional recommendation of 320 µg·d⁻¹, while vitamin C was three times higher among women than the 60 mg·d⁻¹ recommended (Table 2). Increased intake of these nutrients can be attributed to the orange juice since there were no other detectable dietary changes.

One study has shown that vitamin C and folate intakes increased with increasing consumption of orange juice, yet they returned to the original values after a washout period (Kurowska et al., 2000b). Other studies also reported higher vitamin C and folate intakes by individuals who consumed orange juice. The mean
vitamin C and folate intakes of hyperlipidemic and normolipidemic men in one study were 218 ± 139 mg and 488 ± 161 mcg, respectively (Cesar et al., 2010a), and the mean vitamin C intake of normolipidemic men and women in another study was 205 ± 43 mg (Cesar et al., 2010b). These individuals ingested less vitamin C and more folate than the participants of this study (326 ± 81 mg and 444 ± 168 mcg, respectively).

Epidemiological studies have found contradictory results regarding vitamin C and CAD mortality, but the consumption of fruits and vegetables has been consistently associated with reduced CAD risk (Knab et al., 2002; O’Byrne et al., 2002; WHO, 2002). Short-term vitamin C intake improves vascular function in patients with CAD (Levine et al., 1996) and long-term intake improves endothelial function in hyperlipidemic children (Engler et al., 2003).

Folic acid is essential for homocysteine remethylation, in addition to other important metabolic functions. Population studies have shown that homocysteine levels are inversely related to serum folate, both in CAD and healthy individuals. Folate intake also correlates negatively with plasma homocysteine (Hatzijs et al., 2006; Moat et al., 2004). Folate is widely distributed in leafy green vegetables, fruits, especially orange juice, and fortified grains (Atabek et al., 2006). Therefore, the increase in folate intake seen in the studied population was probably due to the consumption of pasteurized orange juice.

Total cholesterol and LDL-C levels decreased significantly in all the participants of this study (Table 1). According to the National Cholesterol Education Program (2001, 2002, 2004), LDL-C reduction decreases CAD risk. It is recommended that the LDL-C level remains below 160 mg·dL⁻¹. A reduction of 43% in the LDL-C level was observed in an experimental study that fed orange juice to hypercholesterolemic rabbits (Kurowska et al., 2000a). Orange juice also reduced hepatic cholesterol ester and TC without increasing fecal cholesterol. Bok et al. (1999) observed that citrus flavonoids promote a reduction of cholesterol biosynthesis in the liver of rats fed a high-cholesterol diet possibly by inhibiting HMG-CoA reductase and ACAT activities. Reduced ACAT activity leads to less esterification of the hepatic cholesterol available for VLDL production, thereby reducing hepatic VLDL secretion (Carr et al., 1992). Two studies reported that the lipid profiles of individuals who consumed 240 mL of orange juice daily for 3 weeks did not change (Devaraj et al., 2004; Franke et al., 2005). However, hypercholesterolemic individuals given citrus flavonoids (270 mg) and tocochromenols (30 mg) saw their TC levels decrease by 20% to 30% and their LDL-C levels decrease by 19% to 27% (Roza et al., 2007). Similar results were found by other studies: a reduction of 10% and 15% in TC and LDL-C levels respectively in normolipidemic individuals who volunteered to consume orange juice (Cesar et al., 2010b), and 5% and 9.4% respectively in individuals who consumed 500 mL of orange juice fortified with phytosterols daily for 8 weeks (Devaraj et al., 2004).

The systolic blood pressure of both men and women did not change significantly from baseline to end of study period (Table 1). Yet, diastolic blood pressure decreased significantly in men. Another study observed that regular consumption of orange juice reduced the systolic and diastolic blood pressure of young adult men (Bonifacio and Cesar, 2009) and also decreased diastolic blood pressure in middle-aged and moderately overweight men (Morand et al., 2011).

Since consumption of orange juice has been associated with better lipid and glycemic profiles, orange juice may possess functional potential to fight atherosclerosis and diabetes. Even though pasteurized orange juice has slightly less vitamin C than fresh orange juice, it contains significant hesperidin content and greater apigenin content than fresh juice because of the contact between juice and peel during processing, which contributes to the functional potential of the juice. However, more studies are needed on the bioavailability and metabolism of the nutritive and non-nutritive components of orange juice to assess the role of each one of them in promoting cardiovascular health. It should be pointed out that daily consumption of pasteurized orange juice could be a practical way of consuming a healthy food item, which is also a source of nutrients and flavonoids. It can also help in the prevention of chronic diseases, including cardiovascular diseases and diabetes.

Consumption of pasteurized orange juice increased the daily dietary intakes of vitamin C and folate in both men and women, as well as the energy and carbohydrate intakes of women. There was also a reduction of abdominal fat among women but no weight
change. There were no significant changes in the anthropometric characteristics of the studied men. There were significant reductions in total cholesterol and LDL-C and a significant increase in HDL-C among women, and significant reductions in total cholesterol, LDL-C, triglycerides, and fasting glucose among men, suggesting that orange juice can aid the prevention of atherosclerosis. Males also experienced a significant decrease in their diastolic blood pressure. Based on the results of this study, daily consumption of pasteurized orange juice may improve biochemical and clinical characteristics such as dyslipidemia, insulin resistance, systemic hypertension and abdominal fat, thereby reducing the risk of cardiovascular diseases and diabetes.

Nowadays, soft drinks are consumed more than orange juice, but they have the same energy value per 100 mL and are considered “empty calories” because they only have simple sugars and don’t have other important nutrients. Therefore, the consumption of fruit or orange juice should be greatly encouraged because of the nutritional and health benefits.

**Literature Cited**


