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## Orange juice improved lipid profile and blood lactate of overweight middle-aged women subjected to aerobic training

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### ABSTRACT

**Objective:** This study investigated how consumption of orange juice associated with aerobic training affected serum lipids and physical characteristics of overweight, middle-aged women.

**Methods:** The experimental group consisted of 13 women who consumed 500 mL/d of orange juice and did 1 h aerobic training 3 times a week for 3 months. The control group consisted of another 13 women who did the same aerobic training program but did not consume orange juice.

**Results:** At the end of the experiment, the control group lost an average of 15% of fat mass ( $P < 0.05$ ) and 2.5% of weight ( $P < 0.05$ ), whereas the experimental group lost 11% of fat mass and 1.2% of weight ( $P < 0.05$ ). Consumption of orange juice by the experimental group was associated with increased dietary intake of vitamin C and folate by 126% and 61% respectively. Serum LDL-C decreased 15% ( $P < 0.05$ ) and HDL-C increased 18% ( $P < 0.05$ ) in the experimental group, but no significant change was observed in the control group. Both groups improved the anaerobic threshold by 20% ( $P < 0.05$ ), but blood lactate concentration decreased 27% in the experimental group compared to the 17% control group, suggesting that experimental group has less muscle fatigue and better response to training.

**Conclusions:** The consumption of 500 mL/d of orange juice associated with aerobic training in overweight women decreased cardiovascular disease risk by reducing LDL-C levels and increasing HDL-C levels. This association also decreased blood lactate concentration and increased anaerobic threshold, showing some improvement in the physical performance.

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### 1. Introduction

In order to reduce the risk of diabetes, coronary heart disease and cancer, middle-aged men and women are currently recommended to maintain a healthy body weight, avoiding overweight and obesity, and proper serum cholesterol levels [1,2]. The strategy to accomplish these goals includes a balanced diet and a regular physical activity plan [3]. Fruits and vegetables are an important part of a balanced and nutritious diet because they provide not only a wide assortment of vitamins, minerals and phytochemicals, but few calories in comparison with other foods [2]. Also, regular exercise, such as walking or jogging, has been demonstrated to positively affect serum lipids and lipoproteins by decreasing total cholesterol, LDL-cholesterol (LDL-C) and triglycerides, and by increasing HDL-cholesterol (HDL-C) in adults with and without coronary heart disease (CHD) [4,5].

Flavonoids are the most common phytochemicals in vegetables and fruits. They have antioxidant and anti-inflammatory properties that protect LDL from oxidation, preventing the development of atherosclerosis [6,7]. How flavonoids benefit humans is not entirely understood since most of the data comes from animal and tissue culture testing [2,8]. Previous studies have shown that flavonoids from citrus fruits, such as hesperidin and naringin, can reduce LDL-C and triglycerides in animals and humans, and increase HDL-C in hypercholesterolemic individuals [9]. It has been suggested that flavonoids from orange juice affect cholesterol metabolism in the liver [9], because they inhibit the production of endogenous lipoproteins [10].

Orange juice is also considered a good source of important essential nutrients such as vitamin C, folate and potassium. It was recently shown that vitamin C protects endothelial cells and LDL from either intra- or extracellular oxidant stress [11] and also may reduce the risk of atherosclerosis [12]. In addition, folic acid can lower plasma homocysteine concentrations and reverse endothelial dysfunction in patients with cardiovascular disease [13], while potassium may contribute to lower blood pressure [14].

Therefore, the association of a flavonoid and essential nutrient-rich diet, obtained by consuming natural sources, such as orange

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juice, with regular aerobic training can be a strategy to prevent coronary heart disease [5]. Based on these assumptions, we evaluated serum lipids and lipoproteins in a group of sedentary middle-aged women before and after subjecting them to a daily consumption of 500 mL of orange juice combined with regular aerobic training.

## 2. Materials and methods

### 2.1. Human subjects

Thirty premenopausal women aged from 30 to 48 years weighing  $75.5 \pm 14.2$  kg were randomly assigned among sixty volunteers to participate in this study, 15 as the control group and 15 as the experimental group. They were recruited by advertisement in local TV and radio stations of the city of Matao, SP, Brazil. The inclusion criteria were: (1) LDL-C < 160 mg/dL and triglycerides < 200 mg/dL; (2) irregular or no consumption of orange juice and lack of regular physical activity; (3) absence of thyroid and/or kidney disorders and diabetes; (4) not taking hormone replacement therapy; (5) nor taking vitamin or mineral supplements; and (6) not taking cholesterol-lowering medication. The study was approved by the Research Ethics Committee of the Faculty of Pharmaceutical Sciences, University of the State of Sao Paulo (UNESP), Araraquara, SP, Brazil, protocol no.12/2002. All the participants signed a free and informed consent form and were advised not to change their regular diet during the study.

### 2.2. Study design

The study aimed to change the sedentary lifestyle of all the participants to a more active lifestyle and provide half of them with a daily orange juice serving that might improve their serum lipid profile and reduce excess body weight. The control and experimental groups were subjected to aerobic training 3 times per week during 3 months and the experimental group also consumed 500 mL of orange juice per day. The aerobic training sessions lasted 1 h and were supervised by a certified trainer. The participants had to walk and run on a 400 m track at a speed that corresponded to 2.2–2.4 mM of blood lactate. Compliance with the training program was monitored and allowed for only one absence in 12 sessions per month, and three absences in 36 sessions in 3 months (8.3% of the total sessions). Four women, two from the control group and two from the experimental group, were dropped from the study based on this criterion. Consequently, from the 30 women originally recruited, 26 finished the study (13 in each study group). Biometric and biochemical measurements were taken from all the participants on the first day (before) and on the last day (after) of the experiment.

### 2.3. Anthropometric measurements

Anthropometric measurements were taken twice from each individual: on the first and ninetieth days of the experiment. Body weight (kg) and height (m) were measured and body mass index (BMI) was determined with the formula:  $\text{weight}/\text{height}^2$  [15]. Triceps, abdominal and thigh skinfold thicknesses were measured three times before and after the study period with a Lange Skinfold Caliper (Cambridge Scientific Industries, Inc.), and the average was used as the reference value. Body fat (%) was assessed early in the morning with a bioelectrical impedance device before the participants broke the overnight fast or exercised (Biodynamic Corp., model 310, Seattle, USA).

### 2.4. Dietary nutrient intake

Daily consumption of foods and beverages was determined with 24-h recalls and food frequency questionnaires, administered before and after the experimental period. Food intakes were described in terms of portion sizes and the individual's sugar and oil intakes were calculated from the questionnaire. Nutritional requirements for all the individuals were estimated according to the National Research Council [3]. A single batch of frozen concentrated orange juice (65° Brix) was provided by Citrusuco S/A (Matao, Brazil) and used throughout the experiment. The orange juice was stored in 500 mL bottles at  $-20^\circ\text{C}$ . The participants were given one bottle per week of the concentrated juice and instructed on how to reconstitute it to 12° Brix for consumption. According to USDA [16], the flavonoid content in 500 mL of orange juice (chilled from concentrate) was estimated at 54.6 mg of hesperitin and 10.6 mg of naringenin. Each daily serving of OJ (500 mL) contained approximately 168 mg of vitamin C, 95  $\mu\text{g}$  of folate, and 1.01 MJ (245 kcal) [17]. Compliance with orange juice consumption in the experimental group was verified indirectly by self-report and all the volunteers confirmed that they drank the preset amount of orange juice daily.

### 2.5. Blood lipid profile

Blood samples were drawn from the antecubital vein of the forearm between 7:00 and 8:00 am after a 12-h fast. The serum was immediately centrifuged and total cholesterol, LDL-C and HDL-C were determined with commercial kits from Bayer (USA) using an automatic analyzer in the Clinical Laboratory of the Faculty of Pharmaceutical Sciences, University of the State of Sao Paulo (UNESP), Araraquara, SP, Brazil. LDL-C was estimated as suggested by Friedewald et al. [18].

A treadmill (Professional Equipment, Movement LX-160) was used to determine the anaerobic threshold, according to a standard protocol [19]. The initial load was 2 km/h, with increments of 1 km/h at every 2 min until voluntary cessation. During this exercise, seven 25  $\mu\text{L}$  samples of blood were taken at 30-s intervals from the ear lobe with a capillary. The blood samples were immediately diluted in 50  $\mu\text{L}$  of 1% NaF and stored at  $-20^\circ\text{C}$ . Blood lactate concentration was measured by a lactate analyzer, YSI Model 1500 Sport Lactate Analyzer, at the Exercise Physiology Laboratory of the Federal University of Sao Carlos, SP, Brazil.

## 3. Statistical analysis

All the values were expressed as means  $\pm$  SD. Estimates of dietary energy and nutrient intakes obtained with the 24-h recalls and food frequency questionnaires were calculated by the NUTRI software, version 2.5 (CIS, School of Medicine, Federal University of Sao Paulo, Sao Paulo, SP, Brazil). The paired Student's *t*-test was used to compare the means obtained before and after the training program for the 2 groups separately. Comparison among control group and experimental group was made for the differences between before and after experimental period using Student's *t*-test. Statistical analysis of the data was done with the Sigma Stat software version 3.11 (Sigma Stat for Windows, Systat Software Inc. San Jose, CA, USA). Statistical significance was set at  $P < 0.05$  [20].

## 4. Results

Women participating in this study were overweight (37%) or obese (63%) [15], and all had a sedentary lifestyle. They agreed to start a physical training program because they wanted to improve their quality of life and health. Both groups (control and

**Table 1**

Energy and nutrient intakes of women enrolled in a 90-day aerobic training program associated with a daily consumption of 500 mL of orange juice.

Groups treatment	Control <sup>a</sup> (n = 13)		Experimental <sup>b</sup> (n = 13)	
	Aerobic training		Aerobic training + orange juice	
	Before	After	Before	After
Dietary intakes				
Energy (MJ <sup>c</sup> )	7.85 ± 1.90	7.70 ± 1.32	8.48 ± 2.09	8.04 ± 1.97
Protein (g d <sup>-1</sup> )	65.6 ± 22.3	73.7 ± 16.5	80.4 ± 18.5	82.7 ± 25.4
Carbohydrate (g d <sup>-1</sup> )	223.7 ± 67.1	217.9 ± 33.7	234.5 ± 78.7	226.5 ± 32.6
Total fat (g d <sup>-1</sup> )	79.9 ± 24.5	75.0 ± 34.5	85.4 ± 23.2	76.2 ± 40.6
SFA (g d <sup>-1</sup> )	28.7 ± 9.91	27.0 ± 13.9	29.1 ± 10.8	22.6 ± 9.69
PUFA (g d <sup>-1</sup> )	39.6 ± 15.9	38.3 ± 20.1	41.6 ± 21.7	35.9 ± 19.3
MUFA (g d <sup>-1</sup> )	11.6 ± 8.79	9.57 ± 4.11	14.6 ± 9.16	17.6 ± 21.9
Cholesterol (mg/d)	254.6 ± 123.0	217.9 ± 81.4	185.7 ± 72.9	239.2 ± 145.7
Vitamin C (mg/d)	101.7 ± 85.5	98.2 ± 129.7	119.1 ± 111.4	269.2 ± 108.4 <sup>*</sup>
Folate (μg/d)	143.6 ± 55.1	152.7 ± 86.3	177.2 ± 41.7	290.1 ± 93.5 <sup>*</sup>

Values are means ± SD. SFA, saturated fatty acids; PUFA, polyunsaturated fatty acids; MUFA, monounsaturated fatty acids.

<sup>a</sup> Control group: subjected to aerobic training, 1 h per day, 3 times per week, for 90 days.<sup>b</sup> Experimental group subjected to the same aerobic training as control group, in addition to a daily 500 mL serving of orange juice for 90 days.<sup>c</sup> To convert MJ to kcal multiply by 230.<sup>\*</sup>  $p \leq 0.05$ , differences before and after drinking 500 mL of orange juice per day for 90 days, tested by the paired Student's *t*-test.

experimental) participated in a physical exercise program for 90 days, but only the experimental group drank 500 mL of orange juice per day during that period. Analysis of the compliance of the consumption of the orange juice showed that the thirteen women of the experimental group followed the recommended daily dose of 500 mL during the experimental period and all the remaining twenty six women from the control and experimental groups met the attendance and participation requirements for the exercise sessions.

Total energy and macronutrient intakes, such as protein, carbohydrate, lipid (saturated, monounsaturated and polyunsaturated fatty acids) and cholesterol intakes, were obtained from the dietary questionnaires administered at the beginning and end of the experiment. The two groups did not differ significantly with regard to energy and macronutrient intakes, before and after the study (Table 1). By using this food consumption information, we could estimate that the vitamin C and folate intake by the experimental group increased 2.3-fold and 1.6-fold respectively, probably because of the orange juice (Table 1).

The women in the control group lost 2.5% of body weight ( $P < 0.05$ ), 15% of body fat ( $P < 0.05$ ), and 2.5% of BMI ( $P < 0.05$ ) (Table 2). Their triceps, thigh and abdominal skinfold thicknesses

were also reduced by 15%, 14% and 15%, respectively ( $P < 0.05$ ). Likewise, women in the experimental group also lost 1.2% in body weight and BMI ( $P < 0.05$ ), body fat (11.5%) and triceps (16%) ( $P < 0.05$ ), thigh (17%) and abdominal (7%) skinfold thicknesses (17.5%) ( $P < 0.05$ ) (Table 2). The change in weight, BMI and body fat percentage was not significantly different between control and experimental groups ( $P < 0.05$ ).

To evaluate the muscle fatigue level caused by acid lactic production during the exercise training, we measured the blood lactate concentration before and after the study period to determine the anaerobic threshold. The usefulness of anaerobic threshold incremental test lies in their ability to identify parameters that have long been considered a hallmark of aerobic fitness [19]. The average anaerobic threshold parameter increased 20% in both groups after the experimental period. Our results showed that after lactic acidosis induction by the incremental exercise, the plasma lactate concentration in the control group dropped 17%, while for the experimental group it dropped significantly by 27% ( $P < 0.05$ ) (Table 2).

Lipid profile was analyzed before and after the trial period for both groups. In the experimental group total cholesterol decreased 5%, LDL-C 15%, LDL/HDL ratio 27%, but HDL-C

**Table 2**

Biometric and biochemical characteristics of women enrolled in a 90-day aerobic training program associated with a daily consumption of 500 mL of orange juice.

Groups treatment	Control <sup>a</sup> (n = 13)		Experimental <sup>b</sup> (n = 13)	
	Aerobic training		Aerobic training + orange juice	
	Before	After	Before	After
<b>Biometric</b>				
Weight (kg)	76.3 ± 15.3	74.5 ± 15.9 <sup>*</sup>	74.6 ± 13.0	73.6 ± 12.4 <sup>*</sup>
BMI (kg/m <sup>2</sup> )	29.0 ± 5.53	28.3 ± 5.81 <sup>*</sup>	28.4 ± 4.46	28.1 ± 4.47 <sup>*</sup>
Body fat (%)	39.3 ± 7.33	33.8 ± 7.98 <sup>*</sup>	37.7 ± 7.56	33.4 ± 7.42 <sup>*</sup>
Skinfold thickness (mm)				
Triceps	32.0 ± 10.1	27.3 ± 9.33 <sup>*</sup>	31.9 ± 7.90	26.6 ± 6.85 <sup>*</sup>
Abdominal	30.2 ± 14.3	25.5 ± 11.9 <sup>*</sup>	32.2 ± 11.8	29.3 ± 9.60 <sup>*</sup>
Thigh	53.0 ± 12.8	45.9 ± 14.9 <sup>*</sup>	52.6 ± 11.5	43.4 ± 9.99 <sup>*</sup>
Anaerobic threshold (km/h)	4.3 ± 0.8	5.5 ± 1.4 <sup>*</sup>	4.6 ± 1.1	5.5 ± 1.3 <sup>*</sup>
<b>Biochemical</b>				
Lactate (mmol/L)	2.38 ± 0.87	1.97 ± 0.73	2.23 ± 0.59	1.64 ± 0.78 <sup>*</sup>
Triglycerides (mg/dL)	89.9 ± 25.9	83.7 ± 23.9	99.9 ± 43.0	98.0 ± 27.8
Total cholesterol (mg/dL)	193.6 ± 27.1	190.3 ± 29.4	185.4 ± 28.5	176.9 ± 28.5 <sup>*</sup>
LDL-C (mg/dL)	134.6 ± 33.3	128.1 ± 32.8	116.5 ± 24.5	99.7 ± 30.5 <sup>*</sup>
HDL-C (mg/dL)	58.8 ± 11.1	55.3 ± 12.5	48.9 ± 10.7	57.5 ± 12.1 <sup>*</sup>
LDL/HDL	2.59 ± 0.95	2.29 ± 0.81	2.51 ± 0.89	1.87 ± 0.92 <sup>*</sup>

Values are means ± SD.

<sup>a</sup> Control group: subjected to aerobic training, 1 h per day, 3 times per week, for 90 days.<sup>b</sup> Experimental group subjected to the same aerobic training as control group, in addition to a daily 500 mL serving of orange juice for 90 days.<sup>\*</sup>  $p < 0.05$ , differences before and after drinking 500 mL of orange juice per day for 90 days, tested by the paired Student's *t*-test.

increased 18% (Table 2). The biochemical parameters of the control group did not change significantly by the end of the study.

## 5. Discussion

Orange juice is a remarkable food because of its high content of vitamin C, and considerable amounts of essential nutrients such as folate and potassium. Orange juice and citrus fruits are also sources of hesperidin and naringin, which have been associated with many benefits for human health [7,9,10]. According to Arabbi et al. [21], 70% of total flavonoids consumed by Brazilians are provided by oranges and orange juice. Orange juice is also a popular drink in most western countries, consumed by the general population as part of a healthy diet and as a source of vitamins and minerals [22].

Although, two cups of orange juice represented an extra 210 kcal/d to the energy intake of the experimental group, the individuals in this group spontaneously decreased the consumption of other foods so their energy intake at the beginning and end of the study remained the same (Table 1). Furthermore, the consumption of orange juice did not compromise the intake of essential nutrients, because consumption of the major food groups, as cereals, fruits, vegetables, milk and dairy, and meats, evaluated by 24-h recalls and frequency food questionnaires, did not change as observed in previous studies [9,22].

Total energy, protein, total fat, fatty acid (saturated, polyunsaturated and monounsaturated) intakes at the beginning and end of the study remained unchanged for both groups. Lipids comprised more than 30% of the energy intake of all the participants, and the consumption of orange juice did not alter this percentage. Likewise, neither group changed significantly their mean cholesterol intake during the course of the study, which remained below 255 mg/d.

As observed in earlier studies [9,3], the orange juice consumption increased the vitamin C and folate intakes of the experimental group (Table 1). Ascorbic acid is the most prevalent vitamin in orange juice and previous studies have suggested that it protects against CHD because of its antioxidant properties, capable of restoring vitamin E and preventing LDL oxidation [23] and also decreases serum cholesterol [24].

Women enrolled in this study had a sedentary lifestyle and were overweight (37%) or obese (63%) when they started the physical training program. As reported elsewhere [25,26] the association between physical inactivity and excess weight in pre- and post-menopausal middle-aged women increases the risk of CHD. According to Ainsworth et al. [27], an average person walking moderately burns approximately 350 kcal/h, which means that this alone can promote weight loss without the need for dietary changes. This is consistent with the weight loss seen in the control group (2.5%) and in the experimental group (1.2%), although these results were not significantly different between the two groups ( $P < 0.05$ ) (Table 2). Energy intake also was not different at the end of the study in the experimental versus control groups ( $P < 0.05$ ) (Table 1).

Physical activity promoted a reduction of total body fat and adipose tissue in both groups. Changes in anthropometric parameters, without major dietary changes, will depend mostly on energy expenditure during exercise, as shown in previous studies [28–30]. Changing lifestyle from sedentary to active also helped reduce the anaerobic threshold of both groups. They did the same exercise program but just the experimental group had a significant decrease in blood lactate concentration. Lower production of lactate during physical activity verified in the experimental group in comparison with control subjects signified an improvement in physical performance with less fatigue. It is possible that the intake of orange juice

influenced this result by providing extra essential nutrients as well as energy.

A previous study [31] reported an improvement in the efficiency of aerobic exercise in male athletes treated with a mix of vitamin E, beta-carotene and vitamin C. In the present research, the orange juice group showed decrease of blood lactate concentration and increase of anaerobic threshold after the maximal exercise test. Thus, only orange juice intake was associated with major decreasing of blood lactate concentration. This finding provided extra benefit to the experimental group. Further studies are necessary to understand the role of the orange juice components on physical performance.

Subjecting normocholesterolemic women to aerobic training and regular consumption of orange juice (500 mL/d) improved their plasma lipoprotein profile by significantly increasing the HDL-C (18%) and reducing the LDL-C (15%) levels. Both changes caused a substantial and significant decline in the LDL/HDL-cholesterol ratio (27%), decreasing the risk of atherosclerosis. It has been shown that subjects with moderate hypercholesterolemia consuming 750 mL/d of orange juice experienced an increase in HDL-C but the decrease in LDL-C was not significant; however, the LDL/HDL ratio dropped significantly [9]. However, feeding orange juice to rabbits [32] or isolated citrus flavonoids to rats and hamsters [8,10] did induce a significant reduction in LDL-C.

In this study, plasma triglycerides were not affected by the consumption of orange juice and this effect could be due to the presence of hesperidin in orange juice, which has been shown to inhibit pancreatic lipase [33]. Similar results were also found in normocholesterolemic men treated with fresh orange juice and consuming a diet high in saturated fatty acids [34]. Conversely, increases in plasma triacylglycerol and HDL-C were produced in subjects with hypercholesterolemia in response to treatment with 750 mL/d of orange juice [9]. The authors suggested that this effect was not due to fructose or sucrose from orange juice, because increases in plasma triacylglycerol induced by these sugars in humans were associated with decreases in HDL-cholesterol. On the other hand, in rats fed a hypercholesterolemic diet plus a naringin and hesperidin mixture, there was no change in plasma triglyceride levels suggesting that hepatic ACAT (acyl-CoA:cholesterol acyltransferase) inhibition did not affect VLDL (very low density lipoprotein) secretion [10].

The strength of the study was the good selection of the volunteers that accomplished the physical training with high adherence for 3 months. Limitation, however, was the dietary information that was obtained through a self-reported questionnaire, which may be subject to underreporting and interviewer bias.

## 6. Conclusion

In summary, our results have demonstrated that the intake of 500 mL/d of orange juice combined with 1 h of aerobic training, 3 times a week, was associated with substantial hypolipidemic responses in normolipidemic women and improved the physical performance of overweight/obese and previously sedentary women. This study may add important insight into the role of orange juice and its components in helping to decrease risk factors associated to coronary heart disease. In addition, further clinical studies that identify the citrus compounds and its metabolites into the body compartments could help to understand their biological action and benefits to the human health.

## Contributors

The authors, Nancy P. Aptekmann and Thais B. Cesar, declare that we participated in this work, as to the selection the volunteers, collection of data, biochemical and nutritional evaluation, statisti-

cal analysis and interpretation of data, writing of the text, and that we have seen and approved the final version of this paper. We also declare that we do not have any conflicts of interest and that the source of funding is independent of the objectives and results found in this study.

### Competing interests

The authors, Nancy P. Aptekmann and Thais B. Cesar, declare that there are no competing interests and that the source of funding is independent of the objectives and results found in this study.

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