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Randomized control trials

Marked antioxidant effect of orange juice intake and its phytomicronutrients in a preliminary randomized cross-over trial on mild hypercholesterolemic men

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SUMMARY

Background & aims: Blond orange juice is the most consumed fruit juice in the world. It is a source of hesperidin, a bioavailable flavonoid reported to exhibit potential vascular protective actions. However, the specific impact on vascular function of Citrus phytomicronutrients, is unknown. For the first time, we investigated the effects of blond orange juice compared with a control beverage mimicking the composition of orange juice (including Vitamin C but no phytomicronutrients), on antioxidant markers, cardiovascular risk factors and endothelial function.

Methods: Twenty five male volunteers with two cardiovascular risk factors (age over 50 years and LDL-cholesterol between 130 and 190 mg/L) were enrolled in a randomized cross-over study. They received 3 times daily 200 mL of either blond orange juice or control beverage for 4 weeks, spaced by a 5-week wash-out. Endothelial function (flow mediated dilatation and plasma markers), oxidative status, lipid profile and inflammatory markers were assessed.

Results: Daily intakes of orange juice significantly led to a marked antioxidant effect which was correlated to hesperetin plasma levels and related with a decrease in reactive oxygen species. A tendency towards reduction of endothelial dysfunction and modest increase in plasma apoA-I concentration were also observed. This allows further experiments demonstrating the specific effect of phytomicronutrients from orange juice.

Conclusions: These findings suggest that daily intake of nutritionally relevant dose of blond orange juice may contribute for a significant antioxidant effect through the phytochemicals contained in. Orange juice may be associated to other healthy foods to achieve a significant effect on the vascular function.

This study is recorded in ClinicalTrials.com as NCT00539916.

1. Introduction

Atherosclerosis is a chronic pathogenic inflammatory-fibroproliferative process leading to cardiovascular diseases (CVD). Previous studies showed that the increase in plasma low-density lipoprotein cholesterol (LDL-C) is one of the main risk factors for atherosclerosis. The most plausible mechanism is related to the LDL oxidation. Oxidized LDL (OxLDL) cause the activation of endothelium, via an early loss of nitric oxide bioactivity. As monocytes and

Non-standard abbreviations: ANCOVA, analysis of covariance; apo, apolipoprotein; BMI, body mass index; CRP, C-reactive protein; CVD, cardiovascular diseases; FCS, fetal calf serum; FMD, flow mediated dilatation; FPLC, fast protein liquid chromatography; FRAP, ferric reducing ability of plasma; GPx, glutathion peroxidase; HDL-C, HDL-cholesterol; HESP, hesperidin; HF-HC, high fat-high cholesterol; IL, interleukin; LDL-C, LDL-cholesterol; Lp(a), lipoprotein (a); NF-κB, nuclear factor-kappa B; ORAC, oxygen radical absorbance capacity; PBS, phosphate buffer saline; sICAM-1, soluble intercellular cell adhesion molecule-1; SMC, smooth muscle cells; SOD, superoxide dismutase; sVCAM-1, soluble vascular cell adhesion molecule-1; TAC, total antioxidant capacity; TC, total cholesterol; TG, triglycerides.

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smooth muscle cells (SMC) migrate into the subendothelial space, oxLDL promote the differentiation of monocytes into macrophages, their proliferation and that of SMC. Their uptake in macrophages and SMC, leads to cholesterol ester accumulation and foam cell formation [1]. Oxidized lipids activate nuclear factor-kappa B (NF-κB), which induces genes coding for adhesion molecules, tissue factors, inflammatory cytokines and chemokines [2].

Food antioxidants seem to reduce the risk of CVD since their consumption through fruits and vegetables is associated with a reduced risk of CVD [3]. Joshipura et al. [4], in a follow-up study of 42,148 men for 8 years and of 84,251 women for 14 years, confirmed an inverse association with risk of coronary heart disease (CHD). Fruits and vegetables seem also to protect against the risk of stroke in men [5]. Antioxidants, like vitamins C and E or carotenoids may be involved in these effects as suggested in [6]. Polyphenols may contribute to the total antioxidant capacity (TAC) of fruits and vegetables [7] but they also can act through other pathways.

Citrus fruit juices are dietary antioxidant sources largely consumed worldwide. Orange juice contains high levels in vitamin C, folates, flavanones namely hesperidin (hesperitin-7-rutinoside) and β-cryptoxanthin, a precursor of Vitamin A. Dauchet et al. [8] found an inverse relationship between *Citrus* fruit consumption and the rates of acute coronary events. Daily intake of orange juice significantly increases plasma vitamin C [9], decreases oxidative stress [9], and CRP, a marker of inflammation [10] in humans. However, *Citrus* fruit consumption appears to have little or no effect on lipid profiles [11]. It is unclear if vitamin C alone or associated to flavonoids and carotenoids is responsible for these beneficial effects. Thereby, anthocyanins from blood oranges were reported to reduce risk of myocardial infarction [12,13].

In the present study, phytomicronutrients effect was assessed using blond orange juice with no anthocyanin and compared to a control beverage containing equal amount of vitamin C. This allows demonstrating the effect of flavanones and β-cryptoxanthin. In a cross-over design including healthy males subjects over 50 years with a LDL-C between 130 and 190 mgL^{-1}, the endothelial function, oxidative stress and antioxidant status, lipid profile and inflammatory markers were investigated. Flow mediated dilatation (FMD) and plasma soluble markers were used to explore the endothelial function.

2. Materials and methods

2.1. Subjects

Male subjects, 50–60 years of age, were recruited via mass advertising from Bordeaux Urban Community (n = 85). Only those presenting a LDL-C between 130 and 190 mgL^{-1} after a 4 week-period of a commended diet following the French National Program for Nutrition and Health (PNNS) were included (n = 25). We excluded participants with family and personal history of CVD, tobacco consumption, hypertension, diabetes, renal and/or liver failures, thyroid abnormalities, known autoimmune disease, infection or inflammation or surgery in the last 3 months, history or presence of cancer, and those unable or unwilling to sign the written informed consent that was systematically required. We also excluded all subjects considered as regular high consumers of dietary polyphenol-rich sources (like tea, coffee, cocoa, *Citrus* fruit and juice). The study protocol was approved by the Committee to Protect Persons from Bordeaux hospital (Nb 2006-A00274-49) and by the French Ministry of Health and Solidarity (Nb DGS2007-0194) and was performed in accordance with the 1964 Declaration of Helsinki. This clinical study has been declared to the website ClinicalTrials.com under the number NCT00539916.

2.2. Study design

Subjects were enrolled in a randomized, single-blinded, cross-over trial (Fig. 1). The juice and control drink were distinguishable. The randomization was designed to compare, into a French traditional diet without *Citrus* fruits, the effect of blond orange juice phytomicronutrients. Therefore, it compared the effect of a regular intake of blond orange juice to the one of a control beverage mimicking the composition of the juice without phytomicronutrients. Fruival (Portes-les-Valence, France) prepared the orange juice. An independent analysis of the juice performed by Eurofin analytics (Nantes, France) allowed PYC laboratories (Aix-en-Provence, France) to prepare the powder mix containing all the juice constituents except flavanones and β-cryptoxhantin. The mix was used by the Coralis Company (Cesson-Sévigné, France) to prepare a reconstituted control drink on a semi-industrial scale. The compositions of the drinks are given in Table 1. Subjects under recommended classical French diet were instructed to drink 200 mL three times a day of either blond orange juice or control beverage during a first 4 week-period. This daily intake of juice provided 212 mg of hesperidin (HESP). After a wash-out period of 5 weeks, they had the alternative drink for the second experimental 4 week-period. This phase was followed by a second wash-out (Fig. 1). All participants completed a monthly 5 day-food record to check dietary status and compliance to food and beverage recommendations and compliance was also checked via plasma vitamin C, hesperetin and β-crypthoxanthin level.

At the entry (baseline) and at the end of each 4-week period, all included 12 h-fasted subjects had an evaluation of the brachial FMD

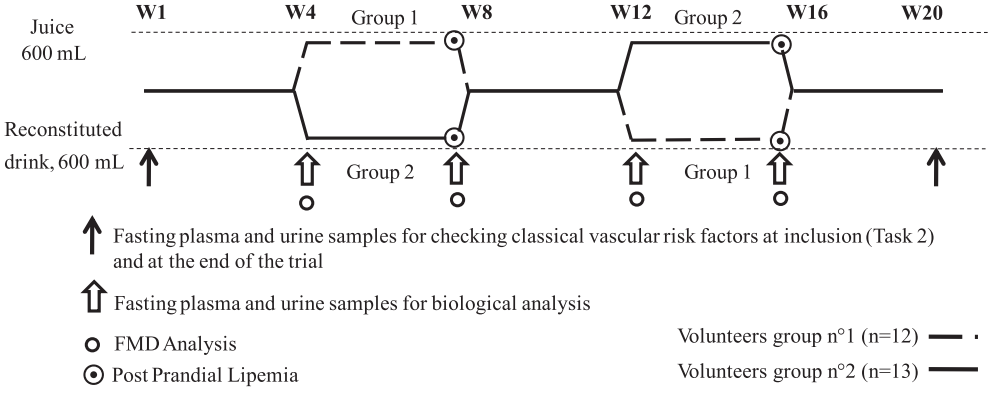

Fig. 1. Cross-over protocol followed during the study. Plasma and urine samples collection, FMD analysis and postprandial lipemia measurements are indicated. W: week of experiment; FMD: flow mediated dilatation.