Flavanones protect from arterial stiffness in postmenopausal women consuming grapefruit juice for 6 mo: a randomized, controlled, crossover trial

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INTRODUCTION

The incidence rates of cardiovascular diseases (CVDs) rise sharply in the years after menopause. Many prospective cohort studies have provided consistent evidence that a higher intake of fruit and vegetables is associated with a lower risk of cardiovascular mortality (1). Plant-based foods are the exclusive sources of flavonoids, a large family of bioactive compounds (2). With a database of flavonoid contents in foods, the total intake of flavonoids has been estimated at ~0.5 g/d in a French cohort (3). These compounds may help to promote the health benefits of plant-based foods; accumulating evidence from cohort studies has indicated that an increased intake of dietary flavonoids may reduce the risk of CVDs and type 2 diabetes (4, 5). This evidence is supported by studies conducted in animal models with nutritionally realistic doses of isolated flavonoids (2) and in humans consuming flavonoid-rich foods (6, 7). The most convincing clinical data are available for few flavonoid-rich products, including tea, cocoa, and soy, which are also the most studied (7, 8). These studies have reported beneficial effects of flavonoid-rich product consumption on some intermediate risk factors for CVD, such as LDL cholesterol, blood pressure (BP), and endothelial function. However, the trials with flavonoid-rich foods often cannot dissociate the specific effect of flavonoid compounds from that of the entire food due to the lack of appropriate controls.

Although fruits have been recognized as major contributors to dietary flavonoid intake in humans, clinical studies focusing on fruit flavonoids remain scarce. Flavanones are almost

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FLAVANONES AND VASCULAR PROTECTION IN HUMANS

Exclusively present at high concentrations in citrus fruits and in citrus fruit–derived food products. The major flavanones are naringenin and hesperetin, which are present as glycosides in grapefruit, orange, or clementine, respectively (9). Data from animal and cell studies indicated that flavanones might exhibit anti-inflammatory and anti-atherogenic properties, improve vascular reactivity, and exert antihypertensive and insulin-sensitizing effects when used for relevant nutritional conditions (9). A large prospective study examining the link between flavonoid intake and CVD mortality in postmenopausal women reported a statistically significant inverse association between flavanone intake and CVD (10). In this study, the intake of grapefruit was associated with a statistically significant reduction in CVD mortality (>1 serving/wk: RR: 0.85; P = 0.001). In another cohort of women, higher flavanone intake was associated with a lower relative risk of ischemic stroke (RR: 0.81; 95% CI: 0.66, 0.99; P = 0.04), and citrus fruit/ juice consumption tended to be associated with a reduced risk of stroke (RR: 0.90; 95% CI: 0.77, 1.05) when comparing extreme quintiles (11). These epidemiologic associations suggest that citrus flavanones may be cardioprotective. However, clinically based evidence remains scare (12, 13), and to our knowledge, the role of grapefruit flavanones in vascular protection has never been addressed through well-designed, randomized controlled trials (RCTs) in humans. Therefore, the present study was designed to investigate whether grapefruit flavanones could affect markers of vascular function in healthy postmenopausal women consuming grapefruit juice for 6 mo.

METHODS

Study population

Subjects were recruited in the Clermont-Ferrand region (France) through several media venues from February through October 2010. Nonsmoking Caucasian women who were 50–65 y old and 3–10 y after menopause were eligible for inclusion if these individuals had a normal to overweight BMI (in kg/m²; 19–30), a waist circumference >88 cm, normal BP levels, normal blood analyses at screening, and normal baseline electrocardiogram examination with a corrected Q-T interval <420 ms. The exclusion criteria included a medical history of cancer or severe metabolic diseases, medical history of major gastrointestinal surgery, therapy with blood lipid-lowering drugs or antihypertensive drugs, therapy with CYP3A4-metabolized drugs or other drugs that may interfere with grapefruit consumption (14), hormone replacement therapy in the previous 3 mo before entering the study, allergic reactions to citrus-containing foods, special dietary habits (e.g., vegetarians and vegans), use of dietary supplements (e.g., phytoestrogens and antioxidants), or physical activity level >5 h/wk. Subjects were also excluded if their usual consumption of one or more flavonoid-rich beverages, such as tea, coffee, wine, cocoa, or fruit juices, exceeded 500 mL/d, as estimated from a diet history interview at screening.

The 52 enrolled subjects provided written informed consent, and all subjects completed the study except for 4 who were lost to follow-up during the first 6-mo intervention period, as outlined in Figure 1. Ethical approval was obtained from the local human...
Characteristics of the intervention and randomization

Concentrate blond grapefruit juice (GFJ) and the analysis of its constituents (Table 1) were provided by the Florida Department of Citrus. A control drink (CD) matched for the macro- and micro-nutrients of the juice but without naringenin glycosides (Table 1) was manufactured from a powder mix (PYC-DENA Laboratory, Eupen, Belgium). Ocean Spray produced and packed both GFJ and CD from the supplied grapefruit concentrate and powder mix, respectively. Throughout the intervention period, bottles of both beverages were blinded at the time of packaging.

Volunteers were required to consume daily one bottle containing 340 mL blond GFJ or the same volume of the isocaloric CD. This daily dose of GFJ corresponds to 2 servings of fruit juice, and it provided ~210 mg naringenin glycosides (corresponding to 105 mg naringenin aglycone). The intake of each drink provided 110 kcal/d. Subjects were asked to consume 170 mL in the morning at breakfast and 170 mL of beverage over lunch. The deliveries of bottles occurred every 1.5 mo, and volunteers were instructed to keep the bottles at home in a dark place and at a low temperature.

Participants were assigned to one of the 2 treatment sequences (GFJ then CD or CD then GFJ) after a blocked randomization procedure by using a random block of 4 participants. An independent person working at the clinical research unit generated a computerized random list. The allocation sequence was concealed from the researchers in opaque sealed envelopes. The envelopes were handed to persons responsible for packing bottles of drinks in opaque bags numbered for each woman according to the random schedule.

Study design

The objective of the present study was to examine the role of flavanones in the chronic effects of GFJ consumption on vascular function in healthy postmenopausal women. This study was a double-blind, crossover RCT that included two 6-mo periods of intervention and a 2-mo washout period.

The primary outcome was the change in endothelium-dependent flow-mediated dilation (FMD) measured from the brachial artery after the intervention period. Secondary outcomes included the effect on BP, carotid-femoral pulse wave velocity (PWV), and digital peripheral arterial tonometry (PAT signal), as well as body composition and metabolic and inflammatory markers. These markers included glycemia, insulinenia, systemic endothelial function biomarkers (endothelin 1, soluble intercellular cell adhesion molecule 1, soluble vascular cell adhesion molecule 1, von Willebrand factor), inflammation (high-sensitivity C-reactive protein, IL-6), and antioxidant status (ferric reducing ability of plasma, oxygen radical absorbance capacity). All measurements were performed in fasted subjects at baseline and at the end of each experimental period.

During the entire study period, participants were requested to maintain their dietary habits and to minimize their intake of citrus foods and their consumption of flavonoid-rich beverages (coffee, tea, cocoa, fruit juices, and wine) to 250 mL/d. At screening, information regarding the habitual food intake of the participants was obtained from a diet history interview. At the beginning and at the end of each experimental period, anthropometric measures were made and nutritional intake was determined by using an in-house developed 3-d estimated dietary record. It has been previously demonstrated that a 3-d dietary record was valid to estimate dietary intakes in adults without cognitive impairments (15). The participants were instructed to record their entire food intake on 3 consecutive days directly after consumption. Each subject was trained in the level of detail required to adequately describe the foods and amounts consumed, including the name of the food, preparation methods, recipes for food mixtures, and portion sizes. For completeness and reliability, a trained dietitian screened all the 3-d food intake questionnaires to clarify entries, to probe for forgotten foods, and to check the portion sizes with the subjects. Participants were assisted with the use of an instruction manual for coding food portions that included validated photographs of >250 foods represented in 3 different portion sizes (16). Nutrient intakes were estimated by using the French food-composition database originally developed for the Supplementation en Vitamines et Minéraux Antioxydants (SU.VI.MAX) study (17).

Adherence to the intervention was assessed by counting the nonconsumed study products (bottles) brought back at the end of each period and by comparing the plasma concentrations of vitamin C at the beginning and end of each period.

The safety and tolerance of the intervention were monitored during the study period by recording clinical and laboratory adverse events. During the intervention, blood was sampled every 3 mo to determine liver markers, including alanine aminotransferase, aspartate aminotransferase, y-glutamyl transferase, and alkaline phosphatase. A control electrocardiogram was performed 10 d after the beginning of each intervention period and at each visit to avoid any risk of a prolonged corrected Q-T interval in response to grapefruit consumption (18).

Body composition analysis

Volunteers underwent a dual-energy X-ray absorptiometry examination at the beginning and at the end of each experimental period.
Noninvasive assessments of vascular function

Vascular function measurements were performed in the morning after an overnight fast in a quiet temperature-controlled room (22–25°C). The subjects laid quietly for several minutes before the measurements were performed. During the study period, the same trained operator who was blinded to the allocation of treatments performed each vascular measurement.

BP was measured on the right upper arm with a validated and automated sphygmomanometer device (SureSigns V3; Philips). The subjects rested 5–10 min in a seated or lying position before each BP assessment. Three consecutive BP readings were recorded at 1-min intervals. The mean of the 3 consecutive readings was considered for statistical analysis.

FMD of the left brachial artery was measured in the longitudinal plane above the antecubital fossa by using a high-resolution ultrasound system with a 7- to 12-MHz linear array transducer (Vivid S5; GE Healthcare). A mechanical arm device (Vascular Imaging), which was equipped with micrometer screws that allowed precise movements in the 3 dimensions, was used to obtain correct and stereotactic positioning of the transducer. After optimally positioning the transducer, images were recorded at baseline for 10 s and for 3 min after the release of forearm ischemia, which was caused by inflating a sphygmomanometer cuff to 220 mm Hg for 5 min (occlusion). Images were coded and recorded on a videotape and then digitized for subsequent blinded analysis by using automated edge detection software (Hemodyn 3M apparatus; Dinap SRL). FMD is expressed as the percentage increase in the brachial artery diameter from baseline to maximal dilation, which occurs 30–90 s after the cuff is released.

Endothelial function in the peripheral arterial beds was assessed by using PAT (EndoPAT 2000; Itamar Medical Ltd.). The measurements of the endothelium-mediated changes in vascular tone were made in the fingertips by using a pair of unique plethysmographic biosensors. The PAT signal was measured from the fingertip by recording the finger arterial pulsatile volume changes before and after a 5-min occlusion of the brachial artery (identical to that applied for FMD). The measurement from the contralateral arm was used as a control for non–endothelial-dependent changes in vascular tone. Therefore, subjects served as their own control. The results are expressed as PAT ratios, which correspond to the mean pulse wave amplitude during hyperemia (60–120 s of the postocclusion period) to the mean pulse wave amplitude during baseline in the occluded hand, divided by the same values in the control hand, and then multiplied by a baseline correction factor. The EndoPAT device also generates the augmentation index, which is a measure of peripheral microvascular stiffness calculated from the shape of the pulse wave recorded by the probes during baseline. To correct for the independent effect of heart rate, we adjusted the augmentation index to a heart rate of 75 beats/min.

The carotid-femoral PWV is an established index of aortic stiffness. Pulse, which travels at a higher velocity in stiff arterial vessels, is calculated from measurements of pulse transit time and the distance traveled between the 2 considered recording sites (i.e., carotid artery and femoral artery). The measurements were performed by using a validated nondivasive device (Sphygmocor; AtCor Medical Pty. Ltd.) that allows online pulse wave recording (requiring an acquisition of ~20 sequential waveforms) and automatic PWV calculation [PWV = distance (m)/transit time (s)]. The R-wave of a simultaneously recorded electrocardiogram was used as a reference frame. The system software calculates 2 variables concerning the variability of the recorded waves, thus providing internal quality control for the recording and minimizing operator bias.

Biochemical analysis

Fasting blood was collected before and after each intervention period. Venous blood was collected into evacuated tubes containing EDTA or Na-heparin. The plasma samples were immediately isolated, processed, and stored at −80°C until analysis. The plasma samples for vitamin C analysis were immediately transferred into Eppendorf tubes containing 2 volumes of meta-phosphoric acid (5%) and stored in the dark at −80°C. The laboratory technicians in charge of blood sample analysis were not aware of the allocation of treatments.

Plasma glucose concentrations were measured according to a standard laboratory procedure, and plasma high-sensitivity C-reactive protein concentrations were measured according to a standard immunonoturbidimetric method at the university hospital laboratory. Plasma insulin, inflammatory cytokine, and endothelial biomarker concentrations were assayed by ELISA with kits from Eurobio AbCys for insulin, IL-6, von Willebrand factor, soluble intercellular cell adhesion molecule 1, and soluble vascular cell adhesion molecule 1, as well as a kit from R&D Systems Europe Ltd. for endothelin 1. Ferric reducibility ability of plasma was measured by spectrophotometry according to the method of Benzie and Strain (20). Oxygen radical absorbance capacity was determined by fluorimetry according to the method as previously described (21). The total nitric oxide pool as measured in plasma was based on the conversion of nitrate to nitrite in the presence of nitrate reductase, followed by the fluorimetric determination of nitrite (Cayman kit; Interchim). Vitamin C was quantified in deproteinized plasma by HPLC with a fluorescent detector (excitation wavelength, 360 nm; emission wavelength, 440 nm) as described previously (22).

HOMA-IR was calculated from fasting plasma glucose and insulin values by using HOMA calculator software developed by the University of Oxford (https://www.dtu.ox.ac.uk/homacalculator), which estimates steady-state β-cell function and insulin sensitivity as percentages of a normal reference population.

Statistical analysis

The variables are presented as means ± SDs. A power analysis based on FMD measurements performed in the laboratory, with an SD of the mean of 2.96 to observe a difference of
25%, indicated that 42 of the 52 subjects were required to complete the study ($\alpha = 0.05$, power of 80%).

All statistical analyses were conducted in a blind manner. Usual descriptive statistics and robust measures were computed. The data for outcome variables were tested for normality and log-normality by using the Shapiro-Wilk test.

According to the statistical guidelines for the analysis of crossover trials (23), the effects of the treatment on the outcome variables were assessed by using mixed models. Treatment (GFJ or CD), period (first or second period), and group/sequence (the order in which the subjects received the treatment) were used as fixed effects. The effects of subjects were included as random effects to account for correlations between the observations made on the same subject. The baseline values (for each period) were also used as covariates. Because hypotheses were prespecified a priori in this study, no adjustment for multiple comparisons was made (24). The treatment effect was considered statistically significant if the P value was <0.05. All statistical analyses were performed with SAS version 9.1.3 software (SAS Institute).

Comparisons of baseline values between the 2 treatment periods were performed by using paired t tests to determine whether any carryover effects were present. This procedure failed to reveal any statistically significant differences, indicating the absence of carryover effects. Therefore, the baseline values presented for all the measured variables were the means of the 2 periods.

RESULTS

Baseline characteristics of the study population, safety, and compliance

The baseline characteristics of the study population are summarized in Table 2. The enrolled subjects were healthy postmenopausal Caucasian women with a mean age of 57.8 ± 3.7 y and with a mean waist circumference of 95.7 ± 5.3 cm. The subjects were normoglycemic, had typical laboratory test results (hematologic, renal, liver, and inflammatory markers), and were clinically normal (data not shown). The volunteers ranged from 47 to 63 y of age, with a mean age of 57.8 ± 3.7 y and a mean time since menopause of 6.3 ± 2.2 y. At screening, the enrolled subjects were slightly overweight (BMI ranging from 21.1 to 29.9) and had a mean waist circumference of 95.7 ± 5.3 cm. The subjects were normoglycemic, had typical laboratory test results (hematologic, renal, liver, and inflammatory markers), and were clinically normal (data not shown). The volunteers ranged from normal to slightly hyperlipidemic, as shown by the screening values for plasma total cholesterol and LDL cholesterol concentrations (Table 2). The recruited subjects exhibited normal to high-normal BP values. Using the Framingham risk equation, the 10-y risk of CVD for the study population was estimated at 8.5 ± 3.9%, which corresponded to a moderate risk compared with the reference value (5.9%) for a population of middle-aged women (25).

The 6-mo consumption of each beverage did not induce any hepatic toxicity, as reflected by the absence of changes in plasma liver enzyme activities for the duration of the study period (data not shown). At the end of the 2 experimental periods, the plasma concentrations of vitamin C were statistically significantly higher compared with baseline values: 74.7 ± 19.7 μmol/L and 71.13 ± 15.2 μmol/L for GFJ and CD, respectively, compared with 61.6 ± 18.4 μmol/L at baseline (P < 0.001). This result reflected the good compliance of the volunteers for the protocol and was consistent with an estimated level of compliance of >95% based on the counting of distributed and unused bottles.

Anthropometric measures, body composition, and dietary intakes

The two 6-mo intervention periods with each beverage did not affect the body weight of volunteers or the distribution of fat mass between the abdominal region and whole body (Table 3). The intake of the 2 study drinks provided the subjects with an extra daily energy intake of 110 kcal but did not induce any statistically significant changes in their total energy intake or in their dietary intake of individual macronutrients (Table 3).

Vascular function

As shown in Figure 2, after daily GFJ consumption for 6 mo, the PWV between carotid and femoral arteries was statistically significantly lower (7.36 ± 1.15 m/s) than that measured after CD consumption (7.70 ± 1.36 m/s), with a P value of 0.019 for the diet effect according to the mixed-model analysis adjusted from baseline values. The corresponding difference in PWV values between the 2 drink periods was estimated at −0.524 m/s by using the mixed model. No statistically significant treatment effect was found on the augmentation index at 75 beats/min, which reflected the peripheral arterial stiffness (27.77 ± 18.39% and 26.13 ± 18.76% for GFJ and CD, respectively; P = 0.735).

As presented in Table 4, the mixed-model analysis did not reveal any statistically significant dietary treatment effect (GFJ vs. CD) on endothelial function when assessed in the brachial artery (FMD) and in the peripheral arterial beds (PAT ratio), with P values of 0.739 and 0.208, respectively. The values for diastolic, systolic, and pulse blood pressures were not significantly different between GFJ and CD treatments, with P values estimated at 0.429, 0.729, and 0.248, respectively (Table 4). No differences in plasma biomarkers of hemodynamic function, nitric oxide, and endothelin 1 were observed between volunteers after the 2 interventions (Table 4).

Glucose metabolism, biomarkers of inflammation, endothelial function, and antioxidant status

As presented in Table 4, the fasting plasma glucose and insulin concentrations, as well as values of HOMA-IR reflecting insulin resistance, were not significantly different between the dietary interventions with GFJ and a matched CD without flavanones.
The plasma concentrations of high-sensitivity C-reactive protein, IL-6, vWF, soluble intercellular cell adhesion molecule 1, and soluble vascular cell adhesion molecule 1 did not statistically significantly differ between the 2 experimental periods (Table 4). The total plasma antioxidant status, as reflected by ferric reducing ability of plasma and oxygen radical absorbance capacity values, was also similar after 6 mo of regular GFJ or CD consumption (Table 4).

### DISCUSSION

The main finding from this RCT is that GFJ consumption statistically significantly lowered arterial stiffness compared with a matched CD without flavanones. Regardless of the significant effect of grapefruit flavonones on PWV, we did not detect statistically significant changes in endothelial function assessed by FMD and PAT ratio as well as in peripheral systolic and diastolic BP.

The consistency of clinical evidence regarding the relation between the consumption of fruit juices rich in polyphenols and improvement in vascular function has been recently reviewed (26, 27). These reviews suggested that improved endothelial function and reduced diastolic BP could be reliable mechanisms by which fruit juice–derived polyphenols can reduce CVD risk. Furthermore, the beneficial effect of flavonoids on arterial stiffness is emerging. Indeed, a recent review has compiled data from studies that have evaluated the association between flavonoid consumption and arterial stiffness (28). These data support an improvement in arterial stiffness related to the intake of isoflavones, anthocyanins, and, to a lesser extent, flavanols. Arterial stiffness is generally related to blood pressure (29). However, several chronic flavonoid-based intervention studies have reported a statistically significant reduction of arterial stiffness independently from blood pressure changes. Several studies performed in normotensive subjects (healthy volunteers and patients with type 2 diabetes or coronaropathy) showed improvement in PWV without any change in peripheral BP (30–32). In overweight men and postmenopausal women, a decrease in both PWV and BP was observed after an isoflavone-based intervention, but changes in these measures were independent (33). Therefore, results from these trials are consistent with those from our study showing a decrease in aortic stiffness without a concomitant decrease in BP in normotensive post-menopausal women.

The PWV measured between the carotid and femoral arteries is considered the gold-standard measurement of central arterial stiffness (34) and strongly correlates with cardiovascular events and all-cause mortality (35). The magnitude of the difference in PWV values between GFJ and CD (−0.524 m/s) after the 6-mo consumption period could correspond to an absolute ~5% reduction in the global CVD risk (35). Although arterial stiffness is a consequence of vascular aging, this condition has been shown to be a reversible process, and several interventions, including physical activity and dietary changes, have been proposed to improve arterial stiffness (34). Arterial stiffness is influenced by vascular tone and the release of vasoactive endothelial mediators, as well as structural alterations that may cause deterioration in vessel elasticity (36, 37). In healthy people, the age-related stiffening of large arteries is estimated at ~0.096 m/s per year (C Heiss, University of Düsseldorf, personal communication).

### TABLE 3
Effect of the intervention on anthropometric measures, body composition, and dietary intakes

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>GFJ</th>
<th>CD</th>
<th>GFJ–CD</th>
<th>P value&lt;sup&gt;1&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight, kg</td>
<td>67.4 ± 6.6</td>
<td>68.3 ± 7.0</td>
<td>67.8 ± 6.6</td>
<td>-0.241</td>
<td>0.624</td>
</tr>
<tr>
<td>Body lean mass, g</td>
<td>42769 ± 4076</td>
<td>43034 ± 4100</td>
<td>42757 ± 4150</td>
<td>252.6</td>
<td>0.229</td>
</tr>
<tr>
<td>Body fat mass, g</td>
<td>25817 ± 4259</td>
<td>26211 ± 4592</td>
<td>26053 ± 4117</td>
<td>-296.1</td>
<td>0.422</td>
</tr>
<tr>
<td>% Body fat</td>
<td>36.4 ± 3.7</td>
<td>36.6 ± 4.0</td>
<td>36.7 ± 3.6</td>
<td>-0.456</td>
<td>0.128</td>
</tr>
<tr>
<td>% Abdominal fat</td>
<td>35.2 ± 4.2</td>
<td>35.6 ± 4.6</td>
<td>35.6 ± 4.2</td>
<td>-0.580</td>
<td>0.152</td>
</tr>
<tr>
<td>Energy intake, kcal</td>
<td>1554 ± 330</td>
<td>1574 ± 415</td>
<td>1549 ± 408</td>
<td>9.065</td>
<td>0.895</td>
</tr>
<tr>
<td>Carbohydrate intake, g/d</td>
<td>171.8 ± 45.9</td>
<td>166.25 ± 54.49</td>
<td>162.2 ± 54.2</td>
<td>-1.956</td>
<td>0.830</td>
</tr>
<tr>
<td>Protein intake, g/d</td>
<td>73.8 ± 17.0</td>
<td>75.2 ± 14.5</td>
<td>73.7 ± 13.2</td>
<td>1.477</td>
<td>0.484</td>
</tr>
<tr>
<td>Fat intake, g/d</td>
<td>59.1 ± 18.5</td>
<td>63.47 ± 23.40</td>
<td>62.62 ± 21.27</td>
<td>1.183</td>
<td>0.736</td>
</tr>
</tbody>
</table>

<sup>1</sup>All values are means ± SDs, n = 48. Mixed models are adjusted for baseline value. Dietary intake was determined by using a self-administered 3-d estimated dietary record. CD, control drink; GFJ, grapefruit juice.

<sup>2</sup>P value for dietary treatment effect (PROC MIXED; SAS Institute).

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**FIGURE 2** Effect of GFJ naringenin on PWV, index of central arterial stiffness. This study was a crossover study, and all participants (n = 48) had values for GFJ and CD. Values are means ± SDs. Mixed-model analysis adjusted for baseline value. CD, control drink; GFJ, grapefruit juice; PWV, pulse wave velocity.
Germany, personal communication, 2014). Therefore, the observed difference in PWV between GFJ and the matched CD without flavanones is equivalent to 5 y of age-related stiffening. The present study does not identify the nature or origin of the observed difference in PWV between GFJ and the matched CD. In this former study, it was suggested that PWV was largely dependent on nitric oxide bioavailability, which is consistent with our results, a previous clinical trial aiming to study whether the chronic consumption of cranberry juice rich in flavonoids may improve vascular function also showed a reduction in PWV in fasted subjects without any changes in FMD (31).

In this study performed in free-living subjects, the daily consumption of 2 servings of GFJ over 6 mo did not alter daily intake of macronutrients, body weight, or body fat distribution. Similarly, no adverse effect on glucose metabolism was observed. This result is consistent with a recent meta-analysis of RCTs evaluating the effects of a variety of fruit juices on glucose control and insulin sensitivity that showed that fruit juice consumption did not significantly affect fasting glucose and insulin concentrations (43).

The present RCT provides the first clinical evidence of the vasculoprotective property of flavanones in subjects who regularly consume GFJ. It should be noted that this effect was observed for an intake of flavanones equivalent to ~40% of the estimated daily intake of flavanoids (3). This finding is consistent with recent epidemiologic studies reporting an inverse association between citrus flavanone intake and the risk of CVDs (10, 11). Previous in vitro and animal studies also support our results suggesting a positive effect of flavanones on vascular function (9).

TABLE 4
Effect of a 6-mo consumption of grapefruit flavanones on vascular function, glucose metabolism, biomarkers of inflammation, endothelial function, and antioxidant status

<table>
<thead>
<tr>
<th>Blood pressure, mm Hg</th>
<th>Baseline</th>
<th>GFJ</th>
<th>CD</th>
<th>GFJ-CD</th>
<th>P value χ²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic</td>
<td>126.04 ± 13.99</td>
<td>125.89 ± 17.08</td>
<td>125.28 ± 14.36</td>
<td>1.413</td>
<td>0.429</td>
</tr>
<tr>
<td>Diastolic</td>
<td>70.75 ± 9.02</td>
<td>69.39 ± 8.66</td>
<td>70.06 ± 8.87</td>
<td>−0.283</td>
<td>0.729</td>
</tr>
<tr>
<td>Pulse pressure</td>
<td>55.3 ± 9.5</td>
<td>56.5 ± 12.4</td>
<td>55.2 ± 10.6</td>
<td>1.5487</td>
<td>0.248</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Endothelial function</th>
<th>FMD dilation, %</th>
<th>Baseline brachial diameter, mm</th>
<th>PAT ratio</th>
<th>Hemodynamics</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4.21 ± 2.33</td>
<td>3.97 ± 2.37</td>
<td>2.6 ± 0.56</td>
<td>NO, μmol/L</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>98.59 ± 36.55</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>FMD dilation, %</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.26 ± 0.34</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Plasma</th>
<th>Glucose, mmol/L</th>
<th>Insulin, μU/mL</th>
<th>HOMA-IR</th>
<th>hs-CRP, mg/L</th>
<th>IL-6, pg/mL</th>
<th>vWF, μUnit/mL</th>
<th>sICAM-1, ng/mL</th>
<th>sVCAM-1, ng/mL</th>
<th>FRAP, μmol Fe²⁺/mL</th>
<th>ORAC, μmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4.8 ± 0.52</td>
<td>8.2 ± 2.93</td>
<td>1.05 ± 0.37</td>
<td>3.24 ± 2.11</td>
<td>0.21 ± 0.28</td>
<td>1440.93 ± 668.3</td>
<td>595.48 ± 121.23</td>
<td>759.79 ± 207.77</td>
<td>718.63 ± 110.44</td>
<td>15188 ± 2616</td>
</tr>
</tbody>
</table>

1 All values are means ± SDs, n = 48. Mixed models are adjusted for baseline CD, control drink; FMD, flow-mediated dilation; FRAP, ferric reducing ability of plasma; GFJ, grapefruit juice; hs-CRP, high-sensitivity C-reactive protein; NO, nitric oxide; ORAC, oxygen radical absorbance capacity; PAT, peripheral arterial tonometry; sICAM-1, soluble intercellular adhesion molecule 1; sVCAM, soluble vascular cell adhesion molecule 1; vWF, von Willebrand factor.

2 P value for dietary treatment effect (PROC MIXED; SAS Institute).
Several limitations should be acknowledged in the present study. Special attention was paid to match the CD to GFJ as closely as possible for a range of components, but we cannot fully exclude the possibility that other known or unknown bioactives present in GFJ might also be causally related to the biological outcomes beyond flavanones. A way to strengthen the contribution of flavanones would have been to look at the correlation between the observed effect on PWV and plasma concentrations of flavanones. However, due to our study design, it is irrelevant to seek such a correlation because all flavanone metabolites disappeared from the circulation due to our study design, it is irrelevant to seek such a correlation given the number of endpoint measures considered in our study, we cannot exclude some inflation of \( \alpha \) risk due to multiple comparisons. To address this issue, a specific study focused on PWV is warranted. Despite these limitations, our trial was the first long-term study examining the impact of GFJ consumption on vascular function in healthy postmenopausal women. This trial provides evidence of an improvement in arterial stiffness over a long time frame of grapefruit consumption as part of a normal diet. It also highlights a possible role of flavanones in mediating this beneficial effect.

In conclusion, our results support that some bioactive compounds abundant in grapefruit, particularly flavanones, may prevent arterial stiffening. These results have potential implications for public health, but dietary recommendations regarding the intake of grapefruit products for the whole population cannot yet be provided. Indeed, grapefruits also contain furanocoumarins, which can interact with the metabolism of a variety of drugs (44). To overcome this difficulty, the citrus industry is making great efforts to evolve citrus products for the whole population cannot yet be provided. Indeed, grapefruits also contain furanocoumarins, which can interact with the metabolism of a variety of drugs (44). To overcome this difficulty, the citrus industry is making great efforts to evolve citrus production, and storage.

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The authors’ responsibilities were as follows—VH: conducted clinical study implementation and monitoring and performed statistical analysis; VH and M-AV: performed data acquisition or analysis; VH, DM, NB-C, AM, CD, and CM: prepared or revised the manuscript; M-AV: implemented protocol; DM, AM, CD, and CM: designed study; DM, NB-C, AM, CD, and CM: interpreted data; and CD: performed volunteer recruitment and clinical study management. None of the authors reported any conflicts of interest concerning the research described in this article. The funders had no role in the study design, data collection and analysis, publication decision, or manuscript preparation.

REFERENCES


