The Journal of Nutrition
Nutrition and Disease

Daily Consumption of Grapefruit for 6 Weeks Reduces Urine F2-Isoprostanes in Overweight Adults with High Baseline Values but Has No Effect on Plasma High-Sensitivity C-Reactive Protein or Soluble Vascular Cellular Adhesion Molecule 1[1–3]

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Abstract

Individuals with obesity and metabolic syndrome (MetS) are at increased risk of cardiovascular disease, in part due to heightened inflammatory/oxidative processes. Results from epidemiologic and experimental studies suggest that citrus, and grapefruit in particular, may have a role in promoting vascular health, although clinical trial data are lacking. Here, we evaluated the anti-inflammatory/antioxidant effects of habitual grapefruit consumption in 69 overweight/obese men and women and in a subsample of participants with MetS ($n = 29$). Participants were randomly assigned to either a grapefruit group in which they consumed a low bioactive diet plus 1.5 grapefruit/d for 6 wk ($n = 37$, $n = 14$ with MetS) or to a control condition in which a low bioactive diet devoid of citrus was consumed ($n = 32$, $n = 15$ with MetS). Plasma soluble vascular adhesion molecule-1 (sVCAM-1), plasma high-sensitivity C-reactive protein (hsCRP), and urinary F2-isoprostanes were evaluated before and after the intervention phase. F2-isoprostane concentrations were not different in the grapefruit versus control arm after the intervention (12.4 ± 6.4 vs. 15.9 ± 9.0 ng/mg creatinine, $P = 0.16$), whereas plasma hsCRP concentrations tended to be lower in the grapefruit versus control arm postintervention (2.1 ± 1.5 vs. 2.8 ± 2.0 mg/L, $P = 0.09$). In adults with MetS, grapefruit consumption tended to result in lower postintervention F2-isoprostane concentrations compared with the control condition (12.0 ± 4.5 vs. 18.3 ± 10.9 ng/mg creatinine, $P = 0.06$). Furthermore, those with high baseline F2-isoprostane concentrations experienced significant reductions in this biomarker in response to grapefruit consumption ($P = 0.021$). Change in sVCAM-1 concentrations did not vary by treatment arm nor were there differences between arms postintervention. These results suggest that intake of grapefruit twice daily for 6 wk does not significantly reduce inflammation and oxidative stress, although there is a suggestion of favorable modulation of oxidative stress in overweight and obese adults with MetS or those with high baseline urine F2-isoprostane concentrations. J. Nutr. 143: 1586–1592, 2013.

Introduction

Atherosclerotic diseases, characterized by endothelial dysfunction, are the global leading cause of death (1). Atherosclerosis is the manifestation of damage to the endothelium due to a dysregulated cardiometabolic state (elevated lipids, glucose, and insulin), inflammation, and oxidative stress (2). Individuals with metabolic syndrome (MetS)[8] are at particularly high risk of developing atherosclerosis due to hyperlipidemia and elevated inflammatory/oxidative processes that develop as a result of visceral adiposity (1,2). Methods to reduce atherosclerotic plaque production via promotion of a healthy cardiometabolic state and a reduction in inflammatory/oxidative processes are warranted.

[1] This project was funded by USDA National Needs Fellowship 2010-38420-20369 and the USDA Cooperative State, Research, Education and Extension Service (USDA-CSREES) grant 2010-34402-20875.
[2] Author disclosures: C. A. Dow, B. C. Wertheim, B. S. Patil, and C. A. Thomson, no conflicts of interest.
[3] The trial is registered at www.clinicaltrials.gov as NCT01452841.
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[8] Abbreviations used: CVD, cardiovascular disease; hsCRP, high-sensitivity C-reactive protein; MetS, metabolic syndrome; NHS, Nurses' Health Study; PAD, peripheral artery disease; sVCAM-1, soluble vascular cellular adhesion molecule 1; WC, waist circumference.

Manuscript February 5, 2013. Initial review completed March 23, 2013. Revision accepted July 8, 2013.
First published online July 31, 2013; doi:10.3945/jn.113.175166.

Large epidemiologic trials suggest that citrus may be an important component of a heart-healthy diet. The Prospective Epidemiological Study of Myocardial Infarction (PRIME) in 50–59-y-old men found that citrus intake, but not intake of other fruits, was associated with a reduction in incidence of ischemic events (3). Data from the Nurses' Health Study (NHS) indicate that citrus and grapefruit intake specifically are associated with lower concentrations of fasting proinflammatory cardiovascular disease (CVD)–associated cytokines (4). Furthermore, an analysis of dietary flavonoid intake in the NHS suggested that high flavonone (flavonoids in citrus) intake, but not intake of other flavonoids, reduced the risk of ischemic stroke (5). Together, these data suggest that both citrus and grapefruit may promote cardiovascular health.

In addition to epidemiologic evidence supporting a role for flavonones in CVD, experimental evidence has shown that flavonones exert significant antioxidant, anti-inflammatory, and antihypertensive properties in cell culture and animal model studies (6–8). Randomized human trials are limited but suggest that flavonones have endothelial protective properties, although flavonones from orange juice have been the most widely tested (6,9). To date, no randomized human trials have been conducted to assess the effect of whole grapefruit, rather than flavonone supplements or orange juice, on outcomes associated with CVD in an at-risk population. Our group recently demonstrated that 6 wk of daily Rio red grapefruit intake significantly reduced total and LDL cholesterol, systolic blood pressure, and waist circumference (WC) in healthy, overweight, and obese adults (10).

The purpose of this secondary analysis was to determine the effect of 6 wk consumption of Rio red grapefruit on biomarkers associated with atherosclerotic plaque production: soluble vascular adhesion molecule 1 (sVCAM-1), high-sensitivity C-reactive protein (hsCRP), and F2-isoprostanes. Furthermore, we examined associations between classic demographic and anthropometric cardiovascular risk factors (age, sex, BMI, and WC) and atherogenic biomarkers at baseline and after the intervention. Last, we assessed the role of grapefruit consumption in participants with elevated risk of cardiometabolic diseases by performing a post hoc analysis on participants classified as having MetS.

Participants and Methods

Study population. The methods and primary results from this randomized controlled trial have been reported previously (10). Briefly, 85 nonsmoking men and premenopausal women over the age of 18 y who were free of inflammatory diseases or a history of cancer, heart disease, hypercholesterolemia (≥225 mg/dL), hepatic or renal disease, or diabetes were enrolled. Eligible participants had a BMI between 25 and 45 kg/m^2. Individuals taking medications metabolized by the cytochrome P450-3A enzyme were excluded due to potential adverse interaction with grapefruit (11). The University of Arizona Institutional Review Board approved this protocol, and all participants provided informed consent.

Study design. This study was completed over 2 grapefruit seasons (winter 2009–2010 and winter 2010–2011). Participants were enrolled in the trial for a total of 9 wk and made visits to the clinic at 4 time points (baseline, wk 3, wk 6, and wk 9). After the baseline visit, participants consumed a washout/run-in diet for 3 wk. The washout diet restricted fruits and vegetables with high polyphenol and carotenoid content including citrus, berries, spinach, carrots, tomatoes, and sweet potatoes. Participants were also provided with a daily multivitamin/mineral supplement (One-A-Day; Bayer Healthcare); all other dietary supplements were restricted for the duration of the study. Seventy-four participants completed the washout diet and were randomly assigned either to the control group (n = 32), in which they continued consuming the low bioactive diet, or to the grapefruit group (n = 42), in which they consumed the low bioactive diet plus half of a fresh Rio red grapefruit (Texas Rio Star Grapefruit; grown by Rio Queen Citrus) before each meal (3 times daily) for 6 wk. Participants received a 1-wk supply of fruit 6 times throughout the intervention period and were instructed to keep the fruit refrigerated at all times. Participants were trained to peel the grapefruit and eat all portions of the fruit, including the innermost white membrane of the fruit (the albedo), without adding caloric sweeteners to the fruit. Those randomly assigned to the grapefruit group were provided with a logbook in which they indicated every time grapefruit was consumed throughout the intervention period. Compliance was calculated as follows: (number of times grapefruit consumed before each meal/number of meals) × 100.

Anthropometric and blood pressure measures. Height, weight, and WC were measured at baseline, wk 6, and wk 9. Height and weight were measured to the nearest quarter inch and the nearest half-pound, respectively. WC was measured at the umbilicus by using a Gulick II measuring tape (Country Technology). Blood pressure was measured at baseline, wk 3, wk 6, and wk 9 by using a ReliOn HEM 780REL (Omron) automated blood pressure cuff following standard procedures (10).

Biosample collection and processing. Urine and blood samples were collected at the end of the washout phase (wk 3) and at the end of the intervention phase (wk 9). Participants collected a first-morning urine sample on 3 consecutive days before their clinic visit in order to capture intraindividual variability in oxidative stress analysis. Urine was stored in ice chests with ice packs until arrival at the research clinic. The 3 collections were then pooled and centrifuged at 1500 × g for 10 min at 4°C. After an 8-h overnight fast, blood was collected into serum and plasma tubes. Plasma and serum were processed following standard procedures (10). After centrifugation, plasma, serum, and urine were aliquoted into storage cryovials and stored at −80°C until analysis. Of note, biosample collection was not coordinated with the menstrual cycle in the female participants, as previous work suggests that the outcomes in this trial (sVCAM-1 and hsCRP) are not significantly modified by menstrual cycle phase (12).

Biosample analysis. Serum samples were analyzed for HDL cholesterol, TGs, and glucose concentrations by using an LDX Cholestech System (Cholestech). Samples were run in duplicate and recorded as an average of the 2 measurements.

Plasma samples were analyzed for hsCRP and sVCAM-1 concentrations by using ELISA kits (United States Biological and R&D Systems, respectively). The mean inter- and intra-assay CVs were 3.3 and 6.3% (hsCRP) and 13.4 and 6.3% (sVCAM-1), respectively.

F2-isoprostanes were measured in 3-d, first-morning, pooled urine samples. Urine samples were purified by adding 3 volumes of ethanol to 1 volume of sample, mixed on a vortex, and cooled to 4°C. Ethanol-sample mixtures were centrifuged for 20 min at 1500 × g. The supernatant was then decanted, and the ethanol was evaporated off by using a speed vacuum. F2-isoprostanes were quantified in the purified samples in duplicate via a competitive ELISA kit (Cayman Chemical). Mean inter- and intra-assay CVs were 10.8 and 2.5%, respectively. F2-isoprostane concentrations were corrected for creatinine concentration (Cayman Chemical).

MetS classification. Participants were classified as having MetS at study entry, post hoc, on the basis of guidelines set by the National Cholesterol Education Program Adult Treatment Panel III (13). MetS classification requires the presence of at least 3 of the following 5 criteria: elevated TGs (>150 mg/dL), reduced HDL cholesterol (<40 mg/dL in men, < 50 mg/dL in women), elevated WC (>102 cm in men, >88 cm in women), elevated blood pressure (≥130/≥85 mm Hg), or elevated fasting glucose (≥100 mg/dL).

Statistical methods. Baseline characteristics of participants in each treatment arm (or for participants with and without MetS) were compared by using χ^2 tests for categorical variables and t tests for

continuous variables. Within each treatment arm (total enrolled and only those with MetS), inflammatory and oxidative stress markers were compared from pre- to postintervention by using paired *t* tests. Postintervention values (total enrolled and only those with MetS) were compared between the 2 arms by using linear regression, controlling for baseline values and cohort (enrolled during the 2009–2010 or 2010–2011 harvest season). A stratified analysis was also conducted to compare changes in biomarkers after the intervention in those with "high" versus "low" baseline values of the biomarkers. Stratification cutoffs were based on median baseline values. Paired *t* tests were used to compare preintervention and postintervention values within each stratification group and randomization arm. Linear regression models were used to compare postintervention biomarker values between stratification groups within each randomization arm, adjusting for cohort group.

Linear regression models were conducted to explore the relation between risk factors of cardiometabolic diseases and concentrations of inflammatory/oxidative stress markers in the entire cohort at baseline and in each arm separately postintervention. Potential interactions between cardiometabolic risk factors and treatment arm on each postintervention biomarker outcome were tested by using likelihood ratio tests.

Post hoc effect sizes for each biomarker were calculated by using the sample sizes available (n = 37 in the grapefruit group and n = 32 in the control group). All tests were set using 80% power and an α of 0.05. Effect sizes of 1.2 mg/L, 80 μg/L, and 6.2 ng/mg creatinine were calculated for hsCRP, sVCAM-1, and F2-isoprostanes, respectively, between the grapefruit and control groups.

Data presented are means ± SDs; α = 0.05 for all tests. All analyses were performed by using Stata 12.1 (StataCorp).

Results

Three participants randomly assigned to the grapefruit group were lost to follow-up during the intervention phase, and blood collections were unsuccessful for 2 additional participants, leaving 37 and 32 participants who were randomly assigned to the grapefruit and control groups, respectively. Participants had a mean (±SD) age of 41.8 ± 10.7 y and a BMI of 32.1 ± 4.1 kg/m^2 (**Table 1**). Of the 69 participants who successfully completed the study, 29 (42%) were classified as having MetS at baseline.

TABLE 1 Baseline characteristics of overweight/obese participants enrolled in a 6-wk grapefruit feeding trial[1]

	Total (n = 69)	Grapefruit (n = 37)	Control (n = 32)
Age, *y*	41.8 ± 10.7	40.6 ± 10.8	43.2 ± 10.6
Female, *n (%)*	48 (69.6)	25 (67.6)	23 (71.9)
Non-Hispanic white race/ethnicity, *n (%)*	43 (62.3)	23 (62.1)	20 (62.5)
Weight, *kg*	91.5 ± 13.9	92.1 ± 15.0	90.8 ± 12.8
BMI, *kg/m^2*	32.1 ± 4.1	32.8 ± 4.2	31.4 ± 3.8
Waist circumference, *cm*	103 ± 10	103 ± 11	103 ± 10
Systolic blood pressure, *mm Hg*	120 ± 17	121 ± 18	119 ± 15
Diastolic blood pressure, *mm Hg*	81 ± 11	82 ± 11	80 ± 10
Serum glucose, *mg/dL*	91.7 ± 1.6	95.2 ± 2.5*	88.6 ± 2.0
Serum TGs, *mg/dL*	123 ± 10	114 ± 12	134 ± 17
Serum HDL-C, *mg/dL*	42.7 ± 1.5	43.2 ± 1.8	42.2 ± 2.5
Metabolic syndrome[2], *n (%)*	29 (42.0)	14 (37.8)	15 (46.9)

[1] Continuous variables are shown as means ± SDs and categorical variables are shown as *n* (%).Some parameters were not detectable in all participants: grapefruit, TGs (n = 35); control, blood pressure and HDL (n = 31), TGs (n = 30). *Different from control, $P < 0.05$. HDL-C, HDL cholesterol.

[2] Metabolic syndrome defined by using National Cholesterol Education Program Adult Treatment Panel III guidelines (13).

There were no significant differences at baseline for any measurement, including inflammatory/oxidative stress measurements between randomization groups. Thirty-two of the 37 participants randomly assigned to the grapefruit group logged their grapefruit consumption. Of those, compliance was calculated at 93 ± 11%. Diet composition during the washout and intervention periods, as measured by 24-h dietary recall, was overall unchanged (10). However, fruit intake increased by 1.2 servings/d and vitamin C intake increased by 100 mg/d, whereas vegetable intake decreased by 0.7 servings/d in those randomly assigned to the grapefruit group during the intervention phase. Fruit intake decreased in the control group by 0.8 servings/d (10).

Changes in inflammation and oxidative stress were analyzed within and between randomization arms. No significant difference was detected with regard to change in plasma sVCAM-1 concentrations from baseline to postintervention between groups (**Table 2**; P = 0.35); changes in sVCAM-1 were also not significant over time within treatment groups (grapefruit: P = 0.11; control: P = 0.26). Plasma hsCRP concentrations did not change in the grapefruit or control groups over time (P = 0.60 and P = 0.14, respectively); the difference in plasma hsCRP change score between groups was marginally significant (P = 0.09). Urinary F2-isoprostanes tended to decrease in the grapefruit group (P = 0.09), and no significant change was observed in the control group (P = 0.45). There was also no difference in isoprostane concentrations between groups postintervention (P = 0.16). Stratification on the median (**Table 3**) revealed no effect of the intervention on plasma sVCAM-1 concentrations, regardless of baseline values in the "high" or "low" stratification group. However, those with high baseline plasma hsCRP concentrations (≥2.18 mg/L) demonstrated a marginally significant reduction in hsCRP after grapefruit consumption (P = 0.10). Similarly, those with high baseline urinary F2-isoprostane concentrations (≥12.7 ng/mg creatinine) demonstrated a significant reduction in F2-isoprostanes after the grapefruit intervention (P = 0.021). In those randomly assigned to the grapefruit arm, the change in isoprostanes in the "high" stratification group was significantly different from those in the "low" stratification group (P = 0.004).

To further explore the effect of grapefruit consumption on cardiometabolic risk factors, post hoc analyses of participants with MetS were performed (n = 14 in the grapefruit arm, n = 15 in the control arm). At baseline, plasma hsCRP concentrations were 2.7 ± 1.6 mg/L in participants with MetS compared with 2.0 ± 1.8 mg/L in participants without MetS (P = 0.08; data not shown). hsCRP concentrations were not different between those randomly assigned to the grapefruit or control arms after the intervention (P = 0.11). Similar to the results from the entire cohort, F2-isoprostane concentrations in participants with MetS tended to be lower in the grapefruit group compared with the control group postintervention (**Table 4**; P = 0.06).

Associations between CVD risk factors (BMI, WC, age, and sex) and inflammatory/oxidative stress markers (sVCAM-1, hsCRP, and F2-isoprostanes) were tested at baseline in the entire cohort and postintervention in each arm separately. At baseline, sVCAM-1 concentrations were associated with age, hsCRP concentrations were associated with BMI and WC, and F2-isoprostane concentrations were associated with only WC (P < 0.05 for all associations; **Table 5**). WC and postintervention F2-isoprostanes were significantly inversely related in the grapefruit arm, but they were positively associated in the control arm (test for WC-by-assignment interaction, P = 0.004). There was a nonsignificant inverse association between male sex and

TABLE 2 Biomarker concentrations before and after 6 wk of intervention in overweight/obese participants enrolled in a grapefruit feeding trial[1,2]

	Grapefruit (n = 37)		Control (n = 32)		
	Pre	Post	Pre	Post	P value[2]
Plasma sVCAM-1, *μg/L*	628 ± 117	614 ± 123	623 ± 125	611 ± 111	0.35
Plasma hsCRP, *mg/L*	2.2 ± 1.5	2.1 ± 1.5	2.4 ± 2.0	2.8 ± 2.0	0.09
Urinary F2-isoprostanes, *ng/mg creatinine*	14.1 ± 7.7	12.4 ± 6.4	14.7 ± 6.5	15.9 ± 9.0	0.16

[1] Values are means ± SDs. A definitive value for biomarkers was not determined in all samples—sVCAM-1, n = 36 and n = 30; hsCRP, n = 36 and n = 30; and F2-isoprostanes, n = 34 and n = 32 for Grapefruit and Control, respectively. No differences were found between groups at baseline for any biomarker. Paired t tests were used to compare Pre and Post biomarker concentrations within each randomization arm, although no significant differences were found; $P < 0.05$. hsCRP, high-sensitivity C-reactive protein; sVCAM-1, soluble vascular cellular adhesion molecule 1.

[2] P values were derived from linear regression models comparing differences between randomization arms, postintervention, after adjustment for baseline value of biomarker and harvest year (2009–2010 or 2010–2011).

F2-isoprostanes in the grapefruit arm (P = 0.08) that was significantly different from the association in the control arm (test for sex-by-assignment interaction, P = 0.015).

Discussion

In this first, to our knowledge, randomized trial assessing grapefruit consumption on biomarkers associated with inflammation/oxidative stress and, more specifically, atherosclerotic plaque production, we found that 6 wk of daily grapefruit consumption did not reduce sVCAM, hsCRP, or F2-isoprostane concentrations, although those individuals with high baseline F2-isoprostane concentrations demonstrated a reduction in concentrations after the grapefruit intervention. In a subsample of participants with MetS, those who consumed grapefruit demonstrated marginally significantly lower F2-isoprostane concentrations (P = 0.06) compared with those assigned to the control condition after the intervention.

VCAM-1 is an adhesion molecule expressed by endothelial cells and vascular smooth muscle cells in response to inflammatory/oxidative stimuli (14), and circulating concentrations correlate with risk of coronary death (15). In vitro work indicates that flavonoids may attenuate inflammatory-induced adhesion molecule expression (16). To date, 2 studies have evaluated the effects of citrus flavonoids (in the form of orange juice or its main flavonoid, hesperidin) on sVCAM-1 concentrations. Daily consumption of orange juice or a hesperidin-enriched beverage (both containing ~290 mg hesperidin) for 4 wk nonsignificantly lowered sVCAM-1 concentrations in overweight men (9). In line with our results, a study of 3 wk daily hesperidin supplementation (500 mg/d) in participants with MetS did not change sVCAM-1 concentrations (6). Our results are also supported by a similar study using high-polyphenol grape powder (~500 mg/d of polyphenols) for 30 d wherein no change in sVCAM-1 concentrations in men with MetS was demonstrated. In that trial, other outcomes associated with endothelial health did improve (e.g., flow-mediated dilation, soluble intercellular adhesion molecule 1), although these measures were not assessed in our study (17). Epidemiologic evidence from the NHS indicated that women with the highest flavonol intake had a 4% reduction in sVCAM-1 concentrations compared with women in the lowest quintile of intake (4). These data, coupled with our own, suggest that sVCAM-1 concentrations are not highly responsive to short-term exposures of flavonoid intake, but long-term, habitual intake may promote healthy sVCAM-1 concentrations. It is also possible that dietary flavonoids, or citrus flavonones in particular, do not substantially modify sVCAM-1 concentrations.

TABLE 3 Biomarker concentrations before and after 6 wk of intervention, stratified by baseline concentration of biomarker in overweight/obese participants enrolled in a grapefruit feeding trial[1]

	Grapefruit (n = 37)			Control (n = 32)		
Stratification cutoff	Pre	Post	P value[2]	Pre	Post	P value[2]
Plasma sVCAM-1, *μg/L*			0.70			0.72
Low: <611 μg/L	628 ± 117	614 ± 123		623 ± 125	611 ± 111	
High: ≥611 μg/L	712 ± 107	704 ± 109		715 ± 118	697 ± 87	
Plasma hsCRP, *mg/L*			0.10			0.97
Low: <2.18 mg/L	1.0 ± 0.7	1.3 ± 1.3		0.6 ± 0.8	1.0 ± 0.9	
High: ≥2.18 mg/L	3.5 ± 1.0	3.0 ± 1.1		4.0 ± 1.1	4.3 ± 1.2	
Urinary F2-isoprostanes, *ng/mg creatinine*			0.004			0.55
Low: <12.7 ng/mg	9.5 ± 3.5	10.2 ± 4.6		9.7 ± 1.7	11.9 ± 5.0	
High: ≥12.7 ng/mg	19.3 ± 7.9	15.0 ± 7.4*		19.7 ± 5.7	19.8 ± 10.3	

[1] Values are means ± SDs. Stratification resulted in the following: n = 34 in the "low" and n = 32 in the "high" groups for sVCAM-1 and F2-isoprostanes; and n = 33 in "low" and n = 33 in "high" groups for hsCRP. Baseline median values of each biomarker were determined in the whole sample (control and grapefruit participants combined), and stratification cutoffs were determined on the basis of this median value. *Different from Pre, $P < 0.05$ (paired t test). hsCRP, high-sensitivity C-reactive protein; sVCAM-1, soluble vascular cellular adhesion molecule 1.

[2] P values were derived from linear regression models comparing differences between stratification groups (low vs. high), post-intervention, after adjusting for baseline value of biomarker and harvest year (2009–2010 or 2010–2011).

TABLE 4 Biomarker concentrations before and after 6 wk of intervention in a subsample of participants with metabolic syndrome enrolled in a grapefruit feeding trial[1]

	Grapefruit (n = 14)		Control (n = 15)		
	Pre	Post	Pre	Post	P value[2]
Plasma sVCAM-1, $\mu g/L$	666 ± 134	650 ± 145	603 ± 86	594 ± 94	0.84
Plasma hsCRP, *mg/L*	2.3 ± 1.7	2.0 ± 1.0	3.1 ± 1.5	3.1 ± 1.6	0.11
Urinary F2-isoprostanes, *ng/mg creatinine*	14.3 ± 6.7	12.0 ± 4.5	15.0 ± 6.9	18.3 ± 10.9	0.06

[1] Values are means ± SDs. No differences were found between groups at baseline for any biomarker. Paired t tests were used to determine the change in biomarker within each randomization group, although no significant differences were found; $P < 0.05$. hsCRP, high-sensitivity C-reactive protein; sVCAM-1, soluble vascular cellular adhesion molecule 1.
[2] P values were derived from linear regression models comparing differences between randomization arms, postintervention, after adjustment for baseline value of biomarker.

A significant body of evidence indicates that CRP is associated with risk of CVD (14,18). CRP was once considered a pathologically insignificant hepatic marker of a larger inflammatory cascade, but more recent evidence demonstrates that CRP is also produced by the endothelium in response to injury (14) and has proatherogenic properties (1). In the epidemiologic NHS, Landberg et al. (4) showed that grapefruit intake (≥1 serving/d) was inversely associated with CRP concentrations, in contrast to findings of our randomized controlled trial that showed no significant reduction in CRP in response to eating 3 servings/d for 6 wk. However, the stratified analysis performed in this study revealed that those with higher baseline concentrations demonstrated a marginally significant reduction in hsCRP concentrations, an effect that was not seen in those with low baseline concentrations. An exploratory analysis was also performed to examine the effect of grapefruit consumption on individuals with baseline hsCRP concentrations ≥3.0 mg/L, the cutoff for increased risk of CVD. Only 10 individuals randomly assigned to the grapefruit group had hsCRP concentrations ≥3.0 mg/L, with a mean concentration of 4.1 ± 0.7 mg/L. After the intervention, this concentration was reduced to 3.3 ± 1.2 mg/L (P = 0.08). These results align with the results from the stratification on the median and suggest that individuals with high baseline hsCRP concentrations are likely to demonstrate a robust response to regular grapefruit intake. A recent randomized crossover study by Dalgård et al. (19) showed an 11% reduction in CRP concentrations after 4 wk consumption of orange and blackcurrant juices compared with a placebo drink in participants with peripheral artery disease (PAD) who had a median baseline CRP concentration of 2.8 mg/L. Of note, a study in healthy adults who consumed 500 mL orange juice for 14 d demonstrated a nonsignificant reduction in CRP (20). These findings suggest that the health status of the cohort may explain differential effects demonstrated across the published studies.

F2-isoprostanes are a validated marker of lipid peroxidation, a key mechanism leading to atheroma formation (21). Previous studies have shown a reduction in plasma F2-isoprostanes after orange juice consumption in healthy adults (20) as well as in patients with PAD consuming orange and blackcurrant juices (19). In contrast to the results from the orange and blackcurrant juice study, our study resulted in no significant change in F2-isoprostanes after 6 wk of daily grapefruit consumption. However, our stratified analysis indicates that those with high

TABLE 5 Relation between inflammatory/oxidative stress biomarkers and common CVD risk factors in overweight/obese participants enrolled in a grapefruit feeding trial[1]

Risk factor	Predicted effect on biomarker at baseline[2] (95% CI)	Predicted change in biomarker[3] (95% CI)		P-interaction[4]
		Control arm	Grapefruit arm	
Plasma sVCAM-1 (μg/L)				
BMI (kg/m^2)	−0.84 (−8.21, 6.54)	−0.26 (−5.75, 5.23)	−1.61 (−5.77, 2.55)	0.68
WC (cm)	−0.16 (−3.12, 2.79)	−0.29 (−2.42, 1.83)	−0.81 (−2.42, 0.81)	0.66
Age (y)	3.15 (0.49, 5.80)	−0.12 (−2.08, 1.84)	1.0 (−0.71, 2.71)	0.18
Male sex	26.5 (−37.0, 89.9)	−36.2 (−78.5, 6.2)	−2.4 (−39.8, 35.0)	0.16
Plasma hsCRP (mg/L)				
BMI (kg/m^2)	0.15 (0.05, 0.25)	0.02 (−0.11, 0.16)	0.02 (−0.08, 0.12)	0.66
WC (cm)	0.06 (0.02, 0.10)	−0.03 (−0.08, 0.02)	0.02 (−0.02, 0.06)	0.22
Age (y)	0.01 (−0.02, 0.05)	−0.02 (−0.06, 0.03)	0.01 (−0.03, 0.04)	0.49
Male sex	0.01 (−0.92, 0.94)	−0.22 (−1.23, 0.83)	0.28 (−0.54, 1.10)	0.43
Urinary F2-isoprostanes (ng/mg creatinine)				
BMI (kg/m^2)	0.10 (−0.33, 0.53)	0.50 (−0.29, 1.28)	−0.20 (−0.59, 0.18)	0.08
WC (cm)	0.19 (0.02, 0.36)	0.32 (0.02, 0.63)	−0.16 (−0.32, −0.01)	0.004
Age (y)	0.10 (−0.06, 0.26)	−0.07 (−0.36, 0.22)	−0.06 (−0.21, 0.10)	0.91
Male sex	2.16 (−1.64, 5.95)	5.78 (−0.74, 12.3)	−2.68 (−6.11, 0.75)	0.015

[1] n = 69. CVD, cardiovascular disease; hsCRP, high-sensitivity C-reactive protein; sVCAM-1, soluble vascular cellular adhesion molecule 1; WC, waist circumference.
[2] β Coefficients derived from linear regression models assessing the predictive nature between risk factors and biomarkers in the entire cohort at baseline, adjusting for baseline values.
[3] β Coefficients derived from linear regression models assessing the predictive nature between groups postintervention, adjusting for baseline values.
[4] P-interaction from a likelihood ratio test including the interaction between the intervention arm and the predictor variable on outcome variables postintervention.

baseline F2-isoprostane concentrations were more responsive to the intervention than those with low baseline concentrations.

Data from the Framingham Heart Study indicate that visceral adiposity is highly correlated with isoprostane concentrations (22). At baseline, WC was positively associated with isoprostane concentrations. Grapefruit consumption modified this relation and resulted in an inverse association between baseline WC and F2-isoprostane concentrations, a result not seen in the control group. However, we cannot ascertain if the reduction in isoprostanes was directly due to grapefruit consumption or if it was an outcome secondary to WC reduction, as we had previously shown a significant reduction in WC after 6 wk of grapefruit consumption (10).

Participants with MetS randomly assigned to the grapefruit intervention demonstrated a nonsignificant reduction in F2-isoprostanes ($P = 0.24$), although those in the control group showed a nonsignificant increase in F2-isoprostanes ($P = 0.26$). Although these very slight differences were clearly not significant within each randomization arm, the difference between randomization arms, postintervention, approached significance ($P = 0.06$). This result may have been an artifact of the methods used, because citrus fruits were restricted in the control group. These findings suggest that antioxidant-rich foods, such as grapefruit or citrus, may be necessary to maintain healthy concentrations or to reduce high circulating concentrations of F2-isoprostanes. However, this hypothesis will need to be prospectively tested in a sample of adequate size.

This study has both strengths and limitations. This randomized controlled trial evaluated the anti-inflammatory and antioxidant effects of Rio red grapefruit, a widely available whole food, in overweight/obese individuals, a population that is subject to high rates of CVD (2). Outcomes were also evaluated in a subsample of participants with MetS, a population with a 2- to 5-fold increased risk of CVD compared with metabolically healthy controls (23). The original trial was powered for change in weight (10), not inflammatory/oxidative biomarkers. Nevertheless, grapefruit consumption did lead to reductions in these markers compared with the control condition, suggesting that these interventions should be tested in a larger sample before definitive conclusions can be drawn.

In our sample, baseline sVCAM-1 concentrations were more similar to those of a healthy population (4), whereas previous studies in patients with MetS (17) or coronary artery disease (15) showed that circulating sVCAM-1 concentrations may be as much as 2–3 times greater in at-risk populations. This may explain why sVCAM-1 concentrations did not change in relation to the grapefruit intervention. A similar intervention in a high-risk population may be beneficial given previous findings demonstrating sVCAM-1's responsiveness to flavonoids (16) or flavonoid-rich foods (24).

Self-reported compliance to the intervention was high, although a biomarker of compliance would strengthen the results of this trial. Metabolites of naringenin would be the most appropriate biomarker in this study; however, naringenin is excreted within 6–12 h after consumption (25), limiting its utility in a study such as this one wherein fasting samples were collected. A nutrient analysis of the fruit may have also strengthened our results. However, previous work has quantified many of the nutrients in the Rio red grapefruit cultivar, including naringenin (26) and ascorbic and citric acids (27).

Last, atherosclerosis develops in response to multiple stimuli and signaling pathways, and the cardioprotective response to grapefruit could potentially be detected by using a variety of biomarkers. Here, we evaluated biomarkers with the most robust preliminary data in relation to the pathogenesis of atheroma formation and responsiveness to dietary flavonoids. Overall, our results cannot support daily citrus and/or grapefruit to significantly modify CVD risk associated with the selected biomarkers.

These hypothesis-generating data, coupled with previous research, suggest that daily grapefruit consumption may promote cardiovascular health. Our trial suggests future research in the field of grapefruit/citrus consumption and cardioprotection should focus on populations at high risk of CVD, including MetS individuals, in a sufficiently large sample size to test these important hypotheses.

Acknowledgments

The authors thank Christina Preece for her work in quantifying sVCAM-1 and F2-isoprostanes. C.A.D., B.S.P., and C.A.T. designed the research; C.A.D. conducted the research and had primary responsibility for final content; B.S.P. provided essential reagents; C.A.D. and B.C.W. performed statistical analysis; and C.A.D., B.C.W., and C.A.T. wrote the manuscript. All authors read and approved the final manuscript.

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