Effects of Consumption of Pomegranate Juice on Carotid Intima–Media Thickness in Men and Women at Moderate Risk for Coronary Heart Disease

Michael H. Davidson, MD[a,*], Kevin C. Maki, PhD[b], Mary R. Dicklin, PhD[b], Steven B. Feinstein, MD[c], MarySue Witchger, RD[b], Marjorie Bell[b], Darren K. McGuire, MD[d], Jean-Claude Provost, MD[e], Harley Liker, MD[f], and Michael Aviram, DSc[g]

This randomized, double-blind, parallel trial assessed the influence of pomegranate juice consumption on anterior and posterior carotid intima–media thickness (CIMT) progression rates in subjects at moderate risk for coronary heart disease. Subjects were men (45 to 74 years old) and women (55 to 74 years old) with ≥1 major coronary heart disease risk factor and baseline posterior wall CIMT 0.7 to 2.0 mm, without significant stenosis. Participants consumed 240 ml/day of pomegranate juice (n = 146) or a control beverage (n = 143) for up to 18 months. No significant difference in overall CIMT progression rate was observed between pomegranate juice and control treatments. In exploratory analyses, in subjects in the most adverse tertiles for baseline serum lipid peroxides, triglycerides (TGs), high-density lipoprotein (HDL) cholesterol, TGs/HDL cholesterol, total cholesterol/HDL cholesterol, and apolipoprotein-B100, those in the pomegranate juice group had significantly less anterior wall and/or composite CIMT progression versus control subjects. In conclusion, these results suggest that in subjects at moderate coronary heart disease risk, pomegranate juice consumption had no significant effect on overall CIMT progression rate but may have slowed CIMT progression in subjects with increased oxidative stress and disturbances in the TG-rich lipoprotein/HDL axis. **(Am J Cardiol 2009;104:936–942)**

Pomegranate juice is a naturally rich source of polyphenols and other antioxidants including tannins and anthocyanidins.[1] In vitro and in vivo studies have supported the ability of pomegranate juice to scavenge free radicals and inhibit low-density lipoprotein oxidation.[2–8] Consumption of pomegranate juice for 2 weeks by hypertensive subjects significantly decreased systolic blood pressure.[3] Pomegranate juice also has been reported to decrease the amount of stress-induced myocardial ischemia in subjects with coronary heart disease after 3 months of consumption.[6] A small pilot study has shown that consumption of pomegranate juice for 1 year by subjects with carotid artery stenosis is associated with decreased low-density lipoprotein susceptibility to oxidation and a significant decrease in carotid intima–media thickness (CIMT).[5] The present trial was designed to further evaluate the effects of pomegranate juice consumption on CIMT progression in subjects at moderate coronary heart disease risk.

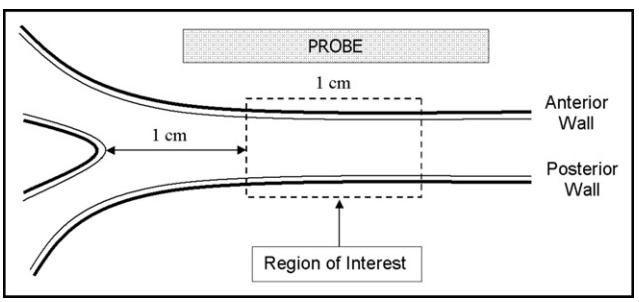

Figure 1. Schematic showing the intima–media thickness region of interest on the common carotid artery.

Methods

The trial was conducted at 2 clinical research sites in the United States (Radiant Research, Chicago, Illinois, and University of Texas Southwestern Medical Center, Dallas, Texas) in accordance with good clinical practice guidelines. The protocol was approved by an institutional review board (Quorum Review, Inc., Seattle, Washington), and all subjects provided written informed consent. To be eligible for participation, men (45 to 74 years old) and women (55 to 74 years old) were required to have ≥1 of the following risk factors: low-density lipoprotein cholesterol (≥130 and <190 mg/dl), low high-density lipoprotein (HDL) cholesterol (<40 mg/dl), increased blood pressure (≥140/90 mm

[a]Radiant Research and University of Chicago, and [c]Rush Medical College, Chicago; [b]Provident Clinical Research, Glen Ellyn, Illinois; [d]University of Texas Southwestern Medical Center, Dallas, Texas; [e]Synarc Imaging, Paris, France; [f]David Geffen School of Medicine, University of California, Los Angeles, California; and [g]Lipid Research Laboratory, Technion Faculty of Medicine and Rambam Medical Center, Haifa, Israel. Manuscript received February 9, 2009; revised manuscript received and accepted May 13, 2009.

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*Corresponding author: Tel: 312-494-2220; fax: 312-494-2217.

E-mail address: michaeldavidson@radiantresearch.com (M.H. Davidson).

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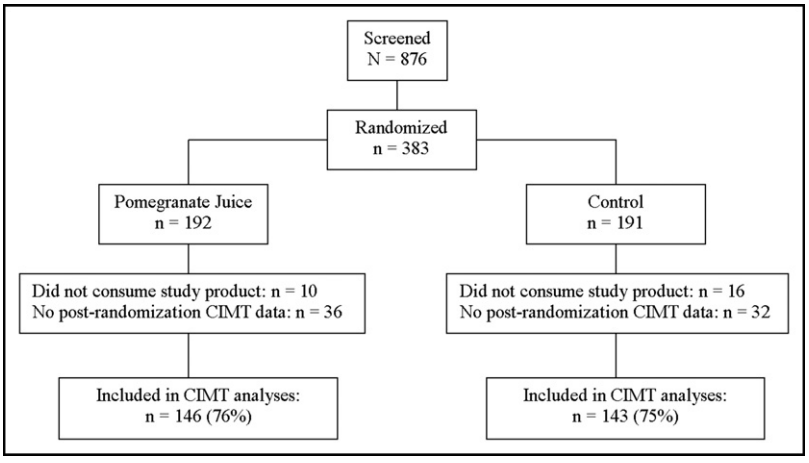

Figure 2. Subject disposition.

Table 1
Baseline characteristics of intent-to-treat sample by treatment group

Parameter	Pomegranate Juice (n = 146)	Control (n = 143)
Men	85 (58%)	79 (55%)
White	108 (74%)	94 (66%)
Black	24 (16%)	34 (24%)
Asian	6 (4.1%)	9 (6.3%)
Hispanic/Latino	5 (3.4%)	4 (2.8%)
Hypertension	70 (48%)	69 (48%)
Angina pectoris	2 (1.4%)	1 (0.7%)
Arrhythmia	5 (3.4%)	5 (3.5%)
Heart failure	1 (0.7%)	0 (0.0%)
Smoker	23 (16%)	29 (20%)
Blood pressure ≥140/90 mm Hg or antihypertensive agent(s) use	69 (47%)	70 (49%)
HDL cholesterol <40 mg/dl	23 (16%)	9 (6.3%)
Family history of premature coronary heart disease	7 (4.8%)	6 (4.2%)
Age ≥45 years (men) or ≥55 years (women)	146 (100%)	142 (99%)
Medication use		
Antihypertensive agent(s)	47 (32%)	41 (29%)
Calcium channel blocker	18 (12%)	20 (14%)
Angiotensin-converting enzyme inhibitor or angiotensin II receptor blocker	34 (23%)	18 (13%)
β blocker	7 (4.8%)	5 (3.5%)
Diuretic	14 (10%)	16 (11%)
α-adrenergic blocker	2 (1.4%)	2 (1.4%)
Lipid-altering agent(s)	32 (22%)	25 (18%)
Statin therapy	24 (16%)	21 (15%)
Aspirin	27 (19%)	28 (20%)
Age (years)	60.8 ± 7.3	60.5 ± 7.8
Body mass index (kg/m^2)	28.6 ± 4.8	28.7 ± 4.5
Systolic blood pressure (mm Hg)	127.7 ± 18.7	129.3 ± 18.4
Diastolic blood pressure (mm Hg)	70.9 ± 10.5	71.5 ± 11.0
Fasting glucose (mg/dl)	94.6 ± 10.0	94.7 ± 8.9
Total cholesterol (mg/dl)	224.3 ± 37.8	227.2 ± 35.7
Low-density lipoprotein cholesterol (mg/dl)	138.8 ± 33.5	142.3 ± 29.6
HDL cholesterol (mg/dl)	55.1 ± 15.4	56.1 ± 13.9
TGs (mg/dl)	152.8 ± 75.4	144.3 ± 65.4
Apolipoprotein-B100 (mg/dl)	109.2 ± 2.0	110.1 ± 2.1
Apolipoprotein-AI (mg/dl)	153.2 ± 2.2	152.9 ± 2.2

Values are numbers of subjects (percentages) or means ± SEMs.

Hg) or medication to treat hypertension, or current cigarette smoking (any cigarette smoking within previous month). They were also required to have a baseline posterior wall common CIMT measurement >0.7 and <2.0 mm on ≥1 side (right or left). Evidence of carotid stenosis ≥50% was exclusionary.

Subjects with coronary heart disease or a risk equivalent, including diabetes,[9] were not enrolled. Also excluded were subjects with a body mass index >40 kg/m^2, hepatic disease or dysfunction, cancer in the previous 2 years (except nonmelanoma skin cancer), human immunodeficiency virus, hepatitis B or C, uncontrolled hypertension, or untreated or unstable hypothyroidism. Use of β-adrenergic blockers, immunosuppressants, or estrogen or progestin therapy was prohibited during the study. Concomitant statin therapy was allowed, but use of any other drug or nondrug lipid-altering agents within 6 weeks before screening was exclusionary. Any subject with a known allergy to pomegranates or who had a history of eating pomegranates or drinking pomegranate juice within the previous 6 months was excluded from the study.

Subjects visited the clinic at screening; weeks 0, 13, 26, 38, 52, and 65; and 1 time between weeks 78 and 90 (end of treatment). At baseline, subjects were randomly assigned to consume pomegranate juice 240 ml/day providing 140 kcal, fat 0 g, protein 1 g, and total carbohydrates 35 g (Wonderful variety, Pom; supplied by Roll International Corporation, Los Angeles, California) or a control beverage of similar color and energy content (150 cal, fat 0 g, protein 0 g, and total carbohydrates 37 g) for 18 months. The study product was packaged in single-serving bottles labeled so that neither subjects nor staff members were aware of treatment assignment. Subjects were instructed that the study product should replace a food or beverage in their diet with approximately the same amount of energy. Adherence to study product consumption was assessed at each visit by reviewing a daily consumption diary maintained by the subject.

Carotid ultrasound measurements were performed at baseline, 12 months, and end of treatment according to methods described previously.[10] Carotid arteries were imaged by high-resolution B-mode carotid artery ultrasound using an HDI 5000 ultrasound system with a linear-array 7.5-MHz transducer (Phillips Medical Systems NA, Both-

Table 2
Indicators of inflammation and oxidative stress at baseline and changes from baseline by time point and treatment group in intent-to-treat sample

Variable	Pomegranate Juice	Control	p Value
High sensitivity C-reactive protein (mg/L)			
Subjects	143	143	
Baseline	3.10 ± 0.24	3.19 ± 0.27	0.960
Change from baseline to 3 months	−0.16 ± 0.18	0.40 ± 0.34	0.231
Change from baseline to 12 months	−0.39 ± 0.21	0.06 ± 0.37	0.298
Change from baseline to end of treatment	−0.50 ± 0.23	−0.44 ± 0.22	0.547
Ferric reducing ability of plasma (μM Fe^{2+})			
Subjects	146	143	
Baseline	112.9 ± 2.1	112.8 ± 2.0	0.965
Change from baseline to 3 months	5.9 ± 1.4	6.9 ± 1.4	0.984
Change from baseline to 12 months	8.5 ± 1.8	11.1 ± 1.9	0.318
Change from baseline to end of treatment	10.8 ± 1.9	11.6 ± 1.9	0.749
Paraoxonase-1 (U/ml)			
Subjects	144	139	
Baseline	112.7 ± 1.9	111.2 ± 2.03	0.688
Change from baseline to 3 months	0.8 ± 1.25	0.4 ± 1.06	0.816
Change from baseline to 12 months	−3.7 ± 1.74	−2.1 ± 1.85	0.677
Change from baseline to end of treatment	−0.7 ± 1.81	0.1 ± 1.61	0.728
PD − AAPH (nmol/ml)			
Subjects	139	135	
Baseline	26.4 ± 1.2	24.7 ± 1.1	0.362
Change from baseline to 3 months	−0.3 ± 0.9	−0.9 ± 1.0	0.251
Change from baseline to 12 months	−7.8 ± 1.3	−2.6 ± 1.4	0.010
Change from baseline to end of treatment	−5.3 ± 1.3	−3.0 ± 1.2	0.159
PD + AAPH (nmol/ml)			
Subjects	146	143	
Baseline	702.5 ± 7.5	696.1 ± 7.9	0.561
Change from baseline to 3 months	−15.3 ± 5.6	−10.1 ± 4.6	0.480
Change from baseline to 12 months	19.7 ± 8.6	25.7 ± 9.0	0.628
Change from baseline to end of treatment	18.2 ± 9.0	28.6 ± 9.4	0.296
Thiobarbituric acid-reactive substances − AAPH (nmol/ml)			
Subjects	146	143	
Baseline	3.45 ± 0.13	3.63 ± 0.15	0.530
Change from baseline to 3 months	−0.04 ± 0.07	0.11 ± 0.07	0.075
Change from baseline to 12 months	−0.56 ± 0.16	−0.76 ± 0.17	0.363
Change from baseline to end of treatment	−0.57 ± 0.15	−0.79 ± 0.16	0.335
Thiobarbituric acid-reactive substances + AAPH (nmol/ml)			
Subjects	146	143	
Baseline	16.1 ± 0.3	16.0 ± 0.3	0.943
Change from baseline to 3 months	−0.1 ± 0.2	−0.2 ± 0.2	0.546
Change from baseline to 12 months	−0.8 ± 0.3	−1.0 ± 0.3	0.600
Change from baseline to end of treatment	−1.1 ± 0.3	−1.1 ± 0.3	0.895

Values are numbers of subjects or means ± SEMs.

ell, Washington). All scanning throughout the study was conducted by a single ultrasonographer at each of the testing sites using the same equipment. All measurements were taken in the same artery region throughout the study. After baseline ultrasound examination, masking software (Io-Mask; Synarc, Paris, France) was used to match follow-up scans to optimize alignment with the baseline scan. Longitudinal scans of the blood–intima and media–adventitia interfaces of the posterior wall of the right and left common carotid arteries were performed. A sequence of images was digitally recorded using end-diastolic electrocardiographic gating, i.e., recording for ≥1 second along with the electrocardiographic tracing to allow for end-diastolic image identification. Digital images were electronically transmitted to a central imaging laboratory (Synarc) that was responsible for quality control, maintaining a database, and prereading (selecting the highest-quality end-diastolic images).

Images were blinded according to visit and treatment group and forwarded to an expert reviewer. A nongain-dependent software program (Io-QIMT, Synarc-IoDP Medical Imaging Research) was used to analyze images and calculate CIMT using automated edge detection to locate the lumen–intima and media–adventitia echocardiographic boundaries at subpixel resolution.[11,12] CIMT was averaged over 70 to 100 individual measurements taken along a 1-cm segment of the common carotid artery proximal to the bifurcation (Figure 1).

Subjects were given the option of being intravenously injected with microbubble contrast agent 0.5 ml (Optison, GE Healthcare, Inc., Princeton, New Jersey), which increases

Table 3
Common carotid intima–media thickness and progression in carotid intima–media thickness by time point, measurement site, and treatment group in intent-to-treat sample

Variable	Pomegranate Juice	Control	p Value
Anterior*			
Subjects	69	82	
Baseline (mm)	0.84 ± 0.02	0.85 ± 0.02	0.591
12 mos (mm)	0.82 ± 0.02	0.84 ± 0.02	0.204
End of treatment (mm)	0.80 ± 0.02	0.83 ± 0.02	0.222
Progression at end of treatment (mm/year)	−0.022 ± 0.014	−0.011 ± 0.012	0.570
Posterior			
Subjects	146	143	
Baseline (mm)	0.77 ± 0.01	0.77 ± 0.01	0.888
12 mos (mm)	0.78 ± 0.01	0.79 ± 0.01	0.128
End of treatment (mm)	0.79 ± 0.01	0.78 ± 0.01	0.945
Progression at end of treatment (mm/year)	0.013 ± 0.003	0.009 ± 0.003	0.587
Composite			
Subjects	146	143	
Baseline (mm)	0.78 ± 0.01	0.79 ± 0.01	0.336
12 mos (mm)	0.79 ± 0.01	0.81 ± 0.01	0.022
End of treatment (mm)	0.79 ± 0.01	0.80 ± 0.01	0.168
Progression at end of treatment (mm/year)	0.005 ± 0.004	0.005 ± 0.004	0.654

Values are numbers of subjects or means ± SEMs.
* Anterior wall measurements were available only for subjects who agreed to the use of intravenous contrast.

visibility of the anterior wall of the common carotid.[13] In subjects who received the injection, the anterior wall was measured using the same analysis techniques used for the posterior wall.

Analyses of fasting lipids, serum chemistry, hematology, and urinalysis at screening and/or baseline and at week 13, 12 months, and end of treatment were performed by PPD Laboratory (Highland Heights, Kentucky). Frozen blood samples were also sent to the Lipid Research Laboratory (Haifa, Israel) for analyses of apolipoprotein-AI, apolipoprotein-B100, high-sensitivity C-reactive protein, ferric reducing ability of plasma, paraoxonase-1, and serum susceptibility to oxidation. Concentrations of apolipoprotein-AI and apolipoprotein-B100 were determined turbidimetrically after agglutination with antisera using a Cobas Integra analyzer (Roche Diagnostics GmbH, Mannheim, Germany). Analysis of high-sensitivity C-reactive protein was performed on a Modular Analytics P800 system (Roche Diagnostics, Indianapolis, Indiana) using Tina Quant CRP high sensitive (Roche Diagnostics GmbH).[14] Ferric reducing ability of plasma was measured spectrophotometrically.[15] Analysis of paraoxonase-1 arylesterase activity toward phenyl acetate was determined as previously described.[16] Susceptibility to oxidation was determined (with and without the free radical generator 2.2′ azo bis [2-amidopropane] dihydrochloride [AAPH])[17] by analyzing the formation of lipid peroxides (PDs)[18] and by thiobarbituric acid-reactive substances assay.[19]

Statistical analyses were generated using SAS 9.1.3 (SAS Institute, Cary, North Carolina). An evaluable sample of 113 subjects per group was expected to provide 80% power to detect a difference in CIMT progression of 0.015 mm/year (pooled SD 0.04). All tests of statistical significance were completed at alpha = 0.05, 2-sided. Assumptions of normality of residuals were investigated for each response measurement. Where it was determined that the distribution was not approximated by a normal curve, values were ranked before final analysis.

Baseline comparability of treatment groups was assessed by chi-square tests (categorical variables) and analysis of variance (continuous variables). Possible differences between treatments in response variables were evaluated by analysis of variance. The primary end point was rate of CIMT progression, which was evaluated in an intent-to-treat population including all subjects with baseline and ≥1 postrandomization CIMT measurement for a given CIMT wall. For subjects who had a value available after 1 year but not at the end of the treatment period, the method of last observation carried forward was used to impute an end-of-treatment value. Baseline, 12-month, and end-of-treatment CIMT values were used in a linear regression model for each subject to determine the slope (progression rate) of the best-fit line. Progression rates were calculated separately for the right and left side anterior and posterior walls (mean for right and left when the 2 were available) and then all available walls were used to calculate the mean composite CIMT value for each subject, which included anterior wall measurements in a subset of the study sample. All CIMT readings were performed by a single reader and variability was assessed by the Bland-Altman method.[20] Mean ± SD for the difference in CIMT values for duplicate readings (all available walls) was 0.007 ± 0.046 mm.

CIMT variables were also evaluated in selected subgroups in exploratory analyses designed to generate hypotheses for future examination. No correction for multiple comparisons was applied for exploratory analyses to minimize the risk of a type II statistical error.

Results

A complete description of subject disposition is shown in Figure 2. Of the 876 subjects screened, 383 were randomized and 289 completed ≥1 postrandomization CIMT measurement. Percentages of randomized subjects included in the intent-to-treat analysis were similar in the pomegranate juice (76%) and control (75%) groups. Table 1 presents baseline characteristics of subjects in the intent-to-treat sample. With the exception of the percentage of smokers (16% of completers and 38% of noncompleters, p = 0.013), there were no significant differences in baseline characteristics between subjects who did and did not complete the study. Treatment groups were well matched and no significant differences at baseline were noted for any variables except the use of angiotensin-converting enzyme inhibitors and/or angiotensin II receptor blockers, which was more common (p = 0.027) in the pomegranate juice group (23%) compared to the control group (13%).

Baseline values and changes from baseline by group and time point for indicators of inflammation and oxidative stress are listed in Table 2. No significant differences in baseline values were present. The pomegranate juice group showed a significantly larger decrease in serum PD − AAPH (levels of PDs measured without adding the free

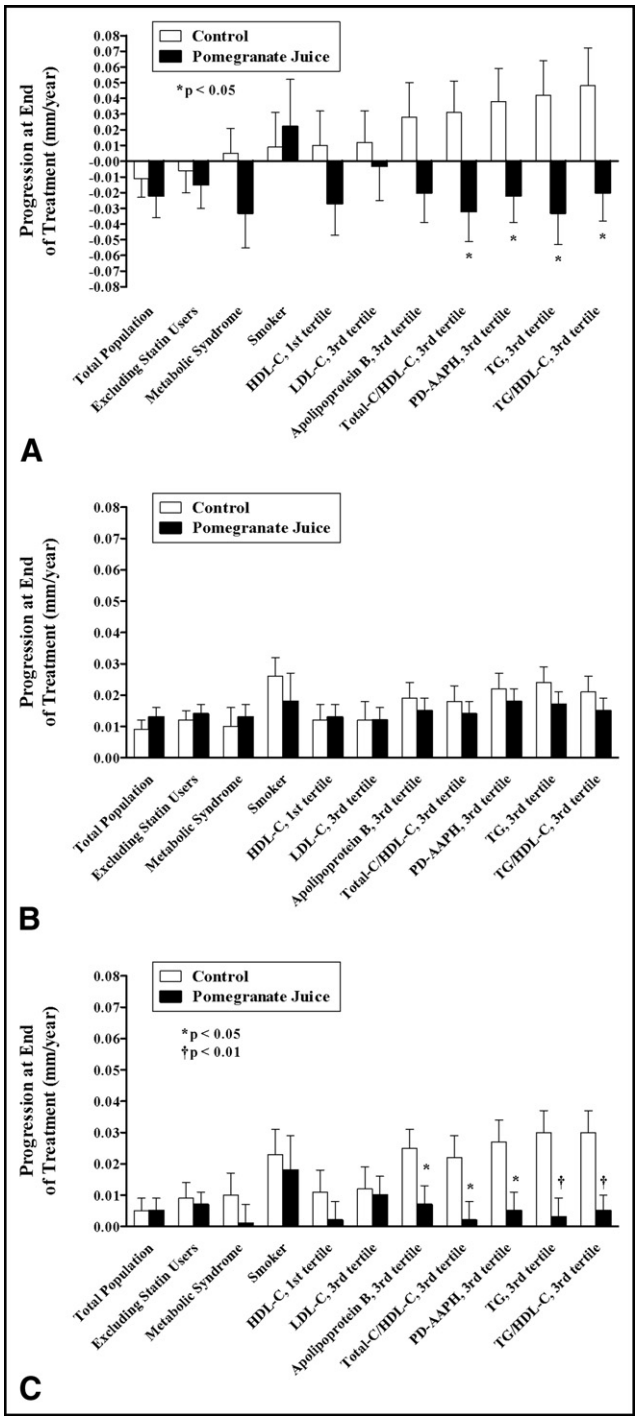

Figure 3. Mean ± SE for anterior wall *(A)*, posterior wall *(B)*, and composite *(C)* CIMT progression at end of treatment in selected subgroups. Number of subjects for anterior wall measurement: total population (control, n = 82; pomegranate juice, n = 69); excluding statin users (control, n = 72; pomegranate juice, n = 56); metabolic syndrome (control, n = 34; pomegranate juice, n = 30); smokers (control, n = 16; pomegranate juice, n = 11); HDL cholesterol (HDL-C), first tertile (control, n = 23; pomegranate juice, n = 33); low-density lipoprotein cholesterol (LDL-C), third tertile (control, n = 23; pomegranate juice, n = 22); apolipoprotein-B, third tertile (control, n = 27; pomegranate juice, n = 25); total cholesterol (Total-C)/HDL cholesterol, third tertile (control, n = 28; pomegranate juice, n = 31); PD − AAPH, third tertile (control, n = 24; pomegranate juice, n = 30); TG, third tertile (control, n = 25; pomegranate juice, n = 30); and TG/HDL cholesterol, third tertile (control, n = 23; pomegranate juice, n = 31). Number of subjects for posterior wall and composite measurements: total population (control, n = 143; pomegranate juice, n = 146); excluding statin users (control, n = 122; pomegranate juice, n = 122); metabolic syndrome (control, n = 52; pomegranate juice, n = 59); smokers (control, n = 29; pomegranate juice, n = 23); HDL cholesterol, first tertile (control, n = 41; pomegranate juice, n = 56); low-density lipoprotein cholesterol, third tertile (control, n = 47; pomegranate juice, n = 48); apolipoprotein-B, third tertile (control, n = 49; pomegranate juice, n = 45); total cholesterol/HDL cholesterol, third tertile (control, n = 44; pomegranate juice, n = 52); PD − AAPH, third tertile (control, n = 40; pomegranate juice, n = 46); TGs, third tertile (control, n = 41; pomegranate juice, n = 55); and TG/HDL cholesterol, third tertile (control, n = 40; pomegranate juice, n = 56).

radical generator AAPH, p = 0.01) at 12 months and a trend toward a greater decrease in thiobarbituric acid-reactive substances − AAPH (levels of thiobarbituric acid-reactive substances measured without adding AAPH, p = 0.075) at 3 months. No other significant differences were present.

With the exception of apolipoprotein-B100, which decreased more with pomegranate juice than with control (−12.0 vs −9.0 mg/dl), there were no differences between treatment groups for changes from baseline in traditional cardiovascular risk markers, including fasting lipoprotein lipids, blood pressures, or smoking status (data not shown). Body weight increased slightly during treatment and to a similar degree (1 to 2 kg) in the 2 groups (data not shown).

Results for CIMT values by treatment group and time point and progression rates during treatment are listed in Table 3. Of the 152 subjects (52%) agreeing to the optional administration of intravenous contrast agent for anterior wall imaging, as expected, baseline values for the anterior wall of the common carotid artery were larger than for the posterior wall. Anterior and posterior wall CIMT values and progression rates did not differ significantly between treatment groups at any time point. The composite measurement of CIMT showed a significantly smaller value at 12 months in the pomegranate juice group compared to the control group (0.79 vs 0.81 mm, p = 0.022). However, this difference was no longer significant at the end of the treatment period (0.79 vs 0.80 mm, p = 0.168).

Exploratory analyses of several subgroups indicated significantly lower values for pomegranate juice versus control after treatment for anterior wall and/or composite CIMT values: subjects in the top tertiles for baseline triglycerides (TGs; anterior, p = 0.007; composite, p = 0.061), total cholesterol/HDL cholesterol ratio (anterior, p <0.001; composite, p = 0.006), TG/HDL cholesterol ratio (anterior, p = 0.005; composite, p = 0.073), and apolipoprotein-B100 (anterior, p = 0.062; composite, p = 0.033) and the lowest tertile for HDL cholesterol (anterior, p = 0.009; composite, p = 0.018). No differences for anterior wall or composite CIMT values were found in smokers or for the top tertiles of low-density lipoprotein cholesterol or PD − AAPH. There were no significant differences between treatments in any of these subgroups at baseline for any CIMT measurements or after treatment in posterior wall CIMT values.

Figure 3 shows progression rates for anterior wall, posterior wall, and composite CIMT measurements by treatment group for the entire intent-to-treat sample and selected subgroups. Results are arranged in ascending order by pro-

gression rate for anterior wall CIMT in the control group. Results suggest that pomegranate juice consumption may have retarded CIMT progression in those subgroups with substantial progression in the control group, while having little or no effect on progression rates in the remainder of subjects. An evaluation of cardiovascular risk factors among subgroups with significant responses shown in Figure 3 suggested a possible pattern for 2 variables, high-sensitivity C-reactive protein and thiobarbituric acid-reactive substances plus AAPH. The level of high-sensitivity C-reactive protein was significantly decreased with pomegranate juice versus control in the top tertiles for TGs ($p = 0.039$), total cholesterol/HDL cholesterol ratio ($p = 0.012$), and apolipoprotein-B100 ($p = 0.014$). Levels of thiobarbituric acid-reactive substances plus AAPH also significantly decreased with pomegranate juice versus control in the top tertiles for TGs ($p = 0.018$), TG/HDL cholesterol ratio ($p = 0.010$), and PD − AAPH ($p = 0.022$).

Discussion

Results of the present study showed no significant influence of ~18 months of pomegranate juice consumption on CIMT progression in the overall study sample. However, results from post hoc exploratory analyses, which should be interpreted with caution, suggest that the rate of CIMT progression may have been slowed in subgroups characterized by more rapid CIMT progression, including those with increased levels of TG-rich lipoproteins, low levels of HDL cholesterol, and greater oxidative stress. Notably, differences were most evident in rate of progression in the anterior wall, which was thicker than the posterior wall at baseline. Because the decrease in CIMT progression in these subgroups was based on analyses that were not preplanned and had no correction for multiple comparisons (increasing the possibility of type I errors), these findings will need to be confirmed in future investigations. However, consumption of pomegranate juice is very safe; thus demonstration of a benefit on atherosclerotic disease progression, even in a subset of the population, would have important public health implications. Greater CIMT is a risk factor for coronary artery disease, stroke, and myocardial infarction and correlates with increased levels of serum oxidized low-density lipoprotein.[21,22]

Results from previous trials have suggested that therapies that are effective for decreasing CIMT progression may not produce evidence of benefit when the baseline CIMT value is low.[23–27] Recent advancements in the use of contrast enhancements have improved the ability to evaluate the anterior wall of the carotid artery.[13,28] In a review of the literature, Macioch et al[13] concluded that the anterior wall is the site of maximum CIMT and is likely to be the location of greatest change after therapeutic intervention. Use of a contrast agent is required to satisfactorily image the anterior CIMT,[13] which increases the cost and invasiveness of the test. However, these trade-offs may prove to be justifiable if the sensitivity for detecting changes is sufficiently increased. Results from the present study are consistent with this possibility.

At the time this study was designed, the greater apparent sensitivity of the anterior wall for demonstrating differences in response was not known. Each subject was given the option of receiving contrast agent due to a concern that an intravenous injection would deter subject participation. Subjects chose whether to undergo contrast agent administration before randomization and those who consented were equally distributed between treatment groups. Furthermore, posterior wall progression rates in either group did not differ between those who did and did not undergo contrast agent injection. Therefore, it is unlikely that inclusion of a contrast agent in a subset of the study sample biased the study findings.

The hypothesized mechanisms through which pomegranate juice consumption might favorably influence atherosclerosis development and progression relate mainly to effects of its antioxidant components (polyphenols, tannins, and anthocyanidins).[1,2] Of the serum indicators of oxidation measured, only PD − AAPH at 12 months decreased to a significantly larger extent with pomegranate juice than with control. Greater changes in oxidative stress markers were expected given the previously demonstrated antioxidant effects of pomegranate juice consumption.[1,2,4,5,7,8] Whether possible benefits of pomegranate juice consumption on CIMT progression in some subgroups relate to antioxidant activity is uncertain. A lack of significant improvements in most markers of oxidative stress argues against an important role for antioxidant activity. However, specific reactive oxygen/nitrogen species may be scavenged by pomegranate-unique polyphenolic hydrolysable tannins. Indeed, a subgroup for whom there was an apparent benefit was the top tertile for baseline PD − AAPH, suggesting that antioxidant effects may have played a role in the protection against CIMT progression by pomegranate juice consumption.

One potential mechanism through which the antioxidant components in pomegranate juice may influence atherosclerosis involves modulation of paraoxonase-1, an HDL-associated esterase that can hydrolyze and decrease lipid peroxides in arterial cells and atherosclerotic lesions.[29] Several in vitro and in vivo studies have demonstrated increased paraoxonase-1 with pomegranate juice consumption,[2,5,29] but in the present study, there were no significant differences in serum concentrations of paraoxonase-1 between the pomegranate and control groups at any time point. This may be due to relatively high baseline levels of paraoxonase-1 (~112 U/ml) suggestive of adequate antioxidant status at baseline compared to previous studies. Aviram et al[5] reported significant increase in paraoxonase-1 levels in subjects with carotid atherosclerosis consuming pomegranate juice. However, baseline paraoxonase-1 was substantially lower in those subjects[5] than those in the present study. Pomegranate juice and/or polyphenol consumption might favorably influence CIMT progression through effects on platelet activity, endothelial function, or shifts in the production of prostacyclin production.[2,30] However, because none of these variables were measured in the present trial, their potential roles here are unknown.

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