

Effects of tomato extract on platelet function: a double-blinded crossover study in healthy humans^{1–3}

Niamh O'Kennedy, Lynn Crosbie, Stuart Whelan, Vanessa Luther, Graham Horgan, John I Broom, David J Webb, and Asim K Duttaroy

ABSTRACT

Background: Aqueous extracts from tomatoes display a range of antiplatelet activities in vitro. We previously showed that the active components also alter ex vivo platelet function in persons with a high response to ADP agonist.

Objective: The objective was to evaluate the suitability of a tomato extract for use as a dietary supplement to prevent platelet activation.

Design: A randomized, double-blinded, placebo-controlled crossover study was conducted in 90 healthy human subjects selected for normal platelet function. Changes from baseline hemostatic function were measured 3 h after consumption of extract-enriched or control supplements.

Results: Significant reductions in ex vivo platelet aggregation induced by ADP and collagen were observed 3 h after supplementation with doses of tomato extract equivalent to 6 (6TE) and 2 (2TE) tomatoes [3 μmol ADP/L: 6TE (high dose), -21.3% ; 2TE (low dose), -12.7% ; $P < 0.001$; 7.5 μmol ADP/L: 6TE, -7.8% , 2TE, -7.6% ; $P < 0.001$; 3 mg collagen/L: 6TE, -17.5% ; 2TE, -14.6% ; $P = 0.007$]. No significant effects were observed for control supplements. A dose response to tomato extract was found at low levels of platelet stimulation. Inhibition of platelet function was greatest in a subgroup with the highest plasma homocysteine ($P < 0.05$) and C-reactive protein concentrations ($P < 0.001$).

Conclusion: As a functional food or dietary supplement, tomato extract may have a role in primary prevention of cardiovascular disease by reducing platelet activation, which could contribute to a reduction in thrombotic events. *Am J Clin Nutr* 2006;84:561–9.

KEY WORDS Tomato, platelet, natural antiplatelet agents, thrombosis, cardiovascular disease

INTRODUCTION

Activation of blood platelets plays a crucial role not only in hemostasis but also in the development of several serious arterial disorders (1–3). Platelet hypersensitivity is thought to be one of the many causal factors for the development and progression of atherosclerosis and an important contributor to the disease mechanism (4–7). Mortality due to coronary artery disease (CAD) can occur as a result of an acute thrombotic event caused by the rupture of an atherosclerotic plaque (2, 8). In support of the pathophysiologic role of platelets, therapy with antiplatelet agents has been shown to significantly decrease the incidence of primary and secondary coronary events related to cardiovascular disease (CVD) in secondary prevention trials (9–13). Studies

also indicate that secondary antiplatelet treatment results in a 15–30% reduction in the incidence of stroke after a transient ischemic event or cerebral infarction (9, 14). In terms of primary prevention of CVD in healthy individuals with low risk status, the situation is more complex. It has been suggested that, in combination with lipid-lowering measures and blood pressure monitoring, the administration of antiplatelet agents to all persons aged >55 y could help to reduce the number of heart attacks and strokes by up to 80% (15, 16). However, whereas there is consensus that prophylactic suppression of platelet activation helps to prevent a prothrombotic state (17), slows the development of atherosclerosis (18, 19), and reduces risk of stroke and myocardial infarction (9, 10), the limited evidence available from primary prevention trials suggests that the side effects of prophylactic drug regimens outweigh their benefits (20–23).

Our previous work established that tomato extracts can influence platelet activity in vitro and ex vivo (24, 25). The tomato's bioactive components inhibit platelet aggregation in response to a range of agonists in vitro and reduce the platelet expression of activation-dependent antigens. In healthy subjects whose platelet response to ADP agonist ex vivo is above average, supplementation with tomato extract results in a reduction of $20.0 \pm 4.9\%$ in platelet aggregation induced by 3 μmol ADP/L after 3 h (25). Following on from this work, we wished to examine the suitability of the tomato extract, administered in the form of a dietary supplement, for use in the primary prevention of CVD. To this end, we conducted a double-blinded crossover study in which changes from baseline hemostasis after consumption of extract- or placebo-supplemented drinks were quantified and compared in a group of healthy subjects.

¹ From Provexis plc, Manchester, United Kingdom (NO, LC, and SW); Rowett Research Services, Aberdeen, United Kingdom (VL); Biomathematics and Statistics Scotland, Aberdeen, United Kingdom (GH); the School of Life Sciences, The Robert Gordon University, Aberdeen, Scotland, United Kingdom/NHS Grampian, Aberdeen, United Kingdom (JIB); the Centre for Cardiovascular Science, Edinburgh, United Kingdom (DJW); and the Department of Nutrition, University of Oslo, Oslo, Norway (AKD).

² Supported by Provexis plc.

³ Address reprint requests to N O'Kennedy, Provexis plc, c/o The Rowett Research Institute, Greenburn Road North, Bucksburn, Aberdeen, AB21 9SB, United Kingdom. E-mail: niamh.okennedy@provexis.com.

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SUBJECTS AND METHODS

Preparation of supplement drinks

A standardized tomato extract was produced as described previously (25). Two treatment supplements were prepared in an orange juice vehicle (orange juice concentrate; Treelinks Ingredients Ltd, Malvern, United Kingdom) to deliver, per 200-mL volume, 18 and 6 g, respectively) of tomato extract syrup (equivalent to the amount of antiplatelet components quantified in 6 and 2 fresh tomatoes, respectively) (25). A placebo supplement drink without tomato extract was also prepared for use as a control treatment. Final concentrations of glucose and fructose in the control and treatment drinks were balanced by the addition of supplementary glucose and fructose as required. Any potentially discernible sensory effects due to the presence of tomato extract in the 2 treatment drinks were masked by the use of flavoring agents (nature-equivalent pineapple and grapefruit flavors; Synergy Flavors Ltd, High Wycombe, United Kingdom) in all 3 drinks. The 3 supplement drinks are referred to throughout as drinks that are equivalent to 6 (6TE) or 2 (2TE) tomatoes and as the control drink.

To allow independent randomization and double blinding, the different supplement drinks were prepared externally by a contract bottling house (Axis Mannerwild PLC, Bedale, United Kingdom). Appropriate amounts of tomato extract syrup were mixed with previously determined quantities of orange juice concentrate, sugar powders, water, and flavoring components. The mixed solutions were then bottled in 200-mL dark glass bottles and pasteurized, and the bottles were coded on-site at the bottling plant in accordance with a randomization protocol from an independent organization [Biomathematics and Statistics Scotland (BioSS), Aberdeen, United Kingdom] and refrigerated until required.

Platelet aggregation studies

Blood collection, platelet-rich plasma preparation, and aggregometry were carried out as described previously (24, 25). The aggregating agents used were ADP and collagen (Helena Biosciences, Sunderland, United Kingdom). Because the nature of the platelet response to ADP is dependent on agonist concentration, data were collected for this agonist under conditions of both optimal and suboptimal platelet stimulation; on the basis of data from screening blood samples, the mean optimal concentration of ADP for the subject group was standardized at 7.5 $\mu\text{mol/L}$, whereas suboptimal ADP was defined as 3 $\mu\text{mol/L}$. The effects on platelet aggregation observed after treatment or control supplementations are expressed as the percentage change from baseline in area under the curve or in lag time (collagen) after consumption of extract or placebo.

Coagulometry

Prothrombin time (PT) and thrombin clotting time (TCT) estimations were performed on a CoaData 4001 coagulometer (Helena Biosciences) as described previously (25).

Supplementary measurements

After each withdrawal of blood, plasma fibrinopeptide A (FPA) concentrations were measured by using a competitive enzyme-linked immunosorbent assay (HYPHEN BioMed,

Neuville-sur-Oise, France) as described previously (25). Baseline plasma C-reactive protein (CRP) concentrations were measured by using a semiquantitative latex agglutination assay (Dade Behring, Milton Keynes, United Kingdom). High-sensitivity CRP was measured by using an enzyme-linked immunosorbent assay (Bender MedSystems, Vienna, Austria). Baseline fasting plasma lipid and plasma glucose concentrations were measured by using a colorimetric autoanalyzer method in specific autoanalyzer analysis kits (KONE Instruments Autoanalyser; Labmedics Ltd, Manchester, United Kingdom). Appropriate controls and standard solutions were supplied by the manufacturer. Baseline fasting plasma homocysteine concentrations were measured by using isotope dilution gas chromatography–mass spectrometry and the method of Calder et al (26).

Subjects

Ninety-three healthy adults of both sexes were recruited into the study. Subjects were aged 45–70 y and had no history of serious disease or hemostatic disorders. Suitability for inclusion onto the study was assessed by using diet and lifestyle questionnaires and by medical screening, during which platelet function was assessed. Screened subjects with normal platelet function, as defined below for the population studied, were recruited onto the study, unless excluded by low hematologic counts (hematocrit below the normal range or platelet count $<170 \times 10^3/\mu\text{L}$). The platelet aggregation response to 3 $\mu\text{mol ADP/L}$ in whole blood was used as an index of normal platelet function. A platelet response corresponding to $>30\%$ aggregation at time $t_1 = 30$ s after the addition of agonist and to $>20\%$ aggregation at time $t_2 = 4$ min after the addition of agonist was defined as normal for the local population. Ninety percent of the subjects screened had an aggregation response above these limits. Subjects whose aggregation response fell below these limits were considered to have an abnormally low platelet response to ADP and were not enrolled in the study. Any subject habitually consuming dietary supplements (eg, fish oils or evening primrose oil) was asked to suspend these supplements for ≥ 1 mo before participating in the study. Subjects were also instructed to abstain from consuming drugs known to affect platelet function for a 10-d period before participation. Of the 93 subjects recruited into the study, 3 were withdrawn from the study due to difficulties with phlebotomy, and 3 completed <3 interventions (because of illness, time pressures, etc).

Written informed consent was obtained from all subjects. The study was approved by Grampian Research Ethics Committee.

Phlebotomy

Subjects recruited into the study were required to give two 35-mL blood samples, 3 h apart, on 3 occasions at 1-wk intervals. For measurements of platelet function, clotting time, and total homocysteine (tHcy), blood was drawn into plastic blood collection tubes (Sarstedt, Leicester, United Kingdom), and coagulation was prevented by mixing 9 volumes of blood with 1 volume of sodium citrate (final concentration, 13 mmol/L). Siliconized 21-gauge needles were used (BD Biosciences, Cowley, United Kingdom). For measurement of CRP and high-sensitivity CRP, plasma lipids, and plasma glucose, a baseline blood sample (5 mL) was drawn into tubes containing EDTA anticoagulant (15% K3E; Sarstedt). For measurement of FPA at each time-point, 5 mL blood was collected into EDTA anticoagulant containing trasyolol and chloromethylketone (HYPHEN BioMed).

Blood samples were incubated at 37 °C in a portable incubator for transfer to the laboratory. As in previous studies, any blood samples that showed evidence of activation, by the presence of FPA at a concentration >6 µg/L, were discarded. Any volunteers with an elevated inflammatory response, as evidenced by a baseline CRP concentration >6 mg/L, were withdrawn from the study temporarily and asked to repeat the appropriate intervention at a later date.

Study design

Trial design, randomization, and supplement coding to ensure double-blinding were independently undertaken by BioSS. A randomized multiple Latin-square design was chosen to take account of potentially high intrasubject variance in baseline platelet function and to allow placebo blinding and measurement of the intrasubject dose response. Experimental variables (eg, peak response time, intersubject variance, or response variance) defined by previous range-finding studies (25) were used in designing the trial. After an initial screening, subjects were asked to attend the Human Nutrition Unit at the Rowett Research Institute (Aberdeen, United Kingdom) on 3 occasions at intervals of ≥1 wk. At each visit, a baseline blood sample was taken, and the subjects received a randomly assigned supplement drink—ie, 6TE, 2TE, or control drink. After a period of ≈3 h, during which subjects remained fasted, a second blood sample was taken and compared with the baseline. Blood pressure was measured before baseline blood sampling at each visit, and volunteers were required to adhere to a (recorded) pattern of early morning activity on each study day. Measurement of the extent of ADP- and collagen-induced platelet aggregation was carried out for each blood sample in platelet-rich plasma by using defined concentrations of agonist (7.5 and 3 µmol ADP/L and 3 mg collagen/L). The different concentrations of ADP were used to approximate different physiologic conditions. Measurements of clotting time were also made at each timepoint. For each subject, the baseline blood sample from the first treatment period was also used to measure some biomarkers of general cardiovascular health. Plasma lipids—total cholesterol, HDL cholesterol, and triacylglycerols—were quantified, as were plasma tHcy, glucose, and high-sensitivity CRP.

Statistical analysis

Data are presented as means ± SEMs. Data from interventions with FPA and CRP values above defined concentrations (6 µg/L and 6 mg/L, respectively) were removed from the set. This resulted in loss of 8% of the data points collected, corresponding to data from 22 interventions, of which 11 were control and 11 were treatment interventions. Preliminary assessment of the data distribution was carried out by inspecting histograms, and data points classified as outliers were removed (2 data points). Data from postintervention timepoints were expressed as differences from baseline values before analysis. Differences between treatment and control groups were analyzed by using a 2-factor ANOVA with the use of treatment (ie, control, 2TE, or 6TE) and sex as factors. Different ADP concentrations were not treated as factors in the analysis; data collected under different conditions were analyzed separately. Interactions between subject group variables were analyzed by linear regression. When >2 means were compared, the Tukey method was used to adjust for multiple comparisons. Values of $P < 0.05$ were considered significant. We used GENSTAT for WINDOWS statistical software

TABLE 1
Subject baseline physical, dietary, and hematologic characteristics¹

	Men (n = 50)	Women (n = 40)
Age (y)	59 (54–64)	56 (49–61)
BMI (kg/m ²)	26.1 (24.7–27.7)	26.7 (24.7–27.9)
Blood pressure (mm Hg)		
Systolic	142 (129–150)	132 (120–140) ²
Diastolic	85 (79–92)	81 (77–87) ²
Dietary variables		
Fruit and vegetables (portions/wk)	31 (20–36)	34 (26–42)
Tomato (g/wk)	395 (170–510)	425 (170–595)
Alcohol (units/wk) ³	12 (4–15)	5 (1–5) ²
WBCs (10 ⁹ /L)	5.6 (4.7–6.5)	5.2 (4.3–6.0)
RBCs (10 ¹² /L)	4.2 (4.1–4.4)	4.1 (3.9–4.3)
Platelets (10 ⁹ /L)	223 (186–249)	226 (207–239)
Hematocrit (%)	38.6 (36.9–40.5)	37.1 (34.9–37.9) ²
MCV (fL)	91.2 (89.2–93.3)	90.5 (88.0–92.5)
MPV (fL)	8.0 (7.6–8.4)	8.5 (7.8–9.1) ²

¹ All values are \bar{x} ; interquartile range in parentheses. $n = 7$ smokers (men) and 3 smokers (women). WBCs, white blood cells; RBCs, red blood cells; MCV, mean cell volume; MPV, mean platelet volume.

² Significantly different from men, $P < 0.01$ (ANOVA).

³ A unit of alcohol = 10 mL pure ethanol (eg, a 125-mL glass of wine containing 8% alcohol or a 25-mL portion of spirits).

(version 8.1; VSN International Ltd, Hemel Hempstead, United Kingdom) for statistical analyses.

RESULTS

Subject baseline profiles

Of the 93 subjects recruited, 3 were withdrawn during the study. The baseline characteristics of the remaining 90 subjects are shown in **Table 1** and **Table 2**; variables that differed significantly between men and women are indicated. All variables measured were within the normal range for the UK adult population (27). Baseline platelet function, as measured by the platelet aggregation response to ADP and collagen at 0 h, was stable

TABLE 2
Concentrations of plasma lipids, glucose, total homocysteine (tHcy), and high-sensitivity C-reactive protein (hsCRP) at baseline¹

	Men (n = 50)	Women (n = 40)
Plasma lipids (mmol/L)		
Total cholesterol	5.17 (4.69–5.66)	5.22 (4.81–5.68)
HDL cholesterol	1.12 (0.90–1.37)	1.35 (1.12–1.53) ²
LDL cholesterol	4.05 (3.52–4.54)	3.87 (3.41–4.37)
Total:HDL	5.0 (3.9–5.8)	4.1 (3.0–4.8) ³
Triacylglycerols	1.55 (0.74–1.64)	1.12 (0.74–1.39) ³
Other plasma constituents		
Glucose (mmol/L)	5.48 (5.01–5.91)	5.23 (4.94–5.46) ⁴
tHcy (nmol/g)	11.30 (8.34–14.95)	9.09 (7.88–10.22) ³
hsCRP (mg/L)	2.42 (1.23–3.76)	2.51 (1.51–3.88)

¹ All values are \bar{x} ; interquartile range in parentheses. $n = 7$ smokers (men) and 3 smokers (women).

^{2–4} Significantly different from men (ANOVA): ² $P < 0.001$, ³ $P < 0.01$, ⁴ $P < 0.05$.

TABLE 3Baseline (0 h) measurements of the hemostatic system in all subjects during treatment periods 1–3¹

	Treatment period		
	1	2	3
Aggregometry			
Mean area under the aggregation curve (AU)			
7.5 μ mol ADP/L	40 611 \pm 1392 (85)	39 205 \pm 1416 (86)	39 410 \pm 1455 (81)
3 μ mol ADP/L	18 483 \pm 1607 (85)	18 542 \pm 1607 (87)	18 112 \pm 1656 (80)
Mean lag time of the aggregation curve (s)			
3 mg Collagen/L	62 \pm 2 (80)	62 \pm 3 (83)	61 \pm 2 (73)
Clotting time measurements			
PT (s)	13.7 \pm 0.1 (88)	13.7 \pm 0.1 (88)	13.6 \pm 0.1 (85)
TCT (s)	20.5 \pm 0.3 (90)	20.6 \pm 0.3 (88)	19.8 \pm 0.2 (86)

¹ All values are $\bar{x} \pm$ SEM; *n* in parentheses. PT, prothrombin time; TCT, thrombin clotting time. Treatment periods refer to the different (randomized) crossover interventions undertaken by each subject. No significant differences were observed between treatment periods, $P > 0.05$ (ANOVA), which indicated that intersubject baseline variations over the study period were not significant.

across treatment periods for individual subjects. The mean within-subject CVs were calculated as 12.1%, 29.9%, and 9.3% for baseline measurements made at treatment periods 1, 2, and 3 (ie, baselines before the first, second, and third interventions, which took place on different dates) using 7.5 μ mol ADP/L, 3 μ mol ADP/L, and 3 mg collagen/L, respectively. Comparison of the mean baseline aggregation and clotting times for each treatment period showed no significant differences between treatment periods (**Table 3**). The large variances in the mean baseline values reflect the range of the baseline data sets collected, a consequence of the heterogeneous subject group.

Effect of the control and 2 or 6 tomato-equivalent supplement drinks on markers of hemostasis

Supplementation with 2TE and 6TE supplements resulted in a significant decrease in the platelet aggregation response at 3 h, whereas the control supplement resulted in no change. Platelet aggregation responses to both ADP and collagen were significantly lower than baseline values (**Figure 1** and **Figure 2**). The mean changes in ADP-induced platelet aggregation observed for the control, 2TE, and 6TE supplements (sexes combined) were 2.6%, -7.6%, and -7.8%, respectively, at 7.5 μ mol ADP/L (optimal concentration; $P < 0.001$) and -2.6%, -12.7%, and -21.3% at 3 μ mol ADP/L (suboptimal concentration; $P < 0.001$). At 7.5 μ mol ADP agonist/L concentration, the men showed significantly greater sensitivity to supplementation with treatments 2TE and 6TE than did the women ($P = 0.044$; **Figure 1**). The difference between sexes was not significant when 3 μ mol ADP/L was used. At both ADP agonist concentrations, the effect of the 2TE and 6TE treatment supplements differed significantly from that of the control drink, but the effects of 2TE differed significantly from 6TE only at 3 μ mol ADP/L (**Figure 1**). The different treatment effects (dose-response) observed for ADP-induced aggregation in one male subject are shown by using suboptimal aggregation curves in **Figure 3**.

The mean lag time of the platelet response to collagen increased after supplementation, which corresponded to changes in collagen-induced aggregation of -7.4%, -14.6%, and -17.5% ($P = 0.003$) with the control, 2TE, and 6TE supplements, respectively (sexes combined). Inhibition of collagen-induced platelet aggregation by the treatment supplements also showed an influence of sex ($P = 0.006$, **Figure 2**). After correction for

multiple comparisons, a significant difference between the effects of control and 2TE supplementation was observed in the women but not in the men. Conversely, the men showed a significant difference between the effects of 6TE and control supplementation, whereas the women did not (**Figure 2**). No significant difference between the effects of 2TE and 6TE supplementation was observed in collagen-induced aggregation in either sex.

Changes in clotting time variables at 3 h did not differ significantly between subjects who ingested the 6TE or 2TE supplement drinks and those who received the control drink. No significant differences were observed in PT or TCT values over time, and no significant difference was detected between treatment groups. Clotting time measurements for each treatment group are summarized in **Table 4**.

Responders and nonresponders

A wide range of subject responses to the 2TE and 6TE supplement drinks was observed, and histograms of the percentage change in platelet aggregation showed evidence of a bimodal distribution. Distinct groups representing responders and nonresponders to the 6TE and 2TE supplements appeared to be present. To examine the characteristics of responder and nonresponder groups, the 2 categories were defined as follows. Subjects were defined as responders at a particular agonist concentration if inhibition of platelet aggregation observed after 6TE or 2TE treatments exceeded that observed after the control drink by an amount greater than the least significant difference for the appropriate treatment effect. Under this classification, 50% (6TE group) and 45% (2TE group) of the subjects were responders, in terms of observed inhibition of 7.5 μ mol ADP/L-induced aggregation. When 3 μ mol ADP agonist/L was used in the ex vivo measurements, 61% (6TE group) and 51% (2TE group) of the subjects were responders, and when collagen was used as the aggregating agent, the corresponding proportions were 52% (6TE group) and 45% (2TE group). Because of the within-subject variance in baseline platelet aggregation, subjects did not always fall into the same category for each treatment or agonist. A smaller proportion of subjects responded to both 6TE and 2TE, and another subset had no response to either treatment; these subgroups represented the most and least sensitive responders to treatment, respectively, and the mean inhibition of aggregation



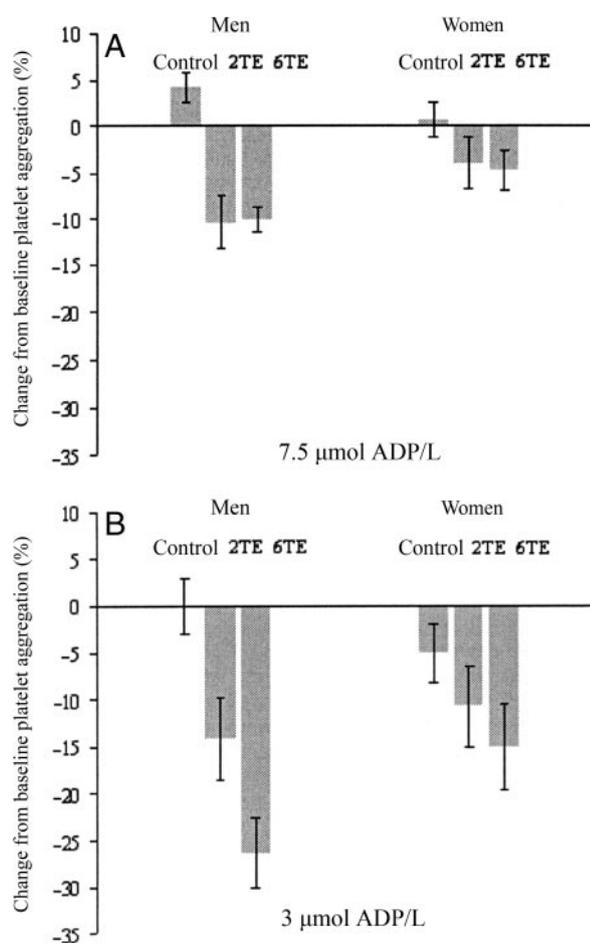


FIGURE 1. Mean (\pm SEM) reduction from baseline platelet aggregation response, as induced by 7.5 μ mol ADP/L and 3 μ mol ADP/L after ingestion of treatment supplement drinks with 2 or 6 tomato equivalents (2TE and 6TE, respectively) or of the control drink. The percentage change was calculated from the area under the platelet aggregation curves at baseline and 3 h. A: Control drink: $n = 39$ M, 37 F; 2TE drink: $n = 47$ M, 36 F; 6TE drink: $n = 46$ M, 37 F. At 7.5 μ mol ADP/L, a significant treatment \times sex interaction was observed ($P = 0.044$, ANOVA). Within sexes, the treatment and control supplement groups differed significantly ($P < 0.001$), but the 6TE and 2TE groups did not differ significantly (ANOVA followed by Tukey's post hoc test). B: Control drink: $n = 37$ M, 34 F; 2TE drink: $n = 48$ M, 35 F; 6TE drink: $n = 43$ M, 36 F. At 3 μ mol ADP/L, the treatment \times sex interaction was not significant ($P > 0.05$, ANOVA). With sexes combined, treatment supplements were significantly different from placebo and from each other ($P < 0.001$, ANOVA followed by Tukey's post hoc test).

observed in these subgroups for each agonist used is shown in **Table 5**. Data for the remaining subjects, defined as an intermediate group, are also shown. ANOVA followed by Tukey's method to correct for multiple comparisons was used to identify significant differences between this intermediate group and the defined high responders and nonresponders ($P < 0.001$; Table 5). A responder status \times sex interaction was observed for only one variable, the percentage change in 6TE at 7.5 μ mol ADP/L ($P = 0.023$), and so, for simplicity, the data shown in Table 5 are not broken down by sex.

Relations between subject baseline characteristics and treatment effects

Regression plots showed that factors other than sex were associated with a strong response to the 2TE and 6TE supplements.

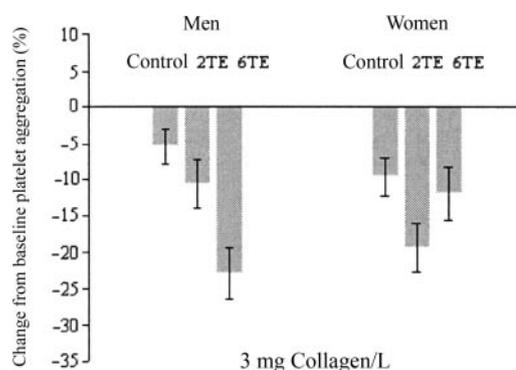


FIGURE 2. Mean (\pm SEM) reduction from baseline platelet aggregation response, as induced by 3 mg collagen/L, after ingestion of treatment supplement drinks with 2 or 6 tomato equivalents (2TE and 6TE, respectively) or of the control drink. Control drink: $n = 37$ M, 34 F; 2TE drink: $n = 43$ M, 35 F; 6TE drink: $n = 43$ M, 34 F. The percentage change was calculated from the lag times of the aggregation curves at baseline and 3 h. A significant treatment \times sex interaction was observed ($P = 0.006$, ANOVA). The 6TE supplement differed significantly from the control drink in men, whereas the 2TE supplement differed significantly from the control drink in women ($P = 0.003$ for both). No significant difference between 2TE and 6TE was detected within sexes after adjustment for multiple comparisons (ANOVA followed by Tukey's post hoc test).

ANOVA (using an unbalanced treatment structure and followed by Tukey's post hoc test) was used to test for possible interactions of subject characteristics (see Tables 1 and 2) with treatment effects in the defined responder status groups described in Table 5. Significant interactions of responder status with plasma tHcy (7.5 μ mol ADP/L: $P = 0.020$; 3 μ mol ADP/L: $P = 0.028$; 3 mg collagen/L: $P = 0.047$) and plasma high-sensitivity CRP (7.5 and 3 μ mol ADP/L: $P < 0.001$ for both; 3 μ mol ADP/L: $P = 0.002$) were observed. The significant differences identified between responder groups are shown for each variable in Table 5. At 3 μ mol ADP/L, the men in the high-responder group had significantly higher plasma tHcy concentrations than did the women, but otherwise the interactions of responder status with plasma variables were sex independent (Table 5). Plasma lipid concentrations, although significantly sex sensitive, did not show significant interaction with treatment effects, because the variance of the dataset was high. No relations between the treatment effects and BMI, blood pressure, or dietary variables—fruit or vegetable intake or alcohol consumption—were observed. Hematologic variables—eg, hematocrit or mean platelet volume—did not differ significantly between responders and nonresponders.

DISCUSSION

In our previous study (25), we showed that, in specifically selected subjects with high platelet responsiveness to ADP, the consumption of tomato extract components led to a reduction of 3% to 20% (at optimal and suboptimal ADP agonist concentrations, respectively) from baseline platelet aggregation response. The primary aim of the current study was to determine whether a similar supplementation would yield similarly significant ex vivo antiplatelet effects in a study group that was more representative of the general population—ie, that had a broader, less-defined range of platelet function. Results were intended to allow evaluation of the potential of the tomato extract as a dietary

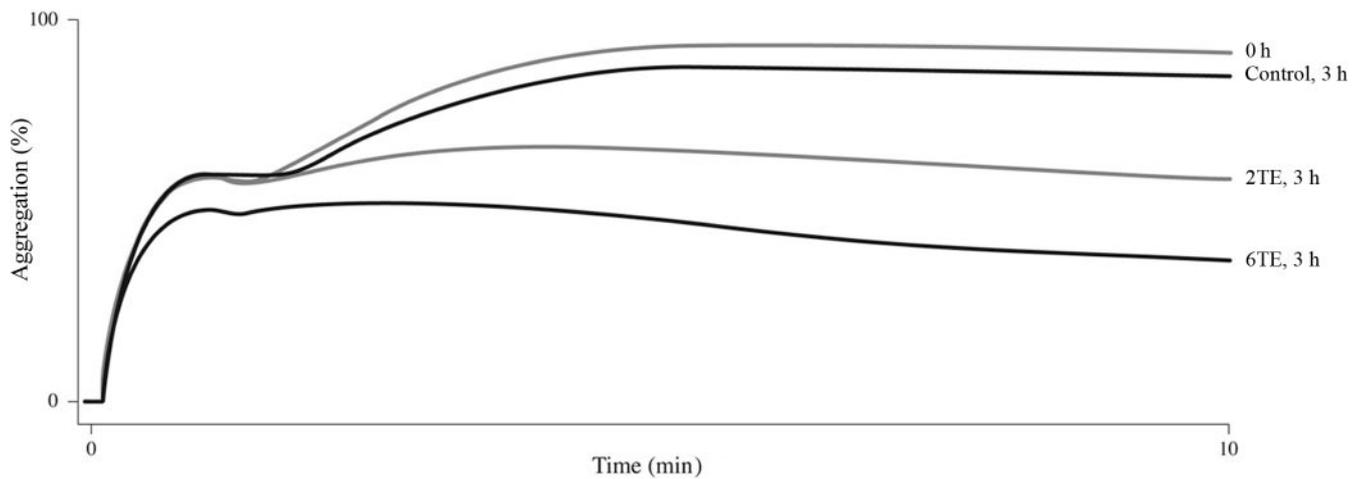


FIGURE 3. Dose-dependent inhibition of ADP-induced platelet aggregation observed in one subject 3 h after consumption of the control drink and treatment supplements containing 2 or 6 tomato equivalents (2TE and 6TE, respectively). Compared with baseline platelet function at 0 h, the aggregation response to 3 μmol ADP/L is significantly lower, as indicated by a reduction in area under the curve, after consumption of 2TE and 6TE supplements ($P < 0.001$, ANOVA). The percentage change in platelet aggregation from baseline at this agonist concentration was -4.2% , -33.7% , and -47.8% with the control, 2TE, and 6TE treatments, respectively.

supplement or functional food, which could help to maintain circulatory health through its effects on hemostasis.

The major outcome of this study was the observation, 3 h after supplementation with tomato extract, of a significant reduction from baseline in the extent of ex vivo platelet aggregation in healthy subjects aged 45–70 y. A similar effect was not observed after the administration of control supplements under identical conditions. Inhibition of aggregation was observed for both ADP- and collagen-mediated aggregation in a dose-dependent manner. The effects of the 6TE supplement on ADP-induced aggregation were comparable to those of an equivalent supplement used during our previous study (25). In terms of platelet function, the subject group was heterogeneous and displayed a wide range of baseline responses to both platelet agonists used. Male subjects showed greater sensitivity to the extract, as evidenced by significantly larger reductions in platelet aggregation in response to ADP or collagen, than did the female subjects. No adverse side-effects of the supplementation were reported, and no effects on clotting time variables were detected after supplementation. These results were in agreement with the outcome of our previous time-course study (25), which showed that the adequacy of the coagulation cascade was maintained after supplementation.

TABLE 4

Percentage change ($\Delta\%$) from baseline clotting times 3 h after supplementation with the control drink or drinks equivalent to 2 (2TE) or 6 (6TE) tomatoes¹

	Supplement drink		
	Control	2TE	6TE
	<i>% change</i>		
$\Delta\%$ from baseline			
PT at 3 h	0.8 ± 0.7 (83)	3.5 ± 2.4 (88)	0.1 ± 0.7 (88)
TCT at 3 h	1.8 ± 1.2 (84)	0.2 ± 1.1 (87)	1.2 ± 1.3 (90)

¹ All values are $\bar{x} \pm \text{SEM}$; n in parentheses. PT, prothrombin time; TCT, thrombin clotting time. No significant differences between treatments were observed, $P > 0.05$ (ANOVA). No treatment \times sex interaction was observed.

The tomato extract is known to contain a wide variety of different types of compounds that have antiplatelet activity in vitro and that affect different mechanisms of activation and aggregation (24, 25, 28, 29). We have now shown that 2 of the observed in vitro antiplatelet mechanisms are operational in vivo. In addition, we have observed a correlation between high responders to tomato extract supplements and plasma concentrations of 2 established risk factors for CVD, tHcy, and CRP (30, 31). Both tHcy and CRP have been reported to affect platelet function (32, 33), and tHcy is reported to contribute to systemic platelet activation directly by disruption of platelet redox status (34, 35) and interference with kinase C cycle activity (36), as well as indirectly by up-regulation of tissue factor expression in endothelium and monocytes (37). CRP has been shown to induce cytokine imbalance (38), which affects many aspects of platelet function. We suggest that the observed relation between ex vivo platelet response to tomato extract supplementation and plasma tHcy and CRP concentrations may imply that some tomato components prevent or reduce the interactions between platelets and these plasma components.

The likely clinical benefits of reducing platelet activity in a healthy population by means of a functional food are currently nonquantifiable because of a lack of suitable published data. This fact reflects the difficulties in defining both platelet hyperreactivity and a target acceptable level of platelet function. However, it is acknowledged that populations whose diet results in a suppression of platelet activation (eg, a high fish diet or a Mediterranean diet) obtain measurable health benefits in terms of reduction of CVD risk (39). The 2TE supplement represents a tomato extract concentration and format appropriate for use as a dietary supplement or functional food. In the most highly responsive persons, who have the highest concentrations of some markers of CVD risk, the 2TE supplement can reduce ADP-induced platelet aggregation by 18.5–24.4% (optimal and suboptimal agonist concentrations), simultaneously lowering collagen-induced aggregation by 24.4%. These highly responsive persons composed 30–35% of the cohort in the current study. In the remaining 65–70% of subjects, the reduction in platelet function that can be

TABLE 5

Comparison of plasma homocysteine (tHcy) and high-sensitivity C-reactive protein (hsCRP) concentrations in groups with different responses to supplementation with tomato extract¹

Agonist and response status ²	$\Delta\%$ 2TE	$\Delta\%$ 6TE	tHcy	hsCRP
	% change from baseline	% change from baseline	nmol/g	mg/L
7.5 $\mu\text{mol ADP/L}^3$				
High	-18.5 ± 3.4^a	-19.3 ± 3.8^a	$10.82 \pm 0.65^{a,b}$	$3.568 \pm 0.126^{4,a}$
Intermediate	-7.3 ± 3.5^b	-9.2 ± 3.2^b	11.30 ± 0.69^b	2.726 ± 0.309^b
None	0.6 ± 1.3^b	1.3 ± 1.3^c	9.17 ± 0.37^a	1.320 ± 0.086^c
3 $\mu\text{mol ADP/L}^5$				
High	-23.8 ± 3.8^a	-33.6 ± 3.5^a	$11.20 \pm 0.62^{6,a}$	3.323 ± 0.201^a
Intermediate	-2.4 ± 8.7^b	-24.1 ± 6.0^a	$10.16 \pm 0.55^{a,b}$	2.157 ± 0.234^b
None	-1.1 ± 4.3^b	-1.4 ± 4.2^b	9.15 ± 0.40^b	1.536 ± 0.157^b
3 mg Collagen/L ⁷				
High	-24.4 ± 4.2^a	-33.1 ± 5.8^a	11.44 ± 0.61^a	3.151 ± 0.228^a
Intermediate	$-12.5 \pm 5.1^{a,b}$	-17.1 ± 4.6^b	$9.95 \pm 0.48^{a,b}$	2.000 ± 0.226^b
None	-8.8 ± 3.2^b	5.6 ± 2.8^b	9.54 ± 0.57^b	2.210 ± 0.237^b

¹ All values are $\bar{x} \pm \text{SEM}$. 2TE and 6TE, supplements with tomato extract equivalent to 2 and 6 tomatoes, respectively. Values in the same column with different superscript letters are significantly different, $P < 0.05$ (ANOVA followed by Tukey's post hoc test).

² High response status was defined as a reduction in platelet function greater than the least significant difference (LSD) for the treatment effect, in response to both 2TE and 6TE. Nonresponse status was defined as an absence of platelet inhibition for both TE doses. Intermediate response status was defined as a response $>$ LSD for 2TE or 6TE but not for both.

³ 7.5 $\mu\text{mol ADP/L}$: high, $n = 30$; intermediate, $n = 22$; none, $n = 34$.

⁴ A significant sex effect was observed in the hsCRP data at 7.5 $\mu\text{mol ADP/L}$ ($P = 0.005$), but the response status \times sex interaction was not significant.

⁵ 3 $\mu\text{mol ADP/L}$: high, $n = 37$; intermediate, $n = 22$; none, $n = 27$.

⁶ A significant response status \times sex interaction was observed in the tHcy data at 3 $\mu\text{mol ADP/L}$ ($P = 0.011$).

⁷ 3 mg Collagen/L: high, $n = 29$; intermediate, $n = 26$; none, $n = 31$.

achieved with this supplementation, although lower (up to 12.7% and 17% reduction of ADP- and collagen-induced aggregation, respectively), still represents a significant reduction, which seems appropriate for the general healthy population at low risk of CVD-related events. The change in platelet function observed is an acute effect, and it does not persist for >18 h (25).

Studies of the use of aspirin in primary prevention are available for comparison (40–48). Aspirin at a dose of 162 mg [the minimum recommended dose for persons with suspected myocardial infarction (40, 41)] can inactivate up to 95% of platelet cyclooxygenase, which results in the blockade of arachidonate-production and thus the inhibition of arachidonate-induced platelet aggregation (42). The precise degree of the acute inhibition achieved is highly variable (values reported from 0–100%), because other aggregation pathways are not affected. Arachidonate is a weak platelet agonist that serves as an amplifier of platelet activation induced in vivo by other agonists, typically collagen. It is now recognized that 20–30% of persons experience the so-called aspirin-resistance syndrome, in which the expected antiplatelet effects are not observed (42, 43). This is partially due to regeneration of aspirin-inactivated cyclooxygenase or induction of a second cyclooxygenase isoform by nucleated cells (44) and partially due to the greater contribution of other platelet aggregation mechanisms, which are not strongly mediated by arachidonate, in these persons (45). In the current study, responders and nonresponders to tomato extract supplementation were observed, which suggests parallels with aspirin resistance. However, subjects classified as nonresponders when ADP agonist was employed as platelet aggregation agent were not always nonresponders when collagen was employed. In fact, only 3 subjects were nonresponders to tomato extract under both ADP- and collagen-stimulated conditions. Thus, 97% of subjects experienced a significant inhibition of one platelet aggregation

pathway after consumption of tomato extract. This finding indicates an advantage of the tomato extract's broad antiplatelet activity profile over single-target drugs such as aspirin. The greater benefits of combined antiplatelet therapies that target more than one mode of platelet aggregation, as compared with single-drug therapeutic strategies, have been shown in clinical trials (46, 47). This finding is of particular interest because in vitro data suggest that some tomato extract components also affect arachidonate- and thrombin-mediated pathways of platelet aggregation, although this possibility was not directly examined in the current study (25). Further ex vivo studies are required to explore the full potential of the tomato-derived antiplatelet supplements.

A daily dose of 162 mg aspirin is associated with increased risk of gastric bleeding and hemorrhagic stroke (14, 21) and therefore is not recommended for prophylaxis (22, 48). Studies using lower doses for primary prevention have not shown efficacy in reducing subsequent CVD events (20, 22), which implies that partial inactivation of the platelet cyclooxygenase enzyme does not affect the overall process of platelet aggregation sufficiently to confer real benefits. Consensus exists that a safer, more efficacious alternative to aspirin as a prophylactic regimen could be significant in improving public health. According to the results in our studies, a dietary supplement or functional food based on tomato extract is a candidate for such a prophylactic regimen.

In summary, the current study showed that consumption of antiplatelet components derived from the tomato, in a supplement drink format suitable for use as a dietary supplement or functional food, led to a significant reduction in ex vivo platelet aggregation after 3 h. The observed acute effects were more wide-ranging than those of aspirin, the only drug widely studied as a potential prophylactic, in that more than one pathway of platelet aggregation is targeted. Persons with high concentrations



of some known markers of CVD showed greater sensitivity to supplementation, and overall the range of responses measured seemed appropriate for a primary prevention regime. We conclude that consumption of such extracts as a food supplement could benefit public health by helping to maintain platelets in an inactivated state and reducing the risk of thrombotic events mediated by platelet activation. 

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