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RESEARCH ARTICLE

Protective activity of processed tomato products on postprandial oxidation and inflammation: A clinical trial in healthy weight men and women

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Scope: This study was designed to evaluate the ability of tomato rich in lycopene to modify postprandial oxidative stress, inflammation, and endothelial function in healthy weight individuals.

Methods and results: Twelve women and 13 men (mean age = 27 ± 8 years; mean body mass index = 22 ± 2) consumed high-fat meals known to induce postprandial oxidative stress on two separate occasions containing either processed tomato product or non-tomato alternative. Blood samples were collected at 0, 30, 60, 90, 120 min, then hourly until 360 min. Flow-mediated dilation (FMD) was performed at 0 and 210 min. Endpoints included changes in glucose, insulin, lipids, oxidized low-density lipoprotein (OxLDL), inflammatory cytokines, and FMD. Both meals induced increases in plasma glucose, insulin, and lipid concentrations ($p < 0.05$). A trend for higher triglycerides at >240 min was observed after the tomato meal ($p = 0.006$). Tomato significantly attenuated high-fat meal-induced LDL oxidation ($p < 0.05$) and rise in interleukin-6 ($p < 0.0001$), a proinflammatory cytokine and inflammation marker.

Conclusion: The data indicate that consuming tomato products with a meal attenuates postprandial lipemia-induced oxidative stress and associated inflammatory response. The relevance of OxLDL and inflammation to vascular injury suggests a potentially important protective role of tomato in reducing cardiovascular disease risk. ClinicalTrials.gov Registration number – NCT00966550.

Keywords:
Inflammation / Lipemia / Lycopene / Oxidized LDL / Postprandial

1 Introduction

Consumption of a high-fat meal results in a postprandial (fed-state) response characterized by hypertriglyceridemia [1, 2]. Postprandial hypertriglyceridemia increases the oxidation of low-density lipoproteins (LDLs) [3, 4] and increases blood concentrations of several biomarkers of inflammation [5, 6]. There is also concurrent impairment of endothelium-dependent relaxation (EDR) indicative of endothelial dysfunction [7, 8]. This postprandial lipemia-induced oxidative stress-mediated response to a high-fat meal has been suggested as a major contributor to the pathogenesis of atherosclerosis along with other chronic disease states of diabetes and obesity [2, 9].

Consumption of foods rich in antioxidant compounds provides a defense source to compliment endogenous defense systems to protect against oxidative damage during pro-oxidant conditions. An important role for dietary antioxidants is to limit and potentially prevent postprandial oxidative stress. Lycopene is a pigment compound in tomatoes known for its antioxidant properties and is considered the most potent antioxidant among the carotenoids efficiently scavenging unstable reactive oxygen species [10, 11]. Lycopene is the most abundant carotenoid in tomatoes and while mixtures of carotenoids are known to be more effective than a single

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Abbreviations: **EDR**, endothelium-dependant relaxation; **FMD**, flow-mediated dilation; **hsCRP**, high-sensitivity C-reactive protein; **IL-6**, interleukin 6; **LSM**, least squares mean; **OxLDL**, oxidized low-density lipoproteins; **PBMC**, peripheral blood mononuclear cells; **TG**, triglycerides; **TNF-α**, tumor necrosis factor alpha

compound, the synergistic effect of overall carotenoid antioxidant activity is more pronounced when lycopene is present [11]. Epidemiological studies describe an inverse relationship between a diet high in lycopene, most notably rich in tomatoes and processed tomato products, and the incidence of cardiovascular disease (CVD) [12, 13]. Lycopene is touted as a major contributor to the cardio-protective effects seen with increased tomato intake [14].

Daily lycopene supplementation or intake of lycopene-containing foods over a period of 1–8 weeks has been shown to reduce biomarkers indicative of oxidative stress and inflammation in healthy individuals [14–18]. Denniss et al. [19] studied the effects of daily lycopene supplementation for 1 week followed by a single high-fat meal challenge; however, failed to show a protective effect of daily lycopene supplementation on biomarkers of oxidative- or inflammatory stress during the 3-h postprandial state. We have recently shown that dietary antioxidants from strawberry effectively protect LDL from oxidation during postprandial lipemia when consumed concomitantly with a high-fat meal [4]. These data suggest that acute protection during the postprandial state may require concurrent consumption of dietary antioxidants with meals. Further, Sesso et al. [20] reported that a decreased risk for developing CVD was more strongly associated with higher tomato intake than lycopene intake, suggesting that the whole food (tomato) as compared to components (lycopene) may be more effective in mitigating disease risk. With this background, we sought to examine the effects of consuming tomato in the form of processed tomato paste incorporated in a single high-fat meal on postprandial oxidative- and inflammatory-stress markers as well as endothelial function in healthy weight men and women.

We hypothesized that tomato paste, a source of concentrated tomato constituents with potent antioxidant activity, would attenuate high-fat meal-induced oxidative stress and concomitant inflammatory and impaired endothelial-dependent relaxation response.

2 Methods and materials

This study was conducted according to good clinical practice guidelines and approved by the Illinois Institute of Technology (IIT) Institutional Review Board. All subjects reviewed and signed an Informed Consent Form approved by the Institutional Review Board prior to screening.

2.1 Subjects

Twenty-nine subjects ($n = 15$ females, $n = 14$ males) from the greater Chicago, IL area were recruited through online advertisements and from the university community to participate in the study. Eligible subjects were required to be nonsmoker and in generally good health with a body mass index (BMI) between 19 and 24 kg/m^2 and high-sensitivity C-reactive protein (hsCRP) <1.0 mg/L. Individuals with clinical evidence and/or history of cardiovascular, respiratory, renal, gastrointestinal, metabolic, or hepatic disease/conditions, who use prescription and/or over the counter medications that may interfere with study endpoints (i.e. antioxidant supplements, anti-inflammatory drugs, lipid-lowering medications), have unusual dietary habits (e.g., pica), are actively losing weight or addicted to drugs and/or alcohol were not eligible for participation. Four subjects dropped out of the study: two due to health issues, one with taste intolerance to the test meals, and one due to catheter placement difficulties. A total of 25 subjects completed the study.

2.2 Study design and treatments

This study was a single-center, randomized, cross-over, two-arm, two-sequence, placebo-controlled, 360-min postprandial trial conducted at the Clinical Nutrition Research Center (CNRC) at the IIT Research Tower, Chicago, IL.

Eligible participants were required to limit tomato product consumption throughout the entire study period starting 1 week prior to the first study visit, while maintaining all other aspects of diet and physical activity constant. Subjects were randomized to receive one of two treatment test meals: tomato-containing meal (Tomato) or non-tomato-containing meal (Control) in a randomly selected sequence (Fig. 1). Each subject served as his/her own control.

Both test meals were prepared as a high-fat meal with or without tomato products (Tables 1 and 2). Both meals provided approximately 850 kcal with 46% of energy from fat, and were matched as closely as possible in terms of sensory qualities, macronutrient composition, and key micronutrients (i.e. sodium, vitamin C, and potassium). Lycopene and other tomato-associated antioxidants (such as flavonoids, carotenoids) were not matched. Test meals were prepared in the metabolic kitchen at the CNRC where strict food safety standards are maintained.

2.3 Procedures

Subjects arrived at the CNRC fasted on two separate occasions not less than 3 days apart and not more than 10 days apart. All study procedures were identical on both postprandial test day visits with the exception of the test meal consumed (Tomato or Control). While fasting and before baseline blood collections, subjects participated in a flow-mediated vascular reactivity assessment (Flow-mediated dilation, FMD) using ultrasound imaging technology conducted by a certified sonographer. After the initial FMD, the postprandial testing protocol was initiated with a fasting blood sample followed by consumption of the test meal. Blood samples were collected postmeal at defined intervals for 360 min as described below. A second FMD was performed starting at 210 min

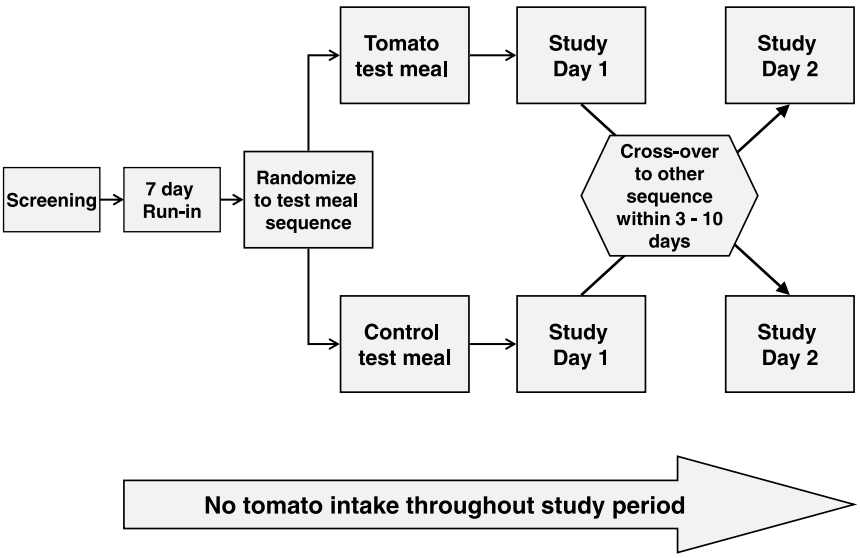

Figure 1. Study schema denoting study flow in healthy weight men and women.

corresponding with expected peak lipemia and between blood draws for logistical purposes. Three-day food records (one set per week) were recorded and reviewed by a dietitian with subjects for compliance to limited tomato intake throughout the study period.

2.4 Postprandial testing protocol

The postprandial test was conducted according to standardized protocols [21]. A Registered Nurse (RN) placed an indwelling catheter in the antecubital vein of the nondominant arm of subjects before test meal consumption and collected blood at baseline (Time 0), then at 30, 60, 90, 120 min, and hourly thereafter to 360 min. Blood was processed for subsequent laboratory analysis including: plasma lipids, glucose, insulin, oxidized LDL (OxLDL), hsCRP, interleukin 6 (IL-6), and tumor necrosis factor-alpha (TNF-α).

2.5 Flow-mediated vascular reactivity

FMD was studied in the brachial artery of all subjects using standardized methods recommended by the American College of Cardiology [22] with minor modifications. Briefly, in a fasted state, the brachial artery was imaged by ultrasound (GE LOGIQ e, UK) and baseline measurements of vessel diameter (mm) collected. A blood pressure (BP) cuff was then applied below the elbow for cuff inflation to a pressure of 50 mmHg above systolic pressure for 5 min. Immediately after cuff deflation, brachial artery vessel diameter was monitored and measured to obtain peak vessel relaxation. Ultrasound measurements were recorded as six individual measurements of vessel diameter. All measurements took place in the morning after an overnight fast and in a dimmed-light, temperature controlled room. Subjects refrained from intense physical activity and consuming caffeinated drinks, tea, coffee, and wine for 24 h prior to their laboratory visit.

2.6 Analytical methods

Various analytical methods were used to assess the effect of tomato product consumption on fasting and postprandial concentrations of plasma lipids, glucose, insulin, OxLDL, hsCRP, IL-6, and TNF-α. Lipids, glucose, and hsCRP were measured using standardized enzyme-based assay kits (Randox, Antrim, UK) on the Randox Daytona Auto Clinical Analyzer (Oceanside, CA). Insulin was measured using the AlphaLISA method (Perkin Elmer, Waltham, MA). Measurement of OxLDL was determined by ELISA assay kits (Mercodia Inc., Winston Salem, NC), and measurements of IL-6 and TNF-α were measured by highly sensitive ELISA assays (R&D Systems, Minneapolis, MN). All assay protocols set forth by the manufacturers were followed with appropriate quality controls as applicable.

2.7 Statistical analysis

Data were analyzed by repeated measures analysis of variance (RM-ANOVA) using PC-SAS (version 9.1.3; SAS Institute Inc, Cary, NC) GLM and MIXED procedure with treatment, time, and sex as main factors, and subject as the blocking variable. Because sex did not significantly impact results, it was dropped from the model. Pearson correlation coefficients were determined using CORR procedure. Absolute and normalized (to subjects' own baseline) values were analyzed; the latter to account for individual baseline variability. Normalizing procedures were used only when variability in fasting values between treatments was significantly different ($p < 0.05$). Clinical laboratory endpoints not

Table 1. Macronutrient composition of the tomato and control test meals[a)]

Item name[b)]	Calories (kcal)	Pro (g)	Carb (g)	Fat (g)	SatFat (g)
Tomato meal					
Plain bagel, enriched[d)]	357.5	13.7	69.4	2.1	0.3
Cream cheese with chives[e)]	91.4	1.5	1.5	9.1	5.3
Tomato Paste[f)]	77.1	4.1	17.8	0.4	0.1
Whole milk[g)]	17.4	0.9	1.3	0.9	0.5
Rice milk[h)]	29.2	0.3	5.3	0.7	0
Coconut milk[i)]	45.3	0.5	0.7	4.9	4.4
Corn oil[g)]	217.7	0	0	25.4	3.6
Apple juice[g)]	12.2	0	3.0	0	0
Total	847.8	21.0	99.0	43.5	14.2
Control meal					
Plain bagel, enriched[d)]	283.3	10.8	55.0	1.7	0.2
Cream cheese with chives[e)]	80.0	1.3	1.3	8.0	4.7
Potato, baked, mashed[j)]	76.0	1.6	17.7	0.1	0
Fiber supplement[k)]	17.6	0	4.4	0	0
Instant nonfat dry milk[c)]	28.7	3.0	4.1	0	0
Whole milk[g)]	57.0	3.1	4.3	3.1	1.8
Rice milk[h)]	12.0	0.1	2.2	0.3	0
Coconut milk[i)]	37.4	0.4	0.5	4.1	3.6
Corn oil[g)]	222.9	0	0	26.0	3.7
Table salt[l)]	0	0	0	0	0
Salt substitute[m)]	0.1	0	0	0	0
Apple juice with Vit C[g)]	9.4	0	2.3	0	0
Granulated sugar[n)]	27.1	0	7.0	0	0
Total	851.5	20.3	98.8	43.3	14.0

a) Nutrient analysis by Food Processor SQL Edition (version 10.6.0 ESHA Research, Salem, OR).
b) All foods were purchased at Jewel-Osco Grocery Store (with the exception of c) Safeway instant nonfat dry milk [Pleasanton, CA] purchased at Dominick's).
d) Thomas' plain bagel (Horsham, PA).
e) Kraft whipped cream cheese spread with chives (Glenview, IL).
f) Contadina tomato paste (San Francisco, CA).
g) Jewel brand whole milk, corn oil, apple juices (Boise, ID).
h) Rice dream rice milk (Boulder, CO).
i) Thai Kitchen coconut milk (Berkeley, CA).
j) Potato, produce section local Jewel-Osco (Arlington Heights, IL).
k) Benefiber powdered fiber supplement (Parsippany, NJ).
l) Morton iodized salt (Chicago, IL).
m) Morton salt substitute (Chicago, IL).
n) Crystal sugar (Moorhead, MN).
Pro = protein, Carb = carbohydrates, SatFat = saturated fat.

conforming to expected distributional assumptions were log transformed and noted accordingly. The postprandial responses with the two treatments (Tomato and Control) were compared in terms of the least squares mean (LSM) as an estimate of the 360-min (6-h) response. Treatment-associated difference was also evaluated in terms of changes in time relative to baseline and at common time points between treatments. The level used to determine statistical significance was $p < 0.05$.

Sample size was determined based on power calculations using estimated mean differences, variance of means, and within-subjects error for FMD from pilot work in our lab and work of Plotnick et al. [23]. FMD was chosen because it was considered as the most variable endpoint measured in this study. Sample size estimate was based on the following assumptions: significance level (adjusted for sidedness) = 0.025, standard deviation (SD) within subjects = 3.2, power > 0.8, attrition = 20%, and difference in means = 3 units corresponding to a 25% improvement in reactivity after consuming the meal with tomato products compared with no tomato. Based on these assumptions, 25 subjects were required to complete this two-treatment crossover study.

3 Results

3.1 Subject characteristics

A total of 25 subjects completed the study ($n = 12$ females, $n = 13$ males). The mean age ± SD and BMI ± SD for the group was 27 ± 8 years and 22 ± 2 kg/m^2, respectively. Subject

Table 2. Nutrient composition of the tomato and control test meals[a)]

Item name[b)]	Fiber (g)	Sodium (mg)	Vit C (mg)	Sugar (g)	Lyc (mcg)	Pot (mg)	Vit E (IU)	B-Caro (mcg)
Tomato meal								
Plain bagel, enriched[d)]	3.0	694.2	0	–	0	131.3	0.1	0
Cream cheese with chives[e)]	0	198.1	0	1.5	–	–	–	–
Tomato paste[f)]	3.9	742.6	20.6	11.5	27,038.2	953.2	6.0	846.9
Whole milk[g)]	0	11.6	0	1.3	0	41.5	0	1.5
Rice milk[h)]	0	17.5	0	4.1	–	0	0.2	–
Coconut milk[i)]	0.3	3.0	0.2	–	0	50.6	0.2	0
Corn oil[g)]	0	0	0	0	0		5.4	0
Apple juice[g)]	0	0.8	0.2	2.8	0	30.9	0	0
Total	7.2	1667.8	21.0	21.2	27,038.2	1207.5	11.9	848.4
Control meal								
Plain bagel, enriched[d)]	2.4	550.0	0	–	0	104.0	0.1	0
Cream cheese with chives[e)]	0	173.3	0	1.3	–		–	–
Potato, baked, mashed[j)]	1.2	5.3	11.5	–	0	312.4	0.1	0
Fiber supplement[k)]	3.3	16.5	0	0	–	16.5	–	–
Instant nonfat dry milk[c)]	0	45.0	1.4	3.8	0	134.3	–	0
Whole milk[g)]	0	38.0	0	4.3	0	135.9	0.1	4.8
Rice milk[h)]	0	7.2	0	1.7	–	0	0.1	–
Coconut milk[i)]	0.2	2.5	0.2	–	0	41.8	0.2	0
Corn oil[g)]	0	0	0	0	0		5.6	0
Table salt[l)]	0	829.4	0	0	0	0.2	0	0
Salt substitute[m)]	–	0.1	–	–	–	437.6	–	–
Apple juice with Vit C[g)]	0	0.6	8.3	2.2	0	23.8	0	–
Granulated sugar[n)]	0	0	0	7.0	0	0.1	0	0
Total	7.1	1667.9	21.4	20.3	0	1206.6	6.2	4.8

a) Nutrient analysis by Food Processor SQL Edition (version 10.6.0 ESHA Research, Salem, OR).
b) All foods were purchased at Jewel-Osco Grocery Store (with the exception of c) Safeway instant nonfat dry milk [Pleasanton, CA] purchased at Dominick's).
d) Thomas' plain bagel (Horsham, PA).
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f) Contadina tomato paste (San Francisco, CA).
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i) Thai Kitchen coconut milk (Berkely, CA).
j) Potato, produce section local Jewel-Osco (Arlington Heights, IL).
k) Benefiber powdered fiber supplement (Parsippany, NJ).
l) Morton iodized salt (Chicago, IL).
m) Morton salt substitute (Chicago, IL).
n) Crystal sugar (Moorhead, MN).
Vit C = vitamin C, Lyc = lycopene, Pot = potassium, Vit E = vitamin E, B-caro = beta-carotene.

ethnicity and baseline mean age, body weight, height, waist circumference, BMI, fasting glucose, BP, and heart rate (HR) are listed in Table 3.

3.2 Postprandial responses

Postprandial changes in glucose, insulin, and biomarkers of oxidative stress and inflammation were measured over the 360-min experimental period at defined intervals after meal consumption. The overall postprandial responses after the tomato and control test meals are shown in Table 4. Treatment differences based on LSM as an estimate of the 360-min response were evident only for the oxidation of LDL. Time by treatment analyses indicated significant increases in plasma glucose and insulin concentrations from baseline (fasting) concentrations after test meal consumption that were resolved within 90 and 240 min, respectively (Figs. 2A, B; $p < 0.05$). No statistically significant difference between meal treatments was observed; however, insulin concentrations were significantly lower at 30 and 90 min after the tomato meal compared to the control meal (Fig. 2B; $p < 0.05$).

Figure 3 illustrates the postprandial triglyceride (TG) response after consumption of the tomato or control meals. Peak lipemia occurred approximately 120 min after meal consumption for both treatments, and remained significantly elevated from baseline for the duration of the testing period ($p < 0.001$). The TG concentrations after the tomato meal tended to be higher than control meal starting at 180 min ($p = 0.1$) to the end of the 360-min experimental period reaching significance only at 300 min ($p = 0.006$). Analysis of area

Table 3. Subject characteristics[a)]

Variables	Units		Total $N = 25$
Gender		Female	12
		Male	13
Ethnicity		African American	1
		Asian	10
		Caucasian	9
		Hispanic	5
			Mean ± SD
Age	Years	Total	27 ± 8
		Female	27 ± 6
		Male	27 ± 9
BMI	kg/m^2	Total	22 ± 2
		Female	22 ± 1
		Male	22 ± 2
Waist circumference	cm	Total	75 ± 14
		Female	73 ± 15
		Male	76 ± 14
Glucose	mg/dL (mmol/L)	Total[b)]	87 ± 10 (5 ± 0.5)
Systolic BP[c),d)]	mmHg	Total	116 ± 10
Diastolic BP	mmHg	Total	70 ± 10

a) Mean (± standard deviation [SD]) for age, BMI, waist circumference, glucose, and systolic/diastolic blood pressure (BP) measurements for subjects at the screening visit.
b) Three subjects did not have fasting glucose measurement at screening ($n = 22$).
c) Values represent the average of two consecutive BP measurements recorded with 5-min rest between measurements.
d) BP measurements recorded on Vital Signs Monitor 300 Series (Welch Allyn, Beaverton, OR).

under the response curves (AUCs) revealed no significant differences in 360-min TG response between two treatments (tomato AUC 46,033.0 ± 26,913 vs. AUC control 41,255.5 ± 17,192; $p = 0.13$), which corroborates with the LSM data in Table 4.

Postprandial OxLDL concentrations were significantly increased compared to baseline (fasting) values in the control group ($p < 0.05$), whereas no differences in OxLDL from baseline were observed after the tomato meal (Fig. 4). The concentrations of OxLDL were significantly different overall by meal treatment ($p = 0.02$), and a time by meal interaction was also evident ($p = 0.03$).

Concentrations of IL-6 were significantly elevated from baseline to 360 min after the control meal ($p < 0.0001$; Fig. 5). In contrast, elevation in IL-6 after the tomato meal was significantly attenuated, although differences between treatments were not statistically evident until 360 min ($p < 0.0001$). No meal-related changes in postprandial hsCRP or TNF-α were evident (data not shown). No significant effect of sex for TG, glucose, insulin, inflammatory variables, and OxLDL was observed (data not shown).

3.3 Flow-mediated vascular reactivity

Baseline/preocclusion brachial artery diameter did not differ between meals at fasting (0 min) or 210 min. Differences in postreactive hyperemia as measured by percent change in FMD did not change significantly between tomato and control meals at 0 min (14.5% ± 1.3 vs. 13.8% ± 1.3; $p > 0.05$). However, at 210 min, relative peak lipemia, a modest difference in flow-mediated vascular reactivity was evident after consuming the tomato meal compared to the control meal

Table 4. Postprandial response to high-fat tomato and control test meals

	Standard (SI Units)	Tomato	Control	*p*-value (Tomato vs. Control)
Glucose	mg/dL (mmol/L)	92.6 ± 2.0 (5.13 ± 0.11)	92.3 ± 2.0 (5.12 ± 0.11)	0.86
Insulin	μU/mL (pmol/L)	38.0 ± 4.7 (263.9 ± 32.6)	41.1 ± 4.7 (285.4 ± 32.6)	0.26
TG	mg/dL (mmol/L)	122.6 ± 11.3 (1.39 ± 0.13)	111.7 ± 11.3 (1.26 ± 0.13)	0.14
OxLDL	U/L	72.0 ± 4.3	77.8 ± 4.4	0.02
IL-6	pg/mL (pmol/L)	2.2 ± 0.3 (0.10 ± 0.01)	2.6 ± 0.3 (0.12 ± 0.01)	0.19

Values represent the least square means (LSM) ± standard error of the mean (SEM) as an estimate of the 360-min postprandial response after tomato and control test meals. Significance $p < 0.05$. TG = Triglycerides, OxLDL = oxidized low-density lipoproteins, IL-6 = interleukin 6.

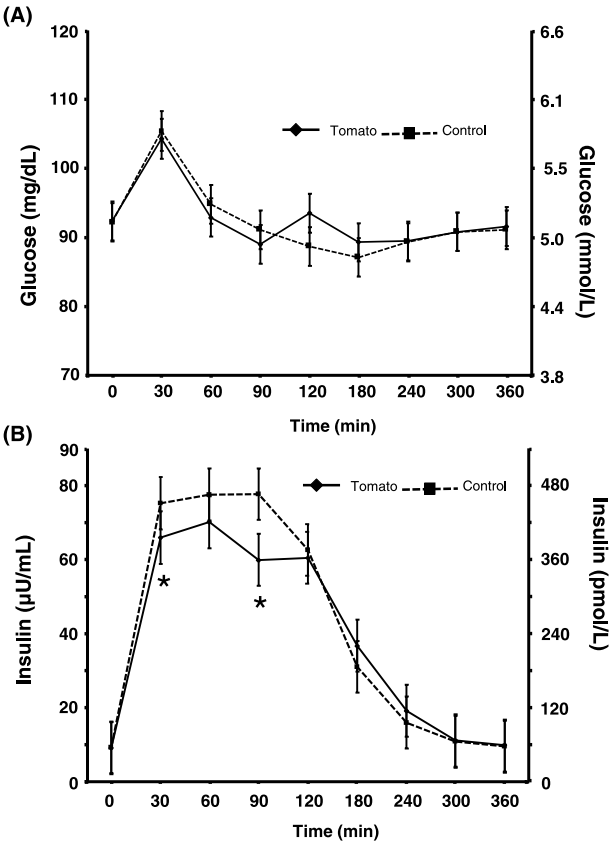


Figure 2. (A) and (B). Glucose and insulin concentrations in healthy weight men and women at baseline and after consumption of the tomato containing meal (Tomato) and the non-tomato-containing meal (Control). Values represent the mean ± SEM of raw data. Data were log transformed prior to final analysis to meet distributional assumptions of normality. $*p < 0.05$ versus control at respective time intervals; $n = 25$.

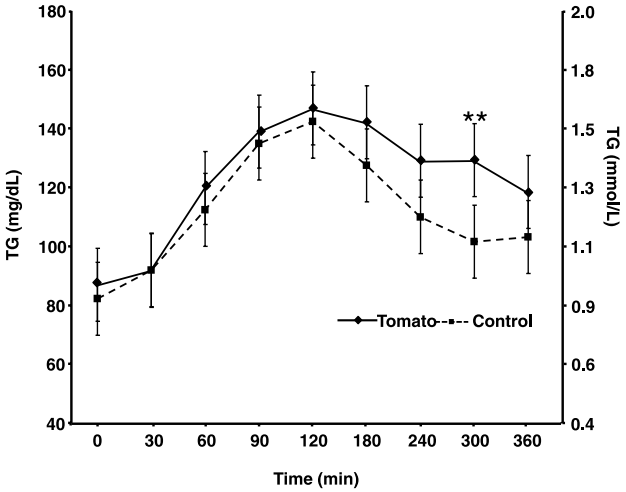


Figure 3. Triglyceride (TG) concentrations at baseline and after consumption of the tomato and control meals. Values represent the mean ± SEM of raw data. Data were log transformed prior to final analysis to meet distributional assumptions of normality. $**p < 0.01$ versus control at respective time intervals; $n = 25$.

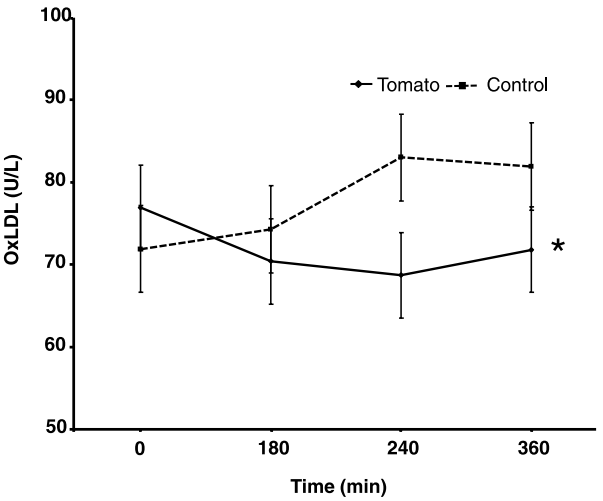


Figure 4. Oxidized low-density lipoprotein (OxLDL) concentrations at baseline and after consumption of the tomato and control meals. Values represent the mean ± SEM of raw data. Data were log transformed prior to final analysis to meet distributional assumptions of normality. $*p < 0.05$ versus control overall by treatment; $n = 25$.

(Fig. 6); although statistical significance was not achieved ($p = 0.18$). A marginal negative correlation ($r = –0.34$, $p = 0.1$) was apparent after the control meal between percent FMD and AUC for the TG response (0–240 min) compared to no relationship between FMD and TG response after the tomato meal ($r = 0.13$, $p = 0.5$).

4 Discussion

The purpose of this study was to test the hypothesis that consuming tomato as part of a high-fat meal attenuates high-fat meal-induced oxidative stress and concomitant inflammatory and impaired endothelial-dependent relaxation responses in healthy weight men and women. The major finding of this study was that consuming 94 g ($1^1/_2$ serving) of tomato paste with a relatively high-fat, pro-oxidative meal significantly attenuated the postprandial increase in LDL oxidation observed when a similar meal was consumed without tomato paste. Additionally, benefits of tomato were observed on meal-induced changes in inflammatory status with minimal impact on endothelial function. Together, these data suggest that tomato products, and potentially other carotenoid/antioxidant rich foods, have a role in modulating oxidative- and inflammatory-stress indicators implicated in the pathogenesis of CVD [12–18].

Postprandial hypertriglyceridemia is the foremost serum lipid indicator of a metabolically nonsteady-state [5]. In our study, plasma TG concentrations increased after both meals

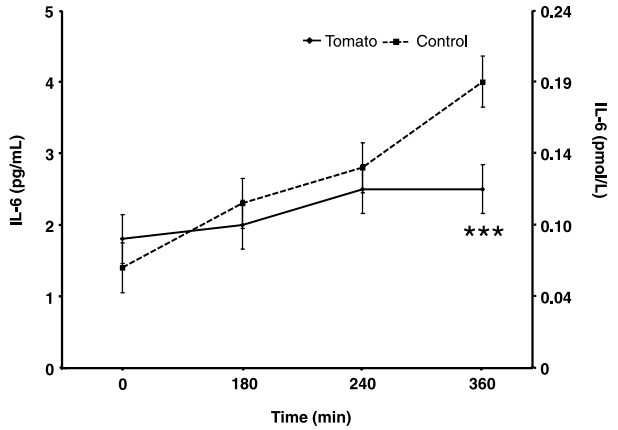
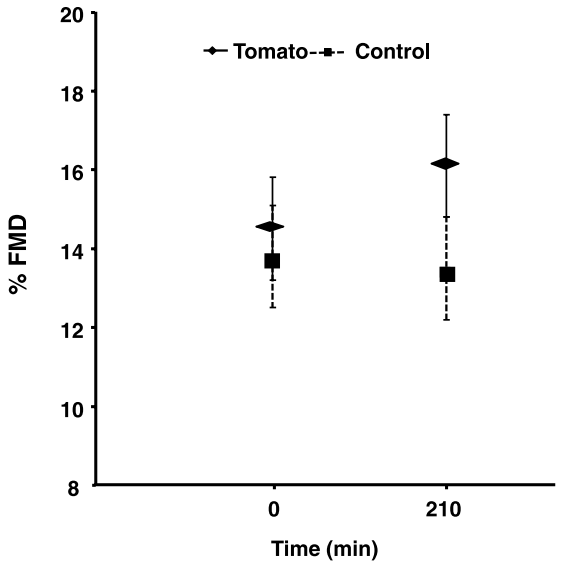


Figure 5. Interleukin 6 (IL-6) concentrations at baseline and after consumption of tomato and control meals. Values represent the mean ± SEM of raw data. Data were log transformed prior to final analysis to meet distributional assumptions of normality. ***$p < 0.001$ versus control at respective time intervals; $n = 25$.



	Control		Tomato	
	0 min	Change at 210 min	0 min	Change at 210 min
FMD %	13.8 ± 1.3	-0.3 ± 1.5	14.5 ± 1.3	1.6 ± 1.5

Figure 6. Flow-mediated dilation (FMD) responses at baseline and after (210 min) consumption of the tomato and control meals. Values represent the mean ± SEM of percent FMD; $n = 25$. Corresponding tabular data show percent change from baseline at 210 min.

peaking at approximately 120 min and remained elevated from baseline throughout the testing period (360 min). As previously shown and as supported in this study, eating 40–50 g of fat in one meal results in significant elevations in serum TG in healthy, normocholesterolic adults [24]. Postprandial hypertriglyceridemia increases the risk of oxidative damage primarily targeting LDL [25]. Considering a significant portion of the day is spent in the postprandial state, an elevated and prolonged presence of oxidative stress and in particular circulating OxLDL increases the susceptibility of cellular activation and damage providing a foundation for atherosclerotic disease initiation and progression [9, 26]. Therefore, dietary strategies to modify postprandial oxidative stress and associated events are likely to have a significant impact on atherosclerotic disease risk reduction. In the present study, we demonstrated that increasing the antioxidant content of an otherwise matched high-fat meal blocked the postprandial rise in OxLDL compared to control. This effect was apparent despite incremental increases in TG after the tomato meal. These data support our hypothesis that consumption of foods rich in compounds known for their antioxidant properties, such as processed tomato products, can favorably impact oxidative stress conditions and serve to protect biological mediators with atherogenic potential, such as LDL.

Oxidative stress is known to impair insulin signaling (decreased insulin sensitivity), which can be restored with concomitant antioxidant compounds/extracts [4, 27]. Our data indicate that at 30 and 90 min, insulin concentrations were significantly lower after the tomato meal compared to the control meal. Lycopene is a well-known and potent antioxidant. A recent study done by Ross et al. [28] indicated that lycopene absorption can occur as early as 30 min after consumption, although the majority is absorbed over a slower time frame consistent with TG absorption via chylomicron pathways. Hence, we suspect that the early increased antioxidant levels after the tomato meal allowed for appropriate insulin signaling, whereas in the control meal insulin signaling was impaired and hence the higher concentrations needed to achieve glucose homeostasis.

A complex inter-relationship is known to exist between oxidative stress, persistent low-grade inflammation, and chronically disturbed vascular homeostasis [29]. In general, oxidative stress results in enhanced vasoconstrictor tone and release of proinflammatory proteins [29]. The second aim of our study was to test the hypothesis that improving oxidative stress status would also attenuate the postprandial inflammatory response typically observed with high-fat meal consumption. IL-6 and TNF-α are widely known cytokines released by macrophages, among other cell types including endothelial cells, and are involved in inflammation, cell proliferation, differentiation, and apoptosis [26, 30]. The use of natural antioxidant products including lycopene as anti-inflammatory agents has been investigated widely [31, 32]. However, the mechanisms underlying in vivo effects and the signal transduction pathway affected by lycopene on anti-inflammatory pathways are not fully understood. In vitro studies clearly indicated that lycopene inhibits the inflammatory response through inhibiting the nuclear factor-kappaB (NF-κB) pathways [33]. In the present study, we detected treatment-related differences in the IL-6 response; however, the differences for IL-6 were not statistically apparent until 360-min postmeal consumption. These data corroborate other work in our lab with strawberries, where TNF-α was not affected by

treatment, but IL-6 was significantly lower by 360 min after strawberry versus control treatment [4, 27]. IL-6 gene expression results, at least in part, due to NF-κB activation. NF-κB activation is increased with oxidants as well as other stimuli such as OxLDL and TNF-α, and inhibited with antioxidants such as pyrrolidine dithiocarbamate and *N*-acetyl cysteine with dithiocarbamates as potent inhibitors of NF-κB activation in intact cells [34]. Further, Blanco-Colio et al. [35] showed that after a fat-enriched meal, NF-κB activation in peripheral blood mononuclear cells (PBMCs) was maximally stimulated at 360 min and remained stimulated through 540 min; whereas red wine consumption with the meal prevented NF-κB activation at 360 and 540 min, an effect not observed with an alternative alcohol (vodka) drink. Hence, decreases in IL-6 at 360 min in response to the tomato meal could be due, at least in part, to the antioxidant effects of tomatoes altering cellular redox status resulting in decreased NF-κB activation. It is also possible that lycopene mediated decreased concentrations of postprandial OxLDL as an inhibitory stimulus of NF-κB activation that may be involved in the reduced IL-6 response after the tomato meal. Oxidative stress can activate a variety of transcription factors including NF-κB signaling pathway [31, 32]. Meal-induced recurrent oxidative stress can lead to chronic inflammation, which in turn could mediate most chronic diseases including diabetes, cardiovascular and neurological diseases.

IL-6 is a known stimulus for the secretion of the acute phase protein and inflammatory cytokine, hsCRP. An elevation of IL-6 would be expected to result in increased concentrations of hsCRP; however, no changes in hsCRP were observed. We suspect that the lack of hsCRP response was due to timing of cytokine (IL-6) messaging and our 360-min postprandial study design, which limited our ability to measure to stimulated hsCRP production and secretion [30]. Future studies will be required to address the timing issue of postprandial induced hsCRP.

FMD reflects endothelium-dependent vasodilator function. FMD is diminished in patients with atherosclerosis and with coronary risk factors, and improves with risk-reduction therapy [22]. Consumption of foods and beverages rich in antioxidant compounds have been shown to improve or block lipemia-induced impaired FMD in patients with documented coronary artery disease and in normolipidemic volunteers [23, 36, 37]. We did not observe a significant improvement in vasodilation with tomato intake and this may be due in part to the enhanced lipemia observed in the tomato group. However, when examining the AUC for TG relative to FMD responses, there was a trend ($p = 0.1$) for reduced FMD with greater TG AUC (0–240 min) after the control meal that was not apparent when tomatoes were consumed with the meal. These data suggest a potential favorable effect of tomato on FMD; however, a recent study by Stangl *et al*. (2011) indicates that tomato products do not affect endothelial function [38]. A major difference in the Stangl study and the present study is the timing of FMD measurements. The former focused on fasting state responses 24 h and 7 days after tomato puree consumption (with buttered roll), whereas the present study focused on FMD responses during the postprandial period (210–240 min after tomato products consumption with a high-fat meal). Hence, additional research is required to better understand the relationship between consumption of tomato/lycopene-rich foods, lipemia, and endothelial function.

In summary, this study demonstrated that the consumption of processed tomato products blocks or attenuates lipemia-induced postprandial oxidative and inflammatory responses with limited effects on endothelial function in healthy men and women. The described effects were demonstrated with a practical dose (serving size) of commercially available, commonly purchased pantry food product that is affordable and easy to incorporate in a variety of menus. These results are noteworthy considering implications for ease of consumer education and use as part of a sustainable preventative dietary strategy to lower risk of CVD.

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5 References

[1] Ursini, F., Sevanian, A., Postprandial oxidative stress. *Biol. Chem.* 2002, *383*, 599–605.

[2] Bowen, P. E., Borthakur, G., Postprandial lipid oxidation and cardiovascular disease risk. *Curr. Atheroscler. Rep.* 2004, *6*, 477–484.

[3] Graner, M., Kahri, J., Nakano, T., Sarna, S. J. et al., Impact of postprandial lipaemia on low-density lipoprotein (LDL) size and oxidized LDL in patients with coronary artery disease. *Eur. J. Clin. Invest.* 2006, *36*, 764–770.

[4] Burton-Freeman, B., Linares, A., Hyson, D., Kappagoda, C. T., Strawberry modulates LDL oxidation and postprandial lipemia in response to high fat meal in overweight hyperlipidemic men and women. *J. Am. Coll. Nutr.* 2010, *29*, 46–54.

[5] Hyson, D., Rutledge, J. C., Berglund, L., Postprandial lipemia and cardiovascular disease. *Curr. Atheroscler. Rep.* 2003, *5*, 437–444.

[6] Lundman P., Boquist S., Samnegard A, Bennermo M. et al., A high-fat meal is accompanied by increased plasma interleukin-6 concentrations. *Nutr. Metab. Cardiovasc. Dis.* 2007, *17*, 195–202.

[7] Lundman, P., Eriksson, M., Schenck-Gustafsson, K., Karpe, F. et al., Transient triglyceridemia decreases vascular reactivity in young, healthy men without risk factors for coronary heart disease. *Circulation* 1997, *96*, 3266–3268.

[8] Edirisinghe, I., Burton-Freeman, B., Varelis, P., Kappagoda, T., Strawberry extract caused endothelium-dependent relaxation through activation of PI3 kinase/Akt. *J. Agric. Food Chem.* 2008, *56*, 9383–9390.

[9] Sies, H., Stahl, W., Sevanian, A., Nutritional, dietary and postprandial oxidative stress. *J. Nutr.* 2005, *135*, 969–972.

[10] Canene-Adams, K., Campbell, J. K., Zaripheh, S. Jeffrey, E. H. et al., The tomato as a functional food. *J. Nutr.* 2005, *135*, 1226–1230.

[11] Heber, D., Lu, Q. Y., Overview of mechanisms of action of lycopene. *Exp. Biol. Med.* 2002, *227*, 920–923.

[12] Bramley, P. M., Is lycopene beneficial to human health? *Phytochemistry* 2000, *54*, 233–236.

[13] Rao, A. V., Agarwal, S., Role of antioxidant lycopene in cancer and heart disease. *J. Am. Coll. Nutr.* 2000, *19*, 563–569.

[14] Jacob, K., Periago, M. J., Bohm, V. Berreuzo, G. R., Influence of lycopene and vitamin C from tomato juice on biomarkers of oxidative stress and inflammation. *Br. J. Nutr.* 2008, *99*, 137–146.

[15] Agarwal, S., Rao, A. V., Tomato lycopene and low density lipoprotein oxidation: a human dietary intervention study. *Lipids* 1998, *33*, 981–984.

[16] Silaste, M. L., Alfthan, G., Aro, A., Kesaniemi, Y. A. Horkko, S., Tomato juice decreases LDL cholesterol levels and increases LDL resistance to oxidation. *Br. J. Nutr.* 2007, *98*, 1251–1258.

[17] Devaraj, S., Mathur, S., Basu, A., Aung, H. H. et al., A dose-response study on the effects of purified lycopene supplementation on biomarkers of oxidative stress. *J. Am. Coll. Nutr.* 2008, *27*, 267–273.

[18] Hadley, C. W., Clinton, S. K., Schwartz, S. J., The consumption of processed tomato products enhances plasma lycopene concentrations in association with a reduced lipoprotein sensitivity to oxidative damage. *J. Nutr.* 2003, *133*, 727–732.

[19] Denniss, S. G., Haffner, T. D., Kroetsch, J. T., Davidson, S. R. et al., Effect of short-term lycopene supplementation and postprandial dyslipidemia on plasma antioxidants and biomarkers of endothelial health in young, healthy individuals. *Vasc. Health Risk Manag.* 2008, *4*, 213–222.

[20] Sesso, H. D., Lui, S., Gaziano, J. M., Buring, J. E., Dietary lycopene, tomato-based food products and cardiovascular disease in women. *J. Nutr.* 2003, *133*, 2336–2341.

[21] Burton-Freeman, B., Davis, P. A., Schneeman, B. O., Interaction of fat availability and sex on postprandial satiety and cholecystokinin after mixed-food meals. *Am. J. Clin. Nutr.* 2004, *80*, 1207–1214.

[22] Corretti, M. C., Anderson, T. J., Benjamin, E. J., Celermajer, D. et al., Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: a report of the International Brachial Artery Reactivity Task Force. *J. Am. Coll. Cardiol.* 2002, *39*, 257–265.

[23] Plotnick, G. D., Corretti, M. C., Vogel, R. A., Effect of antioxidant vitamins on the transient impairment of endothelium-dependent brachial artery vasoactivity following a single high-fat meal. *J. Am. Med. Assoc.* 1997, *278*, 1682–1686.

[24] Sanders, T. A., Dietary fat and postprandial lipids. *Curr. Atheroscler. Rep.* 2003, *5*, 445–451.

[25] Bae, J. H., Bassenge, E., Kim, K. B., Kim, Y. N. et al., Postprandial hypertriglyceridemia impairs endothelial function by enhanced oxidant stress. *Atherosclerosis* 2001, *155*, 517–523.

[26] Libby, P. Inflammation and cardiovascular disease mechanisms. *Am. J. Clin. Nutr.* 2006, *83* (Supp l), S456–S460.

[27] Edirisinghe, I., Banaszewski, K., Cappozzo, J., Sandhya, K. et al., Strawberry anthocyanin and its association with postprandial inflammation and insulin. *Br. J. Nutr.* 2011, *106*, 912–922

[28] Ross, A.B., Vuong le. T., Ruckle, J., Synal, H.A. et al., Lycopene bioavailability and metabolism in humans: an accelerator mass spectrometry study. *Am. J. Clin. Nutr.* 2011 *93*, 1263–1273.

[29] Koh, K. K., Oh, P. C., Quon, M. J., Does reversal of oxidative stress and inflammation provide vascular protection? *Cardiovasc. Res.* 2009, *81*, 649–659.

[30] Biasillo, G., Leo, M., Della Bona, R., Biasucci, L. M., Inflammatory biomarkers and coronary heart disease: from bench to bedside and back. *Intern. Emerg. Med.* 2010, *5*, 225–233.

[31] Reuter, S., Gupta, S. C., Chaturvedi, M. M., Aggarwal, B. B., Oxidative stress, inflammation, and cancer: how are they linked? *Free Radic. Biol. Med.* 2010, *49*, 1603–1616.

[32] Gupta, S. C., Kim, J. H., Prasad, S., Aggarwal, B. B., Regulation of survival, proliferation, invasion, angiogenesis, and metastasis of tumor cells through modulation of inflammatory pathways by nutraceuticals. *Cancer Metast. Rev.* 2010, *29*, 405–434.

[33] Feng, D., Ling, W. H., Duan, R. D., Lycopene suppresses LPS-induced NO and IL-6 production by inhibiting the activation of ERK, p38MAPK, and NF-kappaB in macrophages. *Inflamm. Res.* 2010, *59*, 115–121.

[34] Schreck, R., Meier, B., Mannel, D. N., Droge, W. et al., Dithiocarbamates as potent inhibitors of nuclear factor kappaB activation in intact cells. *J. Exp. Med.* 1992, *175*, 1181–1194.

[35] Blanco-Colio, L. M., Valderrama, M., Alvarez-Sala, L. A., Bustos, C. et al., Red wine intake prevents nuclear factor-kappaB activation in peripheral blood mononuclear cells of healthy volunteers during postprandial lipemia. *Circulation* 2000, *102*, 1020–1026.

[36] Stein, J. H., Keevil, J. G., Wiebe, D. A., Aeschlimann, S. et al., Purple grape juice improves endothelial function and reduces the susceptibility of LDL cholesterol to oxidation in patients with coronary artery disease. *Circulation* 1999, *100*, 1050–1055.

[37] Vogel, R. A., Corretti, M. C., Plotnick, G. D., The postprandial effect of components of the Mediterranean diet on endothelial function. *J. Am. Coll. Cardiol.* 2000, *36*, 1455–1460.

[38] Stangl, V., Kuhn, C., Hentschel, S., Jochmann, N. et al., Lack of effects of tomato products on endothelial function in human subjects: results of a randomised, placebo-controlled crossover study. *Br. J. Nutr.* 2011,*105*, 263–267.

