Attenuation of Meal-Induced Inflammatory and Thrombotic Responses in Overweight Men and Women After 6-Week Daily Strawberry (Fragaria) Intake

— A Randomized Placebo-Controlled Trial

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Aim: A pro-thrombotic, pro-inflammatory diet can play a causative role in atherosclerotic-cardiovascular diseases. Dietary intervention studies provide insight into their pathophysiological manifestations and opportunities for prevention and management. We previously showed in an acute-meal setting that a beverage containing polyphenolic- and antioxidant-rich strawberry (Fragaria) vs placebo attenuated postprandial (fed-state) increases in biomarkers of oxidative and inflammatory stress, and insulin concentrations, induced by a high carbohydrate/fat (HCF) meal.

In the present study, we aimed to extend our findings and investigate hypotheses related to the effects of chronic/6-week (wk) strawberry consumption on HCF meal-induced increases in glucose, insulin, and indicators of inflammation and hemostasis.

Methods: In a crossover design, 14 women and 10 men (mean age, BMI: 50.9 ± 15 years, 29.2 ± 2.3 kg/m², respectively), were randomized to a 6-wk strawberry or placebo beverage followed by an HCF meal with assessments for 6-hours (h) postprandially.

Results: HCF meal responses after 6-wk strawberry beverage showed significantly attenuated postprandial PAI-1 concentrations compared to the placebo (p=0.002); the difference was most notable at 6 h. The IL-1β response was attenuated with strawberry compared to the placebo (p=0.05). IL-6 attenuation was apparent but non-significant; IL-6 rose significantly from baseline to 6 h after the HCF meal following a placebo (p≤0.01), although it remained relatively flat following the strawberry beverage from fasting to 6 h. No significant treatment-related differences were apparent for platelet aggregation, hsCRP, TNF-α, insulin, or glucose.

Conclusion: These data are the first to suggest that regular consumption of strawberry, a polyphenolic- and antioxidant-rich fruit, may provide protection from HCF meal-induced increases in fibrinolytic and inflammatory factors in at-risk men and women.


Key words: Strawberry, Polyphenolics, Postprandial, Thrombosis, Inflammation

Introduction

A predisposing diet can play a causative role in athero-thrombotic cardiovascular diseases. Dietary intervention studies can provide insight into their pathophysiological manifestations, help to quantify the effect of dietary habits on clinical outcomes, and
grant opportunities for prevention and management. Consumption of a meal high in carbohydrates and fat, typifying Western patterns, results in multiple metabolic changes in blood which are potentially detrimental for the maintenance of vascular health. These include oxidative and inflammatory stress, an increase in insulin resistance, transient endothelial dysfunction, and platelet activation with impaired hemostasis. Previous studies reported by us have shown that the postprandial (fed-state) increase in oxidative stress, inflammation and insulin resistance to a high carbohydrate/fat (HCF) ‘test’ meal was attenuated by strawberry, a fruit species of the flowering plant genus Fragaria. Our data suggest that this effect was due to the high anti-oxidant capacity of many of the polyphenolic-structured phytochemicals found in strawberry. Attenuation of these acute responses is consistent with the possible direct effect of the anti-oxidant compounds in strawberry, based on pharmacokinetic data indicating that major strawberry-derived phenolic biomolecules (e.g. anthocyanins and other metabolites) peak in the blood approximately 60-90 minutes (min) post-consumption, clearing within 8 hours (h). Effects persisting after this time, specifically the effects on postprandial metabolic, inflammatory and hemostatic factors, are unknown after strawberry intake.

Previous work has shown reduced fasting lipids and oxidative stress markers after strawberry consumption for 4 wk, suggesting adaptive responses reflective of favorable homeostatic shifts. Research from non-strawberry sources of dietary polyphenols further supports this work showing changes in the expression of anti-oxidant genes. Collectively, these data suggest that chronic consumption of polyphenolic-rich foods may provide protection from meal-induced postprandial metabolic, inflammatory and hemostatic challenges through attenuation of cellular oxidative stress-influenced processes. In the present study, we extended the findings of our acute studies to investigate and define the persistent effects of chronic strawberry consumption on postprandial responses to HCF meal-induced increases in metabolic, inflammatory, and hemostatic factors in at-risk men and women.

**Aim**

Against this background, the primary aim was to test the hypothesis that daily consumption of strawberry in the form of a beverage for 6 wk attenuates postprandial increases in glucose, insulin, c-reactive protein (CRP), interleukin (IL)-1β, IL-6, tumor necrosis factor (TNF)-α, plasminogen activator inhibitor (PAI)-1, and platelet aggregation, induced by a HCF meal in overweight men and women. The secondary aim was to evaluate the effect of daily consumption of strawberry for 6 wk on the fasting concentrations of the same variables.

**Methods, Materials, and Study Subjects**

This study was approved by the University of California, Davis, Human Subjects Review Committee and was funded by a grant from the California Strawberry Commission (CSC).

**Experimental Participants**

Twenty-six overweight and obese men and women (n = 10 and n = 16, respectively) were recruited from the Sacramento, CA community using newspapers and local flyers. All subjects completed an informed consent form approved by the Institutional Review Board prior to screening. The exclusion criteria were the following: i) smokers, ii) those taking medications that would interfere with study endpoints (i.e. lipid-lowering medications, anti-inflammatory drugs, dietary supplements), iii) allergy or intolerance to strawberries, iv) diabetes mellitus or fasting glucose > 110 mg/dL, v) uncontrolled hypertension (> 140/90 mmHg), and vi) documented atherosclerotic, chronic inflammatory or other systemic diseases. Two women dropped out of the study because of work commitments. The final study sample size was 14 women and 10 men (Table 1).

**Experimental Design**

This was a single-center, randomized, single-blinded, placebo-controlled, 6-wk parallel-design dietary intervention trial that extended the findings of another trial using the same subjects to investigate the persistent effects of strawberry consumption on metabolic, inflammatory and hemostatic factors in at-risk men and women.

**Table 1. Subject characteristics at screening visit**

<table>
<thead>
<tr>
<th>Subject characteristics</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>50.9 ± 15.0</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>86.6 ± 12.9</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>171.7 ± 9.8</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>29.2 ± 2.3</td>
</tr>
<tr>
<td>Cholesterol (mg/dL)</td>
<td>210.3 ± 26.9 (5.45 ± 0.70)</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>103.4 ± 52.9 (1.71 ± 0.60)</td>
</tr>
<tr>
<td>HDL-C (mg/dL)</td>
<td>49.1 ± 13.4 (1.27 ± 0.35)</td>
</tr>
<tr>
<td>LDL-C (mg/dL)</td>
<td>142.3 ± 28.3 (3.69 ± 0.73)</td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>86.5 ± 11.8 (4.80 ± 0.65)</td>
</tr>
</tbody>
</table>

Values are the mean ± standard deviation (SD) for 24 subjects (10 male, 14 female). BMI: body mass index, HDL-C: high density lipoprotein-cholesterol, LDL-C: low density lipoprotein-cholesterol.
Table 2. Nutrient content of the strawberry and placebo beverages

<table>
<thead>
<tr>
<th>Beverage</th>
<th>Strawberry</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (g)</td>
<td>305.0</td>
<td>305.0</td>
</tr>
<tr>
<td>Calories (kcal)</td>
<td>248.2</td>
<td>246.8</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>0.3</td>
<td>0.2</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>7.8</td>
<td>7.9</td>
</tr>
<tr>
<td>Carbohydrates (g)</td>
<td>54.8</td>
<td>53.6</td>
</tr>
<tr>
<td>Fiber (g)</td>
<td>1.9</td>
<td>0</td>
</tr>
<tr>
<td>Sugar (g)</td>
<td>24.7</td>
<td>25.9</td>
</tr>
<tr>
<td>Vitamin C (mg)</td>
<td>12.7 ± 0.9</td>
<td>2.7 ± 1.0</td>
</tr>
<tr>
<td>Total phenols (mg)</td>
<td>94.7 ± 2.2</td>
<td>2.4 ± 0.0</td>
</tr>
<tr>
<td>Total anthocyanins (mg)</td>
<td>39.0 ± 1.0</td>
<td>1.7 ± 0.0</td>
</tr>
<tr>
<td>ORAC value (μM Trolox equivalents)</td>
<td>5163 ± 176</td>
<td>1006 ± 79</td>
</tr>
</tbody>
</table>

Beverages (strawberry and placebo) were made with Nesquick strawberry artificially flavored powder, Nestle USA, Inc., Glendale, CA (34 g each). Premium Sanalac Nonfat Dry Milk, Con Agra Foods, Omaha, NE (21 and 22.5 g, respectively). Domino Sugar, Domino Foods, Yonkers, NY (3 and 10 g, respectively). Freeze Dried Strawberry Powder, California Strawberry Commission, Watsonville, CA. (10 and 0 g, respectively) and water to a total volume of 305 mL.

Post-Prandial Studies

The postprandial study included the consumption of an HCF ‘test’ meal which included a plain bagel, margarine, cream cheese, cantaloupe, egg, and a whole-milk-based artificially flavored strawberry beverage (Table 3 and 4). Participants consumed the meal for breakfast in the clinical unit after an overnight fast before and after 6-wk beverage supplementation. Blood samples were collected at timed intervals before and after meal consumption at 0 time (fasting) 30, 60, 90, 120 min (2 h) and then hourly thereafter to 360 min (6 h).

Throughout the study, subjects periodically completed food diaries which were analyzed (Food Processor SQL Edition Version 9.6.2; ESHA Research, Salem, OR) to detect changes in subjects’ diet during the study period. During the 6-wk intervention, participants returned to the testing center at bi-weekly intervals to pick up strawberry or placebo beverages and for a brief assessment of study adherence. Importantly, no significant dietary differences between strawberry and placebo groups were apparent for energy, fat, carbohydrate, protein, fiber, minerals and vitamins analyzed, particularly those with known antioxidant activity (vitamin C, beta carotene, vitamin E).

Experimental Analytical Techniques

Blood samples were centrifuged to obtain plasma, which was stored at −80°C until analyzed for concentrations of glucose, insulin, CRP, IL-1β, IL-6, TNF-α, PAI-1. Platelet function studies were performed on fresh blood samples. High sensitivity (hs) assay systems were used for the measurement of CRP (hsCRP) using the Human C-Reactive Protein ELISA (Enzyme-Linked Immuno-Sorbent Assay)/EIA (Enzyme Immunoassay) kit (catalog number 30-9710s), of IL-1β/
IL-1F2 using the Human IL-1β/IL-1F2 Quantikine hs ELISA kit, of IL-6 using the Human IL-6 Quantikine hs ELISA kit (catalog number HS600B), and of TNF-α/TNFSF1A using the Human TNF-α/TNFSF1A Quantikine hs ELISA kit (catalog number HSTA00D), all of which were purchased from Alpco Diagnostics (Salem, NH). PAI-1 was measured using kits purchased from American Diagnostics (Greenwich, CT). Plasma glucose was analyzed using the 2300 STAT Plus (YSI; Yellow Springs, OH) and endogenous plasma insulin was measured by radioimmunoassay (RIA) according to the basic method described by Yalow RS and Berson SA.²²

Platelet function was assessed using a PFA-100 platelet function analyzer (Dade Behring, Deerfield, IL) as previously reported Rein D et al.²³ Briefly, the PFA-100 measures primary platelet homeostasis as the time it takes for adenosine diphosphate (ADP)-stimulated or epinephrine (EPI)-stimulated whole blood to occlude an aperture in a collagen membrane cartridge under high shear conditions. Whole blood collected (4 mL) into vacutainer tubes containing sodium citrate was used for this assay.

### Statistical Analysis

Data were analyzed by repeated measures analysis of variance RM-ANOVA using PC-SAS (version 9.1; SAS Institute Inc., Cary, NC) GLM and MIXED procedure with treatment, time, and sex as the main factors and subject as the blocking variable. Absolute values were analyzed unless noted otherwise. Clinical laboratory endpoints not conforming to the expected distributional assumptions were log transformed and noted accordingly. Significant differences among treatment means were analyzed by the pair-wise *t*-test and Tukey’s honestly-significant test for appropriate comparisons. Pearson correlations were performed to investigate possible relationships between postprandial responses of PAI-1 and inflammatory markers. The level used to determine statistical significance was *p* ≤ 0.05. Marginal statistical differences were acknowledged at *p* > 0.05-0.1.

### Results

#### Effect of 6-Week Daily Strawberry or Placebo Beverages on Fasting Plasma Glucose and Insulin Concentrations and Inflammatory and Fibrinolytic Factors

Fasting plasma concentrations of glucose, insulin, hsCRP, IL-6, IL-1β, TNF-α and PAI-1 are shown in Table 5. No differences were evident between subjects’ baseline fasting values based on the randomization schedule and therefore were combined (n = 24).

After 6-wk placebo (n = 12) or strawberry (n = 12) consumption, no significant changes in fasting values from baseline (0 wk) to 6 wk were evident; however, a marginal reduction (p = 0.09) from baseline in concentrations of IL-1β was indicated after strawberry intake.

Differences between strawberry and placebo treatments for IL-6, PAI-1, glucose, and insulin at 6 wk were not significant (p > 0.05). Marginal treatment differences for IL-1β (p = 0.08), hsCRP (p = 0.09), and TNF-α (p = 0.07) were apparent (Table 5).

#### Effect of 6-Week Daily Strawberry or Placebo Beverages on Postprandial Plasma Glucose and Insulin Concentrations and Inflammatory and Fibrinolytic Factors After High Carbohydrate/Fat (HCF) Meal

Plasma postprandial concentrations of glucose, insulin, hsCRP, IL-6, IL-1β, TNF-α, and PAI-1 after HCF meal are shown in Table 6.
Consumption of strawberry beverage for 6 wk compared to a placebo beverage in a free-living setting significantly attenuated the overall HCF meal-induced postprandial IL-1β response as assessed by the least squares mean (LSM) as an estimate of the response (Table 6, p=0.05); however, individual differences in IL-1β concentrations measured at specific time points were marginal: 6-wk placebo HCF meal vs 6-wk strawberry HCF meal: T0, p=0.08; T180, p=0.06; T360, p=0.09 (Fig. 1). Pair-wise comparison of HCF meal wk-0 postprandial response vs wk-6 postprandial response after strawberry intervention was not different; however, after placebo intervention, the IL-1β response was significantly higher (p=0.01), although not after controlling for variability in fasting concentrations.

The postprandial IL-6 response after the HCF meal was not different at 6 wk after strawberry vs placebo intervention (Table 6 and Fig. 2); however, attenuation was apparent after controlling for variability in fasting IL-6 concentrations (p=0.07). IL-6 concentrations rose significantly from baseline to 6 h after the HCF meal following placebo intervention (p≤0.01), whereas IL-6 remained relatively flat after the strawberry beverage from fasting to 6 h. Pair-wise comparison of wk-0 HCF meal postprandial responses vs wk-6 postprandial responses corrected for baseline variability was lower (p=0.06) after strawberry intervention and not different after the placebo.

No treatment-related differences were observed for glucose, insulin, hsCRP or TNF-α (Table 6). Pair-wise comparisons of 0-wk postprandial responses vs 6-wk postprandial responses for each treatment were also compared and were not significantly different.

Consumption of a strawberry beverage for 6 wk compared to a placebo beverage significantly attenuated the HCF meal-induced postprandial PAI-1 response (Table 6 and Fig. 3, p=0.002). This differ-

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Table 5. Fasting values for glucose, insulin, inflammatory and hemostatic factors after 6-week daily strawberry or placebo beverages

<table>
<thead>
<tr>
<th>Factor</th>
<th>0-wk HCF MEAL N=24</th>
<th>6-wk Placebo HCF MEAL N=12</th>
<th>6-wk Strawberry HCF MEAL N=12</th>
<th>p-value</th>
<th>Placebo vs. Strawberry</th>
</tr>
</thead>
<tbody>
<tr>
<td>hsCRP (mg/L)</td>
<td>3.0±0.2</td>
<td>3.7±0.4</td>
<td>2.7±0.4</td>
<td>0.09</td>
<td>NS</td>
</tr>
<tr>
<td>IL-6 (ng/L)</td>
<td>2.1±0.02</td>
<td>1.4±0.1</td>
<td>2.1±0.5</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>PAI-1 (ng/L)</td>
<td>3.8±0.3</td>
<td>4.6±0.6</td>
<td>4.5±0.6</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>IL-1β (ng/L)</td>
<td>0.2±0.02</td>
<td>0.2±0.04</td>
<td>0.1±0.01</td>
<td>0.08</td>
<td>NS</td>
</tr>
<tr>
<td>TNF-α (ng/L)</td>
<td>1.2±0.1</td>
<td>1.1±0.04</td>
<td>1.1±0.04</td>
<td>0.07</td>
<td>NS</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>5.1±0.1</td>
<td>5.0±0.1</td>
<td>5.2±0.1</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Insulin (pmol/L)</td>
<td>106.5±11.7</td>
<td>119.8±21.1</td>
<td>113.4±17.9</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

*Values are the mean fasting values ± standard error of mean (SEM) as an estimate of the 6 h response to high carbohydrate/fat challenge meal (HCF MEAL) after 6-wk strawberry or placebo beverage consumption. **Values for IL-6, IL-1β and TNF-α after 6-wk strawberry (n=11) or placebo (n=11) due to insufficient sample for 2 subjects. hsCRP: high sensitivity C-reactive protein, PAI: plasminogen activator inhibitor, TNF: tumor necrosis factor, NS: not significant (p>0.05).

Table 6. Postprandial responses to high carbohydrate/fat ‘test’ meal (HCF MEAL) after 6-week of strawberry or placebo beverages

<table>
<thead>
<tr>
<th>Factor</th>
<th>0-wk HCF MEAL N=24</th>
<th>6-wk Placebo HCF MEAL N=12</th>
<th>6-wk Strawberry HCF MEAL N=12</th>
<th>p-value</th>
<th>Placebo vs. Strawberry</th>
</tr>
</thead>
<tbody>
<tr>
<td>hsCRP (mg/L)</td>
<td>3.1±0.1</td>
<td>3.3±0.2</td>
<td>3.0±0.2</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>IL-6 (ng/L)</td>
<td>3.1±0.2</td>
<td>2.3±0.4</td>
<td>2.2±0.4</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>PAI-1 (ng/L)</td>
<td>3.7±0.2</td>
<td>5.9±0.4**</td>
<td>4.3±0.3</td>
<td>0.002</td>
<td>NS</td>
</tr>
<tr>
<td>IL-1β (ng/L)</td>
<td>0.2±0.0</td>
<td>0.3±0.1</td>
<td>0.1±0.1</td>
<td>0.05</td>
<td>NS</td>
</tr>
<tr>
<td>TNF-α (ng/L)</td>
<td>1.1±0.0</td>
<td>1.1±0.0</td>
<td>1.2±0.0</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>5.5±0.1</td>
<td>5.4±0.1</td>
<td>5.3±0.1</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Insulin (pmol/L)</td>
<td>460.2±16.0</td>
<td>400.9±16.0</td>
<td>415.7±27.2</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

*Values are the least squares means (LSM) ± standard error of mean (SEM) as an estimate of the 6 h response to high carbohydrate/fat challenge meal (HCF MEAL) after 6-wk strawberry or placebo beverage consumption. **Values for IL-6, IL-1β and TNF-α after 6-wk strawberry (n=11) or placebo (n=11) due to insufficient sample for 2 subjects. HCF MEAL: high carbohydrate/fat meal, hsCRP: high sensitivity C-reactive protein, IL: interleukin, PAI: plasminogen activator inhibitor, TNF: tumor necrosis factor, NS: not significant (p>0.05).
ence was most notable 6 h after the meal challenge. At 6 h, PAI-1 concentrations increased ~60% from baseline (0 time, \( p = 0.002 \)) after the HCF meal when the placebo beverage vs strawberry beverage was consumed for 6 wk, which was significantly higher than that observed 6 h after the strawberry beverage (7.34 ± 0.67 ng/L vs 4.79 ± 0.58 ng/L, respectively, \( p = 0.006 \), Fig. 3). Pair-wise comparison of the HCF meal wk-0 postprandial response vs wk-6 postprandial response after strawberry intervention was not different; however, after placebo intervention, the PAI-1 response was significantly higher (\( p = 0.01 \)).

Changes in platelet aggregation after collagen and ADP or EPI stimulation were unremarkable in the subset of subjects studied (data not shown).

Correlations between Postprandial Responses of PAI-1 and Inflammatory Markers

At 0 wk (baseline), no significant correlations were apparent between PAI-1 and inflammatory markers after the HCF meal (\( p > 0.05 \)), although a modest positive relationship was noted for PAI-1 and hsCRP (\( r = 0.22, p = 0.06 \)). After 6-wk placebo or strawberry beverage consumption, the postprandial response of PAI-1 was positively correlated with IL-1\( \beta \) and TNF-\( \alpha \) in the placebo group only (\( r = 0.54 \) and 0.34, respectively, \( p < 0.05 \)).

Discussion

We previously showed that consuming a polyphenolic-rich strawberry (Fragaria) beverage, high in anthocyanins and other anti-oxidant compounds, concurrently with a high carbohydrate/fat (HCF) meal attenuated postprandial (fed-state) increases in oxidized low-density lipoprotein (oxLDL), insulin concentrations and biomarkers of inflammation com-
pared to a non-strawberry macro- and micro-nutrient placebo matched-control beverage. The present study is the first to show that chronic/6-wk daily consumption of strawberry attenuates meal-induced postprandial increases in pro-inflammatory and pro-thrombotic/hypo-fibrinolytic responses, which are major issues in the development of atherosclerotic cardiovascular disease. Daily consumption of a strawberry beverage (vs placebo beverage) for 6 wk, which added ~95 mg total strawberry phenols to subjects' diets per day, significantly attenuated HCF meal-induced postprandial increases in PAI-1 and IL-1β blood concentrations with moderate suppression of IL-6, although IL-6 rose significantly from baseline to 6 h after the HCF meal following the placebo whereas IL-6 remained relatively flat after the strawberry beverage from fasting to 6 h.

Our investigation aimed to address questions and test hypotheses related to inflammation, platelet aggregation/coagulation, and fibrinolysis/thrombosis in response to strawberry consumption as a model of a polyphenolic-, antioxidant-rich fruit. We assessed the effects of chronic strawberry feeding on these endpoints in the basal state (fasting measures) and when the body was in a state of physiological stress (postprandial/fed-state measures); with the latter suggested as a better indicator of disease risk.

An improved inflammatory and thrombotic status has been reported after chronic fruit and vegetable consumption as well as after chronic consumption of specific polyphenol-rich foods, including strawberry. In contrast to these reports, we observed no statistically significant changes in fasting indices of inflammation, platelet function or fibrinolytic activity; although marginal effects (p > 0.05-0.1) were evident for inflammatory indices. Comparing the present study to others, we suspect that our dose of strawberry polyphenols was too low to induce alterations in molecular targets that would result in reduced fasting inflammatory and thrombolytic factors. Changes in fasting lipids and oxidative stress markers after strawberry supplementation were observed when the dose was 2 to 4.5 times that used in the present study [250 g/d frozen strawberries for 3 wk and 454 g/d fresh strawberries for 4 wk] compared to our dose of ~100 g/d (1 serving) fresh strawberries. Our intentions were to test a practical dose of strawberries easily achieved on a daily basis; however, higher intake levels or concentrated sources may be required to shift homeostatic indices of disease risk.

An alternative explanation for our results of fasting variables may be related to the beverage formula, which was dairy-based. Others have suggested that diary proteins interfere with the absorption and bioactivity of polyphenols, as has been shown with tea and blueberry anti-oxidants, however, these suggested chemical phenomena translating to a lack of biological effect have not been confirmed.

The term ‘homeostatic flexibility’ is a relatively new concept combining ideas put forth related to metabolic flexibility and redox homeostasis. Our definition of homeostatic flexibility refers to the ability of biological systems to maintain or re-gain homeostatic balance after exposure to ‘stress’ or physiological insult. In the present study we used an HCF meal to induce acute oxidative and inflammatory stress to challenge and assess changes in biological response mechanisms which we previously have shown to be sensitive to concurrent strawberry polyphenol intake. We found that strawberry supplementation significantly attenuated the postprandial effects of the HCF meal challenge. Our data suggest that while no marked effects of the strawberry beverage intervention were apparent in fasting variables, chronic exposure to strawberries and their associated compounds, namely polyphenols, but possibly also including unidentified bioactive phytochemicals present in strawberries, positively influenced adaptive-stress response signals associated with inflammation and fibrinolytic activity.

PAI-1 attenuates fibrinolysis or the physiological breakdown of blood clots, making it a risk factor for myocardial infarction. Individuals who are obese and/or who have diabetes have increased levels of PAI-1 as well as decreased fibrinolytic activity and platelet aggregability. PAI-1 concentrations are increased with hyper glycemia, -insulinemia and -triacylglycerolemia, which all induce oxidative stress and concomitant increases in inflammatory markers. Hence, the lower postprandial PAI-1 concentrations observed in this study associated with daily strawberry intake suggest influences on oxidative stress-and/or inflammatory-regulated pathways. Candidate pathways include mitogen-activated protein kinase (MAPK) cascades, specifically extracellular signal-regulated kinase (ERK) and jun N-terminal kinase (JNK) signaling pathways, which are redox-sensitive and have been shown to be responsive to polyphenolic anti-oxidant flavonols, catechin and quercetin, in repressing PAI-1 gene expression. Additionally, consensus binding sites for transcription factors in the PAI-1 promoter region include activator protein (AP)-1 and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), the latter particularly known for its responsiveness to changes in cellular oxidative balance and induction of cytokine production. The
timing of our observed effects on PAI-1, IL-1β and IL-6 corroborate (6 h postprandially) NF-kB involvement and are supported by our significant correlations between PAI-1 and inflammatory markers. The present investigation supports links between oxidative, inflammatory and hemostatic processes and modulation by polyphenolic-structured and antioxidant-functioning plant compounds.

Conclusions

This original investigation provides novel insight into the relationship between plant foods, specifically strawberry, and cardiovascular health, and supports research showing that a diet high in fruits and vegetables reduces disease risk. We have now shown in both an acute and chronic experimental paradigm (present study) that strawberry consumption influences adaptive and compensatory oxidative and inflammatory responses that likely underpin the observed effects on insulin metabolism and fibrinolytic/thrombotic activity shown in the present study and by others. The molecular mechanisms supporting these findings are not well described nor are the optimal doses (servings per day) known for maximum benefit; there is therefore much opportunity for research in this area.

The present study used a feeding paradigm that examined effects on fasting as well as postprandial indices of disease risk, the latter reflecting responses to a meal challenge to examine effects on homeostatic flexibility. The data from this study contribute to a growing body of evidence of the clinically-meaningful endpoints sensitive to polyphenolic and antioxidant components of plant foods. The free-living study design further demonstrates that modest inclusion of fruit (1 serving per day) can have meaningful benefits. Collectively, our data, along with others, support the inclusion of polyphenolic-rich fruits in the diet regularly, as demonstrated with strawberry, for disease-risk reduction, particularly athero-thrombotic disease, and maintaining cardiovascular health.

Acknowledgements

The authors would like to acknowledge the contributions of Amanda Linares (A.L.) and Mandeep Cheema who coordinated the trial and performed the hsCRP and cytokine analyses; Prof. John C. Rutledge (J.R.) for mentoring and supporting C.E. financially and providing constructive feedback on the manuscript; Katarzyna Banaszewski who analyzed the phenols using a Triple Quadruple Mass Spectrometer and the vitamin C content of the beverages; Ravi Tadapani who analyzed the ORAC value of the strawberry beverages.

B.B.F and T.K. designed research; C.E., A.L. and I.E. conducted research; B.B.F. and I.E. analyzed data; C.E., I.E., T.K., and B.B.F wrote the paper; B.B.F and T.K. had final responsibility for content; all authors read and approved the final manuscript.

The funding for the study was provided by the California Strawberry Commission. C.E. also received support during the time of working on this manuscript from: The Richard A. and Nora Eccles Harrison Endowed Chair Fund in Diabetes Research (PI: J.R.); the UC Davis T32 Predoctoral Clinical Research Training Program Scholar Award (The NIH -NCRR and -Roadmap for Medical Research Grant Number ULI RR024146); and the UC Davis Professors for the Future Program (Predoctoral Fellowship Award).

Honorarium for invited talks on strawberry nutrition and health has been accepted by the corresponding author. No other financial relationship or conflicts of interest exist with the strawberry industry for any of the authors.

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