Original Research

Strawberry Modulates LDL Oxidation and Postprandial Lipemia in Response to High-Fat Meal in Overweight Hyperlipidemic Men and Women

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Background: Elevated levels of lipids, such as total cholesterol (TC), low-density lipoprotein cholesterol (LDL), and triglycerides (TG), are widely recognized as risk factors for cardiovascular disease (CVD). Oxidized LDL (OxLDL) is an emerging risk factor considered relevant in oxidative stress and endothelial dysfunction, which is implicated in the progression of CVD. Consumption of a diet rich in polyphenols may be cardioprotective through its impact on oxidative stress and protecting LDL from oxidation.

Objectives: This study was designed to test the ability of strawberry phenolic compounds to mitigate the postprandial effects of a high-fat meal on OxLDL as well as investigate the effects of phenolic compounds on lipid metabolism.

Methods: Twenty-four hyperlipidemic men and women (14 women, 10 men; mean age 50.9 ± SD 15 years) were recruited to participate in this randomized, single-blind, placebo-controlled, 12-wk crossover trial. After a 10-day run-in period, subjects consumed either an active strawberry beverage (Str; containing 10 g freeze-dried fruit) or a placebo (Pbo) beverage matched in energy and macronutrient composition for 6 weeks. Twice before randomization and once at the 6-week crossover point, subjects received either Str or Pbo with a high-fat challenge meal (HFM). TC, LDL, high-density lipoprotein cholesterol, TG, and OxLDL were measured at defined intervals for 6 h before and after HFM challenge. Fasting concentrations of blood variables at 0, 6, and 12 weeks were compared to assess chronic intake of Str or Pbo.

Results: After the HFM during the run-in period, TG and OxLDL were lower after Str than Pbo ($p = 0.005$, $p = 0.01$, and $p = 0.0008$, respectively). HFM responses after 6 weeks of Str versus Pbo resulted in decreased lipid levels and a sex by treatment interaction for OxLDL ($p =< 0.0001$, and $p = 0.0002$).

Conclusion: The present results support a role for strawberry in mitigating fed-state oxidative stressors that may contribute to atherogenesis.

INTRODUCTION

Cardiovascular disease (CVD) is the leading cause of morbidity and mortality and recent studies have established that the risk factors for it are similar worldwide [1,2]. Among the modifiable risk factors for CVD, dyslipidemia, hypertension, cigarette smoking, and diabetes mellitus are viewed as being of paramount importance. In addition to these factors, both obesity and the metabolic syndrome are also considered to be markers of high risk [3,4]. The adverse effects of all of these can be significantly ameliorated through changes in lifestyle that incorporate smoking cessation, losing weight, increasing physical activity, and consuming a diet rich in fruits, vegetables, and whole grains and low in saturated fat and *trans*-fat. Dietary recommendations are of particular interest because they have a multifaceted impact on the risk of CVD. An appropriate diet improves weight management in general, but specific constituents in the diet are likely to influence individual cardiovascular risk factors. For instance, dietary fiber improves lipid profiles and promotes glycemic

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control, while omega-3 fatty acids have been found to be beneficial in hypertriglyceridemia. Both clinical observations and epidemiologic studies support a role for phenolic compounds present in certain fruits and vegetables in prevention of CVD [5–7].

Among the common fruits and vegetables consumed in the United States, strawberries rank among those with the highest phenolic content [8,9] and antioxidant activity. The phenolic compounds in the strawberry include quercetin, ellagic acid, anthocyanins, catechins, and kaempferol. Strawberries are also important sources of vitamin C and glutathione, which are potent antioxidants. Thus, strawberries are a candidate food that should be included in a diet designed to promote cardiovascular health.

It is known that a high-fat meal (HFM) causes a postprandial lipemia that is accompanied by biological changes in the plasma and endothelium suggestive of oxidative stress [10,11]. In the study described in this paper, we tested the effect of strawberries on postprandial hyperlipidemia and oxidized low-density lipoprotein cholesterol (LDL) concentrations under 2 experimental conditions: (1) acutely, using a control meal with a strawberry or placebo beverage, and (2) chronically, using a control diet supplemented with the strawberry or placebo beverage for 6 weeks. The crossover design of the study also permitted us to determine the effect of the 6-week use of strawberries on fasting serum lipid and oxidative parameters. The study was placebo controlled, and the subjects were hyperlipidemic and overweight.

MATERIALS AND METHODS

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

Subjects

Twenty-six hyperlipidemic men and women (n = 16 females, 10 males) from the Sacramento, California, community and surrounding region were recruited using newspaper and online advertisements and local flyers. Those meeting the eligibility criteria were enrolled in the study after completing an informed consent form approved by the institutional review board. Subjects were required to have a body mass index between 25 and 33.5 kg/m^2, total cholesterol (TC) $<$300 mg/dL, fasting triglyceride (TG) $<$300 mg/dL, and LDL $<$180 mg/dL. Individuals with the following criteria were excluded: smoking; use of medications that would interfere with outcomes of the study (i.e., lipid-lowering medications, anti-inflammatory drugs, dietary supplements); known allergy or intolerance to strawberries; regular consumption of $>$2 servings per week of strawberries; or presence of documented atherosclerotic disease, chronic inflammatory diseases, diabetes mellitus, uncontrolled hypertension (blood pressure $>$140/90 mm Hg), or other systemic diseases. Two subjects dropped out of the study because of work commitments and data from them were not included in the analysis.

Table 1. Macronutrient Content of the Strawberry and Placebo Beverages

	Beverage	
	Strawberry (Str)	Placebo (Pbo)
Weight (g)	305.0	305.0
Calories (kcal)	248.2	246.8
Fat (g)	0.3	0.2
Protein (g)	7.8	7.9
Carbohydrates (g)	54.8	53.6
Fiber (g)	1.9	0.0
Sugar (g)	24.7	25.9

Study Design

This was a single-center, randomized, single-blind, placebo-controlled, 12-week crossover intervention trial, including 3 acute postprandial tests with a challenge HFM. Each phase of the trial (i.e., recruiting, screening, postprandial test days, beverage-pick-up days, and conclusion visits) took place at the National Institutes of Health–sponsored UC Davis Clinical and Translational Science Center Clinical Research Center at the UC Davis Medical Center–affiliated Northern California Veteran's Affairs Medical Center in Mather, California.

The main intervention in the 2 arms of the study was the daily consumption of a serving of an *active* strawberry beverage (Str) or a *placebo* strawberry-flavored beverage (Pbo). The Str contained a mixture of cultivated strawberry fruit provided by the California Strawberry Commission (Watsonville, CA) in freeze-dried form. The cultivated strawberry mixture comprised public and private varieties of California strawberries. The Str beverages contained 10 g/serving, which was equivalent to 110 g/d of fresh strawberries and delivered ~338 mg total phenolic compounds. The Pbo was prepared from non-strawberry ingredients to provide, as close as possible, the total energy (calories), macro- and micronutrient content, and fiber content, without the phytochemical composition of the Str (Table 1).

Subjects were randomized to 1 of 2 trial sequences: Sequence 1, Str►Pbo or Sequence 2, Pbo►Str, where ► denotes the crossover at 6 weeks (see Fig. 1, study schema). Subjects were transitioned immediately from one beverage to the next based on sequence randomization with no formal (off beverage) washout at crossover. This design was used in order to maintain the subjects' study routine of drinking beverages daily, with the goal of minimizing potential dietary fluctuations associated with taking subjects on and off beverage

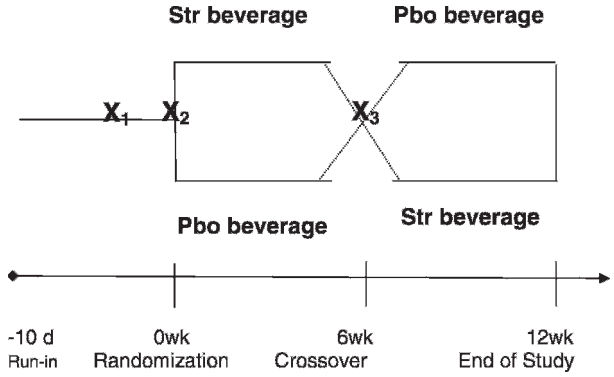

Fig. 1. Study schema: Strawberry (Str) and placebo (Pbo) beverages included in diets of free-living subjects for 6 weeks. Strawberry (10 g freeze-dried powder) intake equivalent to 110 g fresh weight (~2/3 c sliced). ''X'' denotes postprandial meal challenge consisting of high-fat meal with Str or Pbo beverage during run-in and Pbo beverage only at crossover.

intake. Throughout the study, subjects periodically completed food diaries, which were analyzed (Food Processor SQL Edition Version 9.6.2; ESHA Research, Salem, OR) to establish that there were no unanticipated changes in subjects' diets during the study period.

During the two 6-week feeding periods, subjects returned to the testing center at biweekly intervals to pick up the Str or Pbo beverages and for a brief assessment of study adherence. The end of the study evaluation included a short in-clinic visit for collection of a fasting blood sample, body weight check, vital signs, and debriefing.

Acute Effects of Str on Postprandial Lipemia and Oxidative Stress During Berry-Free Background Diet. The acute effects of consuming the Str were tested using a postprandial testing protocol (see Postprandial Testing Protocol) during the initial run-in period after a minimum ''berry-free'' period of 7 days. All subjects were given a high-fat test meal with either Str or Pbo. The HFMs (meals X_1 and X_2, Fig. 1) were given at intervals of 3 days and administration of the accompanied beverage was randomized. The HFM consisted of typical breakfast food items (i.e., bagel, cream cheese, whole milk, egg, margarine, cantaloupe) and reflects a meal of energy and macronutrient proportions typical of American dietary patterns and has been documented in our lab as well as others to induce lipemia, oxidative stress, and inflammatory stress [10,12]. The nutrient composition of the HFM plus Str or Pbo beverages is shown in Table 2 (meals analyzed using Food Processor SQL Edition Version 9.6.2).

Chronic Consumption of Str Beverage on Fasting and Postprandial Lipemia and Oxidative Stress. After the initial run-in period, the subjects were randomly assigned to either the Str group or the Pbo group (n = 12 in each). They

Table 2. Nutrient Breakdown of High-Fat Test Meal, Including Strawberry and Placebo Beverages

Meal + Strawberry Beverage (SM)		Meal + Placebo Beverage (PM)	
Nutrient	Value	Nutrient	Value
Calories (kcal)	962	Calories (kcal)	961
Calories from fat (kcal)	276	Calories from fat (kcal)	275
Calories from sat fat (kcal)	131	Calories from sat fat (kcal)	131
Protein (g)	36.5	Protein (g)	36.6
Carbohydrates (g)	135.5	Carbohydrates (g)	134.3
Dietary fiber (g)	6.1	Dietary fiber (g)	4.2
Total sugar (g)	50.6	Total sugar (g)	51.9
Fat (g)	30.7	Fat (g)	30.6
Saturated fat (g)	14.6	Saturated fat (g)	14.5
Mono fat (g)	9.2	Mono fat (g)	9.2
Poly fat (g)	2.5	Poly fat (g)	2.5
trans-Fatty acid (g)	0.2	*trans*-Fatty acid (g)	0.2
Cholesterol (mg)	272.1	Cholesterol (mg)	272.5
Vitamin C (mg)	53.1	Vitamin C (mg)	45.2
Vitamin E-α-tocopherol (mg)	1.4	Vitamin E-α-tocopherol (mg)	1.4
Calcium (mg)	817.9	Calcium (mg)	824.9
Copper (mg)	0.5	Copper (mg)	0.5
Iron (mg)	4.8	Iron (mg)	4.4
Phosphorous (mg)	558.9	Phosphorous (mg)	573.7
Zinc (mg)	5.9	Zinc (mg)	6.0
ω3 Fatty acid (g)	0.4	ω3 Fatty acid (g)	0.4
ω6 Fatty acid (g)	2.1	ω6 Fatty acid (g)	2.1
Potassium (mg)	1033.7	Potassium (mg)	1059.4
Sodium (mg)	1026.1	Sodium (mg)	1034.1

Mono = monounsaturated; poly = polyunsaturated.

consumed the respective beverage daily together with their regular diet that was otherwise berry-free for 6 weeks. At the end of 6 weeks, subjects returned to the laboratory for a fasting blood draw that was followed by an HFM (meal X_3 in Fig. 1). This protocol enabled us to compare the effects of a berry-plus (Str arm) and a berry-free (Pbo arm) diet on both fasting values and postprandial responses to a high-fat test meal. Both groups ($n = 12$ in each) had the HFM (meal X_3) with only the Pbo beverage. After completion of the HFM meal X_3 test, the groups were crossed over for a further 6 weeks of study in which the alternate beverage supplement was consumed. At the end of the second 6-week period, subjects returned to the laboratory for a final visit when blood was collected for measurement of fasting serum lipids and oxidative stress markers. Thus, all 24 subjects had fasting blood samples collected after 6 weeks of dietary supplementation with either the Str or Pbo beverages.

Postprandial Testing Protocol. The postprandial test was conducted according to standardized protocols [13], whereby subjects consumed a meal over a 20-minute period and blood samples were collected at timed intervals after meal consumption. Blood was collected at 0 time (fasting); 30, 60, 90, and 120 minutes (2 hours); and hourly thereafter to 6 hours, for a total of 9 draws per visit. Blood was processed for subsequent laboratory analysis of clinical endpoints including TC, TG, LDL, high-density lipoprotein cholesterol (HDL), insulin, and oxidized LDL (OxLDL) (see Analytical Methods).

Dietary Analysis. Each subject was required to keep food records throughout the course of the study. During the first 6 weeks of the study, subjects kept 3 records per week (2 weekdays and 1 weekend day) during weeks 0, 3, and 5. Week 0 records reflected their habits prior to any study-specified treatment. Weeks 3 and 5 records reflected the food consumed during the first diet sequence (Str►Pbo or Pbo►Str). For the second 6-week period, subjects kept 3-day food records for weeks 7, 9, and 11. These records reflected the food consumed during the second diet sequence (Pbo►Str or Str►Pbo, respectively). These records were checked for accuracy by the study coordinator and the subjects were consulted for more information when necessary. Food records were entered into Food Processor SQL Edition (Version 9.6.2) by nutrition student interns and checked for accuracy by the study dietitian.

Analytical Methods

Various analytical methods were employed to assess the effect of polyphenolic compounds in Str on fasting and postprandial concentrations of TC, LDL, HDL, TG, and OxLDL. Lipids were assessed by standard enzyme-based kits (PolyMedCo, Cortlandt Manor, NY) following protocols provided by the manufacturer. Measurement of OxLDL was determined by highly-sensitive enzyme-linked immunosorbent assay kits (Mercodia Inc., Uppsala, Sweden) following protocols provided by the manufacturer. Total phenols in strawberry powder were measured by the Folin-Ciocalteu assay [14].

Statistical Analysis

Data were analyzed by repeated-measures analysis of variance using PC-SAS (version 8; SAS Institute Inc., Cary, NC), GLM, and MIXED procedures. Absolute values were analyzed unless noted otherwise, in which case data were normalized (baseline subtracted) to each subjects' baseline/fasting values before analysis. Normalizing procedures were used only when individual variability in fasting values between treatments was significantly different ($p < 0.05$).

The postprandial responses with the 2 treatments (Str and Pbo beverages) were compared in terms of the least-squares mean (LSM) as a composite estimate of the 6-hour response.

The level used to determine statistical significance was $p < 0.05$.

RESULTS

Subject Characteristics

Twenty-six subjects were recruited for this study (10 males and 16 females). During the course of the study, 2 females dropped out, one due to the effects of caffeine withdrawal on postprandial testing days and the other due to an inability to meet the study commitments. A total of 24 subjects completed the study. The results reported are from the remaining 14 women and 10 men. The mean age for the group was 50.9 ± 15 years. The baseline body mass index (kg/m^2) for the group was 29.2 ± 2.3 kg/m^2. The baseline mean body weight, height, TC, TG, HDL, LDL, and glucose are listed in Table 3.

Acute Effects of Single Exposure of Str vs. Pbo on Response to HFM

There were significant increases in TG, HDL, and OxLDL in the postprandial state under both experimental conditions (time, $p < 0.0001$). However, the postprandial TG, HDL, and OxLDL changes were significantly lower when Str was consumed concurrently with the HFM vs. Pbo on a background diet devoid of berries ($p = 0.005$, $p = 0.003$, and $p = 0.0008$, respectively; Table 4, Figs. 2 and 3).

Sex also appeared to influence some of the postprandial responses. The postprandial HDL concentrations were lower in men compared to women (LSM: 37.6 ± 2.6 vs. 50.6 ± 2.2 mg/dL, respectively, $p = 0.0008$), although this was largely driven by lower HDL in men, in general. The postprandial changes in LDL concentrations were affected significantly by the beverage only in men (LSM as estimate of response after

Table 3. Subject Characteristics*

Characteristic	Measurement	Mean ± SD
Age	y	50.9 ± 15.0
Body weight	kg	86.6 ± 12.9
Height	cm	171.7 ± 9.8
BMI	kg/m^2	29.2 ± 2.3
TC	mg/dL (mmol/L)	210.3 ± 26.9 (5.5 ± 0.7)
LDL	mg/dL (mmol/L)	142.3 ± 28.3 (3.7 ± 0.7)
HDL	mg/dL (mmol/L)	49.1 ± 13.4 (1.3 ± 0.3)
TG	mg/dL (mmol/L)	103.4 ± 52.9 (1.2 ± 0.6)
Glucose	mg/dL (mmol/L)	86.5 ± 11.8 (4.8 ± 0.7)

* Characteristics for 24 subjects (10 male, 14 female) at the screening visit.
BMI = body mass index, HDL = high-density lipoprotein cholesterol, LDL = low-density lipoprotein cholesterol, TC = total cholesterol, TG = triglycerides.

Pbo vs. Str: 120.1 ± 0.8 vs. 122.8 ± 0.8 mg/dL, respectively, $p < 0.05$).

Concentrations of OxLDL were lower at all postprandial time points (180, 240, and 360 minutes, $p < 0.05$) after Str compared to Pbo (LSM: $p = 0.0008$, Table 4 and Fig. 3). The postprandial concentrations of OxLDL did not differ by treatment in women (LSM: 1.4 ± 1.9 vs. 1.2 ± 1.9 U/L for Pbo vs Str, respectively, p = NS), but was significantly reduced after Str compared to Pbo in men (LSM: −3.2 ± 2.3 vs. 11.2 ± 2.3 U/L, respectively, $p < 0.0001$). Thus, it appeared that men derived a greater benefit from the Str in protecting their LDL from oxidation (influence of sex interaction, $p = 0.001$),

Chronic Effect of 6-Week Str vs. Pbo on Fasting Clinical Endpoints

Fasting concentrations for TC, LDL, HDL, TG, and OxLDL were determined at randomization (week 0) and after consumption of Str or Pbo beverages for 6 weeks each. No significant treatment-related effects were observed on these fasting values (data not shown).

Chronic Effect of 6-Week Str or Pbo on Modulating Effects of HFM

The diets of the 2 groups of 12 subjects were supplemented with Str and Pbo for 6 weeks. At the end of this period, they were challenged with the HFM (X_3 in Fig. 1) together with the Pbo beverage only. The postprandial responses to the HFM after 6 weeks of Str (PMStr) or 6 weeks of Pbo (PMPbo) are shown in Table 5. Subjects consuming Str for 6 weeks as part of their usual diet had significantly lower mean cholesterol ($p < 0.0001$), LDL ($p = 0.0002$), and TG ($p < 0.0001$) concentrations in response to the HFM.

For OxLDL, 6-week Str vs. 6-week Pbo did not appear to influence OxLDL when only the absolute concentrations of OxLDL ($p > 0.05$) were examined; however, after controlling for variability in fasting OxLDL concentrations by normalizing, significant protection of LDL was apparent postprandially compared to baseline ($p < 0.0001$).

These changes also appeared to be influenced by sex. The data suggested that Str treatment was particularly beneficial for women when presented the HFM (PMStr, Fig. 4); whereas for men, just being in the study appeared to be beneficial (Fig. 5). Women started the study with a fairly neutral postprandial OxLDL response to the HFM. However, after 6 weeks of Str in their usual diet, they experienced a significant reduction in postprandial OxLDL in response to the HFM challenge compared to baseline measures (LSM: 1.4 ± 1.8 U/L at baseline vs. −12.1 ± 3.9 after 6 weeks of Str supplementation, $p = 0.002$). Concentrations of OxLDL declined by −4.6 ± 2.7 U/L ($p = 0.06$) after 6 weeks of Pbo. In contrast, men began the study with an exaggerated OxLDL response to the HFM (LSM,+11.2 ± 2.2 U/L [neutral = 0 U/L]) and, after 6 weeks in the study, men receiving either the Str or Pbo experienced significant improve ment in OxLDL status (LSM of 6-hour response for PMStr, −3.2 ± 3.0 U/L, PMBL vs. PMStr, $p = 0.0001$; and PMPbo, −5.7 ± 5.2 U/L, PMBL vs. PMPbo, $p = 0.003$, respectively).

Table 4. Postprandial Response to High-Fat Meal Challenge with Strawberry and Placebo Beverages*

	Standard (SI) Units	PMBL	SMBL	p Value PMBL vs. SMBL
TC	mg/dL (mmol/L)	194.5 ± 0.6 (5.04 ± 0.02)	193.3 ± 0.6 (5.00 ± 0.02)	0.14
LDL	mg/dL (mmol/L)	118.4 ± 0.5 (3.07 ± 0.02)	119.9 ± 0.5 (3.10 ± 0.02)	0.04
HDL	mg/dL (mmol/L)	44.4 ± 0.2 (1.15 ± 0.01)	43.8 ± 0.2 (1.13 ± 0.01)	0.003
TG	mg/dL (mmol/L)	135.7 ± 1.8 (1.53 ± 0.02)	130.8 ± 1.8 (1.48 ± 0.02)	0.006
OxLDL	U/L	72.0 ± 1.5	74.9 ± 1.5	0.17
OxLDL†	U/L	6.3 ± 1.5	−1.0 ± 1.5	0.0008

* Least-square means (±SEM) as an estimate of the 6-hour response in blood lipids and OxLDL after PMBL (HFM + placebo beverage during run-in) and SMBL (HFM + strawberry beverage during run-in) (n = 24). Significance at $p < 0.05$.
† Normalized to each subject's fasting concentration of OxLDL.
HDL = high-density lipoprotein cholesterol, HFM = high-fat meal, LDL= low-density lipoprotein cholesterol, OxLDL = oxidized LDL, PMBL = placebo meal during run-in, SMBL = strawberry meal during run-in, TC = total cholesterol, TG = triglycerides.

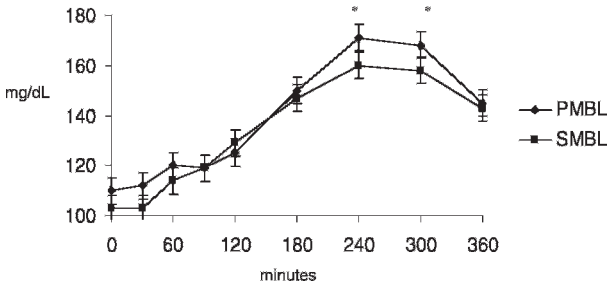

Fig. 2. Triglycerides: Triglyceride (TG) response to high-fat meal (HFM) with strawberry (SMBL) or placebo (PMBL) beverage. Values are the mean ± SEM. Figure represents the main effects of treatment and time ($p < 0.005$, $p < 0.0001$, respectively). Uncommon letters indicate differences in TG concentrations at specified time points ($p < 0.05$).

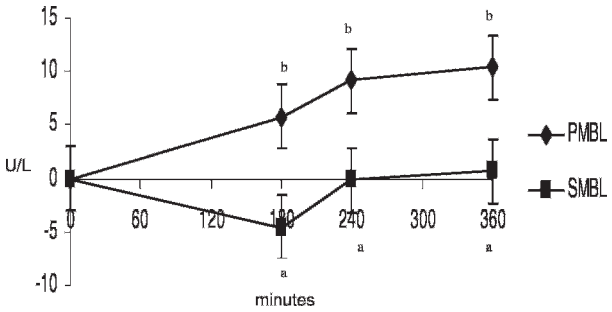

Fig. 3. LDL oxidation: Low-density lipoprotein cholesterol oxidation (OxLDL) in response to a high-fat meal (HFM) with strawberry (SMBL) or placebo (PMBL) beverage. Values are the mean ± SEM change in OxLDL from fasting to 180, 240, and 360 minutes. Figure represents the main effects of treatment ($p < 0.0001$). Uncommon letters indicate differences in OxLDL concentrations at specified time points ($p < 0.05$).

Food Record Analysis

During the course of the study food records were collected and analyzed. Men reported consuming more energy than women (2334 ± 157 vs. 1598 ± 133 kcal, respectively, $p = 0.002$), but energy density of the diet did not differ significantly (1.10 ± 0.06 vs. 1.13 ± 0.07 kcal/g, respectively). No treatment- or time-specific differences were observed for energy or macronutrient intake during the study ($p > 0.05$). However, a change in the intake of some micronutrients was detected among men from study entry to the end, whereas women maintained a fairly stable diet. Specifically, men reported consuming more arginine-containing foods during Pbo and Str interventions compared to baseline food intake assessments (2.7 ± 0.2 and 2.5 ± 0.2 vs. 1.6 ± 0.3 g/d, respectively, $p < 0.05$ for baseline vs. interventions). Vitamin C intake was lower during Pbo intervention compared to baseline intake (88.3 ± 8.3 vs. 114.5 ± 11.1 mg/d, respectively, $p < 0.05$), but was not different from intake during the Str intervention (107.2 ± 9.2 mg/d). Use of beta carotene–containing foods tended to increase during Pbo and Str interventions compared to baseline food intake assessments (3402 ± 543 and 3691 ± 606 vs. 1370 ± 856 μg/d, respectively, $p < 0.05$ for baseline vs interventions).

DISCUSSION

The purpose of our study was to examine the effects of strawberry consumption on circulating concentrations of lipids and LDL oxidation, a marker of oxidative stress, over both long-term (i.e., 6-week) and short-term periods after an HFM challenge in an overweight, hyperlipidemic population. The study aimed to answer 3 main questions. (1) Does Str compared to Pbo beverage alleviate the acute effects of a ''challenge'' HFM on lipids and LDL oxidation? (2) Does Str compared to Pbo beverages included in the usual diet of individuals for 6 weeks provide protection from the acute effects of a high-fat HFM on lipids and LDL oxidation? (3) Does 6-week consumption of Str improve fasting lipid and

Table 5. Postprandial Response to High-Fat Meal Challenge after 6-Week Daily Consumption of Strawberry or Placebo Beverages*

	Standard (SI) Units	PM at Baseline 0 Week	PM after 6 Wk Str in the Diet	PM after 6 Wk Pbo in the Diet	*p* Value 6 Wk PMStr vs. PMPbo
TC	mg/dL (mmol/L)	194.5 ± 0.7 (5.04 ± 0.02)	193.7 ± 1.2 (5.04 ± 0.03)	206.0 ± 1.4 (5.30 ± 0.04)	<0.0001
LDL	mg/dL (mmol/L)	118.4 ± 0.5 (3.07 ± 0.02)	122.0 ± 0.9 (3.19 ± 0.02)	129.6 ± 1.1 (3.34 ± 0.03)	<0.0001
HDL	mg/dL (mmol/L)	44.4 ± 0.2 (1.15 ± 0.01)	43.2 ± 0.3 (1.13 ± 0.01)	45.1 ± 0.4 (1.17 ± 0.01)	0.0005
TG	mg/dL (mmol/L)	135.7 ± 1.5 (1.53 ± 0.02)	137.1 ± 2.7 (1.58 ± 0.03)	155.4 ± 3.1 (1.67 ± 0.04)	<0.0001
OxLDL	U/L	72.0 ± 1.4	76.1 ± 2.4	73.9 ± 2.8	0.58
OxLDL†	U/L	6.3 ± 1.5	−7.7 ± 2.4	−5.2 ± 2.9	0.57

* Least-square means (±SEM) as an estimate of the 6-hour response in blood lipids and OxLDL after HFM at baseline (n = 24) and after 6 weeks (n = 12 each). Significance at $p < 0.05$.

† Normalized to each subject's fasting concentration of OxLDL.

HDL = high-density lipoprotein cholesterol, HFM = high-fat meal, LDL= low-density lipoprotein cholesterol, OxLDL = oxidized LDL, Pbo = placebo, PM = placebo meal, Str = strawberry beverage, TC = total cholesterol, TG = triglycerides.

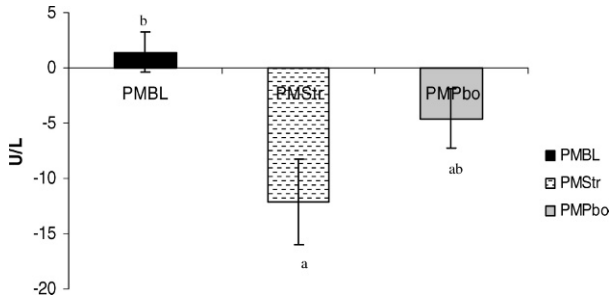

Fig. 4. LDL oxidation—women: Low-density lipoprotein cholesterol oxidation (OxLDL) in response to a high-fat meal (HFM) with placebo beverage during run-in (PMBL) and after 6 weeks of daily strawberry beverage (PMStr) or after 6 weeks of Pbo beverage (PMPbo). Values are the least-squares mean ± SEM of the 6-hour response, representing change in OxLDL from fasting to 360 minutes. Figure represents the main effects of sex by treatment interaction ($p < 0.007$). Uncommon letters indicate differences in OxLDL concentrations among treatments ($p < 0.05$).

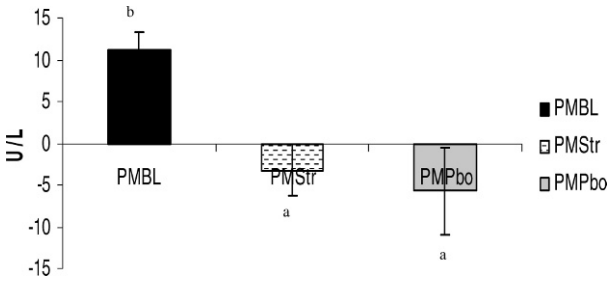

Fig. 5. LDL oxidation—men: Low-density lipoprotein cholesterol oxidation (OxLDL) in response to a high-fat meal (HFM) with placebo beverage during run-in (PMBL) and after 6 weeks of daily strawberry beverage (PMStr) or after 6 weeks of Pbo beverage (PMPbo). Values are the least-squares mean ± SEM of the 6-hour response, representing change in OxLDL from fasting to 360 minutes. Figure represents the main effects of sex by treatment interaction ($p < 0.007$). Uncommon letters indicate differences in OxLDL concentrations among treatments ($p < 0.05$).

oxidative stress endpoints in overweight hyperlipidemic subjects compared to Pbo?

Our results support the hypothesis that strawberry phenolic compounds attenuate postprandial lipemia and LDL oxidation when consumed with a fat-containing meal. TG concentrations increased significantly from baseline after both challenge meals (PMBL vs. SMBL), reaching peak concentrations around 4 hours; however, TG concentrations were lower after the Str-accompanied meal compared to Pbo during peak lipemia (at 4- and 5-hour time points), although statistically, marginally lower ($p = 0.07$ and $p = 0.08$, respectively) at these time points. This translated to a calculated 4% decrease in the overall LSM response over the 6-hour study period. During this same time frame, the concentration of OxLDL was decreased ~116% with the Str versus Pbo beverage with the challenge HFM. The 6-hour response indicated that strawberry blocked the meal-induced increase in OxLDL. This acute protection is likely due to the antioxidant effects of the strawberry phytonutrients. Previous work has shown that dietary phenolic compounds can bind human LDL [15]. Phenolic compounds that bind LDL are likely to provide a relative protection of LDL from reactive oxygen species through their peroxyl-scavenging activity [16]. Preservation of protein thiols and reduced malondialdehyde concentrations after a strawberry- vs. oat bran bread–supplemented portfolio diet further supports an antioxidant protection associated with strawberries [17]. The postprandial state is critical in atherogenesis. As OxLDL is an emerging risk factor in the progression of atherosclerosis, these data support a role for strawberry and other phenolic-rich foods in reducing risk for CVD.

The study also showed that strawberry consumed daily in the form of a beverage for 6 weeks had a persistent beneficial effect on postprandial lipemia. TC, TG, and LDL levels were 5%, 14%, and 5% lower, respectively, in subjects consuming the Str vs. Pbo beverages daily. Strawberry intake also reduced concentrations of OxLDL; however, this strawberry-specific benefit was most apparent in women, as men experienced improvement in OxLDL concentrations regardless of treatment assignment. One reason for this might be that men experienced an ''improvement'' in their diet during the study, in general, compared to women, whose habitual intake was more comparable to the higher phenolic content of the strawberry-supplemented study diet. Analysis of food records suggested that men increased their intake of beta-carotene– and arginine-rich foods while in the study. Beta-carotene intake increased 2.5-fold and arginine intake increased 1.5-fold. It is therefore possible that the beneficial effects specific to the strawberry treatment were masked by the overall dietary improvement in men. Other factors that might account for the variable sex response could be differences in the dose per kilogram of body weight of strawberry phenolic compounds, and/or sex-specific differences in kinetic variables. The kinetic profile of various polyphenolics from food sources, extracts, and aglycone preparations has been described [18], yet sex-specific differences in the bioavailability and kinetics of polyphenolics are not clear. Likewise, dose-response effects of food-associated polyphenolics are not well described. Future studies addressing dose and kinetic variables of bioactive phenolic compounds in strawberry relative to subject characteristics will help elucidate these findings.

In addition to determining the acute and persistent effects of strawberry in the fed state, we also assessed the effects of strawberry on fasting laboratory parameters. In contrast to our postprandial findings, we did not observe a treatment-related effect on fasting lipids or OxLDL at the doses provided in our study population. For 6 weeks, subjects consumed a beverage daily containing 10 g of dried strawberry powder, which was the equivalent of ~110 g of fresh weight strawberry (91%

water content) and delivered ~338 mg total phenols. At this dose level and in a free-living setting with subjects exhibiting many of the risk factors for metabolic syndrome, significant alterations in fasting values of these parameters may not have been expected. Preliminary work in our lab with an aqueous extract of the strawberry powder using a rabbit aorta model of endothelium-dependent relaxation indicated that our dose was active [19]; however, these dose-response data may be more consistent with bioactivity that we observed in the postprandial state, since other than quercetin, most water-soluble phenolic compounds are metabolized and cleared by 8–12 hours postprandially [18]. To shift homeostasis in a population that this subject group represents, our modest intervention may require much longer than 6 weeks for an effect to be apparent [20,21]. More aggressive dietary approaches with increased phenolic compound intake may show changes within this short time frame.

Our results support the role of polyphenolic compounds as potent antioxidants that may help achieve a lowered risk for CVD, particularly through their action in mitigating fed state oxidative stressors that contribute to atherogenesis. In a single day, the systemic stress of lipemia and redox imbalance may seem trivial. Over time, however, these daily insults can lead to complicated atherosclerosis, contributing to the more than 829,000 deaths a year due to diseases of the heart and vasculature [1]. Strawberries, like most plant foods, are rich sources of essential and nonessential bioactive compounds. Strawberries contain a collection of phenolic compounds that have important benefits for CVD risk reduction.

Practical Implications

These data provide an interesting perspective for advising individuals on food choice when consuming a moderate- to high-fat meal is unavoidable. In those times and as the offerings permit, consuming fruits rich in polyphenolic compounds, such as strawberries and other berries, with the meal or as a dessert might be one way of modifying the unfavorable oxidative stress–related events associated with postprandial lipemia. Given the fat content of the current Western diet, regular consumption of phenolic-rich foods, particularly in conjunction with meals, appears to be a prudent strategy to maintain oxidative balance.

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