Incorporating freeze-dried strawberry powder into a high-fat meal does not alter postprandial vascular function or blood markers of cardiovascular disease risk: a randomized controlled trial[1–3]

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ABSTRACT

Background: Postprandial dysmetabolism—an exaggerated spike in triglycerides, glucose, and insulin—increases cardiovascular disease risk by inducing oxidative stress, inflammation, and endothelial dysfunction. Polyphenol-rich foods may blunt these effects when they are incorporated into a high-fat, calorie-dense meal. Strawberries are a rich source of polyphenols, but there is little research on their postprandial effects.

Objective: This study was designed to investigate the effect of adding 40 g freeze-dried strawberry powder (∼1 lb. or 0.45 kg fresh strawberries) to a high-fat (50 g total fat) meal on postprandial vascular function, as well as triglyceride, glucose, and insulin responses.

Design: Healthy, overweight or obese [mean ± SEM body mass index (in kg/m^2): 31 ± 0.5] adults (mean ± SEM age: 28 ± 2 y; 17 men and 13 women) consumed a control meal and a strawberry meal in a randomized crossover design. Testing sessions were separated by ≥1 wk for men and ∼1 mo for women to control for hormonal variations. Blood samples were obtained before the meal and 0.5, 1, 2, and 4 h after the meal. Central blood pressure and arterial stiffness indexes were measured at baseline and 2 and 4 h postmeal with the use of pulse waveform analysis.

Results: There were no significant differences between the strawberry and control meals for any outcomes. Consumption of either meal significantly decreased the augmentation index at 2 and 4 h ($P < 0.002$) and significantly increased triglycerides, insulin, and glucose at all time points ($P < 0.001$) relative to baseline.

Conclusions: The strawberry intervention did not alter vascular function or attenuate postprandial metabolic derangements in triglycerides, glucose, or insulin relative to the control meal. Additional research is needed to clarify whether strawberries or other polyphenol-rich interventions improve postprandial responses, and future studies should take into account the acute meal-induced improvements in measures of vascular function. This trial was registered at clinicaltrials.gov as NCT01989637. *Am J Clin Nutr* 2017;105:313–22.

Keywords: augmentation index, cardiovascular disease, hyperlipidemia, phytochemicals, postprandial dysmetabolism

INTRODUCTION

Postprandial dysmetabolism is characterized by large spikes in and delayed clearance of triglycerides, glucose, and insulin. This causes oxidative stress and transiently impairs vascular function (1, 2). Over time, these repeated insults to the endothelium promote atherogenesis (3, 4). Plant-based whole foods are recommended for reducing cardiovascular disease (CVD)[9] risk (5) and provide bioactive polyphenols. Effects on postprandial metabolism may be one mechanism by which polyphenols improve health (6). However, it has yet to be established whether incorporating polyphenol-rich plant-based foods and/or beverages into a high-fat meal can attenuate postprandial dysmetabolism and/or favorably alter acute changes in vascular function.

Previous studies have demonstrated promising results for certain interventions (e.g., spices, berries, tea, and red wine), but evidence remains limited. For instance, in overweight men, incorporating 14 g antioxidant-rich spices into a high-fat meal significantly attenuated postprandial triglyceride responses (7, 8), potentially via inhibition of lipase enzymes. However, this effect has yet to be conclusively demonstrated with other interventions.

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[3] Supplemental Tables 1–5 are available from the "Online Supporting Material" link in the online posting of the article and from the same link in the online table of contents at http://ajcn.nutrition.org.

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[9] Abbreviations used: AI, augmentation index; AI@75, augmentation index normalized to a heart rate of 75 beats/min; AP, augmentation pressure; CRC, clinical research center; CRP, C-reactive protein; CVD, cardiovascular disease; PL, pancreatic lipase; PWV, pulse wave velocity; 4-NPB, 4-nitrophenyl butyrate.

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Berries have been found to improve the postprandial glycemic profile (9–11), but previous studies have been limited primarily to carbohydrate-based meals. In addition, although there is evidence that polyphenol-rich interventions attenuate transient postprandial impairments in endothelium-dependent vascular function (12), few studies have evaluated whether central blood pressure and indexes of arterial stiffness can be modified in this context.

Strawberries are a rich source of numerous polyphenols and other bioactive compounds (13, 14), and are one of the most commonly consumed fruits in the United States (15). Longer-term strawberry supplementation has been shown to improve multiple CVD risk factors (16–25), but less is known about the acute effects of strawberries in the context of a fat-containing meal. A modest attenuation of the 6-h postprandial triglyceride, insulin, and oxidized LDL response was found in overweight hyperlipidemic adults when 10 g freeze-dried strawberry powder was incorporated into a meal with 30 g total fat (26, 27). In a subsequent study, incorporating 40 g freeze-dried strawberry powder into a high-carbohydrate meal with 25 g total fat significantly reduced the 6-h insulin response in individuals with insulin resistance, but had no effect on postprandial glucose, triglycerides, or oxidized LDL (28). Effects on postprandial central blood pressure and arterial stiffness have yet to be tested, and additional research is needed to evaluate the potential of strawberries to mitigate the metabolic consequences of consuming a high-fat meal in different study populations.

The present study (NCT01989637) was designed to investigate whether incorporating 40 g freeze-dried strawberry powder into a high-fat (50 g total fat) meal could improve postprandial responses relative to a macro- and micronutrient-matched control meal in overweight or obese but otherwise healthy adults. We hypothesized that this 40-g dose of freeze-dried strawberry powder—which is equivalent to ~1 pound or 0.45 kg fresh strawberries and provides a substantial amount of polyphenols—would improve vascular function by attenuating the exaggerated spikes in triglycerides, glucose, insulin, and markers of oxidative stress caused by the high-fat meal.

METHODS

Study population

Men and women 20–50 y of age and free of any serious illness were recruited for the study. Other inclusion criteria consisted of a BMI (in kg/m^2) of 28–39, resting blood pressure <160/100 mm Hg, fasting triglycerides <350 mg/dL, and fasting LDL <160 mg/dL. Exclusion criteria were acute or chronic inflammatory conditions; liver or kidney dysfunction; a history of heart disease; use of tobacco products; intolerance for high-fat meals; allergies or sensitivities to wheat or strawberries; and use of nonsteroidal anti-inflammatories, immunosuppressants, or medications or supplements for elevated lipids, blood pressure, or glucose.

Participant recruitment

Participants were recruited from July 2013 to March 2014 via fliers in the community, campus e-mail lists, and a university research website. Potential subjects sent an e-mail or called to indicate interest in participating in the study, and were then given additional information about the study. If interested, they were asked a series of medical history and lifestyle questions to screen for eligibility. A schematic of participant recruitment for the study is provided in **Figure 1**. Of the 238 initial respondents who provided contact information, 124 elected to complete the initial screening questions by telephone. Forty-four of these volunteers met study criteria and completed a screening appointment at the Pennsylvania State University Clinical Research Center (CRC) to verify eligibility. After written informed consent was obtained, a urine pregnancy test was performed for premenopausal women, and a blood sample was drawn for a complete blood count and standard chemistry profile (lipid panel, glucose, and liver and kidney function) to rule out the presence of illness (autoimmune disease, cancer, and immunodeficiency). Blood pressure was measured according to the guidelines of the Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (29). Briefly, after a 5-min seated rest, 3 readings were taken by nurses in a controlled environment with the use of a calibrated mercury sphygmomanometer. The mean of the last 2 readings was used to determine eligibility. Body weight and height were measured (without shoes and in light clothing) to calculate BMI. Of the 44 individuals who were screened, 34 met eligibility criteria and were enrolled in the study. Four participants withdrew during the study. Thus, data are reported for 30 participants (n = 17 men and n = 13 women). A balanced randomization scheme with a block size of 6 was developed in advance (by ACS-R) with the use of an online randomization generator, and subjects were assigned to a treatment sequence at enrollment (by CKR). Sample size for this preliminary study was based on earlier studies of postprandial metabolic responses that reported a significant reduction in triglycerides with a similar polyphenol-rich intervention (8, 26). No power calculation was performed for our primary outcome of postprandial vascular function because of the scarcity of published studies with the postprandial use of brachial pulse wave analysis to assess the effects of incorporating a polyphenol-rich intervention into a meal. The study was conducted in accordance with the Helsinki Declaration, and was approved by the Institutional Review Board of the Pennsylvania State University.

Study design and intervention

This was a randomized, placebo-controlled, 2-period crossover study in which participants completed 2 postprandial challenges that consisted of single-day visits that lasted ~5 h each. The 2 test conditions were as follows: *1*) a high-fat control meal with a strawberry-flavored placebo powder that was devoid of strawberry bioactives, and *2*) the same high-fat meal with 40 g freeze-dried strawberry powder. The control and strawberry meals were matched for macro- and micronutrients (**Table 1**), and differed only in polyphenol and vitamin C content. The meals consisted of 2 cheese blintzes (248 g) with heavy whipped cream (12 g) and strawberry-flavored syrup (50 g), a hard-boiled egg, and bacon (24 g cooked). The placebo and strawberry powders were mixed into the cheese filling of the blintzes. All meal components were weighed in advance and prepared fresh before each day of testing. No heat was applied to the powders during food preparation to avoid any potential degradation of polyphenols. The control and strawberry meals were matched for taste and appearance to maintain the blinding of participants and researchers to treatment sequence. Both the freeze-dried strawberry powder and the control powder were provided and

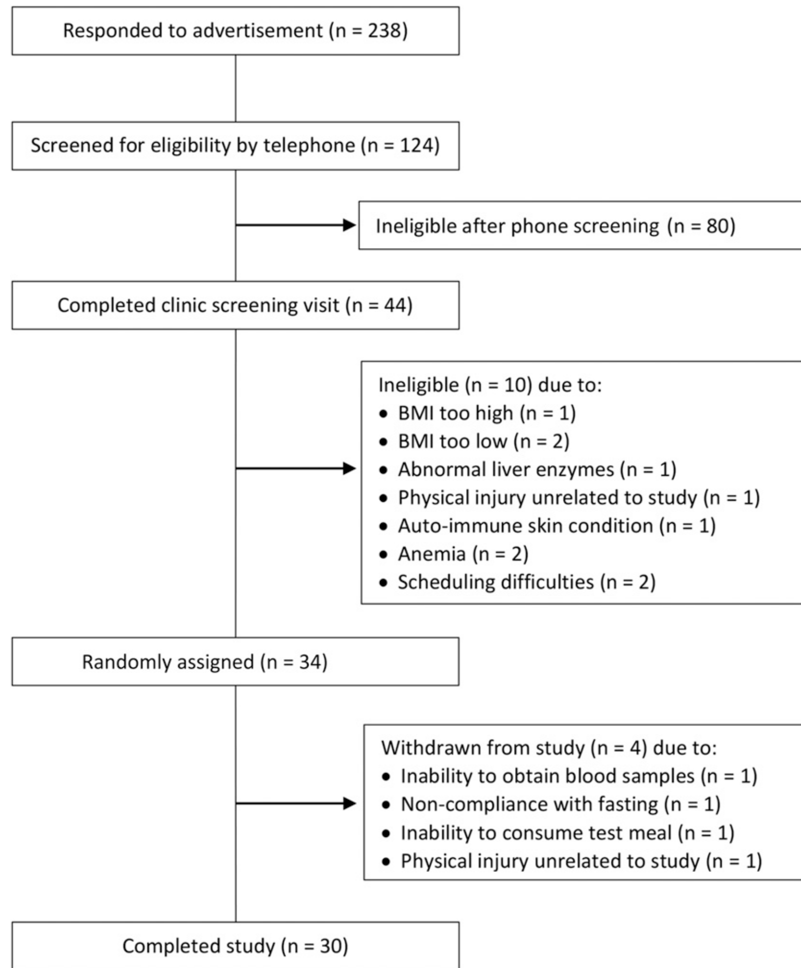

FIGURE 1 Schematic of participant recruitment and reasons for exclusion.

analyzed by the California Strawberry Commission. The detailed nutrient profile and polyphenol content of the strawberry and control powders are available in **Supplemental Tables 1** and **2**.

Postprandial challenges were separated by a minimum of 1 wk for men. Visits for female participants were scheduled during the first 7 d of the menstrual cycle to avoid hormonal effects on vascular measures, and were therefore separated by ∼1 mo. All postprandial challenges were conducted at the CRC according to standardized protocols. During the 48 h before testing visits, participants were instructed to avoid high-antioxidant foods (berries, cocoa and chocolate), strenuous exercise, and alcohol; refrain from taking pain relievers, vitamins, or minerals; and limit their intake of coffee and tea to ≤1 cup/d. Testing visits were conducted after an overnight fast (no food or drink other than water for 12 h).

At each visit, baseline vascular function testing was performed before an intravenous catheter was established for blood sampling. A topical skin anesthetic was offered to numb the area before catheter insertion. After baseline blood sample collection, participants were asked to consume the control or strawberry meal within ∼15 min. No food or drinks (other than water) were allowed for the remainder of the testing period. Additional blood samples and vascular function measures were taken at prespecified time intervals relative to when the meal was finished; blood samples were collected at 0.5, 1, 2, and 4 h postmeal, and vascular function measures were performed at 2 and 4 h after the meal. At the end of the 4-h period, the catheter was removed and participants were briefly evaluated for safety before leaving the CRC. Study procedures began in August 2013 and were completed by May 2014.

TABLE 1
Nutrient composition of test meals

	Control meal	Strawberry meal
Calories, kcal	1000	1000
Calories from fat, kcal	450	450
Total fat, g	50	50
Saturated fat, g	25	25
trans Fat, g	0.5	0.5
Cholesterol, mg	435	435
Carbohydrate, g	106	105
Fiber, g	7	7
Protein, g	30	32
Sodium, mg	1390	1320
Vitamin C, % daily value	2	380

Vascular function measures

Vascular function, in terms of central blood pressure and arterial stiffness indexes, was assessed with the use of the SphygmoCor

System pulse waveform analysis (AtCor Medical). All measurements were performed in a temperature-controlled, quiet, dimly lit room.

Pulse wave analysis: central (aortic) blood pressure and augmentation index

After a 5 min seated rest, central pressures and wave reflection characteristics [i.e., augmentation pressure (AP) and the augmentation index (AI)] were derived from brachial pressure waveforms with the use of a generalized transfer function that is considered by the US FDA to be substantially equivalent to generalized transfer functions for radial tonometry that have been validated against an indwelling catheter (30–32). At each time point, 3 pulse wave analysis measurements were taken while following the guidelines of the Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (29), with 1 min between each reading. The last 2 pulse wave analysis results were averaged and used for analysis. An augmentation index normalized to a heart rate of 75 beats/min (AI@75) was used to correct for the independent inverse effect of heart rate on augmentation of the pulse wave form (33).

Pulse wave velocity

Aortic stiffness was assessed by carotid-femoral pulse wave velocity (PWV). Carotid and femoral arterial pressure waveforms were measured simultaneously via an applanation tonometry sensor manually held in place above the right common carotid artery and a blood pressure cuff placed on the right femoral artery. Distance measurements were taken from the sternal notch to the carotid artery, from the sternal notch to the top of the femoral cuff, and from the femoral artery to the top of the femoral cuff. Based on these measurements, the SphygmoCor System automatically calculates the distance traveled by the pulse wave from the carotid artery to the femoral artery. Transit time between the carotid and femoral pressure waves is determined by the SphygmoCor System with the use of the foot-to-foot method (34). PWV is then calculated as distance over transit time. At each time point, 2 PWV measurements were obtained in the supine position, with 1 min between readings, and averaged for analysis.

Blood sample collection and assay methods

Blood drawn into anticoagulant-coated tubes containing lithium heparin and EDTA was immediately centrifuged for 15 min at $1500 \times g$ at room temperature. Blood drawn into serum separator tubes was allowed to clot for 30 min before centrifugation. Total cholesterol and triglycerides were measured by enzymatic procedures (Quest Diagnostics; CV <2% for both). HDL was estimated according to the modified heparin-manganese procedure (CV <2%). Fasting LDL was calculated with the use of the Friedewald equation [LDL = TC − (HDL + TG/5), where TC is total cholesterol and TG is triglyceride]. Postprandial LDL was not determined, because calculated values are not accurate during nonfasting conditions. Insulin was measured by radioimmunoassay (Quest Diagnostics). Glucose was determined by spectrophotometry procedures (Quest Diagnostics). Serum high-sensitivity C-reactive protein (CRP) was measured by latex-enhanced immunonephelometry (Quest Diagnostics; assay CV <8%). For other endpoints, aliquots of serum and plasma were stored at −80°C for batch analysis.

Biomarkers of oxidative stress

Malondialdehyde was measured in plasma from EDTA-coated tubes with the use of the thiobarbituric acid–reactive substances assay (Cayman Chemical Co.; assay CV <6%). Oxidized LDL was measured in plasma containing EDTA by ELISA (Mercodia; assay CV <8%).

Pancreatic lipase inhibition

Extracts of the freeze-dried strawberry powder were prepared with 10 volumes of acetone:water:acetic acid (80%:20%:0.1%, vol:vol:vol) overnight. The organic solvent was removed under vacuum and the remaining aqueous solution was freeze-dried. Porcine type II pancreatic lipase (PL) and 98% 4-nitrophenyl butyrate (4-NPB) were purchased from Sigma-Aldrich. Stock solutions were prepared in dimethyl-sulfoxide (EMD Chemicals) and stored at −20°C.

Inhibition of PL by the freeze-dried strawberry powder was tested by monitoring the cleavage of 4-NPB to release 4-nitrophenol. PL was suspended in water (10 mg/mL) and incubated at 37°C for 5 min. The solution was centrifuged for 5 min at $664 \times g$ at room temperature, and the supernatant was then used as the enzyme source for subsequent experiments. For each experiment, the PL supernatant was diluted 1:50 in buffer solution (20 mmol/L Tris-HCl and 1.3 mmol/L $CaCl_2$, pH = 8.0). Freeze-dried strawberry powder extracts (0–200 μg/mL) were combined with PL, and 4-NPB (0.2 mmol/L) was added to start the reaction. After 10 min of incubation at 37°C, absorbance was read at 400 nm. Analyses were performed in triplicate. Assay CV was <10%.

Statistical analyses

All statistical analyses were performed with the use of SAS version 9.3. Independent 2-sample t tests were used to assess sex differences at screening (PROC TTEST). Paired t tests (PROC TTEST) were used to compare the premeal baseline characteristics of participants at the strawberry and control meal visits. Postprandial change scores were calculated by subtracting fasting baseline values for each visit from postmeal values. AUC was calculated for postprandial insulin, glucose, and triglyceride responses with the use of the trapezoidal rule. The Matsuda insulin sensitivity index was calculated with the use of the formula developed by Matsuda and DeFronzo (35). Participants with any missing postprandial insulin values because of hemolysis (n = 9) were removed before the calculation of insulin AUC and the Matsuda index. Outcomes were assessed for normality (PROC UNIVARIATE), and positively skewed variables (skew >1; triglycerides, glucose, insulin, glucose AUC, insulin AUC, Matsuda index, central systolic blood pressure, and PWV) were log transformed for analysis. Two participants experienced symptoms of a vasovagal reaction (acute blood pressure decrease and lightheadedness) in response to blood sampling before the postprandial SphygmoCor measurements and were excluded from analyses of vascular outcomes. Two participants with acute inflammation (i.e., CRP >9.5 mg/L) were excluded from all analyses to ensure that the analysis was performed on

the target population of healthy overweight adults. One participant with fasting triglycerides >350 mg/dL before consuming the test meal was excluded from analyses of blood outcomes because he did not meet specified screening criteria (i.e., triglycerides <350 mg/dL). The mixed-models procedure (PROC MIXED) was used to test the effects of meal, time point, and their interaction on outcome measures. Baseline values were included as covariates. Period effects were also assessed to evaluate any habituation to testing and were found to be universally nonsignificant. Selection of model covariance structures was based on optimizing fit statistics (evaluated as lowest Bayesian Information Criterion). Models for postprandial metabolic effects (triglycerides, glucose, and insulin) used a doubly repeated covariance structure that was unstructured for time point and used compound symmetry for period effects. Compound symmetry was used to model both period and time point for all vascular endpoints other than AP and PWV. Models for AP and PWV used a doubly repeated covariance structure that was unstructured for time point and used compound symmetry for period. Compound symmetry was used to model treatment effects for changes in markers of oxidative stress (oxidized LDL and malondialdehyde), which were measured at a single postprandial time point, as well as the Matsuda index and AUC values for insulin, glucose, and triglycerides. Means are reported as least squares means ± SEMs. For all tests, α was set at 0.05.

Unblinded exploratory analyses were also conducted to identify potential predictors (i.e., BMI, sex, and age) of participants' general responsiveness to the strawberry intervention. Participants were designated as "nonresponders" or "responders" based on their response to the strawberry intervention. Individuals who exhibited the hypothesized beneficial effects of the strawberry intervention for glucose, insulin, triglyceride, AP, or AI@75 responses were considered to be responders. These continuous variables were dichotomized by calculating the maximum percentage increase after each meal. For triglycerides, this was calculated as the percentage increase from baseline to the 4-h time point. Because there tends to be more variation in the timing of the maximal glucose and insulin response between individuals, the percentage increase in insulin was calculated based on the change from baseline to the maximum response in each participant, and the percentage increase in glucose was calculated as the change from the minimum value to the maximum response in each participant. If the increase after the control meal was larger than the increase after the strawberry meal, the participant was deemed to be a responder for glucose or insulin. For triglycerides, participants were designated as responders if a ≥10% increase occurred after the control meal compared with the strawberry meal. For AP and AI@75, which tended to decrease postprandially, the percentage change was calculated with the use of the change from baseline to the lowest postprandial value, and participants who experienced a greater reduction after the strawberry meal were deemed to be responders. Logistic regression was performed in SAS with the use of BMI, sex, and age as main effects.

RESULTS

The screening characteristics of participants who completed the study are presented in **Table 2**. There were no significant differences between male and female participants at screening. With regard to premeal baseline characteristics of participants at the strawberry compared with control meal visits, small but statistically significant differences were found for CRP (mean difference: 0.3 mg/L; $P = 0.01$) and BMI (mean difference: 0.1; $P = 0.04$) (**Supplemental Table 3**).

Postprandial vascular function

Meal consumption significantly altered several measures of vascular function, but these effects were not significantly different between the strawberry and control meals (**Figure 2**). AP and the AI@75 were both significantly lower at 2 and 4 h after meal consumption than they were at baseline (Figure 2). There was a significant time point effect for PWV, with values at the 4-h time point being 3% higher than were values at 2 h, although these values were not significantly different from baseline (Figure 2). There was no significant treatment effect for the change in central systolic blood pressure after the strawberry meal (mean 4-h postprandial change: −0.4 ± 0.9 mm Hg) compared with the control meal (mean 4-h postprandial change: 0.4 ± 0.9 mm Hg). Central systolic blood pressure also did not significantly change from baseline at any time point (mean postprandial change at 2 h: −0.5 ± 0.9 mm Hg; mean postprandial change at 4 h: 0.4 ± 0.9 mm Hg).

Postprandial metabolism

Serum triglycerides, insulin, and glucose significantly increased from baseline at all time points after the consumption of both meals ($P < 0.0001$ compared with baseline) (**Figure 3**). There were no significant differences between the 2 meals. The triglyceride response was highest at 4 h postmeal (+86.7 mg/dL relative to baseline), whereas insulin and glucose responses peaked 30 min postmeal (+42.9 μIU/mL and +25.3 mg/dL relative to baseline, respectively), and decreased thereafter. There was no significant treatment effect for the Matsuda insulin sensitivity index [geometric mean (95% CI) after the strawberry meal: 10.8 (8.1, 14.4) compared with after the control meal: 10.5 (8.1, 13.5); $P = 0.7$]. AUC values for postprandial insulin, glucose, and triglyceride responses also were not significantly different after the strawberry meal compared with the control meal ($P = 0.8$, 0.6, and 0.8, respectively; data not shown).

TABLE 2
Screening characteristics of participants who completed the study[1]

	Women (n = 13)	Men (n = 17)
Age, y	28.1 ± 2.7 (20–47)	28.2 ± 2.0 (20–49)
Weight, kg	85.0 ± 3.0 (74.0–111)	98.5 ± 2.2 (83.6–114)
BMI, kg/m^2	31.4 ± 0.8 (28.0–37.9)	31.3 ± 0.6 (28.5–36.5)
SBP, mm Hg	113.8 ± 2.7 (97–135)	123.1 ± 2.4 (109–145)
DBP, mm Hg	77.4 ± 2.0 (68–94)	81.5 ± 1.4 (68–89)
Glucose, mg/dL	85.9 ± 1.5 (77–91)	91.6 ± 0.9 (83–98)
TC, mg/dL	161.1 ± 9.7 (117–228)	164.6 ± 7.2 (110–222)
HDL, mg/dL	54.8 ± 1.9 (44–69)	41.6 ± 1.8 (28–57)
LDL, mg/dL	87.8 ± 9.0 (39–158)	96.8 ± 6.2 (48–147)
TGs, mg/dL	92.8 ± 15.6 (33–186)	131.0 ± 16.7 (35–339)

[1] Values are means ± SEMs (ranges), n = 30. Values were calculated and compared with the use of an independent 2-sample t test (PROC TTEST; SAS version 9.3). There were no significant differences between male and female participants for any variables. DBP, diastolic blood pressure; SBP, systolic blood pressure; TC, total cholesterol; TG, triglyceride.

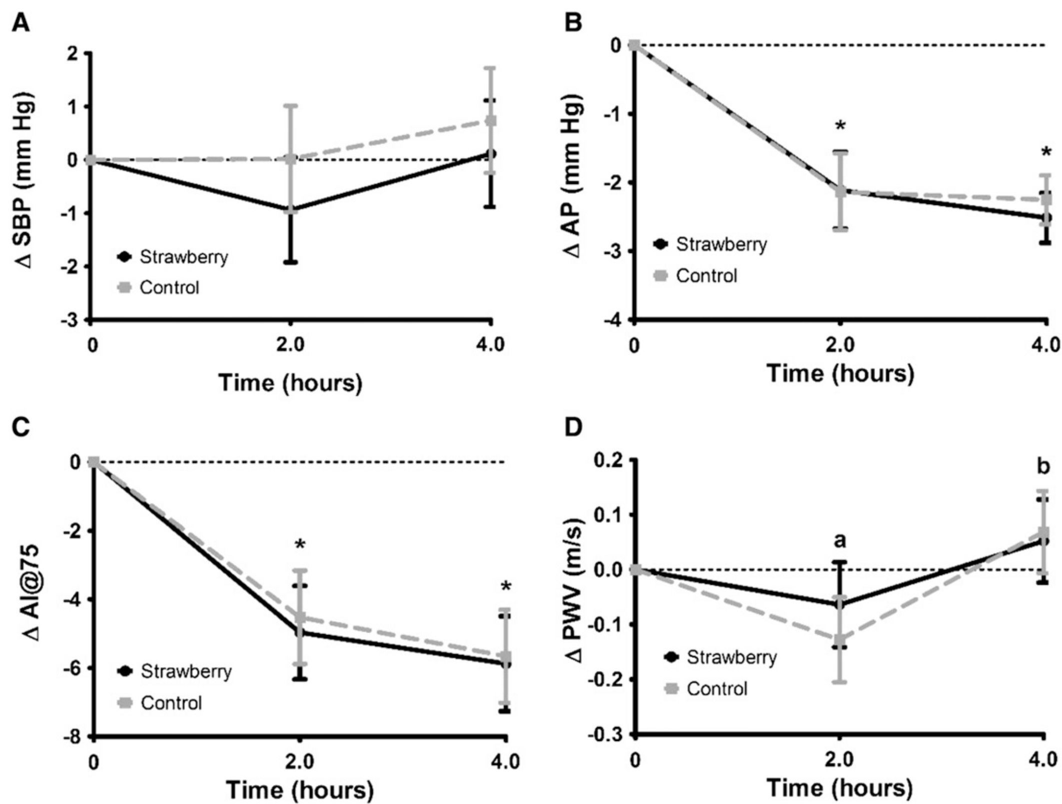

FIGURE 2 Mean ± SEM changes in central SBP (A), AP (B), AI@75 (C), and PWV (D) after consumption of the control and freeze-dried strawberry test meals. Data are presented as unadjusted means ± SEMs, n = 26. Values represent untransformed change scores that were calculated by subtracting the fasting baseline values for each visit from postmeal values, and were compared with the use of the MIXED procedure in SAS version 9.3. For all outcomes, there were no significant interaction terms in the model, and no significant differences between the responses to the strawberry and control meals. Times annotated with different letters were significantly different from one another. *Significantly different from baseline, $P < 0.05$. AI@75, augmentation index normalized to a heart rate of 75 beats/min; AP, augmentation pressure; PWV, pulse wave velocity; SBP, systolic blood pressure.

Predictors of postprandial metabolic and vascular function responses

Hypothesis-generating subset analyses identified age and sex as potential predictors of the effect of the freeze-dried strawberry powder intervention on metabolic (triglycerides, glucose, and insulin) responses. Each additional year of age increased the odds of an individual having an attenuated triglyceride response with the strawberry intervention (OR: 1.2; 95% CI: 1.0, 1.4; $P < 0.05$). However, for glucose responses, each additional year of age was associated with individuals being marginally less likely to respond to the strawberry intervention (OR: 0.9; 95% CI: 0.7, 1.0; $P = 0.06$). A trend for a negative effect of age was also found for insulin responses (OR: 0.9; 95% CI: 0.7, 1.0; $P = 0.1$). Moreover, men were significantly more likely to respond to the strawberry intervention with attenuated glucose responses (OR: 22.2; 95% CI: 2.2, 644.6; $P = 0.03$), but sex had no effect on the triglyceride or insulin response to strawberries. Age, BMI, and sex were not significant predictors of vascular function (AP and AI@75) responses (data not shown).

Markers of oxidative stress and PL activity

There was no significant postprandial change from baseline in malondialdehyde or oxidized LDL, and no significant difference between the response to the strawberry meal and that for the control meal (**Supplemental Table 4**). There was no inhibitory effect on PL activity in vitro at any concentration of strawberry powder extract ≤200 μg/mL (**Supplemental Table 5**).

DISCUSSION

This study investigated the postprandial effects of incorporating freeze-dried strawberry powder into a high-fat meal. We observed marked elevations in triglycerides, glucose, and insulin, whereas the AI@75 was significantly reduced after meal consumption. However, there was no change in malondialdehyde and oxidized LDL postprandially, and no significant treatment effect of the strawberry intervention for any outcomes.

In contrast to the detrimental effect of postprandial lipemia on nitric oxide–dependent vasodilation (36), the AI and other indexes of arterial stiffness are reduced by meal consumption (37–40), potentially because of the vasodilatory effect of insulin (38). However, this may be a paradoxical effect whereby meal-induced elevations in insulin are acutely beneficial, but sustained hyperinsulinemia (resulting in insulin resistance and related conditions) is associated with arterial stiffness (41–45). Given that these postprandial reductions can be maintained hours after a meal, they also may be due in part to increased blood flow to digestive organs, with compensatory reductions in flow to other organs (46). Measurement of vascular function beyond the 4-h time point in future studies would help to clarify how long these reductions are maintained.

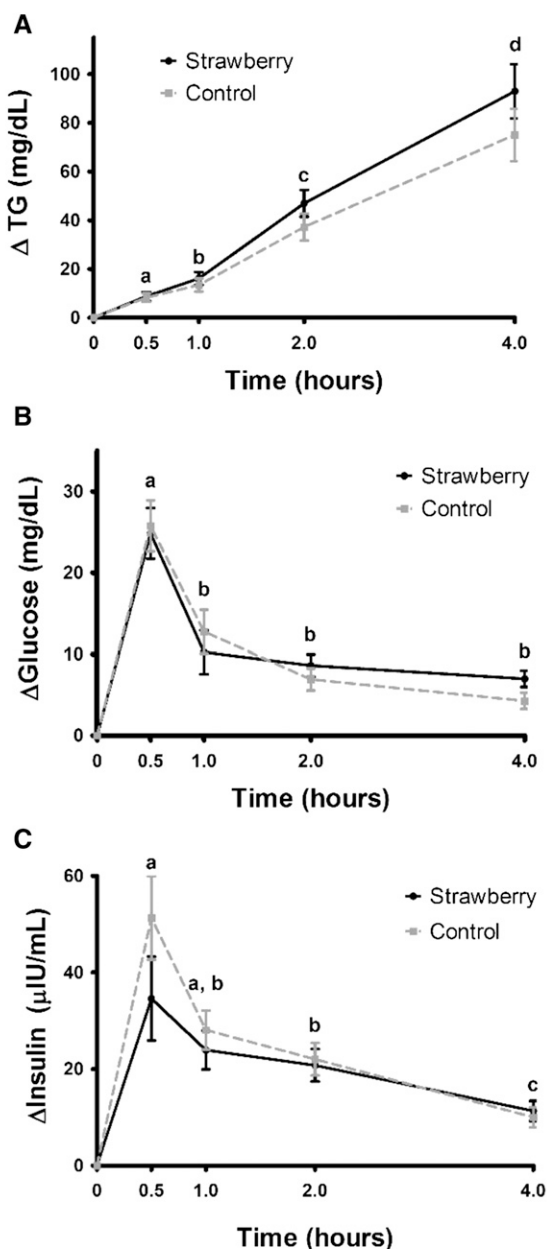

FIGURE 3 Mean ± SEM changes in TGs (A), glucose (B), and insulin (C) after consumption of the control and freeze-dried strawberry test meals. Data are presented as unadjusted means ± SEMs, $n = 27$. Values represent untransformed change scores that were calculated by subtracting the fasting baseline values for each visit from postmeal values, and were compared with the use of the MIXED procedure in SAS version 9.3. All time points were significantly different from baseline. For all outcomes, there were no significant interaction terms in the model, and no significant differences between the responses to the strawberry and control meals. Times annotated with different letters were significantly different from one another, $P < 0.05$. TG, triglyceride.

Postprandial insulin-induced reductions in indexes of arterial stiffness also have important implications for study design. For instance, a higher-carbohydrate test meal that produces a greater insulin response may falsely appear to have beneficial vascular effects compared with a higher-fat meal with a less robust insulin response. Few studies investigating postprandial changes in AI and/or PWV after a mixed meal have assessed potential associations with metabolic responses (40). We timed vascular function measurements to coincide with peak polyphenol and triglyceride concentrations (2 and 4 h postmeal, respectively), but insulin peaks much earlier and has largely declined by 2 h. We found no significant relation between peak insulin (at 30 min) and peak AI@75 (at 4 h), or the 2-h change in AI@75 and insulin, when excluding several outlier responses (data not shown). Vascular function measurements at earlier time points are needed to further explore this relation.

To our knowledge, few studies have investigated whether administering a polyphenol-rich food or beverage with a meal alters measures of vascular function derived from pulse waveform analysis (47–49). In healthy adults, a high-nitrate spinach meal (∼90 kcal and <1 g fat) produced a greater reduction in the AI@75 at 3 h than did a matched low-nitrate meal (48), but the AI did not change in older individuals after a test meal (∼475 kcal) with less nitrate (220 compared with 845 mg inorganic nitrate) (47). Conversely, adding oats to a high-fat meal abolished the 3-h postprandial reduction in the AI@75 (49). This may have been due to a blunted postprandial insulin response from the β-glucan in oats (50), but insulin was not measured. Strawberries do not provide the β-glucan found in oats and contain much less nitrate than spinach contains (51). Thus, divergent postprandial effects on the AI may depend on the unique bioactive profile of the intervention, and additional research is needed. These studies also did not measure postprandial changes in triglycerides, glucose, and insulin. Assessment of these metabolic outcomes, as well as vascular function measurements later in the postprandial period (i.e., after 4 h), would help clarify potential effects on the AI and indexes of arterial stiffness. Given the vasodilatory effect of insulin, future studies should also use test meals with similar insulin-inducing properties.

Other polyphenol-rich interventions also have not attenuated postprandial lipemia and/or glycemic responses to a meal (52–55). It is unclear why our results differ from the significant reductions in postprandial insulin (27, 28), as well as triglycerides and oxidized LDL (26), found in the 2 previous postprandial strawberry studies. Dairy proteins and the timing of meal intake may diminish the effects of polyphenols (56, 57); however, significant benefits have been achieved with the use of a milk-based beverage consumed with a test meal (26, 28). Although the amount of strawberries required to optimize effects on clinical outcomes is unknown, our 40-g dose of freeze-dried powder was equivalent to ∼3 cups of fresh strawberries [13 g freeze-dried powder is equivalent to 1 cup fresh (C Christian California Strawberry Commission, personal communication, 2014)] and provided a substantial amount of polyphenols (Supplemental Table 2). A similar amount of total anthocyanins was provided in the postprandial study by Park et al. (28), although the freeze-dried strawberry powder used by Burton-Freeman and colleagues (26, 27) had a higher oxygen radical absorbance capacity value and a higher concentration of select polyphenolic compounds, including quercetin and pelargonidin-3-glucoside. These differences may account, in part, for the discrepancies between our results and those of Burton-Freeman et al. (26). Based on our exploratory analyses, characteristics of our study population such as age and sex also may have influenced responses. Moreover, compared with the 2 previous postprandial strawberry studies (26, 28), our participants tended to be younger, did not have elevated total or LDL cholesterol, and were not insulin resistant. Thus, the potential benefits of strawberries may be

more likely in individuals at greater CVD risk, and our hypothesis-generating findings should be investigated in future studies.

We further speculate that the unique polyphenol profile of an intervention and its subsequent ability to inhibit specific digestive enzymes may also determine outcomes. For instance, spices, in which phenolic acids, flavones, and flavonols tend to predominate (58), potently inhibit PL in vitro (7). Alternatively, strawberry polyphenols, which are largely anthocyanins, ellagitannins, and ellagic acid (59), may be more effective at altering carbohydrate metabolism, because they consistently inhibit carbohydrate digestive enzymes (60–62), and improved the insulin response to white bread in healthy women (11). Although an extract of strawberry polyphenols has been shown to inhibit PL in vitro (63), our analysis of the freeze-dried strawberry powder showed no inhibitory effect. Future studies are needed to clarify whether effects on postprandial metabolism depend on the phenolic profile of the intervention and/or the macronutrient composition of the test meal.

Longer-term strawberry intake may be required to alter CVD risk factors. Although we found no effect within 4 h of meal consumption, multiple studies have found that 3–12 wk of strawberry supplementation improved oxidative stress and the lipid profile of individuals with elevated CVD risk. Therefore, although it remains unclear whether strawberries alter postprandial dysmetabolism, there is promising evidence that strawberries improve other CVD risk factors.

Our participants were recruited specifically on the basis of BMI (28–39) and were representative of the general population; however, they were otherwise relatively healthy, and effects may be more apparent in individuals at greater CVD risk. Providing the strawberry intervention as part of a meal is reflective of real-world dietary habits, and the strawberry and control meals were well-matched. Hormonal effects on vascular function were minimized by testing female participants in the first week of their menstrual cycle. Postprandial metabolism and vascular function were also measured concurrently. The study meals induced a robust postprandial triglyceride, glucose, and insulin response, but it is possible that significant attenuation of the glycemic response might have been achieved with a carbohydrate-based lower-fat test meal. Our study was limited to a 4-h postprandial period, and it is possible that changes may have occurred at later time points. Malondialdehyde also has limited reliability as a marker of lipid peroxidation (64, 65), and more specific and sensitive biomarkers should be used in future studies. Our sample size was consistent with previous studies; however, as is typically the case with postprandial studies, our study may have been underpowered, given the inherent inter- and intraindividual variability in postprandial responses.

In conclusion, the consumption of a high-fat meal significantly reduced the AI@75, indicating an acute improvement in vascular function, despite marked elevations in postprandial triglycerides, glucose, and insulin. Incorporating 40 g freeze-dried strawberry powder did not further improve vascular function or attenuate postprandial dysmetabolism. Additional research is needed to determine whether polyphenol-rich foods and beverages reduce CVD risk by modifying postprandial vascular function and dysmetabolism. Future postprandial studies should consider the influence of meal-induced insulin elevations on vascular outcomes.

The authors' responsibilities were as follows—CKR, ACS-R, DNP, and PMK-E: designed the research; ACS-R: coordinated the implementation of the study procedures and provided assistance with data collection; CKR: conducted the research; JDL: selected and performed the oxidative stress and pancreatic lipase inhibition assays; CKR, ACS-R, and TLG: performed the statistical analyses; CKR, ACS-R, and PMK-E: wrote the manuscript; and all authors: took responsibility for the manuscript's final content and read and approved the final manuscript. None of the authors reported a conflict of interest related to the study.

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