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Freeze-Dried Strawberries Lower Serum Cholesterol and Lipid Peroxidation in Adults with Abdominal Adiposity and Elevated Serum Lipids[1–3]

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Abstract

Dietary flavonoid intake, especially berry flavonoids, has been associated with reduced risks of cardiovascular disease (CVD) in large prospective cohorts. Few clinical studies have examined the effects of dietary berries on CVD risk factors. We examined the hypothesis that freeze-dried strawberries (FDS) improve lipid and lipoprotein profiles and lower biomarkers of inflammation and lipid oxidation in adults with abdominal adiposity and elevated serum lipids. In a randomized dose-response controlled trial, 60 volunteers [5 men and 55 women; aged 49 ± 10 y; BMI: 36 ± 5 kg/m^2 (means ± SDs)] were assigned to consume 1 of the following 4 beverages for 12 wk: *1*) low-dose FDS (LD-FDS; 25 g/d); *2*) low-dose control (LD-C); *3*) high-dose FDS (HD-FDS; 50 g/d); and *4*) high-dose control (HD-C). Control beverages were matched for calories and total fiber. Blood draws, anthropometrics, blood pressure, and dietary data were collected at screening (0 wk) and after 12-wk intervention. Dose-response analyses revealed significantly greater decreases in serum total and LDL cholesterol and nuclear magnetic resonance (NMR)–derived small LDL particle concentration in HD-FDS [33 ± 6 mg/dL, 28 ± 7 mg/dL, and 301 ± 78 nmol/L, respectively (means ± SEMs)] vs. LD-FDS (−3 ± 11 mg/dL, −3 ± 9 mg/dL, and −28 ± 124 nmol/L, respectively) over 12 wk (0–12 wk; all $P < 0.05$). Compared with controls, only the decreases in total and LDL cholesterol in HD-FDS remained significant vs. HD-C (0.7 ± 12 and 1.4 ± 9 mg/dL, respectively) over 12 wk (0–12 wk; all $P < 0.05$). Both doses of strawberries showed a similar decrease in serum malondialdehyde at 12 wk (LD-FDS: 1.3 ± 0.2 μmol/L; HD-FDS: 1.2 ± 0.1 μmol/L) vs. controls (LD-C: 2.1 ± 0.2 μmol/L; HD-C: 2.3 ± 0.2 μmol/L) ($P < 0.05$). In general, strawberry intervention did not affect any measures of adiposity, blood pressure, glycemia, and serum concentrations of HDL cholesterol and triglycerides, C-reactive protein, and adhesion molecules. Thus, HD-FDS exerted greater effects in lowering serum total and LDL cholesterol and NMR-derived small LDL particles vs. LD-FDS in the 12-wk study. These findings warrant additional investigation in larger trials. This trial was registered at clinicaltrials.gov as NCT01883401. J. Nutr. 144: 830–837, 2014.

Introduction

More than two-thirds of U.S. adults are overweight or obese, conditions that have been associated with increased prevalence of cardiovascular risk factors and with dyslipidemia, hypertension, and impaired glucose tolerance (1). Obesity, especially abdominal adiposity, has been correlated with several metabolic impairments and endothelial dysfunction, conditions that lead to advanced vascular complications (2,3). Thus, reducing waist size and related lipid and vascular abnormalities have been used as surrogate targets in many nutrition intervention studies whose ultimate goal is the prevention of cardiovascular disease (CVD).[7] Nutritional epidemiology provides accumulating evidence on the inverse associations between dietary intakes of polyphenolic flavonoids and CVD. Among the popular sources of dietary flavonoids, berries have gained considerable attention because of

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[7] Abbreviations used: C, control; CVD, cardiovascular disease; CRP, C-reactive protein; FDS, freeze-dried strawberries; HD, high-dose; hs-CRP, high-sensitivity C-reactive protein; LD, low-dose; LSP, lipoprotein subclass profile.

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their inverse associations with CVD events (4) and CVD risk factors, including elevated C-reactive protein (CRP) (5), hypertension (6), and type 2 diabetes (7), demonstrated in large prospective studies. These associations have been mostly attributed to the consumption of blueberries and strawberries, which are important sources of polyphenols in the U.S. diet (8).

In a recent systematic review, Rodriguez-Mateos et al. (9) summarized the evidence from mechanistic studies and clinical trials on the efficacy of berries in lowering CVD risk. However, there remain several gaps in our knowledge about the preventive role of berries in CVD, especially regarding the optimal dose, forms of delivery (fruit vs. extracts), and effects on specific surrogate markers and "events" related to CVD. The role of dietary blueberries and strawberries in CVD management has been addressed by only a few clinical studies in U.S. adults. Furthermore, cardiometabolic effects of berry supplementation may be influenced by post-harvest processing methods that substantially affect nutritional quality, including polyphenol and vitamin content, and antioxidant activity of berries (10–12). In this regard, freeze-dried berries, equivalent to commercially available frozen berries, were shown to retain maximum nutritional value and were used previously in feeding trials (13–17). Therefore, we aimed to evaluate the effects of the supplementation of low and high doses of dietary freeze-dried strawberries (FDS) with standardized polyphenol content on glucose and lipid profiles, lipid peroxidation, CRP, and adhesion molecules in adults with abdominal adiposity and dyslipidemia. We hypothesized that the FDS, reconstituted in water, will be effective in ameliorating elevated serum lipids and surrogate markers of atherosclerosis in obese adults in a dose-response manner and also compared with calorie- and fiber-matched control beverages.

Materials and Methods

Participants and recruitment criteria. Men and women [aged 49 ± 10 y (means ± SDs)] with abdominal adiposity and elevated serum lipids and who were free of any chronic diseases were enrolled in this randomized controlled study. Inclusion was based on the presence of elevated waist circumference (>35 inches for women and >40 inches for men) and fasting serum lipids that met any 2 of the following 4 criteria: *1*) total cholesterol > 200 mg/dL; *2*) LDL cholesterol > 100 mg/dL; *3*) TGs > 150 mg/dL; and *4*) HDL cholesterol < 50 mg/dL for women and < 40 mg/dL for men (18,19). Recruitment and study procedures were conducted at the Clinical Research Center at the University of Oklahoma Health Sciences Center and the Nutritional Sciences Clinical Assessment Unit at Oklahoma State University. Participants were recruited using flyers and campus e-mail advertisements at both sites. Each potential recruit received an initial telephone screening before the screening visit. They were excluded for the following: *1*) they were younger than 21 y of age; *2*) they were taking medications for any chronic disease, including hypoglycemic, hypolipidemic, anti-inflammatory, or steroidal medications; *3*) they had liver, renal, or thyroid disorders or anemia; *4*) they were consuming antioxidants or fish oil supplements on a regular basis; *5*) they were current smokers; *6*) they were consuming alcohol on a regular basis (except social drinking, 1–2 drinks/wk); or *7*) they were pregnant or lactating. The study was approved by the Institutional Review Board at the University of Oklahoma Health Sciences Center and at Oklahoma State University. All participants provided written informed consent.

Intervention. FDS (10% weight of fresh strawberries) provided by the California Strawberry Commission were produced from individually quick frozen kosher, conventional (nonorganic) whole strawberries with no additives (**Table 1**). The mixture of strawberries used to generate the powder contained the University of California public cultivars as follows: Camarosa (37%), Ventana (13%), Diamante (13%), and 2 proprietary varieties (37%) in production in 2010. The macronutrient and micronutrient composition of the strawberry powder was analyzed by the Robert M. Kerr Food & Agricultural Products Center at Oklahoma State University, and the polyphenol and phytosterol content were analyzed by Brunswick Laboratories and Covance, respectively. Participants were randomly assigned to consume 1 of the following 4 beverages for 12 wk: *1*) low-dose FDS [LD-FDS; 25 g reconstituted in 2 cups (474 mL) of water]; *2*) low-dose calorie- and fiber-matched control [LD-C; 4 g of fiber and 5 teaspoons (20 g) of cane sugar, blended in 2 cups (474 mL) of water]; *3*) high-dose FDS [HD-FDS; 50 g reconstituted in 2 cups (474 mL) of water]; or *4*) high-dose calorie- and fiber-matched control [HD-C; 8 g of fiber and 9 teaspoons (36 g) of cane sugar, blended in 2 cups (474 mL) of water]. The fiber used in control beverages was composed of vegetable fibers and natural gums and contained both insoluble and soluble fiber (1:2) per serving (Fiberstir). In addition, the control beverages contained added red food color (McCormick & Company) and artificial strawberry-flavored Kool-Aid (Kraft Foods) to mimic the color and flavor of the FDS beverages. The FDS powder was stored at −20°C until used for the study. The dose administered in the HD-FDS group (50 g/d) was used previously in our pilot study and was reported to be safe and well tolerated by all participants (13). Participants were asked to consume 1 cup in the morning and the second in the evening at least 6–8 h apart. The participants were instructed to add the strawberry or control beverages as a snack to their usual diet and not to replace it with any meals. Because the strawberry and control beverages had a thick and sticky consistency, participants were also instructed to rinse their drinking cups and storage containers with additional water to prevent any wastage.

TABLE 1 Composition of strawberry and control beverages administered in the 12-wk study[1]

Composition	LD-FDS	LD-C	HD-FDS	HD-C
FDS, *g*	25	—	50	—
Fiber, *g*	4.0	4.0	8.0	8.0
Calories, *kcal*	75	80	150	144
Protein, *g*	1.8	—	3.5	—
Fat, *g*	0.3	—	0.5	—
Carbohydrates, *g*	16	20	32	36
Ash, *g*	1.5	—	3.2	—
Vitamin C, *mg*	55	—	109	—
Total phenolics, *mg gallic acid equivalents*	1001	—	2005	—
Total anthocyanins, *mg cyanidin-3-glucoside equivalents*	78	—	155	—
Ellagic acid, *mg*	106	—	220	—
Phytosterols, *mg*	23	—	50	—

[1] C, control; FDS, freeze-dried strawberries; HD, high-dose; LD, low-dose.

Study design and procedures. Participants were randomly assigned to consume strawberry (LD-FDS, HD-FDS) and calorie- and fiber-matched control (LD-C, HD-C) beverages for 12 wk. All participants were required to make 3 visits/wk to their study site (Monday, Wednesday, and Friday) to ensure compliance by supervised consumption on these days. Participants were instructed to keep the beverage refrigerated, to avoid exposing it to direct heat or light, and to avoid consuming it within 2–3 h after any snack, lunch, or dinner because other foods might interfere with the absorption of the strawberry bioactive compounds. Participants were asked to return any unconsumed strawberry and control beverages. In addition, during these mandatory visits, participants had brief discussions with the study dietitian concerning their participation in the study and any issues related to the test beverages. Participants also received monetary compensation during these weekly visits. They were asked to refrain

from consuming any other source of berries or related products derived from berries, such as juices, jams, and desserts. They were also asked to refrain from consuming green tea, cocoa, and soy products while participating in the study, because these were the most commonly consumed flavonoid-rich foods by the enrolled participants as identified by a screening FFQ specific for flavonoids based on the USDA flavonoid database (20). Participants were instructed to maintain their usual diet, physical activity, and lifestyle while in the study. They were asked to record their food intake throughout the study. Body weight, height, waist circumference, and systolic and diastolic blood pressure were measured by trained personnel, and blood draws were performed by registered nurses at screening and 12 wk of the study. In case of premenopausal women, blood draws were conducted between days 10 and 20 of the menstrual cycle. Safety measures included checks of liver enzymes and kidney function at 6 wk of the study. All laboratory staff were unaware of the treatment groups.

Anthropometrics and blood pressure. Body weight was recorded on an uncarpeted surface with the SECA 644 Multifunctional Hand Rail Scale (SECA) and recorded to the nearest 0.1 kg. Height was measured without shoes using the Accustat Genentech Stadiometer and recorded to the nearest 0.1 cm. Systolic and diastolic blood pressure were measured in millimeters of mercury using the Spot Vital Signs Device (Welch Allyn). Participants were asked to lie down and relax for ~8–10 min, after which 3 blood pressure measurements were recorded at intervals of 5 min. Waist circumference was measured at the superior iliac crest using the Gulick II Tape Measure (Vital Signs).

Dietary analyses. Three-day food records were collected at screening and 6 and 12 wk of the study. Three-day averages (2 weekdays and 1 weekend day) of micronutrient and macronutrient intakes were analyzed using Nutritionist Pro (version 3.2, 2007; Axxya Systems). At the beginning of the study, all enrolled participants met with the study dietitians and were instructed on how to record their food and beverage intakes using appropriate food models. All data entry was performed by a registered dietitian trained and certified in using the software. All dietary data entry was verified by a second registered dietitian for quality control. If a participant consumed a food that was not in the database, a food with very similar nutrient composition was chosen. Nutrient information was also obtained through food labels or recipes from participants, online sources, or grocery stores.

Clinical analyses. Fasting blood samples were collected, and serum was promptly transported to the University of Oklahoma Medical Center Laboratory for analyses of glucose, insulin, lipid profiles (total cholesterol, TGs, LDL cholesterol, and HDL cholesterol), high-sensitivity CRP (hs-CRP), and other blood variables, including safety variables (hemoglobin, platelets, white blood cells, liver enzymes, creatinine, blood urea nitrogen, electrolytes, albumin, total protein, and thyroid-stimulating hormone), using automated diagnostic equipment (Abbott Architect Instruments) following standard protocols at the University of Oklahoma Medical Center. Serum hemoglobin A1c was analyzed using a DCA 2000+ (Bayer). Insulin resistance was evaluated by the HOMA-IR calculated as follows: [fasting insulin (mU/L) × fasting glucose (mmol/L)]/22.5 (21). NMR-determined lipoprotein subclass profiles (LSPs) was performed in first-thaw plasma specimens using a 400-MHz proton NMR analyzer at LipoScience as described previously (22). Plasma ellagic acid was measured using HPLC-UV procedures as described previously by Seeram et al. (23). The minimum detectable concentration of ellagic acid in our assay was 3.5 ng/L. The interassay CV was 7.8%.

Lipid peroxidation and adhesion molecules. Lipid peroxidation was measured in serum as combined malondialdehyde and hydroxynonenal using a colorimetric assay according to the protocol of the manufacturer (LPO-586; Oxis Health Products). The mean intra-assay CV for combined malondialdehyde and hydroxynonenal was 5.2%. Plasma concentrations of soluble intercellular adhesion molecule-1 and soluble vascular cell adhesion molecule-1 were determined using commercially available ELISA kits (Human sICAM-1 Quantikine ELISA and Human sVCAM-1 Quantikine ELISA Kits; R&D Systems) according to the protocols of the manufacturer. The minimum detectable concentrations were 15.6 and 12.6 ng/L, respectively. The inter-assay CVs were 3.5% and 7.6%, respectively.

Statistical analyses. For all measures, descriptive statistics were calculated and graphs drawn to look for outliers; no data points were determined to be outliers. Our primary groups of comparisons were as follows: LD-FDS vs. HD-FDS and LD-FDS and HD-FDS vs. calorie- and fiber-matched control groups. For each variable, mean differences between strawberry and control groups at baseline and 12 wk were assessed using the multivariate analysis of variance, followed by Bonferroni's post hoc analyses. Changes in measurements over the 12-wk study period were assessed by calculating the difference between the pre-intervention and post-intervention measurements (0–12 wk) in each group. For each variable, significance was assessed by comparing the change over the 12-wk study period between the strawberry and control groups. Target sample size was calculated to include 15 participants per group to detect minimum differences of 0.3 mmol/L in serum total cholesterol and 0.2 mmol/L in LDL cholesterol with 80% power based on our previous feasibility study (13). All statistical tests were 2 tailed, with significance level set at 0.05. SPSS for Windows (version 15.0; SPSS) was used for the statistical calculations.

Results

Among the 85 participants screened for the study, 66 met the inclusion and exclusion criteria and were enrolled in the study. Among the 66 enrolled, 6 participants withdrew from the study because of their time constraints and inability to make the mandatory 3 visits/wk to the study centers. Thus, 60 participants completed the 12-wk study in the strawberry and control arms. Among these participants, compliance was 100% for the strawberry groups and 97% for the control groups, as assessed by mandatory weekly visits (3 d/wk) and return of any unconsumed beverages on the days the participants did not come to the clinic. No adverse events were reported in the study. Furthermore, plasma ellagic acid was detectable in 14 of 15 participants in the HD-FDS group (means ± SEMs, 25.7 ± 1.6 ng/mL) and 12 of 15 participants in the LD-FDS group (11.3 ± 0.5 ng/L) at 12 wk, whereas concentrations were nondetectable at baseline and also in the controls.

LD-FDS vs. HD-FDS. In the dose-response analyses, no significant differences were noted at baseline between the 2 doses (**Table 2**). As shown in **Table 3**, the 12-wk changes (0–12 wk) in serum total and LDL cholesterol, as well as in NMR-derived small LDL particles were significantly greater in the HD-FDS when compared with the LD-FDS (all $P < 0.05$). At 12 wk, the absolute values of NMR-derived small LDL particles remained significantly lower ($P < 0.05$), whereas total and LDL cholesterol concentrations tended to be lower in the HD-FDS vs. LD-FDS ($P < 0.1$) (Table 3). In addition, plasma ellagic acid, a biomarker of compliance, was significantly higher in the HD-FDS vs. LD-FDS ($P < 0.05$), and the observed significant findings in serum total and LDL cholesterol and NMR-derived small LDL particles, between the 2 strawberry doses, persisted when adjusted for ellagic acid as a covariate.

LD-FDS vs. LD-C. At baseline, no significant differences in clinical and demographic characteristics were noted in the LD-FDS (25 g/d) vs. LD-C (Table 2). No differences were noted in blood pressure, anthropometrics, and measures of glycemic control and conventional lipid profiles, as well as in NMR-derived small LDL particles between the 2 groups (Table 3). Also, NMR-derived VLDL, LDL (total, IDL, large), and HDL subclasses

TABLE 2 Baseline characteristics of the study participants[1]

Variable	LD-FDS	LD-C	HD-FDS	HD-C
Age, *y*	50 ± 10	48 ± 10	49 ± 11	48 ± 10
Men/women, *n/n*	1/14	1/14	2/13	1/14
Waist, *cm*	104 ± 7.6	109 ± 8.1	114 ± 12.7	107 ± 6.6
Height, *cm*	165 ± 5.8	165 ± 6.7	164 ± 6.5	169 ± 7.6
Weight, *kg*	95 ± 14	100 ± 12	101 ± 18	99 ± 15
BMI, kg/m^2	34.5 ± 4.4	37.0 ± 4.4	38.0 ± 7.1	35.0 ± 5.2
BUN, *mg/dL*	14 ± 2.3	16 ± 4.5	16 ± 4.2	17 ± 5.0
Creatinine, *mg/dL*	0.8 ± 0.2	0.8 ± 0.2	0.8 ± 0.2	0.9 ± 0.3
AST, *U/L*	29 ± 10	26 ± 7	25 ± 4	25 ± 7
ALT, *U/L*	34 ± 12	34 ± 12	30 ± 11	31 ± 12
WBC, $n \times 10^{-3}$	6.6 ± 1.2	6.7 ± 1.4	6.7 ± 1.7	7.1 ± 1.5
RBC, $n \times 10^{-3}$	4.6 ± 0.5	4.5 ± 0.3	4.6 ± 0.6	4.8 ± 0.4
Hb, *g/dL*	14 ± 1.4	14 ± 1.3	14 ± 1.4	14 ± 1.6
Multivitamin users, *%*	2.0	2.0	2.0	1.0
Fruit servings, *n/wk*	1.2	1.0	1.2	1.0
Vegetable servings, *n/wk*	1.0	1.1	1.1	1.2

[1] Values are means ± SDs unless noted otherwise, $n = 15$. No significant differences were noted among any groups using the multivariate analysis of variance at baseline for each variable. ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; C, control; FDS, freeze-dried strawberries; Hb, hemoglobin; HD, high-dose; LD, low-dose; WBC, white blood cell.

were not significantly affected by the low-dose strawberry intervention (**Supplemental Table 1**). Conversely, among measures of inflammation and lipid oxidation, only malondialdehyde and hydroxynonenal concentrations were significantly lower in the LD-FDS vs. LD-C ($P < 0.01$) (**Table 4**). Finally, analyses of the change data over 12 wk (0–12 wk) revealed no significant differences in any of these variables between LD-FDS vs. LD-C.

HD-FDS vs. HD-C. As shown in Table 2, baseline clinical and demographic characteristics were not significantly different in the HD-FDS (50 g/d) vs. HD-C. At 12 wk, mean serum total and LDL cholesterol concentrations tended to be lower in the HD-FDS vs. HD-C ($P < 0.1$) (Table 3), although no differences were noted in HDL and VLDL cholesterol and TG concentrations. No differences were noted in systolic and diastolic blood pressure, anthropometrics, and measures of glycemia between the 2 groups at 12 wk (Table 3). Among the NMR-derived lipoprotein subclasses, mean concentrations of small LDL particles were significantly lower in the HD-FDS vs. HD-C at 12 wk ($P < 0.01$) (Table 3), whereas no changes were noted in other LDL-, HDL-, and VLDL-related subclasses (Supplemental Table 1). Among measures of inflammation and lipid oxidation, malondialdehyde and hydroxynonenal concentrations were significantly lower in the HD-FDS vs. HD-C ($P < 0.001$) (Table 4), although mean concentrations of hs-CRP and adhesion molecules were not affected by the high-dose strawberry intervention (Table 4). Furthermore, the changes over 12 wk (0–12 wk) were statistically significant only in case of total and LDL cholesterol concentrations between the HD-FDS vs. HD-C ($P < 0.5$) (Table 3). These significant observations persisted when adjusted for ellagic acid as a covariate.

Dietary intakes. Dietary intakes of macronutrients and micronutrients were not significantly different between the strawberry and control groups at baseline and 12 wk (**Supplemental Table 2**). No significant differences in dietary intakes were noted between baseline and 6 wk and between 6 and 12 wk of the study (data not shown at 6 wk).

Discussion

In a 3-mo randomized controlled trial, we investigated the effects of dietary strawberry supplementation using 2 different feasible doses [LD-FDS: ~25 g of freeze-dried powder daily (~250 g of fresh strawberries); and HD-FDS: ~50 g of freeze-dried powder daily (~500 g of fresh strawberries)] on cardiovascular risk factors in free-living adults with abdominal adiposity and above-optimal serum lipids. Our main findings in the dose-response analyses revealed significantly greater decreases in conventional total and LDL cholesterol concentrations and NMR-derived small LDL particle concentrations in the HD-FDS vs. LD-FDS groups. When compared with the calorie- and fiber-matched control groups, these differences remained significant only between the HD-FDS and HD-C groups. Low and high doses of strawberries were effective in lowering lipid peroxidation (malondialdehyde and hydroxynonenal) compared with the matched control groups. Conversely, neither dose of supplementation affected body weight, waist circumference, blood pressure, measures of glycemic control, HDL cholesterol and TG concentrations, hs-CRP, and adhesion molecules after 12 wk of intervention. Our study findings on the effects of high-dose dietary strawberries in lowering serum cholesterol may be of clinical utility, especially in light of the observed positive correlations between elevated LDL cholesterol and CVD "events" (24).

Berries and their constituent polyphenolic compounds have been shown to exert lipid-lowering effects in adults with CVD risk factors. Some of these findings can be summarized as follows: *1*) they increase HDL cholesterol (25–29); *2*) they lower total and/or LDL cholesterol (13,14,27–30); and *3*) they have no effect on TGs in most of these reported clinical studies. Conversely, studies also reported no effects of berry polyphenols on serum lipid profiles in adults with the metabolic syndrome or other CVD risks (15,17,31–33). These discrepancies may be explained by the sample size and study duration, dose and type of intervention (fresh or processed fruit vs. extracts or purified anthocyanins), and baseline lipid profiles and clinical characteristics of the enrolled participants. Again, among these reported studies, only a few examined the effects of berry fruits that are of relevance to the U.S. diet, thus making dietary recommendations problematic and justifying the need for additional studies.

Strawberries are a popular berry fruit consumed in the United States (8), although only a few studies examined previously their effects on CVD risk factors, including lipid profiles in adults. In a 3-wk randomized crossover trial, Zunino et al. (14) reported the effects of FDS (~320 g of frozen strawberries) in decreasing plasma concentrations of total cholesterol, but they observed no effects on LDL and HDL cholesterol or on TGs in obese individuals with above-optimal LDL cholesterol concentrations. This study also controlled for background dietary intake by providing prepared meals and supervised meal visits for all participants for the study duration (14). In another 6-wk study, FDS (~110 g of fresh strawberries) were shown to suppress postprandial increases in serum TGs in hyperlipidemic adults (16). Our 12-wk study shows the total and LDL cholesterol lowering effects of strawberries, which were observed only at high doses, in obese adults with above-optimal and/or borderline high LDL concentrations. Our findings, in concert with previous studies, provide evidence on the safety and efficacy of FDS, as a well-tolerated low-calorie dietary supplement in the nutritional management of elevated lipid profiles. Furthermore, berry anthocyanins in animal and cell models were shown to inhibit gastric lipase (34) and cholesteryl ester transfer protein

TABLE 3 Anthropometrics, blood pressure, and serum metabolic profiles after 12 wk of low-dose (25 g/d) and high-dose (50 g/d) strawberry supplementation vs. controls in obese adults with elevated serum lipids[1]

Variable	LD-FDS	LD-C	HD-FDS	HD-C
Body weight, *kg*				
0 wk	95 ± 4	100 ± 3	101 ± 5	99 ± 4
12 wk	95 ± 3	100 ± 3	101 ± 5	99 ± 4
Δ (0–12 wk)	−0.1 ± 2.0	0.5 ± 0.7	0.2 ± 2.5	−0.2 ± 3.2
BMI, *kg/m²*				
0 wk	34.6 ± 1.1	37.0 ± 1.1	38.0 ± 1.8	35.0 ± 1.3
12 wk	34.5 ± 1.1	36.9 ± 1.1	38.0 ± 1.9	35.0 ± 1.3
Δ (0–12 wk)	0.0 ± 0.7	0.1 ± 0.3	0.1 ± 0.6	−0.1 ± 1.5
Waist circumference, *cm*				
0 wk	104 ± 2.0	109 ± 2.0	114 ± 3.3	107 ± 1.8
12 wk	104 ± 2.0	109 ± 1.8	114 ± 2.2	109 ± 2.0
Δ (0–12 wk)	0.0 ± 1.0	0.5 ± 0.5	0.0 ± 2.0	−0.8 ± 1.0
Systolic blood pressure, *mm Hg*				
0 wk	128 ± 3	131 ± 3	136 ± 2	134 ± 3
12 wk	128 ± 3	134 ± 3	134 ± 3	134 ± 2
Δ (0–12 wk)	0.2 ± 2.0	−2.7 ± 2.1	1.5 ± 3.8	−0.2 ± 3.6
Diastolic blood pressure, *mm Hg*				
0 wk	83 ± 2	88 ± 1	86 ± 2	85 ± 1
12 wk	81 ± 2	86 ± 1	83 ± 2	86 ± 1
Δ (0–12 wk)	2.7 ± 0.7	1.7 ± 1.2	2.9 ± 3.1	−0.7 ± 1.3
Glucose, *mg/dL*				
0 wk	99 ± 2.6	103 ± 3.3	94 ± 3.5	95 ± 4.6
12 wk	97 ± 3.3	99 ± 2.7	94 ± 3.6	97 ± 5.2
Δ (0–12 wk)	2.1 ± 1.9	3.9 ± 2.7	0.0 ± 2.4	−2.1 ± 2.4
HbA1c, *%*				
0 wk	5.6 ± 0.1	5.8 ± 0.2	5.6 ± 0.1	5.8 ± 0.1
12 wk	5.5 ± 0.1	5.7 ± 0.1	5.6 ± 0.2	5.7 ± 0.1
Δ (0–12 wk)	0.0 ± 0.1	0.0 ± 0.1	0.0 ± 0.1	0.2 ± 0.1
Insulin, *mU/L*				
0 wk	19.6 ± 4.6	17.3 ± 1.9	16.5 ± 1.9	23.1 ± 3.7
12 wk	17.7 ± 4.9	15.7 ± 1.8	14.5 ± 1.7	17.3 ± 1.6
Δ (0–12 wk)	1.9 ± 1.2	1.6 ± 1.9	2.1 ± 2.7	5.7 ± 3.3
HOMA-IR				
0 wk	4.9 ± 1.2	4.5 ± 0.6	3.8 ± 0.5	5.5 ± 0.9
12 wk	4.5 ± 1.3	3.9 ± 0.5	3.4 ± 0.4	4.2 ± 0.5
Δ (0–12 wk)	0.4 ± 0.3	0.6 ± 0.6	0.4 ± 0.6	1.3 ± 0.8
Total cholesterol, *mg/dL*				
0 wk	201 ± 11	200 ± 5	214 ± 7	205 ± 9
12 wk	205 ± 12	196 ± 9	181 ± 5[*†]	204 ± 10
Δ (0–12 wk)	−3.3 ± 11.0	4.3 ± 6.2	33.0 ± 5.8[‡§]	0.7 ± 11.7
LDL cholesterol, *mg/dL*				
0 wk	120 ± 9	121 ± 6	130 ± 7	124 ± 8
12 wk	123 ± 11	119 ± 7	103 ± 5[*†]	122 ± 8
Δ (0–12 wk)	−3.2 ± 9.1	1.9 ± 4.8	27.5 ± 7.2[‡§]	1.4 ± 9.4
NMR-derived small LDL particles, *nmol/L*				
0 wk	773 ± 117	712 ± 106	697 ± 106	792 ± 84
12 wk	801 ± 115	648 ± 88	396 ± 69[‡§]	716 ± 85
Δ (0–12 wk)	−28 ± 124	60 ± 110	301 ± 78[‡]	76 ± 75
HDL cholesterol, *mg/dL*				
0 wk	47 ± 4	49 ± 3	49 ± 4	46 ± 3
12 wk	47 ± 4	48 ± 4	47 ± 3	48 ± 3
Δ (0–12 wk)	−0.3 ± 1.0	1.5 ± 1.9	2.5 ± 2.4	−1.7 ± 3.2
LDL:HDL ratio				
0 wk	2.7 ± 0.3	2.5 ± 0.2	2.9 ± 0.3	2.8 ± 0.2
12 wk	2.8 ± 0.3	2.6 ± 0.2	2.5 ± 0.3	2.7 ± 0.2
Δ (0–12 wk)	−0.1 ± 0.2	−0.1 ± 0.1	0.4 ± 0.2	0.1 ± 0.2

(Continued)

TABLE 3 *Continued*

Variable	LD-FDS	LD-C	HD-FDS	HD-C
VLDL cholesterol, *mg/dL*				
0 wk	32 ± 5	28 ± 3	34 ± 4	35 ± 4
12 wk	30 ± 4	28 ± 4	31 ± 3	35 ± 4
Δ (0–12 wk)	2.0 ± 3.3	−0.5 ± 2.8	2.8 ± 2.9	0.4 ± 4.6
TGs, *mg/dL*				
0 wk	153 ± 20	127 ± 15	173 ± 18	176 ± 19
12 wk	144 ± 14	142 ± 21	165 ± 15	166 ± 13
Δ (0–12 wk)	8.8 ± 16.5	−15.0 ± 12.0	8.0 ± 12.0	10.4 ± 21.5

[1] Values are means ± SEMs, n = 15. P values are derived from the multivariate analysis of variance when assessing differences between the strawberry and control groups at 0 and 12 wk of the study and the changes (0–12 wk) for each variable. $^{*}P < 0.1$ vs. LD-FDS; $^{\dagger}P < 0.1$ vs. HD-C; $^{\ddagger}P < 0.05$ vs. LD-FDS; $^{\S}P < 0.05$ vs. HD-C. C, control; FDS, freeze-dried strawberries; HbA1c, hemoglobin A1c; HD, high-dose; LD, low-dose.

(27) and decrease body fat deposits (35). These mechanisms may explain improved serum lipid profiles observed in clinical trials and warrant additional investigation, especially in context of caloric restriction and/or statin therapy.

Among techniques used to classify lipoprotein subclasses in greater detail, NMR quantifies particles according to density and particle size, and NMR-LSP characteristics were shown to predict vascular complications in observational and clinical studies (36,37). However, clinical data on the effects of berries or purified anthocyanins on NMR-LSP are scarce. In reference to the noteworthy study by Zunino et al., FDS (~320 g of frozen strawberries) were shown to decrease the concentrations of NMR-derived small HDL cholesterol particles and increase LDL particle size, whereas no effects were noted on VLDL- and LDL-related particle concentrations in obese adults (14). Compared with these findings, our 12-wk intervention shows the effects of a higher dose of FDS to decrease only the small LDL particle concentrations, when compared with the control group. Small LDL particles were significantly correlated with increased atherogenicity (38), and thus our findings may have implications in the primary prevention of atherosclerosis in obese adults with above-optimal total and LDL cholesterol concentrations.

In contrast to these findings in serum lipids, we did not observe any significant differences in measures of glycemic control, especially fasting glucose, HOMA-IR, and hemoglobin A1c, as a result of 12-wk strawberry supplementation. Similar effects were also noted for measures of obesity, including waist circumference, BMI, and body weight, as well as blood pressure in our study participants. Our findings are in accordance with other clinical studies showing no effects of berry or polyphenol supplementation on adiposity, blood pressure, and/or glycemic control (14,15,17,25,26). Together, these data suggest that strawberry intervention per se may not be effective in lowering obesity and elevated blood pressure and improving glycemia but must be combined with other dietary strategies and physical activity to address these CVD risk factors.

Berries, as fruits or juice or in the form of purified anthocyanin supplements, were shown to decrease markers of lipid oxidation (13,15,39), whereas effects on inflammatory biomarkers, such as CRP, ILs, and adhesion molecules associated with atherosclerotic CVD, have been addressed in only a few studies that showed inconsistent, mostly neutral effects (13–15,33, 40,41). We report that both low and high doses of strawberries lower index of lipid peroxidation (malondialdehyde) but have no effects on CRP and adhesion molecules. However, we did not measure the effects of FDS on circulating antioxidants, such as glutathione and tocopherols, which form the first line of antioxidant defense mechanisms and could also mediate effects on lipid peroxidation (42). Thus, the role of berries in modulating antioxidant and inflammatory biomarkers needs to be more rigorously examined in future trials.

Our study has the following limitations: *1*) small sample size in each arm; *2*) a general inclusion criteria of above-optimal serum lipids vs. specific criterion of hypertriglyceridemia or hypercholesterolemia that might yield greater power to detect differences in lipid measures; *3*) absence of a placebo agent that is identical to the FDS powder and could be used in a double-blind treatment; and *4*) lack of data on plasma and urinary anthocyanins. Furthermore, our control intervention was matched for total fiber and calories in strawberries with the aim of identifying the lipid-lowering effects of strawberries beyond the function of fiber content as reported previously (43). Our findings are not supportive of this hypothesis because the control groups showed no significant lipid-lowering effects, and these effects

TABLE 4 Serum markers of inflammation and lipid oxidation after 12 wk of low-dose (25 g/d) and high-dose (50 g/d) strawberry supplementation vs. controls in obese adults with elevated serum lipids[1]

Variable	LD-FDS	LD-C	HD-FDS	HD-C
hs-CRP, *mg/L*				
0 wk	4.2 ± 1.1	5.1 ± 1.3	8.1 ± 1.8	4.7 ± 1.0
12 wk	4.4 ± 1.0	6.4 ± 1.8	6.9 ± 1.5	6.5 ± 1.8
Δ (0–12 wk)	−0.2 ± 0.5	−1.3 ± 1.5	1.2 ± 0.9	−1.8 ± 1.5
sVCAM-1, *ng/L*				
0 wk	709 ± 66	608 ± 33	610 ± 44	584 ± 36
12 wk	803 ± 53	712 ± 39	768 ± 63	776 ± 61
Δ (0–12 wk)	−94 ± 61	−104 ± 29	−157 ± 41	−192 ± 46
sICAM-1, *ng/L*				
0 wk	258 ± 19	226 ± 15	256 ± 23	243 ± 16
12 wk	257 ± 14	230 ± 15	278 ± 26	274 ± 15
Δ (0–12 wk)	1 ± 21	−4 ± 17	−22 ± 15	−31 ± 17
MDA and HNE, μ*mol/L*				
0 wk	1.9 ± 0.3	2.3 ± 0.3	1.8 ± 0.3	2.4 ± 0.3
12 wk	1.3 ± 0.2*	2.1 ± 0.2	1.2 ± 0.1*	2.3 ± 0.2
Δ (0–12 wk)	0.6 ± 0.3	0.2 ± 0.4	0.6 ± 0.4	0.0 ± 0.3

[1] Values are means ± SEMs, n = 15. P values are derived from the multivariate analysis of variance when assessing differences between the strawberry and control groups at 0 and 12 wk of the study and the changes (0–12 wk) for each variable. $^{*}P <$ 0.05 vs. control (LD-C or HD-C). C, control; FDS, freeze-dried strawberries; HD, high-dose; HNE, hydroxynonenal; hs-CRP, high-sensitivity C-reactive protein; LD, low-dose; MDA, malondialdehyde; sICAM-1, soluble intercellular adhesion molecule-1; sVCAM-1, soluble vascular cell adhesion molecule-1.

noted in the berry group may be explained by the synergism of several bioactive compounds, including fiber in strawberries. Also, we did not measure other biochemical variables of lipid metabolism, such as apo and lipid enzymes, as well as other markers of lipid oxidation, such as oxidized LDL, which could provide a more comprehensive approach to the effects of strawberries on lipid profiles. Finally, our study participants, although obese, prediabetic, and with borderline high total and LDL cholesterol, were not taking any lipid-lowering medications or other dietary supplements. This may limit the generalizability of our findings to the larger population of adults taking multiple medications and dietary supplements.

To the best of our knowledge, this is the first report of a 12-wk randomized controlled trial showing the effects of a higher dose of strawberries in lowering conventional serum total and LDL cholesterol and NMR-derived small LDL particle concentrations when compared with a lower dose in obese adults. In general, strawberries exerted antioxidant effects by decreasing lipid peroxidation, although no effects were noted on adiposity, blood pressure, glycemic control, or inflammation. Thus, strawberries as a popular fruit in the U.S. diet deserve additional investigation concerning their effects on biomarkers of CVD risk in adults, including those with type 2 diabetes and related lipid and vascular abnormalities.

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