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The effect of strawberries in a cholesterol-lowering dietary portfolio

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Abstract

Effective diets reduce blood lipids and oxidative damage, both of which have been linked to the complications of diabetes and coronary heart disease. Our objective was to assess the effect of adding strawberries, as a source of antioxidants, to improve the antioxidant effect of a cholesterol-lowering diet (dietary portfolio). To this end, 28 hyperlipidemic subjects who had followed the dietary portfolio consisting of soy, viscous fiber, plant sterol, and nuts for a mean of 2.5 years were randomized to receive supplements of strawberries (454 g/d, 112 kcal) or additional oat bran bread (65 g/d, 112 kcal, ≈2 g β-glucan) (control) in a randomized 1-month crossover study with a 2-week washout. Strawberry supplementation resulted in a greater reduction in oxidative damage to low-density lipoprotein (LDL) measured as thiobarbituric acid–reactive substances in the LDL fraction ($P = .014$). At the end of the strawberry period, reductions in LDL cholesterol and in the ratio of total to high-density lipoprotein cholesterol were maintained close to 1-year values at −13.4% ± 2.1% and −15.2% ± 1.7%, respectively ($P < .001$), and were similar to the post–oat bran bread values. Strawberries also improved the palatability of the diet. We conclude that strawberry supplementation reduced oxidative damage to LDL while maintaining reductions in blood lipids and enhancing diet palatability. Added fruit may improve the overall utility of diets designed to lower coronary heart disease risk.

1. Introduction

Antioxidant supplementation has largely been unsuccessful in delivering the anticipated health benefits in terms of cardiovascular disease [1,2] and cancer [3]. Nevertheless, a significant body of theory and basic scientific evidence continues to accumulate supporting plausible reasons for a benefit of reduced oxidative damage to lipids, lipoproteins, and DNA [4-6].

In general, the randomized controlled trials so far undertaken have not assessed the degree of reduction of oxidative damage achieved by antioxidant supplementation [1-3]. It may be that the reduction in the level of oxidative damage was insufficient to produce a measurable improvement or that the key cellular elements that require protection from oxidative stress were not protected by the antioxidant measures used [1-3]. Furthermore, the distinction between the effect of foods as opposed to the use of large amounts of single antioxidants or combinations of antioxidants has been raised as a reason for their lack of beneficial effect.

Certain foods are rich sources of antioxidants, including fruits, seeds, nuts, and pulses [7,8]; but their antioxidant activity in vivo has only recently begun to be explored. Consumption of these foods has also been associated with

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reduction in cardiovascular disease, stroke, diabetes, and certain cancers [9-16], diseases for which, on theoretical grounds, antioxidants may have a benefit [4-6]. However, many of the data on the health benefits come from epidemiologic studies [9-16]. No randomized controlled trials evaluating disease outcome exist in this area, with the possible exception of the Women's Health Initiative, which had advice that included increased fruit and vegetable consumption. Although no significant reduction was seen for coronary heart disease (CHD) risk in the Women's Health Initiative study, the increase in fruit and vegetable consumption was relatively small at 1.1 servings per day [17].

In terms of antioxidants in fruits, berries, including blueberries and strawberries, have been shown to have antioxidant effects in vivo [18,19]. Strawberries on a per calorie basis are good sources of antioxidants, including ellagic acid and anthocyanins [20].

We have therefore assessed the effect of strawberry supplementation on oxidation of circulating low-density lipoprotein (LDL). The dose used was substantial (1 lb/d); but no dose-response data are available, and strawberries were the only antioxidant food to be increased in the diet.

2. Methods

2.1. Participants

A total of 85 hyperlipidemic participants was recruited by newspaper advertisements for a 5-year, open-label, self-selected (ad libitum) study of a dietary portfolio of cholesterol-lowering foods [21]. One subject was recruited by direct physician referral to the Risk Factor Modification Center (Fig. 1). Before the start of this study, 17 men and 17 postmenopausal women had completed between 2 and 3 years of the long-term study, whereas 4 men and 4 women had completed between 1 and 2 years. These 42 subjects were invited to take part in the strawberry substudy, of whom 30 agreed (Fig. 1). The participants were largely of European origin (n = 26). There was also 1 Filipino, 1 East Indian, 1 African, and 1 Hispanic participant. Participants' baseline characteristics are shown in Table 1. At screening, all participants had raised LDL cholesterol (LDL-C) levels (>4.1 mmol/L) [22]. No participants had a history of cardiovascular disease, diabetes, or renal or liver disease. Before the start of the long-term study, a mean of 2.5 years previously, 10 subjects were taking statins on a regular basis but had stopped taking them at least 2 weeks before the long-term study baseline visit. At recruitment, no subject had untreated hypertension (blood pressure ≥140/90 mm Hg). Three participants were taking antihypertensive medications at a constant dose before and during the study. One subject increased the dosage of an existing blood pressure medication and 1 other subject added a new blood pressure medication in addition to having increased the dosage of an existing medication during the first and second years of the study, respectively. During the strawberry substudy, all lipid-lowering and antihypertensive medications were held constant.

2.2. Study protocol

The background intervention throughout the long-term study for the previous 1 to 3 years (mean, 2.5 years) was a single-phase, open-label, self-selected (ad libitum) dietary portfolio of cholesterol-lowering foods. All subjects had been instructed to follow a low–saturated fat therapeutic diet (National Cholesterol Education Program Adult Treatment Panel III) for 2 months before commencing the long-term study [22]. After a mean of approximately 2.5 years, subjects were randomized to receive either a daily dietary strawberry supplement of 1 lb (454 g) per 2000 kcal of diet or a calorie-equivalent amount of oat bran bread for 1 month. This phase was followed by a 2-week washout where subjects returned to the dietary portfolio they had been following before taking strawberries or additional oat bran bread. They then crossed over to receive the alternate treatment for a further 1-month period. Before the strawberry substudy, participants had been seen at 2-month intervals during the long-term study. During the strawberry substudy, they were seen at 2-week intervals. At each visit, fasting body weights were checked; and blood samples were obtained after 12-hour overnight fasts. Blood pressure was measured 3 times in the nondominant arm using a mercury sphygmomanometer by the same observer after subjects had been seated for at least 15 minutes, and the average blood pressure was used. Seven-day diet histories were obtained for the week before the clinic visit and checked by the dietitian. The records were discussed with the dietitian, and suggestions were made to enhance compliance. The previous week's exercise was also recorded, and the dietitian encouraged each participant to hold this constant over the prestudy and study periods. Compliance was estimated from the 7-day diet histories. Satiety was assessed at week 4 using a bipolar semantic scale where −3 was extremely hungry, 0 was neutral, and +3 was satiated. At the end of the second phase, participants were asked to rate the palatability of both dietary treatments (strawberries and oat bran bread) on a semantic scale of 1 to 10 where 1 was unpalatable and 10 was extremely palatable.

The Ethics Committee of St Michael's Hospital, the University of Toronto, and the Therapeutic Products Directorate of Health Canada approved the study. Written informed consent was obtained from the participants. The clinical trials registration number is NCT00345722.

2.3. Diets

Before the start of the long-term study, 1 to 3 years (mean, 2.5 years) previously, participants had been instructed to eat their routine therapeutic low-fat diets with mean macronutrient profiles that were close to current National Cholesterol Education Program Adult Treatment Panel III guidelines (<7% energy from saturated fat and <200 mg/d dietary cholesterol) (Table 2) [21,22].

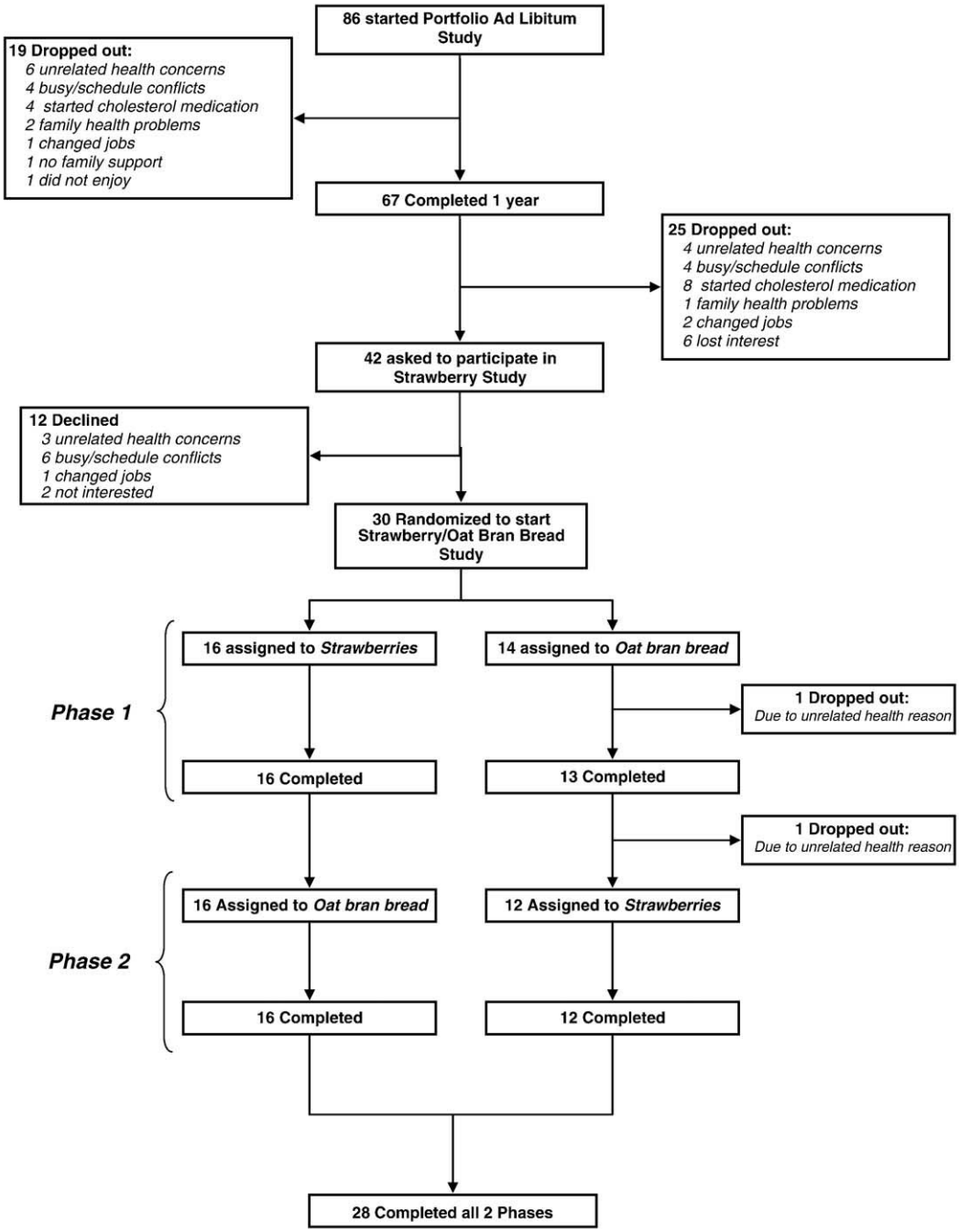

Fig. 1. Study flow diagram showing progress of participants through the trial.

Dietary advice for the long-term ad libitum study was based on the consumption goals for the same 4 dietary components that had been emphasized in previous metabolic dietary portfolio studies [23]. Participants were advised to consume 1.0 g plant sterols per 1000 kcal of diet from a plant sterol ester–enriched margarine; approximately 10 g viscous fibers per 1000 kcal of diet from oats, barley, psyllium, and the vegetables okra and eggplant; 22.5 g soy protein per 1000 kcal as soy milk, tofu, and soy meat analogues; and 22.5 g whole almonds per 1000 kcal of diet in addition to their ongoing low-fat diet. To the extent acceptable to individual participants, advice was given to take a vegetarian diet without the use of dairy foods or eggs.

During the strawberry phase, participants were advised to buy and consume 1 lb per 2000 kcal (454 g providing 112 kcal) of fresh strawberries from local stores on a daily basis and were reimbursed based on their receipts. Strawberries were advised to be eaten in a variety of ways: as snacks, with oat bran at breakfast, as desserts, and blended with soy milk as “smoothies.” During the oat bran

Table 1
Substudy baseline characteristics of study participants (n = 28)

	Mean ± SEM	Range
Age (y)	62 ± 1	(38-75)
Weight (kg)	71.5 ± 2.2	(50.0-98.2)
BMI (kg/m^2)	26.5 ± 0.6	(19.8-32.3)
TC (mmol/L)	5.62 ± 0.14	(4.41-7.52)
LDL-C (mmol/L)	3.61 ± 0.13	(1.97-5.16)
HDL-C (mmol/L)	1.36 ± 0.07	(0.88-2.60)
TG (mmol/L)	1.43 ± 0.12	(0.59-4.17)
Systolic blood pressure (mm Hg)	111 ± 2	(95-141)
Diastolic blood pressure (mm Hg)	68 ± 1	(56-84)

To convert cholesterol and TG to milligrams per deciliter, multiply by 38.67 and 88.57, respectively. BMI indicates body mass index.

bread phase, participants were advised to consume an additional 2 1/2 oz per 2000 kcal (65 g providing 112 kcal) per day of oat bran bread that was provided on a biweekly basis. The general advice given to participants was to eat the strawberries or oat bran bread supplements where possible as replacements for luxury food items such as desserts, cakes, muffins, pastries, and cookies.

Self-taring electronic scales (Salter Housewares, Kent, England) were provided to all participants. They were asked to weigh all food items consumed in the week when diet histories were recorded.

2.4. Analyses

Serum was analyzed according to the Lipid Research Clinics protocol [24] for total cholesterol (TC), triglycerides (TG), and high-density lipoprotein cholesterol (HDL-C) after dextran sulfate–magnesium chloride precipitation [25] in the J Alick Little Lipid Research Laboratory of St Michael's Hospital. Low-density lipoprotein cholesterol was calculated by the method of Friedewald et al [26] (LDL-C = TC − [TG/2.2 + HDL-C]) in millimoles per liter.

Low-density lipoprotein oxidation was estimated by measuring in the LDL fraction both conjugated dienes, based on the method of Ahotupa et al [27], and thiobarbituric acid–reactive substances (TBARS), using the malondialdehyde (MDA)–thiobarbituric acid assay adopted from Jentzsch et al [28]. The coefficients of variation for these analyses were 5.7% and 6.4% for TBARS and conjugated dienes, respectively.

Protein oxidation was measured using the 5,5′-dithiobis (2-nitrobenzoic acid) assay [29] to assess the loss of reduced thiol (−SH) groups as a measure of oxidation. The coefficient of variation of serum samples analyzed in triplicate was 3.0%.

Additional analyses included sodium, potassium, chloride, urea, creatinine, alanine and aspartate transaminases, alkaline phosphatase, albumin, globulin, and total bilirubin (Synchron LX 20; Beckman Coulter Canada, Mississauga, Ontario). The 24-hour urine collections were also analyzed for creatinine, urea, and electrolytes in the hospital's clinical biochemistry laboratory by an autoanalyzer technique (Clinitek Atlas Automated Urine Chemistry Analyzer; Siemens Medical Solutions Diagnostics, Tarrytown, NY).

Complete blood counts were determined on fresh blood samples collected in EDTA tubes (Becton Dickinson, Mississauga, Ontario) in the routine hospital hematology laboratory of St Michael's Hospital using a Coulter LH 700 Series Hematology System (Beckman Coulter Canada).

Diets were analyzed for macronutrients, micronutrients, fatty acids, cholesterol, and fiber using a computer program based on US Department of Agriculture data [30]. Adherence to the ad libitum dietary portfolio was assessed from the completed 7-day food records; consumption of the 4 main components—soy, viscous fiber, almonds, and plant sterol margarine—was measured, with each component eaten at the prescribed dose contributing 25% to the total compliance of 100%.

Table 2
Nutritional profiles (mean ± SEM) of ad libitum portfolio and during both oat bran bread and strawberry phases

	Initial 1-y study		Oat bran bread		Strawberry		*P* value
	Wk 0 (n = 27) mean ± SEM	Wk 52 (n = 28) mean ± SEM	Substudy baseline (n = 28) mean ± SEM	Wk 4 (n = 28) mean ± SEM	Substudy baseline (n = 28) mean ± SEM	Wk 4 (n = 27) mean ± SEM	Wk 4 vs 4
Energy	1593 ± 106	1745 ± 98	1639 ± 90	1782 ± 111	1729 ± 116	1719 ± 93	.057
Total fat (% of energy)	24.5 ± 1.4	29.4 ± 0.8	31.0 ± 1.0	31.5 ± 0.9	30.5 ± 0.9	31.2 ± 1.0	.841
MUFA (% of energy)	9.6 ± 0.6	12.7 ± 0.5	13.5 ± 0.6	13.1 ± 0.5	13.0 ± 0.6	13.1 ± 0.6	.971
PUFA (% of energy)	5.9 ± 0.6	9.4 ± 0.4	9.9 ± 0.6	10.4 ± 0.5	9.8 ± 0.4	9.9 ± 0.3	.292
SFA (% of energy)	6.3 ± 0.5	5.5 ± 0.3	5.9 ± 0.3	6.2 ± 0.3	6.0 ± 0.4	6.2 ± 0.3*	.587
Dietary cholesterol (mg/1000 kcal)	82.3 ± 9.9	47.9 ± 9.8	44.1 ± 9.0	49.4 ± 9.0	52.5 ± 7.6	53.5 ± 5.9	.060
Total protein (% of energy)	17.8 ± 0.7	19.9 ± 0.5	19.1 ± 0.6	19.9 ± 0.6	18.9 ± 0.7	19.1 ± 0.6	.059
Plant protein (% of energy)	7.8 ± 0.3	15.3 ± 0.8	13.7 ± 0.7*	14.4 ± 0.6	13.8 ± 0.7	13.5 ± 0.7	.010
Soy protein (% of energy)	0.3 ± 0.1	4.6 ± 0.5	4.1 ± 0.5	4.0 ± 0.5	4.0 ± 0.5	4.1 ± 0.4	.988
Carbohydrates (% of energy)	57.5 ± 1.4	51.3 ± 1.2	58.2 ± 1.3*	57.3 ± 1.2*	58.4 ± 1.3*	59.1 ± 1.3*	.056
Fiber (g/1000 kcal)	18.3 ± 1.3	27.9 ± 1.4	24.5 ± 1.3*	25.9 ± 1.0	24.2 ± 1.1*	27.1 ± 1.2	.129
Soluble (g/1000 kcal)	4.5 ± 0.3	9.2 ± 0.7	7.6 ± 0.6*	8.3 ± 0.5	7.4 ± 0.5*	9.4 ± 0.5	.024
Alcohol (% of energy)	1.5 ± 0.5	1.2 ± 0.4	1.3 ± 0.5	1.3 ± 0.4	1.8 ± 0.6	1.3 ± 0.4	.861

MUFA indicates monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid.
* Significant difference from week 52 ($P < .05$).

2.5. Statistical analysis

All results are expressed as mean ± SE. Two questions were addressed. First were the gains in blood lipid and blood pressure reduction seen at 1 year retained after the addition of strawberries to the diet, and second were the effects of strawberries different from those of additional oat bran bread. To answer the first question, changes at week 4 from the long-term study baseline were calculated as percentages for serum lipids and as absolute differences (reductions) for blood pressure and body weight and compared with 1-year values by least squares means. To address the second question, the significance of differences in blood lipids, oxidation products, blood pressure, hematology, serum electrolytes, renal and liver function tests, and urinary markers between strawberry and oat bran bread treatments at 4 weeks was assessed using PROC MIXED in SAS (Cary, NC) with the week-4 value as the response variable; treatment, sex, sex by treatment, and a subject ID nested in sex by order as main effects; and substudy baseline as a covariate. Only week-4 values were used for compliance, dietary, satiety, and palatability data.

Because all 30 subjects from the long-term portfolio study who were willing to participate in the strawberry substudy were recruited, only a post hoc power analysis was undertaken. With n = 30 subjects and a standard deviation of the effect of 10% for LDL-C, 18.2% for protein thiols, 15% for conjugated dienes, and 14.4% for TBARS, the respective effect sizes that would be considered significant ($\alpha = .05$, $\beta - 1 = .8$) were, respectively, 5.3% for LDL-C, 9.6% for protein thiols, 8.3% for conjugated dienes, and 7.6% for TBARS.

The intention-to-treat analysis was not used in this study. Because this was a crossover study, we wished to compare treatment results directly among completers who actually took the supplements to determine the potential cardiovascular protective mechanisms of fruit supplementation.

Pearson correlations were also used to determine the relation between week-4 dietary variables and measures of compliance combining data from both treatments [31].

3. Results

Of the 30 subjects who started the study, 28 completed both treatments. Body weight was constant over the course of the 4-week intervention for both strawberry and oat bran phases (Table 3).

Adherence was good on the strawberry phase as reflected by an increase in mean intake of strawberries from 3.9 ± 2.4 g/d before taking strawberries to 393.8 ± 16.7 g/d at week 4 (or 98.5% ± 2.0% compliance) (Fig. 2). For oat bran bread, intake increased from 51.3 ± 10.6 g/d at week 0 to 131.1 ± 12.5 g/d at week 4. Because the long-term study recommendation for oat bran bread was 130 g/d, the supplementation of additional oat bran bread for the substudy of 65 g/d simply achieved the long-term study unsupplemented intake goal.

There were no differences in satiety (−3 to +3) reported between the treatment groups (strawberry, 0.9 ± 0.2; oat bran bread, 1.0 ± 0.2; $P = .861$). However, the strawberry intervention resulted in a higher palatability rating (scale, 1-10) than was seen for the oat bran bread treatment (8.7 ± 0.3 vs 6.2 ± 0.4, $P < .001$).

3.1. Blood lipids

No differences between treatments were seen in absolute lipids and lipoprotein values at 4 weeks for TC, LDL-C, HDL-C, TG, or the ratios TC/HDL-C and LDL-C/HDL-C (Table 3).

At the end of the strawberry treatment, the LDL-C and TC/HDL-C percentage reductions from the long-term study baseline were −13.4% ± 2.1% ($P < .001$) and −15.2% ± 1.7% ($P < .001$), respectively; and the corresponding values for the oat bran bread treatment were −13.9% ± 2.3% ($P < .001$) and −14.2% ± 2.0% ($P < .001$). Similar reductions of −20.7% ± 4.0% and −20.4% ± 4.8% in TG were also seen on both strawberries and oat bran bread treatments, respectively ($P < .001$). These changes were not significantly different from the lipid reductions seen at 1 year (Table 4).

3.2. Oxidation products

No baseline differences were seen in serum protein thiols or in conjugated dienes or TBARS in the LDL fraction expressed either as total conjugated dienes and TBARS or as a molar ratio of LDL-C. Serum protein thiols were increased on both strawberry ($P < .001$) and oat bran bread ($P = .001$) treatments, an indication of less oxidative damage to protein sulfur bonds (Table 5); but no difference between treatment

Table 3
Mean (±SEM) body weight, blood lipids, CRP, and blood pressure during both the oat bran bread and strawberry phases (n = 28)

	Oat bran bread phase			Strawberry phase			*P* value
	Substudy baseline	Wk 2	Wk 4	Substudy baseline	Wk 2	Wk 4	Wk 4 vs 4
Weight (kg)	71.1 (2.2)	71.1 (2.2)	71.0 (2.2)	71.3 (2.2)	71.0 (2.2)	71.0 (2.2)	.983
Cholesterol (mmol/L)	5.84 (0.14)	5.66 (0.16)	5.67 (0.15)	5.63 (0.14)	5.62 (0.10)	5.72 (0.14)	.641
LDL-C (mmol/L)	3.81 (0.12)	3.59 (0.11)	3.63 (0.12)	3.60 (0.13)	3.55 (0.10)	3.66 (0.11)	.784
HDL-C (mmol/L)	1.36 (0.08)	1.33 (0.07)	1.36 (0.07)	1.38 (0.07)	1.35 (0.07)	1.38 (0.08)	.297
TG (mmol/L)	1.47 (0.13)	1.64 (0.12)	1.50 (0.14)	1.42 (0.13)	1.58 (0.13)	1.49 (0.13)	.932
CRP (mg/L)	1.34 (0.22)	1.25 (0.20)	1.54 (0.28)	1.20 (0.20)	1.57 (0.33)	1.23 (0.18)	.277
Systolic blood pressure (mm Hg)	110.8 (2.2)	112.5 (2.1)	108.6 (1.9)	112.1 (2.2)	109.4 (2.3)	110.2 (2.2)	.242
Diastolic blood pressure (mm Hg)	67.8 (1.3)	69.0 (1.4)	67.2 (1.2)	68.6 (1.3)	68.2 (1.4)	67.5 (1.3)	.739

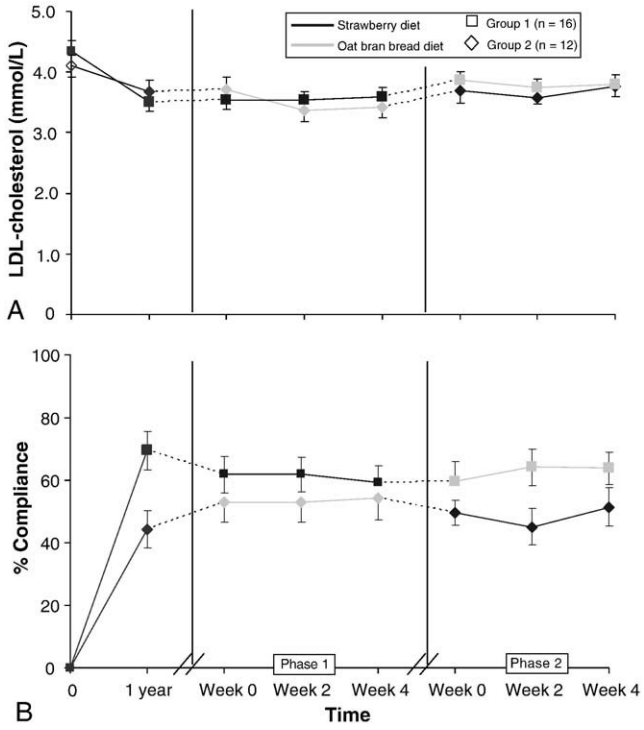

Fig. 2. Mean (A) LDL-C and (B) compliance to the dietary portfolio at the long-term study baseline, 1 year, and during phase 1 and 2 of the strawberry/oat bran bread study. The number of subjects randomized to start the strawberry (group 1, □) and oat bran bread (group 2, ◊) treatments at phase 1 was n = 16 and n = 12, respectively.

was seen in protein thiols. Concentrations of TBARS in the LDL fraction were lowered on the strawberry phase only, but not on the oat bran bread phase; and there was a significant difference both for absolute TBARS concentrations in the LDL fraction ($P = .016$) and for TBARS expressed as a ratio of LDL-C ($P = .014$) (Table 5). There were no significant differences in conjugated dienes either within or between treatments (Table 5).

3.3. Blood pressure, hematology, and biochemistry

No significant treatment differences were seen for blood pressure (Table 3). The absolute reductions from long-term study baseline while on the strawberry and oat bran bread treatments were similar (strawberry, −10.7 ± 1.8 mm Hg systolic and −5.0 ± 1.1 mm Hg diastolic; oat bran bread, −12.3 ± 2.1 mm Hg systolic and −5.4 ± 1.4 mm Hg diastolic; n = 26), although both showed greater reductions than those seen at 1 year ($P < .05$).

No significant treatment differences were seen in hematologic measurements (data not shown) or in C-reactive protein (CRP).

No significant treatment differences were seen in serum electrolytes, fasting glucose, and renal and liver function indices (data not shown). Notably, serum potassium concentration on the strawberry treatment was almost identical to that on the oat bran bread treatment (3.80 ± 0.05 mmol/L vs 3.88 ± 0.06 mmol/L). Although unadjusted 24-hour week-4 urinary potassium output was not significantly different on the strawberry compared with the oat bran bread treatment (mean difference = 8.0 ± 5.0 mmol/d, n = 28, $P = .124$), the creatinine-adjusted potassium output was significantly higher on the strawberry treatment ($P = .004$).

4. Discussion

The antioxidant activity of strawberries was reflected in the lower levels of TBARS in the LDL fraction as an indicator of reduced oxidative damage to LDL-C and thus a potential reduction in its atherogenicity [4,32,33]. At the same time, addition of a significant amount of fruits such as strawberries improved the palatability of a long-term cholesterol-lowering diet without reducing its effectiveness in lowering blood lipids or blood pressure.

Many fruits contain so-called functional components. Strawberries are sources of the antioxidants ellagic acid and anthocyanin [20], which may possibly improve vascular reactivity postprandially [34]. In laboratory cell culture and animal studies, strawberry extracts and their antioxidant components have been shown to have anticancer properties [35-37]. Their effect in minimizing oxidative damage to LDL lipids, shown in the present study as lowered concentrations of TBARS, has not been demonstrated previously after strawberry consumption. It has been proposed that oxidized LDL enhances accumulation of monocytes in the subintimal space and the conversion of

Table 4
Percentage changes (mean ± SEM) from long-term study baseline in blood lipids after 1 year on the dietary portfolio and at the end of both the oat bran bread and strawberry treatments (n = 28)

	Cholesterol	LDL-C	HDL-C	TG	TC/HDL-C
1-y	−11.6% ± 1.6%‡	−15.1% ± 2.7%‡	4.8% ± 2.4%*	−13.9% ± 5.9%‡	−14.5% ± 2.3%‡
Oat bran bread	−11.6% ± 1.7%‡	−14.0% ± 2.3%‡	4.0% ± 2.2%	−20.4% ± 4.8%‡	−14.2% ± 2.0%‡
Strawberry	−10.9% ± 1.5%‡	−13.4% ± 2.1%‡	6.0% ± 2.3%†	−20.7% ± 4.0%‡	−15.2% ± 1.7%‡

No significant difference between treatments ($P > .05$).
Significant difference from long-term study baseline:
* $P = .05$.
† $P < .01$.
‡ $P < .001$.

Table 5
Mean (±SEM) protein thiol, conjugated diene, and MDA equivalent (TBARS) concentrations at substudy baseline and week 4 for the oat bran bread and strawberry phases (n = 26)

	Oat bran bread		Strawberry		*P* value
	Substudy baseline mean ± SEM	Wk 4 mean ± SEM	Substudy baseline mean ± SEM	Wk 4 mean ± SEM	Wk 4 vs 4
Protein thiols (mmol/L)	351.9 ± 10.1	394.4 ± 10.8†	352.8 ± 11.8	397.6 ± 7.9†	.802
Conjugated dienes					
Absolute concentration (μmol)	52.6 ± 3.6	52.7 ± 3.7	51.0 ± 3.2	51.8 ± 3.3	.649
Molar ratio of LDL-C (μmol/mmol)	14.7 ± 1.0	15.3 ± 1.1	15.2 ± 1.0	15.1 ± 1.1	.775
TBARS					
Absolute concentration (μmol)	0.57 ± 0.03	0.55 ± 0.03	0.56 ± 0.03	0.51 ± 0.03*	.016
Molar ratio of LDL-C (μmol/mmol)	0.16 ± 0.01	0.16 ± 0.01	0.17 ± 0.01	0.15 ± 0.01*	.014

Significant difference from substudy baseline:
* $P \leq .05$.
† $P \leq .001$.

macrophages to foam cells with increased uptake of oxidized LDL by the scavenger receptors [4]. The subsequent subintimal lipid accumulation and cell death have been considered to be part of the process of atherogenesis [4,32,33]. Consequently, in the longer term, lower levels of circulating oxidized LDL after strawberry consumption may help reduce the risk of CHD [4,32,33].

An advantage of the berries may be their novelty and palatability compared with the oat bran bread. It is therefore possible that some of the differential metabolic or antioxidant effects between strawberries and oat bran bread could be an adherence phenomenon. Another potential advantage of strawberries and temperate climate fruits in general is that, because of their relatively low glycemic indices, they will tend to lower the glycemic load of the diet. However, no reduction was seen in blood glucose; and insulin was not measured in this study.

Increases in fruit and vegetable intake have been associated with reductions in diseases such as CHD, ascribed in part to their soluble (viscous) fiber content, which has been noted to reduce serum cholesterol [38-41], and also in part to their source of potassium, which has been suggested to reduce blood pressure [42]. However, no effect was seen in this study on blood lipids or blood pressure with either the additional strawberries or oat bran bread. Furthermore, no effect was seen on body weight, despite the calorie value of the supplementation of 112 kcal/d. The lack of weight gain may have been due to the small increase of approximately 450 g for the study, which might have been expected at 1 month. This increase may have been difficult to detect and may also have been minimized by the dietary approach to make strawberries substitute for "luxury" foods including calorie-rich desserts. Reduction in CHD risk has been difficult to demonstrate for specific foods. In a recent cohort study, strawberry intake was not associated with a reduced risk of cardiovascular disease in women; but the study had a median consumption level of only 1 to 3 servings per week [43]. Higher levels of strawberry intake approaching those administered in the present study may be required to demonstrate cardiovascular benefits.

Direct measurement of oxidized LDL, for example, by an antibody technique, as opposed to measurement of lipid oxidation products, would have been informative. Measurement of TBARS as indicators of oxidative damage have been

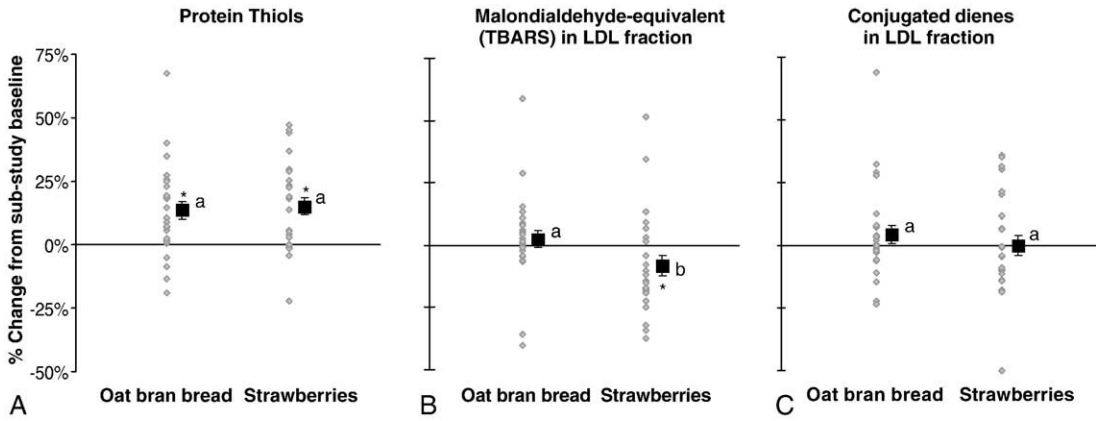

Fig. 3. Individual (♦) and mean (■) percentage reductions at week 4 from substudy baseline of (A) protein thiols, (B) MDA equivalents (TBARS) in LDL fraction, and (C) conjugated dienes in LDL fraction (n = 26). Mean data points within the same graph having different letter designations indicate significant treatment difference ($P < .05$). *Significantly different from substudy baseline ($P < .05$).

criticized because the assay relies on the reaction of thiobarbituric acid with MDA as a marker of oxidative damage to lipids. The reaction is carried out in a water bath at high temperature and in acidic conditions at which thiobarbituric acid may react with other oxidized products. Nevertheless, despite these limitations, in studies such as those where subjects act as their own controls, differences in TBARS between treatments may still have relevance.

The lack of effect of significant treatment difference in conjugated dienes may have been due to insufficient power because the observed effect size of the treatment difference (Fig. 3) for TBARS was approximately double that for conjugated dienes.

The health implications of the study relate to the potential ability of strawberries and possibly fruits in general to increase the palatability of therapeutic diets to make them more acceptable for long-term use without increasing serum lipids. There was also a reduction of TBARS in the LDL fraction, an indication of reduced oxidized lipids associated with LDL. A relation has been seen between TBARS and the development and severity of CHD [44,45]. In this way, despite no change in serum lipids, increased fruit consumption may reduce the risk of CHD. It is not yet possible to extrapolate antioxidant measurements directly to changes in CHD risk as may be done for established risk factors (ie, LDL-C, blood pressure). All that can be said at present is that there is indirect evidence that oxidative stress may play a role in increasing CHD risk.

For the maintenance of health and reduction of risk for chronic disease, Western populations have been advised to increase their intake of fruits and vegetables [46]. However, a limited number of intervention trials have studied the impact of fruit and vegetable intake [17,47]. The present study suggests that targeted advice to eat more fruits is effective in reducing oxidation of circulating LDL and in increasing the palatability of the dietary portfolio without losing the other health advantages gained.

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