Effects of dairy products naturally enriched with *cis*-9,*trans*-11 conjugated linoleic acid on the blood lipid profile in healthy middle-aged men[1–3]

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ABSTRACT

Background: Interest in the development of dairy products naturally enriched in conjugated linoleic acid (CLA) exists. However, feeding regimens that enhance the CLA content of milk also increase concentrations of *trans*-18:1 fatty acids. The implications for human health are not yet known.

Objective: This study investigated the effects of consuming dairy products naturally enriched in *cis*-9,*trans*-11 CLA (and *trans*-11 18:1) on the blood lipid profile, the atherogenicity of LDL, and markers of inflammation and insulin resistance in healthy middle-aged men.

Design: Healthy middle-aged men ($n = 32$) consumed ultra-heat-treated milk, butter, and cheese that provided 0.151 g/d (control) or 1.421 g/d (modified) *cis*-9,*trans*-11 CLA for 6 wk. This was followed by a 7-wk washout and a crossover to the other treatment.

Results: Consumption of dairy products enriched with *cis*-9,*trans*-11 CLA and *trans*-11 18:1 did not significantly affect body weight, inflammatory markers, insulin, glucose, triacylglycerols, or total, LDL, and HDL cholesterol but resulted in a small increase in the ratio of LDL to HDL cholesterol. The modified dairy products changed LDL fatty acid composition but had no significant effect on LDL particle size or the susceptibility of LDL to oxidation. Overall, increased consumption of full-fat dairy products and naturally derived *trans* fatty acids did not cause significant changes in cardiovascular disease risk variables, as may be expected on the basis of current health recommendations.

Conclusion: Dairy products naturally enriched with *cis*-9,*trans*-11 CLA and *trans*-11 18:1 do not appear to have a significant effect on the blood lipid profile. *Am J Clin Nutr* 2006;83:744–53.

KEY WORDS Cholesterol, conjugated linoleic acid, high-density lipoprotein, low-density lipoprotein, triacylglycerol

INTRODUCTION

Conjugated linoleic acid (CLA) is a collective term for a mixture of positional and geometric isomers of linoleic acid (18:2n–6) in which the 2 double bonds are conjugated. CLA is found in the meat, fat, milk, and milk products of ruminant animals. Some animal studies suggest health benefits of *cis*-9,*trans*-11 CLA, including anticarcinogenic (1, 2) and antiatherogenic (3) effects and improvements in blood lipid concentrations (4), although most of these are not unequivocally supported by consistent data from human studies (5). This appears to be largely due to differences in the isomer blends used and biological effects of specific CLA isomers.

Human studies have examined the effects of CLA supplementation on plasma lipid concentrations (6–12). However, the main aim of most of these studies was to examine the antiobesity effects of CLA, which are normally attributed to *trans*-10,*cis*-12 CLA, and the results for blood lipids are highly inconsistent. Some of the discrepancies could be associated with the fact that the studies used different mixtures of CLA isomers at different doses. Tricon et al (13) recently reported that when highly purified *cis*-9,*trans*-11 and *trans*-10,*cis*-12 CLAs were supplemented to healthy subjects, they had opposing effects on the blood lipid profile: *cis*-9,*trans*-11 CLA decreased the ratio of LDL to HDL cholesterol and of total to HDL cholesterol, whereas *trans*-10,*cis*-12 CLA increased these ratios. This would

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suggest a beneficial influence of cis-9,*trans*-11 CLA relative to *trans*-10,*cis*-12 CLA.

The *cis*-9,*trans*-11 CLA isomer is the principal dietary form of CLA, accounting for as much as 85–90% of the total CLA content in dairy products (14). However, the contribution of CLA to the human diet is relatively small (15), and there has been growing interest in the potential for naturally increasing the *cis*-9,*trans*-11 CLA content of milk and dairy products (16). In a study by Malpuech-Brugere et al (17), purified CLA isomers were added to the dairy products postproduction, rather than being enhanced naturally through altered feeding regimens, but there was no effect of the products on body composition.

Concentrations of CLA are higher in milk fat from cows that are given fresh forages than in milk fat from cows that are given conserved forages, and CLA concentrations can also be enhanced with whole oilseeds or oil supplements (14). Fish oil is more effective at enhancing milk fat CLA content than are plant oils (18), and the CLA content can be additionally increased when fish oil is fed in combination with supplements rich in linoleic acid (19). Nutritional strategies for natural enhancement of the CLA content of milk also result in milk fat containing substantially increased *trans* 18:1 concentrations [particularly *trans*-11 18:1 (*trans* 18:1n–7)], lowered saturated fatty acid concentrations, and a slightly higher n–3 polyunsaturated fatty acid content (20). Recent animal studies suggest that CLA-enriched dairy products improve the plasma lipoprotein profile and reduce markers associated with atherogenic risk (21, 22). However, there is little data on the effects of modified dairy products with this type of fatty acid profile on human health, but it is important to consider the human health effects if the potential for CLA-enriched dairy products is to be explored. Desroches et al (23) showed that consumption of foods containing a CLA-enriched butter had little effect on the blood lipid profile, although there was a smaller reduction in plasma total cholesterol and in the ratio of total to HDL cholesterol than that observed with a control diet. The aim of the present study was to examine the influence of naturally modified dairy products enriched in *cis*-9,*trans*-11 CLA and *trans*-11 18:1 on the blood lipid profile, the atherogenicity of LDL, and markers of inflammation and insulin resistance in healthy middle-aged men.

SUBJECTS AND METHODS

Dairy products

Forty-nine early lactation Holstein-British Friesian cows were fed total mixed rations containing 0 (control) and 45 g/kg (on dry matter basis) of a mixture (1:2, wt:wt) of fish oil and sunflower oil during 2 consecutive 7-d periods to produce control and CLA-enhanced milks, respectively. Because the inclusion of oils in the diet was expected to increase the unsaturated fatty acid content of milk, vitamin E (*dl*-α-tocopheryl acetate; Roche Vitamins Ltd, Welwyn Garden City, United Kingdom) was incorporated (500 IU/kg dry matter) into both rations. All experimental procedures that were used were licensed, regulated, and inspected by the UK Home Office under the Animals (Scientific Procedures) Act of 1996. Milk produced from all cows was collected in a refrigerated bulk tank over the last 2 d of both feeding periods. The milk collected on day 6 of each period was used to prepare the ultra-heat-treated (UHT) milk, and that collected on day 7 was used for the manufacture of cheese and butter. After cooling to 4 °C, the control and modified milks were transported to the Pilot Processing Plant at the University of Reading and pasteurized at 72 °C for 15 s with a continuous High Temperature Short Time plant (Plate heat exchanger type JUNIOR; Invensys APV, Crawley, United Kingdom). Batches of milk were treated in an indirect UHT plant (Plate heat exchanger type JHE; Invensys APV) at 142 °C for 2 s and packaged in 250-mL Tetra Paks (Tetra Pak Ltd, Wrexham, United Kingdom).

For making the butter, pasteurized milk was separated with a centrifugal separator. The cream obtained was then standardized to a fat content of 400 g/kg, chilled, and stored overnight at 5 °C. The following day, the cream was transferred to a sterilized butter churn (50 kg capacity; Melotte, Gascoigne, United Kingdom) and rotated at slow speed for 2 min before venting. Thereafter, the churn was rotated at high speed and inspected every 10 min until small butter grains were formed. The buttermilk was then drained away, cold potable water (4 °C) was added, and the churn was rotated at low speed for 15 s and then drained away. Both the washing and draining stages were then repeated. Finally, salt (10 g NaCl/kg, wt:wt) was sprinkled over the surface of the butter grains and the churn was rotated at slow speed for 2 min to allow the salt and butter to consolidate. The butter was then blended with a Stefan mixer (Stefan Machinery Ltd, Middlesex, United Kingdom), packaged into 200-g pots, and stored at −18 °C.

For making cheese, pasteurized milk was incubated for 30 min at 30 °C with a Mesophilic aromatic culture (CHR HANSEN, Hungerford, United Kingdom) and stirred continuously. Thereafter, 0.03% (wt:wt) rennet (CHY-MAX PLUS Fermentation Produced Chymosin; CHR HANSEN, Horsholm, Denmark) was added. Stirring was stopped after 2 min. Coagulation was monitored after 40 min, and, once firm, the curd was cut by using vertical and horizontal knives. The cut curd was then stirred at 32.5 °C until titratable acidity reached 0.01% (wt:wt) lactic acid, at which point the whey was drained. After consolidation for 20 min, the cut curd was divided into sections of ≈12 cm wide, inverted, and left for 20 min. Once the titratable acidity of the whey exceeded 0.2% (wt:wt) lactic acid, cheese sections were cut into halves every 10–15 min until the acidity reached 0.35% (wt:wt) lactic acid. The curd was milled, salted (25 g NaCl/kg milk), transferred into cylindrical molds (height and diameter were 22.0 and 9.2 cm, respectively), and pressed at room temperature for 24 h. The following day, the cheeses were removed from the press, packaged under vacuum, and stored at 8 °C for a 3-wk maturation period. After maturation, the cheeses were stored at −18 °C. The fatty acid composition of the dairy products was analyzed as previously described (20). The composition of the dairy products is shown in **Table 1**.

Subjects and study design

The study was conducted at the University of Southampton and the University of Reading, with approval from the Research and Ethics Committee of the University of Reading and the South and West Hampshire Local Research Ethics Committee. Healthy male volunteers aged 34–60 y were recruited by advertisements. Potential volunteers were selected after confirming that they were healthy; had a body mass index (BMI; in kg/m^2) $>$ 18 and $<$ 32; had no diagnosed cardiovascular disease, diabetes, liver or endocrine dysfunction, or chronic inflammatory disease; were not taking any medication; were not vegetarians or vegans; were not heavy smokers ($>$10 cigarettes/d); were not heavy consumers of alcohol; and were not consuming dietary

TABLE 1
Composition of study products[1]

	UHT milk			Butter			Cheese		
	Control	CLA	Pooled SEM	Control	CLA	Pooled SEM	Control	CLA	Pooled SEM
	g/kg product			*g/kg product*			*g/kg product*		
Total fat	33.7	35.4[2]	0.33	822	802[2]	3.4	297	244[2]	5.2
Total protein	31.8	28.8[2]	0.14	4.7	3.7	0.41	186	189	0.6
4:0	1.01	0.96	0.014	29.5	26.6[2]	0.52	10.9	6.2[2]	0.28
6:0	0.59	0.53	0.014	16.2	14.6[2]	0.27	6.2	3.6[2]	0.16
8:0	0.36	0.31	0.011	9.4	8.8	0.17	3.6	2.3[2]	0.10
10:0	0.77	0.66	0.026	19.6	18.1[2]	0.29	7.6	4.8[2]	0.18
12:0	0.91	0.77[2]	0.027	22.6	20.1[2]	0.28	8.4	5.4[2]	0.12
14:0	3.16	2.86[2]	0.052	78.7	72.1[2]	0.66	27.6	19.9[2]	0.18
cis-9 14:1 (14:1n–5)	0.87	0.65[2]	0.011	0.90	0.64[2]	0.008	0.87	0.59[2]	0.005
16:0	9.30	8.19[2]	0.034	223	185[2]	0.5	78.1	55.7[2]	0.30
cis-9 16:1 (16:1n–7)	0.69	0.53[2]	0.004	15.6	10.9[2]	0.04	5.4	3.6[2]	0.02
18:0	2.86	1.30[2]	0.029	66.2	27.7[2]	0.31	24.5	9.1[2]	0.19
cis-9 18:1 (18:1n–9)	6.20	3.39[2]	0.061	146	71[2]	1.1	54.3	22.8[2]	0.30
trans-11 18:1	0.31	4.49[2]	0.018	6.7	98.8[2]	1.09	2.6	33.3[2]	0.21
Total *cis*-18:1	6.53	3.87[2]	0.068	153.5	81.3[2]	1.10	57.1	26.2[2]	0.32
Total *trans*-18:1	0.76	6.08[2]	0.042	16.9	134.4[2]	1.12	6.7	44.5[2]	0.22
cis-9, *cis*-12 18:2 (18:2n–6)	0.52	0.48[2]	0.009	12.0	9.7[2]	0.09	4.24	3.15[2]	0.026
cis-9, *trans*-11 CLA	0.15	1.40[2]	0.014	3.4	29.2[2]	0.16	1.18	9.80[2]	0.073
Total CLA	0.16	1.47[2]	0.014	3.9	31.3[2]	0.23	1.39	10.51[2]	0.080
Total SFA	20.27	16.82[2]	0.128	496	402[2]	1.6	178	116[2]	0.6
Total MUFA	8.37	10.87[2]	0.100	196	235[2]	1.3	71.8	76.8[2]	0.55
Total *n*–6 PUFA	0.63	0.61	0.013	14.1	12.2[2]	0.22	5.01	4.03[2]	0.042
Total *n*–3 PUFA	0.16	0.42[2]	0.017	3.31	7.74[2]	0.092	1.21	2.75[2]	0.034

[1] All values are $\bar{x}$ ($n = 4$ determinations). UHT, ultra-heat-treated; CLA, conjugated linoleic acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid.
[2] Significantly different from control, $P < 0.05$ (*t* test).

supplements (such as vitamins, fish oils, or evening primrose oil). The subjects fitting these criteria were then screened for fasting plasma concentrations of cholesterol (3.0–8.0 mmol/L), triacylglycerols (0.5–3.0 mmol/L), and glucose (3.9–6.8 mmol/L). All subjects gave informed consent and completed a health and lifestyle questionnaire before entering the study.

The study had a randomized, double-blind, placebo-controlled, crossover design. Randomization was stratified for age, BMI, and fasting triacylglycerol concentrations. Thirty-two subjects participated in the study. The subjects' characteristics are shown in **Table 2**. One subject withdrew in the second phase of the study due to illness not related to participation in the study. Before the study commenced, there was a run-in period that was designed to enable subjects to adjust to consumption of the required targets for dairy product intake. During this period, the subjects received shop-bought dairy products and consumed 500 mL full-fat UHT milk, 12.5 g butter, and 28 g cheese per day.

TABLE 2
Subject characteristics at screening[1]

	Value
Age (y)	45.5 ± 8.7 (34–60)
BMI (kg/m^2)	25.0 ± 3.4 (18–31)
Cholesterol (mmol/L)	5.4 ± 0.8 (4.0–7.2)
Triacylglycerol (mmol/L)	1.1 ± 0.6 (0.5–2.7)
Glucose (mmol/L)	5.2 ± 0.4 (4.4–6.1)

[1] All values are $\bar{x}$ ± SEM; range in parentheses. $n = 32$ subjects.

Thereafter, the subjects were randomly allocated to one of the intervention groups. For the next 6 wk of the study (intervention period), the subjects were asked to consume either CLA-enriched dairy products (500 mL UHT full-fat milk, 12.5 g butter, and 36.3 g cheese per day) or control dairy products (500 mL UHT full-fat milk, 12.5 g butter, and 28 g cheese per day) before crossing over to the other treatment (after a 7-wk wash out period followed by a 1-wk run-in period). The milk was packaged in 250-mL cartons, the butter was packaged into fortnightly portions, and the cheese was packaged in weekly portions. The subjects collected their dairy products every 2 wk. The subjects on the CLA phase of the intervention were required to consume a slightly greater quantity of cheese, because the fat contents of the control and modified products differed (Table 1) and the study aimed to provide equivalent total fat intakes from the study products. During the control phase, the subjects consumed 35.4 g fat/d from the study products and during the CLA phase they consumed 36.5 g fat/d.

A fasting blood sample was obtained from the subjects at the beginning and end of each intervention phase. The subjects were weighed to the nearest kg and their BMIs were calculated at each visit. The subjects also recorded their dietary intake 3 times during the study period: before entering the study (to record habitual intake of dairy products) and during each intervention phase. Food intake was recorded for 3 d, including 2 weekdays and 1 weekend day. All dairy products used in the intervention were analyzed for their fatty acid compositions and this information was added to the Foodbase database of UK food tables

(Institute of Brain Chemistry, London, United Kingdom). Diet diaries were analyzed for nutrient composition by using Foodbase, with amounts of foods consumed quantified from household measures, standard food portion sizes, and weights of foods provided in manufacturer's information.

Biochemical analysis

Blood samples were collected in heparinized evacuated tubes between 0800 and 1000 h after the subjects had fasted ≥10 h. Plasma was prepared and stored at −20 °C before analysis. Plasma triacylglycerol, cholesterol, HDL-cholesterol, glucose (Instrumentation Laboratories Ltd, Warrington, United Kingdom), and nonesterified fatty acid (Wako NEFA C kit; Alpha Laboratories Ltd, Eastleigh, United Kingdom) concentrations were measured with an ILab 600 clinical chemistry analyser (Instrumentation Laboratories Ltd). LDL-cholesterol concentrations were measured with the Friedewald formula (24). Plasma insulin concentrations were measured by using a specific enzyme immunoassay (Dako Diagnosis Ltd, Cambridge, United Kingdom). Fasting insulin and glucose concentrations were used to calculate insulin resistance from the homeostasis model for insulin resistance model [(insulin$_0$× glucose$_0$)/22.5] (25), and insulin sensitivity was calculated with the use of the revised quantitative insulin sensitivity check index formula [1/(log glucose$_0$ + log insulin$_0$ + log nonesterified fatty acids$_0$)] (26). Plasma vascular cell adhesion molecule 1, intercellular adhesion molecule 1, and E-selectin concentrations were measured by using specific human enzyme immunoassays (R&D Systems Europe Ltd, Abingdon, United Kingdom). Plasma interleukin 6 concentrations were measured with the use of the Quantikine high sensitivity interleukin 6 immunoassay kit (R&D Systems Europe Ltd).

Serum prepared from fasting blood samples was collected into evacuated tubes and stored at −20 °C before analysis. Serum C-reactive protein was measured in an ILab 600 clinical chemistry analyser (Instrumentation Laboratories Ltd) with high sensitivity C-reactive protein kits (Biokit SA, Barcelona, Spain).

LDL subclass analysis

Plasma (1.52 mL) was adjusted to a density of 1.067 g/mL by mixing with 60% iodixanol (0.4 mL Optiprep; Axis-Shield Diagnostics, Dundee, United Kingdom) and stained with Coomassie Blue (Sigma, Poole, United Kingdom). A 9% solution of iodixanol (3.4 mL at 1.050 g/mL) was underlayered with 1.5 mL of the adjusted plasma in a 4.9-mL Beckman Optiseal ultracentrifuge tube (Beckman Coulter Ltd, Abingdon, United Kingdom) and centrifuged at 370 752 × g_{av} for 2.5 h at 16 °C in an NVT65.2 rotor (Beckman Coulter, High Wycombe, United Kingdom). LDL subclasses were analyzed with a previously described rapid method (27). Briefly, after centrifugation, Optiseal tubes (Beckman Coulter Ltd) containing the stained bands were photographed against a vertical light box with a Nikon Coolpix 950 digital camera (Nikon UK Ltd, Kingston upon Thames, United Kingdom) set to the highest resolution. The tubes were photographed in a fixed position relative to the camera to allow standardization, and the photographs were downloaded to a personal computer and incorporated into Total-Lab 1D gel-scan software (Pharmacia UK, Milton Keynes, United Kingdom). The software converted the photographs into LDL profiles with an x axis of distance (mm) against a y axis of pixel intensity. The software automatically assigned relative electrophoretic migration distance (R_f) values to the primary LDL peak, and this was converted to density by cross-reference to a photograph of the blank tube calibrated in density intervals. A diagnostic cutoff density of 1.028 g/mL was also used to calculate the relative percentage of small dense LDL.

LDL oxidation

LDL was isolated from plasma by a 2-step density gradient ultracentrifugation process. Plasma was adjusted to a density of 1.21 g/mL with KBr and overlayered with precooled 0.15 mol NaCl/L and 297 mmol EDTA/L at pH 7.4 (density = 1.006 g/mL) in 11.2-mL Beckman Optiseal polyallomer centriguge tubes (Beckman Instruments Ltd, High Wycombe, United Kingdom). The tubes were centrifuged in a near-vertical rotor (NVT 65.2) at 341 650 × g_{av} for 50min at 4 °C. The LDL band was collected; adjusted to a density of 1.15 g/mL with 2.62 mol NaCl/L, 2.98 mol KBr/L, and 297 mmol EDTA/L at pH 7.4 (density = 1.33 g/mL); loaded into a fresh Beckman Optiseal tube (Beckman Instruments Ltd); and overlayered with 1.056 g/mL density solution. The tubes were then centrifuged at 341 650 × g_{av} for 3 h at 4 °C to remove contaminating albumin and to concentrate the LDL. The LDL was recovered from the top of the tube and run down a series of 2 PD10 columns (Amersham Pharmacia Biotech, Bucks, United Kingdom) that were preequilibrated with 25-mL chelex-treated phosphate buffered saline (Dulbecco's; Autogen Bioclear, Calne, United Kingdom) supplemented with 10 mmol EDTA/L. The LDL was filtered through a 45-mm pore disposable filter (Sartorius Ltd, Surrey, United Kingdom) to remove any aggregates, adjusted to 50 mg protein/mL with phosphate buffered saline, and heated to 37 °C in a water bath. Oxidation was initiated by addition of $CuSO_4$ to a final concentration of 5 mmol/L, and the increase in absorbance at 234 nm at 37 °C was monitored every minute until it reached a plateau. LDL oxidizability was assessed as lag time for oxidation (the tangent of the maximum rate of propagation intercept at the x axis), oxidation rate during the propagation phase, and maximum concentrations of conjugated dienes (28).

LDL fatty acid composition analysis

The fatty acid composition of LDL was measured according to procedures developed for the analysis of the fatty acid composition of plasma lipids (29).

Statistical analysis

All statistical tests were performed with SPSS version 12.0 (SPSS Inc, Chicago, IL), and a value of $P < 0.05$ was taken to indicate statistical significance. Prestatistical analyses were used to measure whether there were any effects of treatment sequence as a result of the crossover design. This analysis was simplified by calculating the absolute change for each variable tested (mean value at baseline subtracted from the mean value after supplementation of each treatment for each subject) with independent samples t tests. No period effect and no treatment-by-period interaction were observed (data not shown). Additionally, no carry-over effect was observed. Thus, all data were treated as paired samples from a crossover study. Data that were not normally distributed were logarithmically transformed or analyzed by the nonparametric Wilcoxon signed-ranks test. Data were

TABLE 3
Intake of dairy products before and during the intervention[1]

	Habitual consumption[2]	Control phase	CLA phase
Milk (mL/d)	212.0 ± 23.8	470.2 ± 14.4[3]	491.4 ± 8.6[3]
Butter (g/d)	14.1 ± 1.9	15.7 ± 0.9	15.7 ± 1.3
Cheese (g/d)	28.3 ± 6.5	32.6 ± 2.1	40.4 ± 2.8[3,4]

[1] All values are $\bar{x}$ ± SEM. n = 27–29 subjects. CLA, conjugated linoleic acid. During the CLA phase, study products daily contributed 500 mL milk, 12.5 g butter, and 36.3 g cheese. During the control phase, study products daily contributed 500 mL milk, 12.5 g butter, and 28 g cheese. The total consumption (ie, study products plus other dairy products) is shown above. Milk consumption did not reach 500 mL/d in either study phase because a few subjects did not reach their target intake and no subject consumed extra milk.
[2] Consumption of dairy products before the subjects entered the study (immediately after screening and before the first run-in period).
[3] Significantly different from habitual intake, $P < 0.05$ (one-way ANOVA with Tukey's test).
[4] Significantly different from control phase, $P < 0.05$ (one-way ANOVA with Tukey's test).

analyzed by using paired t tests for significant differences between control and CLA phases. Baseline measurements between the groups were also compared.

RESULTS

Habitual dietary intakes of the subjects and intakes during intervention

The subjects habitually consumed ≈212 mL milk/d, 14 g butter or spread/d, and 28 g cheese/d (**Table 3**). During the intervention, the subjects' intake of milk increased to just below 500 mL/d, which was the target intake (Table 3). A few subjects were unable to reach this target, but were not excluded from the study because they achieved a CLA intake of ≥80% of the target. No significant differences in milk, butter or spread, or cheese intakes were observed between the 2 randomized groups at baseline (data not shown). Cheese consumption was significantly higher during the CLA phase than during habitual intake and the control phase (Table 3). The higher intake of cheese during the CLA phase was necessary because of the naturally low-fat content of the modified cheese and the need to ensure equivalent fat intakes between groups. Total fat intake was significantly higher during the CLA phase than during habitual intake, but total fat and percentage energy from fat were similar for the control and CLA phases (**Table 4**). Intakes of short-chain fatty acids were significantly higher during the intervention than during habitual intake, but were lower during the CLA phase than the control phase (Table 4). Because many *trans* 18:1

TABLE 4
Dietary intake of subjects before and during intervention[1]

	Screening	Control phase	CLA phase
Energy (kcal/d)	2525 ± 96[2]	2562 ± 99	2715 ± 108
Total fat (g/d)	100 ± 5	107 ± 6	114 ± 5[3]
Protein (g/d)	102 ± 9	106 ± 8	110 ± 5
Carbohydrate (g/d)	299 ± 13	287 ± 14	310 ± 15
Fat (% of energy)	35.9 ± 1.2	37.9 ± 1.1	38.1 ± 0.9[3]
Protein (% of energy)	16.3 ± 0.9	16.8 ± 0.9	16.5 ± 0.7
Carbohydrate (% of energy)	47.8 ± 1.1	45.3 ± 0.9	45.4 ± 0.9
4:0 (mg/d)	811 ± 94	1562 ± 57[3]	1334 ± 50[3,4]
6:0 (mg/d)	502 ± 59	911 ± 35[3]	763 ± 31[3,4]
8:0 (mg/d)	315 ± 35	553 ± 21[3]	465 ± 24[3,4]
10:0 (mg/d)	735 ± 82	1211 ± 48[3]	1027 ± 42[3,4]
12:0 (mg/d)	1149 ± 119	1661 ± 93[3]	1457 ± 92[3]
14:0 (mg/d)	3810 ± 319	5264 ± 197[3]	4128 ± 261[4]
16:0 (mg/d)	17958 ± 1009	18425 ± 1005	19168 ± 968
18:0 (mg/d)	8275 ± 553	8321 ± 418	6509 ± 388[3,4]
cis-9 14:1 (14:1n–5) (mg/d)	263 ± 27	427 ± 16[3]	325 ± 18[3,4]
cis-9 16:1 (16:1n–7) (mg/d)	1463 ± 111	1325 ± 68	1529 ± 82
cis-9 18:1 (18:1n–9) (mg/d)	24329 ± 1684	25066 ± 1420	21229 ± 1280
cis-9, *cis*-12 18:2 (18:2n–6) (mg/d)	13611 ± 1870	13288 ± 2254	10800 ± 1310
cis-5, *cis*-8, *cis*-11, *cis*-14 20:4 (20:4n–6) (mg/d)	175 ± 20	201 ± 18	186 ± 16
Total SFA (g/d)	29.6 ± 2.8	35.8 ± 2.3	34.1 ± 1.8
Total *cis* MUFA (g/d)	24.0 ± 2.4	25.9 ± 1.9	24.3 ± 1.6
Total *cis* n–6 PUFA (g/d)	12.0 ± 1.8	12.7 ± 2.2	10.7 ± 1.3
Total *cis* n–3 PUFA (g/d)	1.6 ± 0.2	2.0 ± 0.3	1.6 ± 0.2
cis-9, *trans*-11 CLA (mg/d)[6]	NA	151[5]	1421
Total CLA (mg/d)[6]	NA	168	1508
trans-11 18:1 (mg/d)[6]	NA	312	4689
Total *trans 18:1* (mg/d)[6]	NA	779	6335

[1] n = 26–30 subjects. CLA, conjugated linoleic acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid.
[2] $\bar{x}$ ± SEM (all such values).
[3] Significantly different from screening, $P < 0.05$ (one-way repeated-measures ANOVA with post hoc Tukey's test).
[4] Significantly different from control phase, $P < 0.05$ (one-way repeated-measures ANOVA with post hoc Tukey's test).
[5] $\bar{x}$ (all such values).
[6] Intakes from the study products only.

TABLE 5
Effects of control and modified dairy products on plasma lipids[1]

	Control phase	CLA phase	P^2
Cholesterol (mmol/L)			
Baseline	4.50 ± 0.13	4.46 ± 0.15	
End of phase	4.47 ± 0.14	4.61 ± 0.13	
Change	−0.04 ± 0.08	0.15 ± 0.10	0.125
Triacylglycerols (mmol/L)			
Baseline	1.09 ± 0.13	1.02 ± 0.10	
End of phase	1.05 ± 0.12	1.19 ± 0.13	
Change	−0.03 ± 0.05	0.17 ± 0.10	0.086
HDL cholesterol (mmol/L)			
Baseline	1.09 ± 0.04	1.10 ± 0.04	
End of phase	1.09 ± 0.04	1.09 ± 0.03	
Change	0.01 ± 0.02	−0.01 ± 0.02	0.640
LDL cholesterol (mmol/L)			
Baseline	2.93 ± 0.11	2.90 ± 0.13	
End of phase	2.90 ± 0.12	2.98 ± 0.11	
Change	−0.03 ± 0.06	0.09 ± 0.07	0.223
NEFA (μmol/L)			
Baseline	276 ± 24	322 ± 33	
End of phase	283 ± 21	269 ± 21	
Change	7.13 ± 21.38	−53.31 ± 33.78	0.236
LDL:HDL cholesterol			
Baseline	2.87 ± 0.20	2.75 ± 0.18	
End of phase	2.77 ± 0.17	2.86 ± 0.17	
Change	−0.10 ± 0.09	0.11 ± 0.06	0.023
Total:HDL cholesterol			
Baseline	4.38 ± 0.26	4.21 ± 0.22	
End of phase	4.25 ± 0.21	4.41 ± 0.23	
Change	−0.13 ± 0.11	0.19 ± 0.09	0.068

[1] All values are $\bar{x}$ ± SEM. n = 31–32 subjects. CLA, conjugated linoleic acid; NEFA, nonesterified fatty acid. There were no significant differences between the baseline values (paired t test).
[2] Comparisons between absolute changes induced by the 2 diets (paired t test or Wilcoxon signed-ranks test).

isomers and CLA are not present in the Foodbase database (or other nutrient databases), it was not possible to determine the CLA intake or the total *trans* 18:1 intake from the background diet. The figures shown for these fatty acids in Table 4 therefore refer to contribution from the study products only. The total CLA intake during the control phase was 0.168 g/d, whereas that during the CLA phase was 1.508 g/d. *cis*-9,*trans*-11 CLA contributed >90% of the total CLA intake (Table 4).

Effects of control and modified dairy products on body weight, insulin sensitivity, and inflammatory markers

No significant effect of the intervention was observed on body weight or BMI; on plasma glucose, insulin, interleukin 6, soluble vascular cell adhesion molecule 1, soluble intercellular adhesion molecule 1, soluble E-selectin, or serum C-reactive protein concentrations; or on homeostasis model for insulin resistance or revised quantitative insulin sensitivity check index (data not shown).

Effects of control and modified dairy products on blood lipids

No significant effect of modified dairy products was observed on plasma triacylglycerols, total cholesterol, LDL-cholesterol, HDL-cholesterol, or nonesterified fatty acid concentrations (**Table 5**). However, after the consumption of the modified products, the change in the ratio of LDL to HDL cholesterol was significantly different between the diets (Table 5). A trend toward a difference in the ratio of total to HDL cholesterol was observed between the diets, but this was not statistically significant (Table 5).

TABLE 6
Effect of control and modified dairy products on LDL density[1]

	Control phase	CLA phase	P^2
LDL cholesterol >1.028 g/mL (%)			
Baseline	46.1 ± 3.4	43.2 ± 2.8	
End of phase	45.1 ± 3.0	44.9 ± 3.0	
Change	1.0 ± 1.7	−1.6 ± 1.7	0.222
Peak density (g/mL)			
Baseline	1.0269 ± 0.0007	1.0263 ± 0.0006	
End of phase	1.0268 ± 0.0006	1.0271 ± 0.0007	
Change	0.0000 ± 0.0004	0.0007 ± 0.0004	0.069

[1] All values are $\bar{x}$ ± SEM. n = 29–31 subjects. CLA, conjugated linoleic acid. There were no significant differences between the groups at baseline or at the end of the study (paired t tests).
[2] Comparisons between absolute changes induced by the 2 diets (Wilcoxon signed-ranks test).

Effect of control and modified dairy products on LDL subclasses

The proportion of LDL above the density cutoff of 1.028 g/mL for small dense LDL and the peak density of LDL are shown in **Table 6**. No significant effect of the modified products was observed on the proportion of LDL classified as small and dense. Also, no significant difference in the peak density of LDL was observed between the control and CLA phases (Table 6).

Effects of control and modified dairy products on the fatty acid composition of LDL and on the susceptibility of LDL to oxidation

Both CLA and *trans*-11 18:1 were incorporated into LDL lipids (**Table 7**). No significant effect of the intervention was observed on the lag time for oxidation, the oxidation rate during the propagation phase, or the maximum concentrations of conjugated dienes produced (**Table 8**).

DISCUSSION

The present study examined the effects of control and CLA-enriched dairy products on several human health outcomes. The modified dairy products were previously shown to have acceptable sensory and storage characteristics (20). The dietary intake data suggested that the compliance of the subjects was very good. The expected difference in the daily intake of *cis*-9 18:1 between the control and CLA periods, based on the data in Tables 1 and 4 and assuming that the dairy products were the sole source of the difference, was ≈3 g/d. The actual difference, as calculated from the food diaries, was 3.8 g/d. Additional evidence of compliance was shown by substantial incorporation of both *cis*-9,*trans*-11 CLA and *trans*-11 18:1 into plasma phospholipids and peripheral blood mononuclear cells (30).

Antiatherogenic properties have been attributed to CLA (3, 22) and are believed to be due, at least in part, to changes in lipoprotein metabolism (5, 21). However, the reported effects of CLA on blood lipids in humans are equivocal. A recent study

TABLE 7
Effect of control and modified dairy products on the fatty acid composition of LDL cholesterol (g/100 g total fatty acids)[1]

Fatty acid	Control phase	CLA phase	P^2
16:0			
Baseline	22.2 ± 1.4	21.6 ± 1.1	
End of phase	21.1 ± 1.2	21.6 ± 1.1	
Change	−1.1 ± 0.3	0.0 ± 0.1	0.001
cis-9 16:1 (16:1n–7)			
Baseline	2.2 ± 0.7	2.0 ± 0.6	
End of phase	2.0 ± 0.7	2.0 ± 0.6	
Change	−0.3 ± 0.4	−0.0 ± 0.1	0.013
18:0			
Baseline	7.1 ± 0.1	6.9 ± 0.4	
End of phase	6.9 ± 0.4	6.6 ± 0.5	
Change	−0.3 ± 0.1	−0.1 ± 0.1	0.242
trans-11 18:1			
Baseline	0.2 ± 0.2	0.2 ± 0.1	
End of phase	0.2 ± 0.2	0.4 ± 0.2	
Change	0.0 ± 0.1	0.2 ± 0.2	0.003
cis-9 18:1 (18:1n–9)			
Baseline	18.5 ± 4.0	19.2 ± 1.3	
End of phase	19.6 ± 1.7	18.6 ± 1.3	
Change	1.2 ± 0.9	−0.6 ± 0.2	0.088
cis-9, *cis*-12 18:2 (18:2n–6)			
Baseline	36.5 ± 4.2	37.6 ± 2.7	
End of phase	36.8 ± 3.7	37.1 ± 3.1	
Change	0.3 ± 0.8	−0.1 ± 0.3	0.628
cis-9, *trans*-11 CLA			
Baseline	0.3 ± 0.1	0.2 ± 0.1	
End of phase	0.3 ± 0.1	0.7 ± 0.2	
Change	0.0 ± 0.1	0.7 ± 0.2	< 0.0001
cis-9, *cis*-12, *cis*-15 18:3 (18:3n–3)			
Baseline	0.5 ± 0.2	0.5 ± 0.2	
End of phase	0.4 ± 0.2	0.5 ± 0.2	
Change	−0.1 ± 0.0	0.0 ± 0.0	0.485
cis-8, *cis*-11, *cis*-14 20:3 (20:3n–6)			
Baseline	1.8 ± 0.4	1.7 ± 0.3	
End of phase	1.8 ± 0.3	1.7 ± 0.3	
Change	0.0 ± 0.0	0.0 ± 0.0	0.313
cis-5, *cis*-8, *cis*-11, *cis*-14 20:4 (20:4n–6)			
Baseline	5.8 ± 1.2	5.8 ± 1.0	
End of phase	5.9 ± 1.2	5.7 ± 1.0	
Change	0.1 ± 0.1	−0.0 ± 0.1	0.582
cis-4, *cis*-7, *cis*-10, *cis*-13, *cis*-16, *cis*-19 22:6 (22:6n–3)			
Baseline	1.3 ± 0.3	1.3 ± 0.4	
End of phase	1.3 ± 0.3	1.4 ± 0.5	
Change	0.1 ± 0.1	0.0 ± 0.1	0.204

[1] All values are $\bar{x}$ ± SEM. n = 22–24 subjects. CLA, conjugated linoleic acid. There were no significant differences in CLA or *trans*-11 18:1 concentrations between the groups at baseline (paired *t* tests).
[2] Difference from control phase (paired *t* test)

conducted by our group examined the effects of highly enriched preparations of *cis*-9,*trans*-11 CLA and *trans*-10,*cis*-12 CLA on blood lipids and showed that plasma triacylglycerol concentrations, the LDL-to-HDL cholesterol ratio, and percentage change from baseline for the total-to-HDL cholesterol ratio were significantly higher during supplementation with *trans*-10,*cis*-12 CLA than with *cis*-9,*trans*-11 CLA (13). This suggested opposing effects of the 2 isomers, with a detrimental effect of *trans*-10,*cis*-12 CLA, relative to *cis*-9,*trans*-11 CLA, on the blood lipid profile. This was the first evidence showing relative hyperlipidaemic properties of *trans*-10,*cis*-12 CLA and hypolipidemic properties of *cis*-9,*trans*-11 CLA in humans, although the effects did not appear to be dose-responsive.

Interest in the potential for naturally increasing the *cis*-9,*trans*-11 CLA content of milk and dairy products has increased. Such a product may be expected to have beneficial effects on the blood lipid profile. However, nutritional strategies for the natural enhancement of the CLA content of milk also result in milk fat containing substantially increased *trans*-18:1 concentrations (particularly *trans*-11 18:1), lowered saturated fatty acid concentrations, and a slightly higher n–3 polyunsaturated fatty acid content (20). The increased concentrations of *trans* fatty acids

TABLE 8
Effect of control and modified dairy products on the susceptibility of LDL cholesterol to oxidation[1]

	Control phase	CLA phase	P^2
Lag time (min)			
Baseline	54.1 ± 4.1	54.0 ± 3.7	
End of phase	52.1 ± 3.1	48.7 ± 3.6	
Change	−2.08 ± 2.93	−6.03 ± 3.06	0.428
Oxidation rate (nmol dienes · mg LDL protein^{-1} · min^{-1})			
Baseline	13.5 ± 1.6	13.3 ± 1.0	
End of phase	18.2 ± 5.3	12.8 ± 1.2	
Change	−0.24 ± 1.01	−0.61 ± 1.85	0.884
Maximum dienes (nmol/mg LDL protein)			
Baseline	573 ± 52	598 ± 49	
End of phase	609 ± 47	638 ± 52	
Change	30.38 ± 42.26	7.69 ± 70.32	0.802

[1] All values are $\bar{x}$ ± SEM. $n = 16–18$ subjects. CLA, conjugated linoleic acid. There were no significant differences between baselines (paired *t* test).
[2] Comparisons between absolute changes induced by the 2 diets (paired *t* test).

could be predicted to counteract any beneficial effects of *cis*-9,*trans*-11 CLA on the blood lipid profile. On the other hand, there is likely to be some endogenous synthesis of *cis*-9,*trans*-11 CLA from *trans*-11 18:1 (31), and the reduced saturated fatty acid content could act in concert with this CLA isomer. Consumption of butter naturally enriched with CLA and *trans*-11 18:1 was shown to lower the ratio of atherogenic lipoproteins (VLDL + intermediate-density lipoprotein + LDL) to antiatherogenic HDL in hamsters that were fed a cholesterol-rich diet (21). Similarly, a CLA-rich oil reduced aortic lipid deposition and reduced the nonHDL-to-HDL cholesterol ratio in a hamster model (22). Thus, animal studies suggest that CLA-rich dairy products have a beneficial effect on the blood lipid profile and in atherogenesis. Desroches et al (23) reported a lack of effect of dietary CLA naturally incorporated into butter on the lipid profile of overweight and obese men. However, they noted that consumption of the CLA diet resulted in a significantly smaller reduction in plasma total cholesterol and in the ratios of total to HDL cholesterol and of LDL to HDL cholesterol than did consumption of the control diet. In their study, CLA intakes were almost double those in the present study (2.59 g/d compared with 1.421 g/d), although the duration of each arm of the study was shorter in the Desroches study (4 wk compared with 6 wk) (23). At either of these doses, highly purified supplements of *cis*-9,*trans*-11 CLA have been shown to significantly lower the ratios of LDL to HDL cholesterol and total to HDL cholesterol (13).

The present study found no significant effect of CLA-enriched dairy products on several markers associated with atherogenic risk, including body weight and plasma concentrations of glucose, insulin, and inflammatory markers. CLA-enriched dairy products also had no significant effect on the less well-established putative risk markers, ie, LDL density and the susceptibility of LDL to oxidation. Importantly, there were no detrimental effects of the control dairy products on the blood lipid profile, despite the fact that the subjects doubled their consumption of milk and that the milk was full fat. This suggests that a substantially increased consumption of full-fat milk per se does not result in any detrimental effects on the blood lipid profile. However, the effects of the CLA-enriched dairy products on blood lipids were more complex. After the consumption of the CLA-enriched products, plasma triacylglycerol, cholesterol, LDL-cholesterol, and HDL-cholesterol concentrations and the ratio of total to HDL cholesterol were not significantly changed, whereas the change in the ratio of LDL to HDL cholesterol was significantly different between the diets. The magnitude of the effect was small, particularly compared with the variation in the measurement of this variable. It is interesting to note, however, that the magnitude of the difference in the ratio of LDL to HDL cholesterol between the control and CLA diets described in the study by Desroches et al (23) was similar. Although the ratios of LDL to HDL cholesterol and of total to HDL cholesterol are used as estimates of coronary artery disease risk in epidemiologic studies (32, 33), the marginal nature of the effects of CLA-rich dairy products means that their potential influence on the risk of coronary artery disease is not clear. In the present study, there was a substantial decrease in plasma cholesterol between screening and the study (Tables 2 and 5). The reason for this is not clear, although there were several months between the screening and the start of the study. A change in blood lipid variables often occurs when subjects enter a nutritional study, perhaps due to adjustments in dietary behavior, but it was for this reason that we included a 1-wk run-in period before each arm of the study. During this period, the subjects were given shop-bought dairy products, which they consumed in the same amounts as they would during the study. This allowed them to make any necessary adjustments before the first baseline blood sample was taken. Therefore, the difference in plasma cholesterol between screening and the study reflects this adjustment, although it is difficult to attribute it to any aspect of the dietary intake data, because the subjects were consuming more fat during the study than they did habitually. It has to be acknowledged, however, that this prestudy change was substantially greater than were the effects observed during the study.

The data from human studies that examined the effects of consumption of CLA-rich dairy products on blood lipid profile clearly do not support the results of the animal studies. This may be due to a difference in approach; the animal studies examined the lipid-lowering effects of CLA-enriched products in hyperlipidemic animals that were fed atherogenic diets (21, 22), whereas the study by Desroches et al (23) and the present study involved subjects who had blood lipid profiles within the normal range.

An important consideration is that the increase in the *trans* fatty acid content of the dairy products may counteract any benefits associated with increasing the content of CLA or decreasing the content of saturated fatty acids (34). Unfortunately, all food analysis databases contain an insufficient range of *trans* fatty acids to allow calculation of the total intake of *trans* fatty acids in the background diet, so it is not possible to calculate the increase in *trans* fatty acid intake during the intervention accurately. However, *trans*-11 18:1 was the predominant *trans* fatty acid in the CLA-enriched milk, and the intake of this fatty acid increased substantially during the CLA phase of the intervention. The total *trans*-18:1 isomers in the control products contributed ≈0.3% dietary energy during the control phase (without taking into account any contribution from the background diet), whereas *trans*-18:1 isomers from the modified dairy products contributed ≈2.1% dietary energy during the CLA phase. Zock and Katan (35) suggested that a 1% increase in the dietary intake of *trans*

fatty acids raises LDL cholesterol by ≈0.03 mmol/L and lowers HDL cholesterol by ≈0.015 mmol/L relative to oleic acid or linoleic acid. Thus, it could be predicted that the estimated 1.8% difference in *trans*-11 18:1 should increase LDL cholesterol by 0.06 mmol/L. However, neither LDL cholesterol nor HDL cholesterol were significantly changed by consumption of the modified dairy products in the present study, which is perhaps not surprising given the small size of the predicted effect. Epidemiologic studies have observed an association between *trans* fatty acid intake and increased risk of coronary artery disease for industrially derived *trans* fatty acids, but not for *trans* fatty acids from animal sources (36, 37). This difference may relate to differences in the *trans* isomer profile between the 2 sources. However, Weggemans et al (38) suggested that differences could be related to the level of *trans* fatty acid intake rather than the food source, because industrially derived *trans* fatty acids were consumed at a much higher level in all of the published epidemiologic studies.

Case-control studies show strong associations between the presence of small, dense LDL and cardiovascular disease, and it is thought that this is because small dense LDL undergoes oxidation more readily (39) and binds more avidly to the subendothelial matrix (40). The present study showed that naturally modified dairy products, enriched in both CLA and *trans*-11 18:1, did not significantly affect the peak density of LDL particles or the proportion of small dense LDL compared with the control products. A lack of effect of CLA-enriched butter on LDL peak diameter was also reported by Desroches et al (23). The reported effects of *trans* fatty acids on LDL size are inconsistent. Kim and Campos (41) reported that a 1% increase in *trans* fatty acid intake in the background diet of a Costa Rican population was associated with a 0.244 nm increase in LDL size. However, the overall intake of *trans* fatty acids in this population was very low (<1.5% dietary energy). Furthermore, Mauger et al (42) showed that increasing intakes of *trans* fatty acids result in a dose-dependent decrease in LDL particle size in mildly hypercholesterolemic subjects who were given experimental diets for 35 d. Nevertheless, similar to the present study, the distribution of particle sizes (ie, the proportion classified as small, medium, or large) was not changed by *trans* fatty acids (42).

In the present study, consumption of the modified dairy products did not significantly affect the susceptibility of LDL to copper-induced oxidation, as assessed by lag time, oxidation rate, or maximum diene production. Sargis and Subbaiah (43) suggested that *trans* fatty acids incorporated into LDL are less oxidizable than are their *cis* counterparts and protect endogenous lipids against oxidation, although they did not specifically study the effects of CLA. On the other hand, Flintoff-Dye and Omaye (44) enriched LDL with individual CLA isomers in vitro and examined the subsequent effect on LDL oxidation. Their results are somewhat inconsistent, because CLA isomers were prooxidant at low concentrations, protective against oxidation at medium concentrations, and prooxidant again at high concentrations. Furthermore, because the fatty acid composition of the LDL was not reported in the study by Flintoff-Dye and Omaye (44), it is not possible to compare the degree of enrichment of LDL with CLA achieved in vitro with that obtained in vivo in the present study. Perhaps the most relevant study in the context of pro- versus antioxidant effects of CLA is that of Basu et al (45), who reported increased concentrations of both 8-iso-prostaglandin $F_2\alpha$ and 15-oxo-dihydro-prostaglandin $F_2\alpha$ in urine after consumption of 4.2 g/d of a 50:50 mixture of *cis*-9,*trans*-11 and *trans*-10,*cis*-12 CLA for 4 wk by middle-aged men with abdominal obesity. However, it has been suggested that the *trans*-10,*cis*-12 isomer of CLA is chiefly associated with peroxidation and, indeed, other adverse effects of CLA (46, 47). Because the dairy products used in the present study were enriched with *cis*-9,*trans*-11 CLA, it is important to relate the effects specifically to this isomer where possible.

In conclusion, there is considerable interest in strategies for naturally increasing the *cis*-9,*trans*-11 CLA content of milk and dairy products, which also increase *trans*-18:1 concentrations, particularly *trans*-11 18:1. This combination does not appear to have a significant effect on the blood lipid profile; indeed, an increased intake of naturally derived *trans* fatty acids did not result in a detrimental effect on most of the cardiovascular disease risk markers assessed.

PCC, PY, CMW, and RFG designed the study and supervised the experimental work. ST, GCB, JJR, and ELJ screened, recruited and sampled the volunteers. ST, GCB, EM, and SE-K conducted the experimental work. ABG and DSL supervised the LDL oxidation experiments, and WLH assisted with the LDL subclass analysis. ST and PY analyzed the data. ST and PY wrote the manuscript, with input from all authors. None of the authors has any financial or personal interest in any company or organization sponsoring the research, including advisory board affiliations.

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