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Saturated Fats from Butter but Not from Cheese Increase HDL-Mediated Cholesterol Efflux Capacity from J774 Macrophages in Men and Women with Abdominal Obesity

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Abstract

Background: Recent evidence suggests that the association between dietary saturated fatty acids (SFAs) and coronary artery disease risk varies according to food sources. How SFAs from butter and cheese influence HDL-mediated cholesterol efflux capacity (CEC), a key process in reverse cholesterol transport, is currently unknown.

Objective: In a predefined secondary analysis of a previously published trial, we have examined how diets rich in SFAs from either cheese or butter influence HDL-mediated CEC, compared with diets rich in either monounsaturated fatty acids (MUFAs) or polyunsaturated fatty acids (PUFAs).

Methods: In a randomized crossover controlled consumption trial, 46 men and women with abdominal obesity consumed 5 isocaloric diets, each for 4 wk. Two diets were rich in SFAs either from cheese (CHEESE) or butter (BUTTER) [12.4–12.6% of energy (%E) as SFAs, 32%E as fat, 52%E as carbohydrates]. In 2 other diets, SFAs (5.8%E) were replaced with either MUFAs from refined olive oil (MUFA) or PUFAs from corn oil (PUFA). Finally, a lower fat and carbohydrate diet was used as a control (5.8%E as SFAs, 25.0%E as fat, 59%E as carbohydrates; CHO). Post-diet HDL-mediated CEC was determined ex vivo using radiolabelled J774 macrophages incubated with apolipoprotein B–depleted serum from the participants.

Results: Mean (±SD) age was 41.4 ± 14.2 y, and waist circumference was 107.6 ± 11.5 cm in men and 94.3 ± 12.4 cm in women. BUTTER and MUFA increased HDL-mediated CEC compared with CHEESE (+4.3%, $P = 0.026$ and +4.7%, $P = 0.031$, respectively). Exploring the significant diet × sex interaction ($P = 0.044$) revealed that the increase in HDL-mediated CEC after BUTTER compared with CHEESE was significant among men (+6.0%, $P = 0.047$) but not women (+2.9%, $P = 0.19$), whereas the increase after MUFA compared with CHEESE was significant among women (+9.1%, $P = 0.008$) but not men (–0.6%, $P = 0.99$).

Conclusion: These results provide evidence of a food matrix effect modulating the impact of dairy SFAs on HDL-mediated CEC with potential sex-related differences that deserve further investigation. This trial was registered at clinicaltrials.gov as NCT02106208. *J Nutr* 2018;148:573–580.

Keywords: high-density lipoproteins, diet, dairy, cheese, butter, saturated fatty acids, monounsaturated fatty acids, polyunsaturated fatty acids, cholesterol efflux capacity, J774 macrophages

Introduction

The association between dietary SFAs and the risk of coronary artery disease (CAD) has become controversial over the years (1–5). Epidemiologic studies have suggested that the association between SFAs and CAD risk varies according to food source (6, 7), with high dairy SFA intake being associated with a lower

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risk of CAD. Accordingly, results from our group (8) and others (9) have shown that the LDL-cholesterol-raising effect of SFAs is attenuated when their food source is cheese rather than butter. Previous investigations about dairy SFAs have focused primarily on their LDL-cholesterol-raising effects. However, SFAs also increase serum HDL-cholesterol concentrations when substituted for carbohydrates (10). The extent to which such an increase in HDL-cholesterol concentrations with SFAs relates to longer-term CAD risk is unclear.

Indeed, the association between HDL-cholesterol and CAD risk has also become contentious. While epidemiologic studies have shown that higher HDL-cholesterol concentrations are almost invariably associated with a lower risk of CAD (11), data from Mendelian randomization studies suggest that low HDL-cholesterol is not causal in the etiology of CAD (12). Large clinical trials showing failure of HDL-cholesterol-raising drugs to reduce CAD risk further support the notion that HDL-cholesterol may not be a causal risk factor for CAD (13). It is stressed that the cholesterol content of HDLs represents only 1 of numerous features of these lipoproteins, and may only poorly reflect HDL functional properties. In this regard, it is well established that HDLs play an important role in the reverse cholesterol transport process by promoting cholesterol efflux from cholesterol-loaded cells (14). Studies have suggested that HDL-mediated cholesterol efflux capacity (CEC) is inversely associated with the risk of CAD (15), although this has not been a unanimous finding (16).

To the best of our knowledge, no study has yet assessed the impact of different dietary fats and dairy SFAs from different foods on HDL-mediated CEC measured ex vivo. How the food source modifies the effect of SFAs on HDL physical and functional characteristics is also unknown.

This randomized, fully controlled consumption trial was designed (1) to compare the effect of diets rich in SFAs from either cheese or butter on HDL-mediated CEC and several physicochemical features of HDL and (2) to assess how diets rich in SFAs from butter and cheese influence these study outcomes compared with diets rich in monounsaturated (MUFAs) or in polyunsaturated fatty acids (PUFAs). We hypothesized that consumption of SFAs increases HDL-mediated CEC and that the magnitude of these effects varies according to the SFA food source.

Methods

Study design and population. This paper presents secondary analyses of a study for which methods have been detailed elsewhere (8). Briefly, 92 men and women completed ≥1 dietary phase of this multicenter randomized crossover controlled consumption trial. To be eligible, participants had to be 18–65 years old and to have a waist circumference ≥94 cm and ≥80 cm for men and women, respectively. Participants also had to have HDL-cholesterol concentrations below an age- and sex-specific 75th percentile value (≤1.34 mmol/L and ≤1.53 mmol/L for men and women, respectively) in order to exclude individuals with high HDL-cholesterol concentrations, which was the primary outcome of the original study. Participants were otherwise healthy, had no history of cardiovascular disease, type 2 diabetes, or monogenic dyslipidemia, and did not use lipid-lowering, anti-diabetic, or anti-inflammatory medications. A subsample of 46 subjects from 1 of the participating centers (Institute of Nutrition and Functional Foods in Québec City) who had completed at least the carbohydrate and the cheese diets were included in this sub-study (**Supplemental Figure 1**). Their screening characteristics were similar to those of the original study (data not shown). This protocol is registered at http://www.clinicaltrials.gov (NCT02106208).

Diets. The dietary intervention consisted of 5 isocaloric diets allocated in random order. Dietetic technicians prepared all recipes and meals in the metabolic kitchen of the Institute of Nutrition and Functional Foods. Diets were provided under isoenergetic conditions to maintain a constant body weight. As indicated previously (8), energy (E) levels at baseline were estimated using validated equations and with the use of a quantitative web-based food-frequency questionnaire completed before the beginning of the first dietary phase.

Detailed nutritional composition of the experimental diets consumed by participants in this controlled feeding study is shown in **Supplemental Table 1**. Two diets were rich in SFAs from either cheese (CHEESE) or butter (BUTTER) (12.4–12.6%E as SFAs, 32%E as fat, 52%E as carbohydrates). In the other 2 higher-fat diets, SFAs (5.8%E) were replaced by MUFAs (MUFA) or PUFAs (PUFA). The grams of fat from cheese in CHEESE were replaced by corresponding amounts of butter fat, refined olive oil, and corn oil in BUTTER, MUFA, and PUFA, respectively. The fifth diet in which SFAs were substituted for by carbohydrate was used as a control reference diet (5.8%E as SFAs, 25.0%E as fat, 59%E as carbohydrates; CHO). Total energy, protein, dietary cholesterol, fiber, and sodium were matched across diets. CHEESE was higher in calcium and potassium than all other diets.

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Supplemental Figure 1 and Supplemental Tables 1 and 2 are available from the "Supplementary data" link in the online posting of the article and from the same link in the online table of contents at https://academic.oup.com/jn/.
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Abbreviations used: ABCA1, ATP-binding cassette transporter 1; BUTTER, diet rich in SFAs from butter; CAD, coronary artery disease; CEC, cholesterol efflux capacity; CHEESE, diet rich in SFAs from cheese; CHO, diet in which SFAs were substituted for by carbohydrate (control reference diet); E, energy; MUFA, diet in which SFAs were replaced by MUFAs; PUFA, diet in which SFAs were replaced by PUFAs; SR-B1, scavenger receptor class B type 1.

Each diet lasted 4 weeks with a minimum washout of 4 weeks between diets. Previous studies have shown that 4 weeks of diet supplementation and of washout are sufficient to maximize effects on the HDL pool (17, 18). Alcohol consumption was forbidden to participants 48 h before and during all dietary phases. Compliance to treatments was assessed by checklists filled out by participants on a weekly basis, which allowed the identification of foods that were consumed and foods not consumed. Checklists provided information on beverage intake as well as on current medication. Participants were asked to notify the coordinator in charge of the project before starting any new medication.

Blood sampling. Blood samples were taken from the antecubital vein at the beginning and end of each diet after a 12-h fast. Most analyses in the present study were performed on samples taken at the end of each treatment phase. All laboratory analyses were carried out by staff blinded to study treatments.

HDL isolation. HDLs were isolated by sequential ultracentrifugation of fresh and whole plasma (5 mL) at consecutive densities (*d*) of 1.063 g/mL for 22 h and 1.21 g/mL for 24 h at 312,200 × *g* at 4°C in a Beckman 50.4 Ti rotor (Beckman Instruments Inc., Missisauga, Ontario, Canada) and then dialyzed overnight at 4°C in a buffer containing 0.15 M NaCl, 0.01 M Tris-base, 0.01% EDTA, and 0.03 mM NaN_3 (pH 8.0). Isolated HDLs were rapidly frozen at –80°C until use.

HDL composition and apoA-I. Fatty acid composition in the isolated HDL fraction was assessed as described previously (19). Serum HDL-cholesterol concentration was assessed on a Roche/Hitachi Modular system (Roche Diagnostics, Mannheim, Germany) according to the manufacturer's specifications and using proprietary reagents. Serum LDL-cholesterol concentrations were estimated using the Friedewald equation. Plasma apoA-I concentrations were measured with the use of the DuoSet ELISA (R&D Systems #DY3664-05; (Minneapolis, MN).

Mean HDL size and HDL subclasses. Mean HDL size and HDL subclasses were measured by nondenaturing 4–30% polyacrylamide gradient gel electrophoresis and image analysis as previously described (20). Plasma concentrations of apoA-I–containing HDL subclasses were measured by 2-dimensional nondenaturing agarose-polyacrylamide gel electrophoresis, immunoblotting, and image analysis (Tufts University) as described previously (21).

CEC. Ex vivo CEC was assessed using serum samples stored at –80°C immediately after sampling and taken specifically for that purpose. Serum samples were first depleted of apoB using 40% polyethylene glycol-6000. In that context, CEC is considered to be mediated primarily by HDL (22). The J774 macrophages used in this experiment were cultured in basal condition in the absence of cyclic adenosine monophosphate (cAMP). Hence, HDL-mediated CEC reflects total efflux from ATP-binding cassette transporter 1 (ABCA1), scavenger receptor class B type 1 (SR-B1), and non-receptor dependent efflux (aqueous diffusion) (23). J774 macrophages were plated and incubated in Roswell Park Memorial Institute media containing 1% bovine growth serum and 2 μCi of 3H-cholesterol/mL for 24 h. Cells were then equilibrated for 16–18 h in media containing 0.2% endotoxin-free, low free fatty acids bovine serum albumin. Subsequently, cells were incubated with efflux medium containing 2.8% apoB-depleted serum of study participants for 4 h. Media were then collected and cells were harvested in 0.5 N NaOH. Liquid scintillation counting was used to measure the efflux of radiolabelled 3H-cholesterol from the cells. HDL-mediated CEC was calculated by the following formula: [cpm in media/(cpm in media + cpm in cell lysates)]. HDL-mediated CEC was also measured in serum-free media. The latter was subtracted from HDL-mediated CEC using sera from the study participants. A control sample of pooled plasma from healthy volunteers was used as an internal standard in each assay to normalize values across batches. All measurements were performed in triplicates and all samples from 1 individual were tested on the same plate. CVs for each assay were calculated. Triplicates with CV greater than 20% were further examined. Within a triplicate, a single measure that digressed by >15% from the mean of the other 2 measures was considered as an outlier and was discarded. The mean of the 3 measures was used in the analysis when the CV within the triplicate could not be ascribed to 1 single measure. Mean intra-assay CV based on triplicates was 10.2 ± 9.3% and 8.0 ± 4.6% before and after removal of outliers, respectively.

Sample size calculations and statistical analyses. Based on data from de Vries et al. (24), we had estimated that a sample size of 50 would detect a 2.2% difference in HDL-mediated CEC between diets, the primary outcome of this study, assuming a mean baseline CEC value of 16% and an SD of 3.6%. Based on preliminary analyses, we found that the SD of the HDL-mediated CEC was lower than anticipated. We therefore used samples from only 1 center ($n = 46$ participants) of the original study to assess the impact of the various diets on HDL-mediated CEC.

As indicated earlier, the primary objective of this study was to assess how different sources of SFAs influence HDL-mediated CEC compared with other dietary fats. In this context, CHO was used as a reference, control diet in the analyses. Changes in study outcomes with each of the 4 high-fat diets versus CHO were compared among themselves using mixed models for repeated measures in SAS (v9.4, Cary, NC). Pairwise comparisons of the 4 high-fat diets were considered only when the overall *P* for the main treatment effect in the mixed models was <0.05. Treatment, sex, treatment × sex, sequence of treatments, age, and BMI or waist circumference were defined as fixed effects and subjects as the random effect. The analysis of the treatment × sex interaction was predetermined in our analytic plan. Using a most parsimonious modeling approach, potential confounders of the changes in outcome measures with treatment were included in the final mixed models only when they were found to be significant at $P < 0.05$. The Holm–Bonferroni procedure was used to adjust for multiple comparisons of the high-fat diets (25), as previously described (8). The multiple comparisons under consideration were defined a priori to reflect the study objectives: CHEESE compared with BUTTER; MUFA, PUFA, and BUTTER compared with MUFA and PUFA. The MUFA and PUFA comparisons were not considered because they were not part of the primary aim of this analysis. Although not part of the study objectives per se, the statistical comparison of the high-fat diets with CHO was directly provided by the least squares means (LSMEANS) statement in the mixed model. Characteristics of men and women at screening and self-reported compliance during the intervention were compared using 2-sided Student's *t* tests and Wilcoxon's Signed Rank test, respectively. Spearman rank correlation coefficients were calculated between variation (compared with CHO) in serum lipids and variation (compared with CHO) in HDL-mediated CEC. The normality in the distribution of residual of all study outcome models was considered and BMI, TG, fasting blood glucose, HDL-mediated CEC, and HDL-TG were log-transformed.

Results

Participants' characteristics. Screening characteristics of the 46 subjects included in this study are shown in Table 1. Men and women had HDL-cholesterol concentrations around the 50th percentile of their age group and had large waist circumferences as per the eligibility criteria. Mean ± SD self-reported compliance to the diets was high (99.5 ± 1.1%), with no difference among diets ($P = 0.69$) or between men and women (Wilcoxon's Signed Rank test $P = 0.99$). There was no difference in mean body weight ($P = 0.37$) or waist circumference ($P = 0.11$) among treatments (data not shown).

HDL cholesterol, size, and subclasses. Table 2 shows the changes in serum HDL-cholesterol concentration, HDL size, and HDL subclass distribution after CHEESE, BUTTER, MUFA, and PUFA relative to CHO. No significant differences were observed in HDL-cholesterol concentrations after

TABLE 1 Screening characteristics of the 46 men and women[1]

	Men ($n = 21$)	Women ($n = 25$)	P
Postmenopausal women, % (n)		36.0 (9)	
Ethnicity, % (n)			
Caucasian	100 (21)	92.0 (23)	—
Asian	0	0	—
African	0	0	—
Hispanic	0	4.0 (1)	—
Other	0	4.0 (1)	—
Age, y	40.6 ± 13.2	42.0 ± 15.2	0.73
Body weight, kg	97.5 ± 18	75.8 ± 16.8	0.0001
BMI,[2] kg/m^2	30.7 ± 5.1	29.0 ± 6.3	0.34
Waist circumference, cm	107.6 ± 11.5	94.3 ± 12.4	0.0005
Blood pressure, mmHg			
Systolic	120.6 ± 10.6	107.9 ± 10.7	0.0002
Diastolic	71.5 ± 9.2	68.0 ± 10	0.22
Serum lipids, mmol/L			
Total C	5.26 ± 1.02	5.16 ± 1.06	0.73
LDL-C	3.34 ± 0.88	3.18 ± 0.84	0.51
HDL-C	1.11 ± 0.2	1.30 ± 0.17	0.001
TGs[2]	1.63 ± 0.66	1.30 ± 0.7	0.11
Total C:HDL-C ratio	4.79 ± 0.87	4.02 ± 0.95	0.007
Fasting blood glucose,[2] mmol/L	5.3 ± 0.37	5.12 ± 0.55	0.15

[1] Values are means ± SDs unless otherwise indicated. P values between men and women were determined by a Student's t test. C, cholesterol.
[2] Analyses were performed on log-transformed data.

consumption of the 4 high-fat diets, which all resulted in similar increases in HDL-cholesterol concentrations compared with CHO (all P values <0.01). BUTTER, MUFA, and PUFA similarly increased apoA-I compared with CHEESE (all P values <0.01). CHEESE, BUTTER, and PUFA had relatively similar effects on HDL particle size distribution. Compared with CHO, CHEESE increased the proportion of HDL_{2b} ($P = 0.019$) and reduced the proportion of HDL_{3b} ($P = 0.004$), while BUTTER increased total HDL_2 and HDL_{2b} ($P < 0.05$) and decreased total HDL_3, HDL_{3a}, and HDL_{3b} ($P < 0.05$). Some of these changes with BUTTER were also significant compared with MUFA.

The 2-dimensional gel electrophoresis characterization of HDL showed that BUTTER increased the concentration of all α HDLs compared with CHEESE (all P values <0.05). The MUFA diet also increased the concentration of α-2, α-3, and α-4 HDLs compared with CHEESE (all P values <0.03). BUTTER but not CHEESE increased the proportion of pre-β-1 and pre-β-2 HDLs compared with CHO (both $P < 0.02$).

HDL composition. CHEESE, BUTTER, MUFA, and PUFA had similar effects on the TGs, free cholesterol, cholesteryl esters, phospholipids, or total protein content of HDLs (**Supplemental Table 2**). BUTTER and PUFA slightly decreased TGs in HDLs compared with CHO (both $P < 0.05$).

HDL-mediated CEC. As shown in **Figure 1**, there was a significant difference among the 4 high-fat diets on HDL-mediated CEC ($P = 0.029$). Specifically, BUTTER (+4.3%, $P = 0.026$)

TABLE 2 Changes in endpoint HDL characteristics after consuming the 4 high-fat diets compared with CHO (reference diet) in men and women with abdominal obesity[1]

		Δ vs. CHO				
	CHO ($n = 46$)	CHEESE ($n = 46$)	BUTTER ($n = 44$)	MUFA ($n = 44$)	PUFA ($n = 43$)	P[2]
HDL-C, mmol/L	1.08 ± 0.18	0.05 ± 0.10[a]	0.08 ± 0.09[a]	0.05 ± 0.09[a]	0.06 ± 0.10[a]	0.29
ApoA-I, mg/mL	1.37 ± 0.25	−0.02 ± 0.18	0.12 ± 0.19[a,b]	0.08 ± 0.19[a,b]	0.06 ± 0.21[a,b]	0.0002
HDL size, nm	9.35 ± 0.36	0.06 ± 0.12	0.06 ± 0.20	0.02 ± 0.17	0.07 ± 0.21[a]	0.37
1-dimensional gradient gel electrophoresis subclasses, %						
HDL_2 (total)	55.1 ± 9.5	1.4 ± 2.7	1.9 ± 4.5[a]	0.5 ± 4.0	2.2 ± 4.7[a]	0.09
HDL_{2b}	34.5 ± 8.7	1.6 ± 2.3[a]	2.5 ± 4.6[a]	0.9 ± 3.8[c]	2.6 ± 4.4[a]	0.03
HDL_{2a}	20.7 ± 2.8	−0.2 ± 1.3	−0.6 ± 1.4[a]	−0.4 ± 1.5	−0.4 ± 1.3[a]	0.32
HDL_3 (total)	44.9 ± 9.5	−1.4 ± 2.7	−1.9 ± 4.5[a]	−0.5 ± 4.0	−2.2 ± 4.7[a]	0.09
HDL_{3a}	17.2 ± 2.2	−0.4 ± 0.8	−0.7 ± 1.8[a]	0.0 ± 1.6[c]	−0.7 ± 1.5[a]	0.01
HDL_{3b}	11.5 ± 2.5	−0.6 ± 0.9[a]	−0.6 ± 1.2[a]	0.0 ± 1.1[c,b]	−0.6 ± 1.1[a]	0.003
HDL_{3c}	16.2 ± 6.0	−0.4 ± 1.9	−0.6 ± 2.5	−0.4 ± 2.2	−0.9 ± 2.7	0.70
2-dimensional gel electrophoresis subclasses, mg ApoA-I/mL						
pre-β-1	8.4 ± 2.9	0.4 ± 1.9	1.0 ± 2.6[a]	0.4 ± 2.7	0.1 ± 2.7	0.12
pre-β-2	3.6 ± 1.5	0.1 ± 0.9	0.3 ± 0.8[a]	0.3 ± 1.1[a]	0.1 ± 0.9	0.32
α-1	17.3 ± 6.8	0.7 ± 3.8	2.7 ± 4.1[a,b]	1.0 ± 4.5	2.0 ± 4.4[a]	0.03
α-2	54.1 ± 10.2	−0.9 ± 7.2	3.7 ± 9.2[a,b]	2.8 ± 8.7[a,b]	0.8 ± 9.4	0.002
α-3	23.1 ± 5.9	−1.3 ± 5.2[a]	0.7 ± 4.8[b]	0.8 ± 4.6[b]	0.2 ± 4.8	0.008
α-4	15.6 ± 4.5	−0.2 ± 3.9	1.5 ± 3.8[a,b]	1.4 ± 4.1[a,b]	1.2 ± 4.0[b]	0.01
pre-α-1	4.0 ± 2.1	0.0 ± 1.3	0.7 ± 1.2[a,b]	0.3 ± 1.1[c]	1.0 ± 1.3[a,b]	<0.0001
pre-α-2	6.4 ± 2.0	−0.2 ± 1.6	0.6 ± 1.5[a,b]	0.3 ± 1.2[b]	0.7 ± 1.7[a,b]	0.0004
pre-α-3	2.4 ± 0.8	−0.3 ± 0.6	0.1 ± 0.7[a,b]	0.1 ± 0.5[b]	0.2 ± 0.8[a,b]	<0.0001
pre-α-4	1.1 ± 0.5	−0.1 ± 0.5	0.0 ± 0.4	0.1 ± 0.4[b]	0.1 ± 0.4[b]	0.01

[1] Values are expressed as means ± SDs. Number of participants for apoA-I and 2-dimensional gel electrophoresis subclasses were 42 for all diets. [a] Significantly different from CHO, $P < 0.05$. [b] Significantly different from CHEESE, $P < 0.05$. [c] Significantly different from BUTTER, $P < 0.05$. BUTTER, diet rich in SFAs from butter; CHEESE, diet rich in SFAs from cheese; CHO, diet in which SFAs were substituted for by carbohydrates; HDL-C, HDL cholesterol; MUFA, diet in which SFAs were replaced by MUFAs; PUFA, diet in which SFAs were replaced by PUFAs; Δ, change.
[2] P values are for the main treatment effects in mixed models, comparing the effects of the 4 high-fat diets among themselves. As indicated in the Methods section, values after CHO were used as reference in the analyses. Pairwise comparisons of the high-fat diets were examined only when the P value of the main treatment effect was <0.05. Covariates (baseline values of the selected variable when available, sequence, sex, age, BMI or waist circumference) were included in the mixed models only when they were shown to be significant at $P < 0.05$. The MUFA and PUFA diets were not compared specifically because they were not part of the primary objectives of the study (see Methods).

FIGURE 1 Change in endpoint HDL-mediated CEC measured in J774 macrophages after consuming the 4 high-fat diets compared with CHO (reference diet) in men and women with abdominal obesity. Results are expressed as the post-intervention mean (±SEM) HDL-mediated CEC differences between CHEESE, BUTTER, MUFA, or PUFA and CHO. Analyses were performed on log-transformed data. n = 46 (CHEESE, CHO), n = 44 (BUTTER, MUFA), n = 43 (PUFA). *Significantly different from CHO, $P < 0.05$. #Significantly different from CHEESE, $P < 0.05$. BUTTER, diet rich in SFAs from butter; CEC, cholesterol efflux capacity; CHEESE, diet rich in SFAs from cheese; CHO, diet in which SFAs were substituted for by carbohydrates; MUFA, diet in which SFAs were replaced by MUFAs; PUFA, diet in which SFAs were replaced by PUFAs; tx, treatment.

and MUFA (+4.7%, $P = 0.031$) increased HDL-mediated CEC compared with CHEESE, while the variation in HDL-mediated CEC after PUFA was not statistically different from the other high-fat diets. BUTTER (+3.3%, $P = 0.043$) and MUFA (+3.8%, $P = 0.048$) also significantly increased HDL-mediated CEC compared with CHO, which was not the case for CHEESE and PUFA. The pre-specified test for a diet × sex interaction on HDL-mediated CEC was significant ($P = 0.044$, Figure 2). The increase in HDL-mediated CEC after BUTTER compared with CHEESE was significant among men (+6.0%, $P = 0.047$) but not women (+2.9%, $P = 0.19$), while the increase after MUFA compared with CHEESE was significant among women (+9.1%, $P = 0.008$) but not men (–0.6%, $P = 0.99$).

As shown previously, BUTTER increased serum LDL-cholesterol concentrations compared with the other diets in the entire sample of participants (8) and also in this sample except when compared to CHEESE ($P = 0.066$, Table 3). The increase in LDL-cholesterol after BUTTER (compared with CHO) was significantly correlated with the concurrent increase in HDL-mediated CEC in men ($r = 0.45$, $P = 0.048$), but not in women ($r = 0.27$, $P = 0.20$, Figure 3**A, B**). Finally, variations (compared with CHO) in HDL-cholesterol, apoA-I, and apoB after BUTTER or CHEESE were not correlated with concurrent variations in HDL-mediated CEC in men, women, or in both groups combined (data not shown).

Discussion

To the best of our knowledge, this study is the largest controlled consumption trial to date examining the effects of different dietary fats on HDL-mediated CEC. Moreover, it is the first time that the impact of SFAs from 2 dairy sources, namely butter and cheese, on this important HDL function is compared. The diet rich in SFAs from butter significantly increased HDL-mediated CEC compared with SFAs from cheese. Consumption of MUFAs also increased HDL-mediated CEC, while PUFAs had no effect. Finally, data suggested a sex dimorphism in the HDL-mediated CEC response to dietary fat, with men being more responsive to SFAs from butter and women being more responsive to MUFAs.

Cheese consumption has not been associated with an increased risk of CAD in epidemiologic studies (26), despite the fact that cheese is an important source of dietary SFAs and sodium (27). The neutral effect of SFAs from cheese on HDL-mediated CEC as well as on LDL-cholesterol concentrations (28) is consistent with the absence of an association between cheese consumption and CAD risk. On the other hand, SFAs from butter significantly increased HDL-mediated CEC compared with the same amount of SFAs consumed as cheese and also compared with CHO. This is consistent with the emerging concept that the food matrix may modify the impact of SFAs on cardiometabolic outcomes (8, 9). As indicated earlier, results of several trials (9) and from our group (8) have shown an attenuation of the LDL-cholesterol-raising effects of SFAs when the latter are consumed as cheese rather than as butter.

Cholesterol derivatives, such as oxysterols, have been shown to upregulate ABCA1 expression through activation of the nuclear liver X receptor and retinoid X receptor (29). Higher circulating LDL-cholesterol (8) and, possibly, cholesterol derivatives (30) seen after consumption of SFAs from butter may stimulate

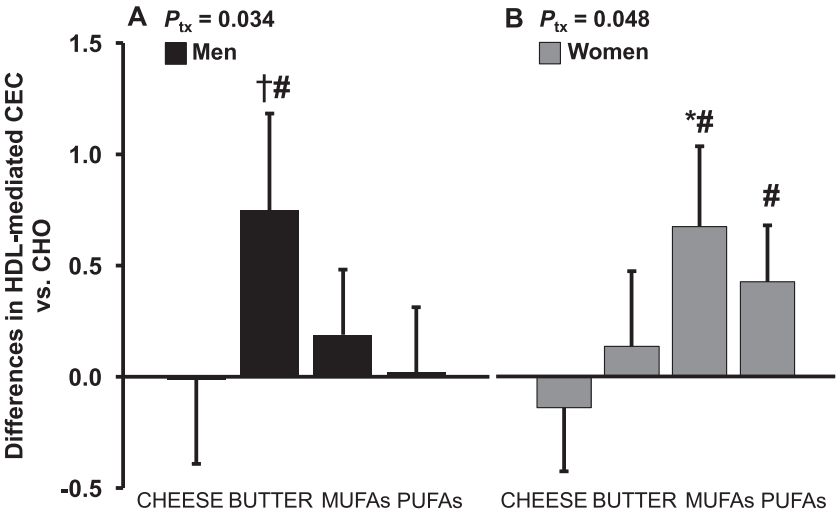

FIGURE 2 Change in endpoint HDL-mediated CEC measured in J774 macrophages after consuming the 4 high-fat diets compared with CHO (reference diet) in men (A) and women (B) with abdominal obesity. Results are expressed as the post-intervention mean (±SEM) HDL-mediated CEC differences between CHEESE, BUTTER, MUFA, or PUFA and CHO in men (n = 21) and women (n = 25). Analyses were performed on log-transformed data. P-treatment by sex interaction = 0.044 (mixed model). *Significantly different from CHO, $P < 0.05$. #Significantly different from CHEESE, $P < 0.05$. †Significantly different from PUFA, $P < 0.05$. BUTTER, diet rich in SFAs from butter; CEC, cholesterol efflux capacity; CHO, diet in which SFAs were substituted for by carbohydrates; MUFA, diet in which SFAs were replaced by MUFAs; PUFA, diet in which SFAs were replaced by PUFAs; tx, treatment.

TABLE 3 Changes in endpoint serum lipid and apoB concentrations after consuming the 4 high-fat diets compared with CHO (reference diet) in men and women with abdominal obesity[1]

		Δ vs. CHO				
	CHO ($n = 46$)	CHEESE ($n = 46$)	BUTTER ($n = 44$)	MUFA ($n = 43$)	PUFA ($n = 43$)	P^2
Total cholesterol, mmol/L	4.87 ± 0.95	0.22 ± 0.35[a]	0.34 ± 0.33[a]	−0.05 ± 0.42[b,c]	−0.18 ± 0.46[a,b,c]	<0.0001
LDL cholesterol, mmol/L	3.09 ± 0.81	0.18 ± 0.33[a]	0.28 ± 0.30[a]	−0.07 ± 0.33[b,c]	−0.16 ± 0.39[a,b,c]	<0.0001
ApoB, g/L	1.67 ± 0.46	0.07 ± 0.25	0.15 ± 0.26[a]	−0.03 ± 0.24[b,c]	−0.11 ± 0.24[a,b,c]	<0.0001
TGs,[3] mmol/L	1.39 ± 0.58	−0.03 ± 0.29	−0.07 ± 0.37[a]	−0.10 ± 0.36[a]	−0.19 ± 0.28[a,b]	0.04
non-HDL cholesterol, mmol/L	3.78 ± 0.93	0.17 ± 0.32[a]	0.26 ± 0.33[a]	−0.11 ± 0.39[a,b,c]	−0.24 ± 0.42[a,b,c]	<0.0001

[1]Values are means ± SDs. These are data of a subset of participants from a larger trial. Serum lipid data of all participants in the trial have been published elsewhere (8). [a]Significantly different from CHO, $P < 0.05$. [b]Significantly different from CHEESE, $P < 0.05$. [c]Significantly different from BUTTER, $P < 0.05$. BUTTER, diet rich in SFAs from butter; CHEESE, diet rich in SFAs from cheese; CHO, diet in which SFAs were substituted for by carbohydrate; MUFA, diet in which SFAs were replaced by MUFAs; PUFA, diet in which SFAs were replaced by PUFAs; Δ, change.

[2]*P* values are for the main treatment effects in mixed models, comparing the effects of the 4 high-fat diets among themselves. As indicated in the Methods section, values after CHO were used as reference in the analyses. Pairwise comparisons of the high-fat diets were examined only when the *P* value of the main treatment effect was <0.05. Covariates (sex, age, sequence, BMI or waist circumference) were included in the mixed models only when they were shown to be significant at $P < 0.05$. MUFA and PUFA were not compared specifically because they were not part of the primary objectives of the study (see Methods).

[3]Analyses were performed on log-transformed data.

ABCA1 expression in various tissues, which in turn results in increased efficiency of HDL interacting with this receptor given the crucial role of ABCA1 in HDL biogenesis (29). Interestingly, the significant correlation between increased LDL-cholesterol and increased HDL-mediated CEC after consumption of SFAs from butter is consistent with such a compensatory mechanism. A recent meta-analysis of epidemiologic studies has shown relatively small or neutral associations between butter consumption and risk of mortality, cardiovascular disease, and diabetes (31). Moreover, consumption of SFAs from butter increased the concentrations of large HDL particles in the present study (HDL_{2b} or α-1 and α-2), which in turn has been associated with increased HDL-mediated CEC via the SR-B1 pathway (32). The extent to which a compensatory increase in HDL-mediated CEC contributes to attenuate the effects on risk of LDL-cholesterol-raising SFAs from butter is an intriguing hypothesis to pursue.

Few studies have compared the effects of dietary fats on HDL-mediated CEC. Hernáez et al. (33) investigated HDL-mediated CEC changes from THP-1 human monocyte-derived macrophages after 1 year on a traditional Mediterranean diet rich in either virgin olive oil or walnut or a low-fat diet in 296 subjects from the PREDIMED (Prevención con Dieta Mediterránea) study. Both supplemented Mediterranean diets similarly increased HDL-mediated CEC relative to baseline values, but not compared to the low-fat diet. In our trial, MUFA, which was rich in refined olive oil, increased HDL-mediated CEC more than CHO and CHEESE. The controlled consumption and crossover nature of our study may have yielded greater capacity to detect the effects of MUFAs on HDL-mediated CEC, compared with PREDIMED.

Our data also suggested that HDL-mediated CEC in men might be more responsive to changes in dietary SFAs while HDL-mediated CEC in women may be more sensitive to changes in dietary MUFAs. Others have also observed a potential sex dimorphism in the CEC response to dietary fat. Montoya et al. (34) have shown that consumption of diets enriched in n–3 PUFAs or in n–6 PUFAs compared with a diet rich in SFAs from palm oil increased whole serum-mediated CEC from Fu5AH hepatoma cells in women, whereas only n–3 PUFAs increased CEC in men compared with the high-SFA diet. Recent data have shown that pro-inflammatory remodeling of the HDL proteome impairs CEC (35). In our trial, men had higher screening waist circumference compared with women (Table 1), which is consistent with greater visceral adipose tissue levels (36) and with a higher degree of subclinical inflammation (36). The extent to which potential differences in the pro-inflammatory status between men and women may have modulated the HDL-mediated CEC responses to various dietary fats is unclear and merits further consideration in future studies on this topic.

Cholesterol efflux via the ABCA1 pathway has been associated primarily with smaller HDL particles, notably HDL_{3b}, HDL_{3c}, and lipid-poor apoA-I like pre-β-1 (37). In the present study, consumption of SFAs from CHEESE increased the proportion of the large HDL_{2b} fraction and reduced the proportion of smaller HDL_{3b} compared with CHO, consistent with the lack of change in HDL-mediated CEC. This remodeling of

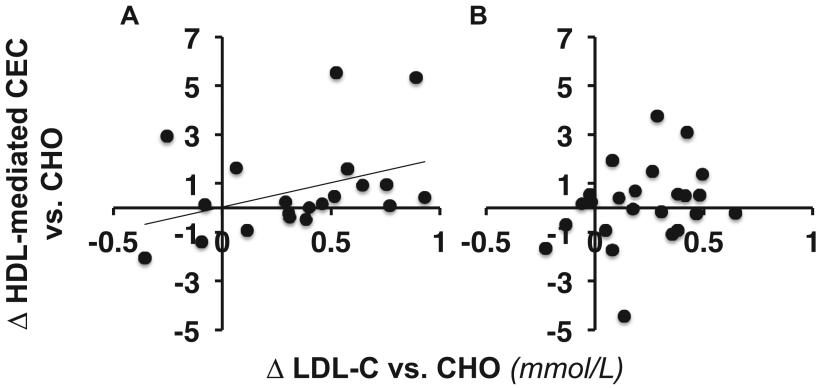

FIGURE 3 Spearman rank correlation coefficients between the changes in HDL-mediated CEC from J774 macrophages and concurrent changes in LDL cholesterol (SFAs from butter compared with CHO) in men (A) ($n = 20$) and women (B) ($n = 24$). Correlation was significant in men ($r_s = 0.45$, $P = 0.048$) but not in women ($r_s = 0.27$, $P = 0.20$). CEC, cholesterol efflux capacity; CHO, diet in which SFAs were substituted for by carbohydrate; LDL-C, LDL cholesterol; Δ, change.

HDL from small to large HDL particles was amplified after consumption of SFAs from BUTTER although concentrations of the smaller pre-β-1 HDL particles were also increased, along with a significant increase in HDL-mediated CEC, at least in men. There was no significant correlation between the change in HDL particle distribution after BUTTER and the change in HDL-mediated CEC (data not shown). MUFA did not alter HDL subclass distribution compared with CHO, but induced significant increase in HDL-mediated CEC, at least among women. Overall, these results suggest that the pattern of change in HDL subclasses observed via 2-dimensional gel electrophoresis after CHEESE and BUTTER is also consistent with an attenuation of the effect of dairy SFAs by the cheese matrix.

This study has several strengths, including its controlled consumption conditions and crossover design, according to which multiple diets were examined. The careful statistical handling of HDL-mediated CEC measurements and the relatively larger sample size compared with previously published studies on this topic are also unique elements of this trial. This study was not conducted under metabolic ward conditions, which reduce the risk of noncompliance and deviation from the protocol. However, compliance assessed using checklists was high across all diets, indicating that nearly all foods and caloric drinks provided were consumed. Furthermore, a large proportion (approximately 30–40%) of the prescribed diets was consumed on-site, under supervision of the research staff (8). In this context, the risk of deviance and noncompliance is very low, and the effects seen are very likely to be due to the interventions per se. Studies suggest that the capacity of HDL to promote CEC is inversely associated with carotid intima-media thickness, angiographically diagnosed coronary artery disease, and incidence of cardiac events, independent of variations in HDL-cholesterol, but not in pre-β-1 concentrations (15, 38, 39). In these studies, HDL-mediated CEC was assessed using J774 macrophages in which the ABCA1 transporters had been upregulated by cAMP (15, 38, 39). Of note, the J774 macrophage cells used in this study were cultured in basal condition, i.e., the ABCA1 transporter was not upregulated with cAMP. Thus, the ex vivo measure of HDL-mediated CEC in the present study reflects the contributions of the ABCA1 transporters as well as of SR-B1 and aqueous diffusion (23). Finally, HDL-mediated CEC and overall reverse cholesterol transport are highly complex pathways that are measured ex vivo. The extent to which such measurements reflect in vivo physiology remains to be determined.

In conclusion, data from this randomized controlled trial suggest that SFAs from butter have a greater influence on ex vivo HDL-mediated CEC and on HDL physical characteristics than SFAs from cheese. This provides further support to the hypothesis that the food matrix modifies the association between SFAs and CAD risk. The increase in HDL-mediated CEC seen with SFAs from butter paralleled the increase in LDL-cholesterol among men, but not among women, which may reflect a sex-dependent compensatory mechanism required for the management of excess cholesterol in the circulation.

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