Sex differences in postprandial responses to different dairy products on lipoprotein subclasses: a randomised controlled cross-over trial

Patrik Hansson¹, Kirsten B. Holven^{1,2}, Linn K. L. Øyri¹, Hilde K. Brekke¹, Gyrd O. Gjevestad³, Magne Thoresen⁴ and Stine M. Ulven^{1*}

¹Department of Nutrition, Institute of Basic Medical Sciences, University of Oslo, P.O. Box 1046, Blindern, 0317 Oslo, Norway

²Norwegian National Advisory Unit on Familial Hypercholesterolemia, Department of Endocrinology, Morbid Obesity and Preventive Medicine, Oslo University Hospital, P.O. Box 4950 Nydalen, 0424 Oslo, Norway

³TINE SA, Centre for Research and Development, P.O. Box 7, Kalbakken, 0902 Oslo, Norway

⁴Oslo Center for Biostatistics and Epidemiology, Department of Biostatistics, Institute of Basic Medical Sciences, University of Oslo, P.O. Box 1122, Blindern, 0317 Oslo, Norway

(Submitted 15 February 2019 – Final revision received 3 June 2019 – Accepted 7 June 2019)

Abstract

Men have earlier first-time event of CHD and higher postprandial TAG response compared with women. The aim of this exploratory sub-study was to investigate if intake of meals with the same amount of fat from different dairy products affects postprandial lipoprotein subclasses differently in healthy women and men. A total of thirty-three women and fourteen men were recruited to a randomised controlled cross-over study with four dairy meals consisting of butter, cheese, whipped cream or sour cream, corresponding to 45 g of fat (approximately 60 energy percent). Blood samples were taken at 0, 2, 4 and 6 h postprandially. Lipoprotein subclasses were measured using NMR and analysed using a linear mixed model. Sex had a significant impact on the response in M-VLDL ($P=0.04$), S-LDL ($P=0.05$), XL-HDL ($P=0.009$) and L-HDL ($P=0.001$) particle concentration (P), with women having an overall smaller increase in M-VLDL-P, a larger decrease in S-LDL-P and a larger increase in XL- and L-HDL-P compared with men, independent of meal. Men showed a decrease in XS-VLDL-P compared with women after intake of sour cream ($P<0.01$). In men only, XS-VLDL-P decreased after intake of sour cream compared with all other meals (*v.* butter: $P=0.001$; *v.* cheese: $P=0.04$; *v.* whipped cream: $P=0.006$). Meals with the same amount of fat from different dairy products induce different postprandial effects on lipoprotein subclass concentrations in men and women.

Keywords: Dairy matrix: Postprandial metabolism: Lipoprotein subclasses: VLDL: LDL: HDL: Sex differences

TAG-rich lipoproteins and their remnants are strongly associated with CVD and all-cause mortality^(1–6). The postprandial TAG response after a high-fat meal has been shown to be larger in men compared with women^(7,8), which may be a contributing factor to why men have earlier first-time event, and higher mortality rate, of CHD^(9,10). TAG-rich lipoproteins include all lipoproteins of various sizes that are enriched with TAG, that is, chylomicrons and VLDL^(11,12), and men have also been shown to have higher postprandial response of TAG-rich VLDL particles compared with women⁽⁸⁾.

Lipoproteins can be divided into subclasses based on their altered particle size as they undergo lipolysis. In epidemiological studies, all VLDL subclasses have been associated with increased CVD risk, and in particular smaller VLDL particles^(13–17).

LDL-cholesterol is a causal CVD risk factor⁽¹⁸⁾, and all LDL subclasses are associated with increased CVD risk^(13,14,19). Plasma TAG concentration is inversely correlated with HDL-cholesterol (HDL-C) concentration^(2,5,20), and there is epidemiological evidence that HDL-C is protective against CVD^(21–23). There is a well-known sex difference in fasting HDL-C concentration, with women having higher HDL-C than men⁽²⁴⁾. HDL particle concentration (HDL-P) has lately been shown to be a better marker for residual CVD and mortality risk than HDL-C^(25,26), but it is unclear to what extent HDL particle size matters as literature shows inconsistent results^(13,14,26,27).

Both long-term interventional studies and epidemiological data indicate that dairy products are a heterogeneous food group with various effects on blood lipids⁽²⁸⁾ and all-cause

Abbreviations: HDL-C, HDL-cholesterol; iAUC, incremental AUC; IDL, intermediate-density lipoprotein; L, large; M, medium; P, particle concentration; S, small; XL, very large; XS, very small; XXL, extremely large.

* Corresponding author: Stine M. Ulven, email smulven@medisin.uio.no

Fig. 1. Flow chart of the study participants. (A) Meal rich in fat from butter; (B) meal rich in fat from medium-hard cheese; (C) meal rich in fat from whipped cream; (D) meal rich in fat from sour cream.

mortality^(29,30). For the first time, we recently demonstrated divergent postprandial TAG and HDL-C responses (measured as the 0–6 h incremental AUC (iAUC_{0–6h})) between different dairy products in healthy adults⁽³¹⁾, with a borderline significant effect of sex on serum TAG-iAUC_{0–6h}. We have also shown that dietary fat quality affects the peak time of larger VLDL subclasses postprandially in healthy adults and adults with familial hypercholesterolemia⁽³²⁾. How intake of different dairy products affects postprandial lipoprotein subclass concentrations in women and men has, to the best of our knowledge, not been reported. Thus, the aim of this sub-study was to investigate if men and women respond differently to high-fat dairy meals, with the same amount of fat from different dairy products, with respect to postprandial lipoprotein subclass concentrations. The primary outcomes for this exploratory sub-study were the total 6 h postprandial particle concentration responses of six VLDL subclasses and four HDL subclasses, and secondary

outcomes were the corresponding particle concentration responses of intermediate-density lipoprotein (IDL) and LDL subclasses, all measured as the iAUC_{0–6h}.

Subjects and methods

Subjects

A total of forty-seven healthy subjects (thirty-three women and fourteen men) who accomplished at least one visit in a postprandial study at the University of Oslo between September 2016 and April 2017 were included in this exploratory sub-study. A total of twenty-one women and ten men completed all four study visits (Fig. 1). As abdominal obesity is associated with an elevated postprandial TAG response⁽³³⁾, subjects between 18 and 70 years of age with BMI 18.5–25 kg/m² and waist circumference <80 cm for women and <94 cm for men, or BMI ≥25 kg/m² and waist

circumference ≥ 80 cm for women and ≥ 94 cm for men were recruited to the postprandial study. Complete inclusion and exclusion criteria have been described previously⁽³¹⁾.

Study design

A randomised controlled cross-over study was conducted with four high-fat dairy meals as intervention, as has been described earlier⁽³¹⁾. Briefly, each meal consisted of three toasted slices of white bread (*Pågen Rosta*), raspberry jam (*Nora Bringebersylltetøy*) and either butter (*TINE Smør*), medium-hard cheese (*TINE Gräddost*), whipped cream (*TINE Kremfløte*) or sour cream (*TINE Seterrømme*), corresponding to 45 g of fat and approximately 60 energy percent of fat with similar fatty acid profiles. The energy content of each meal was 629 kcal (2632 kJ) for the butter meal, 715 kcal (2992 kJ) for the cheese meal, 652 kcal (2728 kJ) for the whipped cream meal and 655 kcal (2741 kJ) for the sour cream meal. The subjects were randomly allocated to one of the four test meal orders (order 1: A_B_C_D, order 2: B_C_D_A, order 3: C_D_A_B and order 4: D_A_B_C; A = butter, B = cheese, C = whipped cream and D = sour cream) by block randomisation performed by the principal investigator. Allocation ratio was 1:1:1:1. Each test day was separated by a 3- to 5-week-long washout period for premenopausal women not taking contraceptives, and a minimum washout period of 2 weeks for other participants. Before each test day, subjects received a reminder to fast for 12 h (with no fatty food for 14 h) and not perform any strenuous physical activity or drink alcohol the last 24 h before visit. Blood samples were drawn fasting, and 2, 4 and 6 h after the meal. Subjects were encouraged to be physically inactive during the 6 h period of blood sampling.

Clinical measurements

Weight was measured by the Medical Body Composition Analyzer seca 515/514 (seca, software version 1.1). Blood pressure was measured three consecutive times in a sitting position in the subjects' non-dominant arm by a Dinamap Carescape v100 (GE Medical System) at a screening visit.

Blood sampling and lipoprotein subclass measurement

Serum was collected in silica gel tubes (Becton Dickinson Vacutainer Systems) and kept in room temperature for 30–60 min to ensure complete blood coagulation before 15 min centrifugation at 1500 *g* (Thermo Fischer Scientific). Serum samples were then stored in a refrigerator until being analysed. Standard blood biochemical measurements were performed at an accredited medical laboratory (Fürost Medical Laboratory)⁽³¹⁾. Plasma was collected in EDTA tubes (Becton Dickinson Vacutainer Systems) and kept on ice for less than 15 min before being centrifuged at 2000 *g* for 15 min at 4°C (Thermo Fischer Scientific). The samples were distributed into smaller tubes and frozen at -80°C for lipoprotein subclass analysis. Lipoprotein subclass profiling was achieved using a commercial proton NMR metabolomics platform (Nightingale Health Ltd) with the following classifications: extremely large

(XXL) VLDL with particle diameters of at least 75 nm (including chylomicrons), five VLDL subclasses (very large (XL), large (L), medium (M), small (S) and very small (XS), with average particle diameters of 64.0, 53.6, 44.5, 36.8 and 31.3 nm, respectively), one IDL subclass with an average particle diameter of 28.6 nm, three LDL subclasses (L, M and S, with average particle diameters of 25.5, 23.0, and 18.7 nm, respectively) and four HDL subclasses (XL, L, M and S, with average particle diameters of 14.3, 12.1, 10.9 and 8.7 nm, respectively). Details about the NMR metabolomics platform have been described previously^(34,35).

Ethics

The study was approved by the Regional Committees for Medical and Health Research Ethics (2016/418/REK sør-øst B) and conducted according to the principles of the Declaration of Helsinki. Written informed consents were obtained from all subjects. The study was registered at www.clinicaltrials.gov as NCT02836106.

Statistics

The primary outcome of the original study was the serum TAG-iAUC_{0–6h}, and the sample size calculation has been described previously⁽³¹⁾. In this exploratory sub-study, differences in characteristics between women and men at baseline were analysed by the Mann–Whitney *U* test using IBM SPSS Statistics for Windows 24.0 (IBM Corp.). Baseline characteristics are presented as medians (25th–75th percentiles). Data from the postprandial measurements were analysed with a linear mixed model using Stata Special Edition 15.1 (StataCorp LLC). All subjects who completed at least one test day were included in the analysis. The response variable was the iAUC_{0–6h}, calculated from the different time points using the trapezoid method^(36,37), and the model included the variables meal, visit number, age, BMI, sex and a meal $\times$ sex interaction as fixed effects in addition to a random intercept at subject level. Response differences between meals were stratified by sex when the meal $\times$ sex interaction was significant. Non-significant meal $\times$ sex interactions were excluded from the model, and as the meal $\times$ sex interaction represents sex-specific meal differences, response differences between meals were calculated for the whole study group independent of sex in these cases. All results beyond baseline characteristics originate from this model. The significance level was set to $\alpha=0.05$. To adjust for multiple testing when performing comparisons on each lipoprotein subclass, the Bonferroni correction was applied (i.e. all *P* values for differences between meals were multiplied by the number of meal comparisons, and all *P* values for meal response differences between sexes were multiplied by the number of meals). Only Bonferroni-corrected *P* values are presented in the text and the figures. Pairwise meal comparisons within each sex were performed by combining the appropriate regression coefficients from the linear mixed model. All data that were analysed by the linear mixed model are shown as mean values with standard errors in the figures.

Table 1. Baseline characteristics of the fasting study subjects* (Medians and interquartile ranges (IQR))

	Female subjects (n 33)		Male subjects (n 14)		P
	Median	IQR	Median	IQR	
Descriptives					
Age (years)	30.0	25.0–42.0	33.5	27.0–46.0	0.50
BMI (kg/m ²)	21.8	20.8–24.9	24.2	23.5–31.0	0.007
Blood pressure (mmHg)					
Systolic	110	105–114	118	113–123	0.001
Diastolic	66	59–70	70	65–73	0.14
Biochemical measurements					
TAG (mmol/l)	0.86	0.68–1.05	0.89	0.80–1.09	0.43
Total cholesterol (mmol/l)	4.8	4.3–5.4	5.0	4.1–5.5	0.95
LDL-cholesterol (mmol/l)	2.8	2.5–3.2	3.4	2.8–3.9	0.08
HDL-cholesterol (mmol/l)	1.7	1.5–2.1	1.3	1.1–1.5	<0.001
Apo B (g/l)	0.9	0.8–1.0	1.0	0.8–1.1	0.20
Apo A-1 (g/l)	1.6	1.5–1.8	1.5	1.4–1.6	0.005
Glucose (mmol/l)	4.7	4.5–5.0	5.0	4.7–5.3	0.14
Insulin (pmol/l)	42	26–63	43	35–68	0.34
Micro-CRP (mg/l)	0.6	0.4–1.6	0.8	0.5–1.1	0.84
Lipoprotein subclass particles					
XXL-VLDL (mol/l (10 ⁻¹⁰))	0.80	0.67–1.06	0.98	0.78–1.38	0.05
XL-VLDL (mol/l (10 ⁻¹⁰))	2.23	1.52–3.79	3.70	2.78–5.55	0.03
L-VLDL (mol/l (10 ⁻⁰⁹))	1.92	1.32–2.84	2.62	2.21–3.86	0.01
M-VLDL (mol/l (10 ⁻⁰⁸))	0.95	0.73–1.16	1.14	1.02–1.61	0.01
S-VLDL (mol/l (10 ⁻⁰⁸))	1.80	1.49–2.07	2.07	1.78–2.40	0.03
XS-VLDL (mol/l (10 ⁻⁰⁸))	2.92	2.53–3.05	2.84	2.44–3.46	0.84
IDL (mol/l (10 ⁻⁰⁸))	8.36	7.51–9.36	8.69	7.21–9.64	0.69
L-LDL (mol/l (10 ⁻⁰⁷))	1.33	1.19–1.52	1.46	1.18–1.65	0.49
M-LDL (mol/l (10 ⁻⁰⁷))	1.04	0.94–1.21	1.20	0.94–1.38	0.28
S-LDL (mol/l (10 ⁻⁰⁷))	1.24	1.13–1.44	1.39	1.11–1.64	0.42
XL-HDL (mol/l (10 ⁻⁰⁷))	5.02	4.13–6.87	3.49	2.67–4.04	<0.001
L-HDL (mol/l (10 ⁻⁰⁶))	1.22	0.91–1.53	0.82	0.60–0.88	<0.001
M-HDL (mol/l (10 ⁻⁰⁶))	1.77	1.62–1.94	1.67	1.50–1.79	0.04
S-HDL (mol/l (10 ⁻⁰⁶))	4.42	4.19–4.53	4.48	4.30–4.59	0.24

CRP, C-reactive protein; XXL, extremely large; XL, very large; L, large; M, medium; S, small; XS, very small.

* Statistical analyses were performed with the Mann–Whitney *U* test.

Results

Baseline characteristics

The median age was 30 years for women and 33.5 years for men, with no significant difference between sexes ($P=0.50$). Women had higher baseline fasting serum HDL-C ($P<0.001$) and apo A-1 ($P=0.005$), and higher fasting plasma XL-HDL-P ($P<0.001$), L-HDL-P ($P<0.001$) and M-HDL-P ($P=0.04$) compared with men (Table 1). Women also had lower BMI ($P=0.007$) and systolic blood pressure ($P=0.001$). Men, on the contrary, had higher fasting plasma XXL-VLDL-P ($P=0.05$), XL-VLDL-P ($P=0.03$), L-VLDL-P ($P=0.01$), M-VLDL-P ($P=0.01$) and S-VLDL-P ($P=0.03$) compared with women (Table 1).

Postprandial differences between sexes

The meal by sex interaction, representing sex-specific meal differences, was only statistically significant for XS-VLDL-P ($P=0.008$). Significant sex effects on other lipoprotein subclass responses were independent of meal.

VLDL subclasses. After intake of each of the four test meals, men tended to have larger iAUC_{0–6h} particle concentrations for all VLDL subclasses, except XS-VLDL, compared with women. However, a significant sex effect, independent of meal,

was only found for M-VLDL ($P=0.04$), whereas a borderline significant sex effect was found for XXL-VLDL ($P=0.05$) and S-VLDL ($P=0.06$) (Fig. 2, online Supplementary Table 1). In contrast, men showed a decrease in XS-VLDL-P, whereas women showed a small increase after intake of sour cream ($P=0.01$) (Fig. 2, online Supplementary Tables 1 and 2).

HDL subclasses. Sex had a significant impact on the increase in XL-HDL-P ($P=0.009$) and L-HDL-P ($P=0.001$) with larger increases observed in women compared with men, independent of meal (Fig. 3, online Supplementary Table 1).

Intermediate-density lipoprotein and LDL subclasses. Sex had a significant impact on the decrease in S-LDL-P ($P=0.05$) with overall larger decreases observed in women compared with men, independent of meal (Fig. 4, online Supplementary Table 1).

Meal-induced differences

VLDL subclasses. Intake of cheese induced the largest increase in XXL-VLDL-P (77% larger *v.* butter: $P<0.001$; 76% larger *v.* whipped cream: $P<0.001$), XL-VLDL-P (77% larger *v.* butter: $P=0.006$; 76% larger *v.* whipped cream: $P=0.006$) and L-VLDL-P (94% larger *v.* butter: $P=0.04$) (online Supplementary Table 3). In men only, intake of sour cream

Fig. 2. Plasma 0–6 h incremental AUC (iAUC_{0–6h}) particle concentrations of VLDL subclasses after intake of meals with butter, cheese, whipped cream and sour cream in healthy men and women. Values are means, with standard errors represented by vertical bars. The original linear mixed model included meal, age, BMI, sex, visit number and a sex $\times$ meal interaction as fixed effects. All subclasses presenting a P value for sex as a main effect (P_{sex}) had no significant sex $\times$ meal interaction, and the interaction was consequently excluded from the model for those subclass analyses. † Bonferroni-corrected P value. (a) n 26, (A) n 10, (b) n 23, (B) n 12, (c) n 26, (C) n 12, (d) n 25, (D) n 11. (a) Response in women after intake of meal rich in fat from butter; (A) response in men after intake of meal rich in fat from butter; (b) response in women after intake of meal rich in fat from cheese; (B) response in men after intake of meal rich in fat from cheese; (c) response in women after intake of meal rich in fat from whipped cream; (C) response in men after intake of meal rich in fat from whipped cream; (d) response in women after intake of meal rich in fat from sour cream; (D) response in men after intake of meal rich in fat from sour cream. L, large; M, medium; S, small; XL, very large; XS, very small; XXL, extremely large.

Fig. 3. Plasma 0–6 h incremental AUC (iAUC_{0–6h}) particle concentrations of HDL subclasses after intake of meals with butter, cheese, whipped cream and sour cream in healthy men and women. Values are means, with standard errors represented by vertical bars. The original linear mixed model included meal, age, BMI, sex, a sex × meal interaction and visit number as fixed effects. All subclasses presenting a P value for sex as a main effect (P_{sex}) had no significant sex × meal interaction, and the interaction was consequently excluded from the model for those subclass analyses. (a) n 26, (A) n 10, (b) n 23, (B) n 12, (c) n 26, (C) n 12, (d) n 25, (D) n 11. (a) Response in women after intake of meal rich in fat from butter; (A) response in men after intake of meal rich in fat from butter; (b) response in women after intake of meal rich in fat from cheese; (B) response in men after intake of meal rich in fat from cheese; (c) response in women after intake of meal rich in fat from whipped cream; (C) response in men after intake of meal rich in fat from whipped cream; (d) response in women after intake of meal rich in fat from sour cream; (D) response in men after intake of meal rich in fat from sour cream. L, large; M, medium; S, small; XL, very large.

induced a significant decrease in XS-VLDL-P ($v.$ butter: $P=0.001$; $v.$ cheese: $P=0.04$; $v.$ whipped cream: $P=0.006$) (online Supplementary Table 3).

HDL subclasses. Intake of sour cream induced the largest increase in XL-HDL-P (69 % larger $v.$ butter: $P=0.04$; 87 % larger $v.$ cheese: $P=0.03$; 113 % larger $v.$ whipped cream: $P<0.001$), L-HDL-P (241 % larger $v.$ cheese: $P=0.01$; 110 % larger $v.$ whipped cream: $P=0.04$) and M-HDL-P ($v.$ cheese: $P<0.001$). In contrast, intake of cheese induced the largest decrease in S-HDL-P (101 % larger decrease $v.$ butter: $P=0.03$; 103 % larger decrease $v.$ whipped cream: $P=0.01$) (online Supplementary Table 4).

Intermediate-density lipoprotein and LDL subclasses. Intake of sour cream induced the largest decrease in L-LDL-P (219 % larger decrease $v.$ whipped cream: $P=0.04$), M-LDL-P (153 % larger decrease $v.$ whipped cream: $P=0.006$) and S-LDL-P (158 % larger decrease $v.$ whipped cream: $P=0.02$) (online Supplementary Table 5).

Discussion

The two major findings from the present study are that men and women have different postprandial lipoprotein subclass responses after intake of similar meals, and that different dairy products cause different responses within men and women, respectively. To the best of our knowledge, this is the first study to investigate sex differences in postprandial lipoprotein subclass responses to high-fat meals with different dairy products.

Regarding the postprandial iAUC_{0–6h} particle concentrations of the HDL subclasses, sex had a significant effect on the response in XL-HDL-P and L-HDL-P with a consistently larger response seen in women compared with men. Intake of sour cream induced the largest increase in XL-, L- and M-HDL-P, which is in accordance with our previous findings from the same study where sour cream induced the largest postprandial increase in HDL-C⁽³¹⁾. This could be due to sour cream being

Fig. 4. Plasma 0–6 h incremental AUC (iAUC_{0–6h}) particle concentrations of Intermediate-density lipoprotein (IDL) and LDL subclasses after intake of meal with butter, cheese, whipped cream and sour cream in healthy men and women. Values are means, with standard errors represented by vertical bars. The original linear mixed model included meal, age, BMI, sex, a sex × meal interaction and visit number as fixed effects. All subclasses presenting a P value for sex as a main effect (P_{sex}) had no significant sex × meal interaction, and the interaction was consequently excluded from the model for those subclass analyses. (a) n 26, (A) n 10, (b) n 23, (B) n 12, (c) n 26, (C) n 12, (d) n 25, (D) n 11. (a) Response in women after intake of meal rich in fat from butter; (A) response in men after intake of meal rich in fat from butter; (b) response in women after intake of meal rich in fat from cheese; (B) response in men after intake of meal rich in fat from cheese; (c) response in women after intake of meal rich in fat from whipped cream; (C) response in men after intake of meal rich in fat from whipped cream; (d) response in women after intake of meal rich in fat from sour cream; (D) response in men after intake of meal rich in fat from sour cream. L, large; M, medium; S, small.

a homogenised dairy product, which means that sour cream consists of more and smaller fat droplets that generate increased initial lipid digestion in the gastrointestinal tract^(38,39). This increase in lipolysis may potentially lead to escalated formation of nascent pre- β HDL particles in the enterocytes, which could partly explain the increase in HDL particles after intake of sour cream⁽⁴⁰⁾. Interestingly, even though there was no significant meal by sex interaction for any of the HDL subclasses, the figures indicate that the strong increase in XL-, L- and M-HDL-P after intake of sour cream mainly occurred in the women. Holmes *et al.* found that the particle concentrations of non-fasting XL-, L-HDL and M-HDL were inversely associated with myocardial infarction in Chinese adults, whereas S-HDL-P had neutral effect on myocardial infarction but was positively associated with ischaemic stroke. Furthermore, the correlations between HDL subclasses and coronary artery calcification have been studied in both women and men with or without type 1 diabetes⁽⁴¹⁾, showing an inverse association between large HDL subclasses

(corresponding to M-, L- and XL-HDL in the present study) and coronary artery calcification in both women and men without diabetes. Thus, it could be that intake of sour cream generates a more favourable postprandial HDL profile than butter, cheese and whipped cream, especially in healthy women. This provides one possible explanation for the neutral and sometimes positive epidemiological associations between intake of fermented dairy products and cardiovascular health. However, the potential health benefits of increased postprandial HDL particle concentrations remain to be investigated.

Regarding the postprandial iAUC_{0–6h} particle concentrations of the VLDL subclasses, men showed an overall larger response in M-VLDL-P and a borderline larger response in XXL-VLDL-P and S-VLDL-P compared with women. These findings are in line with the observed borderline significant effect of sex on the serum TAG response in our previous publication⁽³¹⁾. Based on the response patterns in Fig. 2, the lack of significant sex effects could potentially be due to the lower number of men compared

with women in the study. Intake of cheese induced the largest $iAUC_{0-6h}$ for XXL-, XL- and L-VLDL-P, which is a deviating finding from our previous result showing a significantly larger TAG- $iAUC_{0-6h}$ from intake of sour cream⁽³¹⁾. This could be explained by the different methods of measurements. Firstly, the NMR technology only measures the TAG content inside the lipoproteins, which gives a somewhat incomplete picture of the total postprandial TAG concentration, as the early postprandial phase is characterised by high lipoprotein lipase activity and, thus, a substantial amount of hydrolysed or partly hydrolysed TAG in the circulation, which are not captured with NMR. Secondly, the NMR technology has difficulties measuring very large and TAG-rich chylomicrons that can be present after a high-fat meal. It may therefore be that the sour cream generated more of these very large TAG-rich chylomicrons. This has been supported by Vors *et al.* who found that emulsified fat (similar to homogenised fat droplets) induced a larger increase in chylomicrons/apo-B48 and fatty acid spillover⁽⁴²⁾. The most apparent deviation in this sub-study, though, may be the observed decrease in XS-VLDL-P in men, but not in women, induced by intake of sour cream. This lipoprotein has an average diameter close to the one defining IDL and is therefore more of a remnant particle than a TAG-rich particle, with an assumed atherogenic capacity to enter the arterial wall⁽⁴³⁾. Non-fasting XS-VLDL-P has been linked to increased odds for myocardial infarction and ischaemic stroke in a nested case-control study⁽¹⁴⁾. In addition, findings from the JUPITER (Justification for the Use of Statins in Prevention) trial showed reduced residual risk for CVD when lowering the fasting concentration of small-sized VLDL particles (with diameters corresponding to XS-VLDL measured in the present study) in statin-treated subjects^(15,16). Interestingly, lowering the concentrations of medium and large-sized VLDL particles did not result in further risk reduction in that study. Applying these findings to our study indicates that sour cream may generate a more favourable postprandial VLDL profile than butter, cheese and whipped cream in men, potentially through a higher clearance rate of XS-VLDL particles.

Regarding the postprandial $iAUC_{0-6h}$ particle concentrations of IDL and the LDL subclasses, men showed an overall larger response in S-LDL-P compared with women, which is one of the lipoprotein subclasses considered to be the most atherogenic^(14,44). Intake of sour cream induced significantly larger decreases in L-, M- and S-LDL-P compared with whipped cream. The LDL-cholesterol concentration is expected to temporarily decrease 5–10 % in the postprandial state due to the competition of lipoprotein lipase between intestinally derived chylomicrons and hepatic VLDL particles, where chylomicrons are the preferred lipoproteins^(45–47). As whipped cream induced overall smaller increases in the largest VLDL subclasses (and thus possibly less chylomicrons) compared with sour cream, this may possibly partly explain the smaller decrease in L-, M- and S-LDL-P after intake of whipped cream.

We found that men and women responded differently to the dairy meals for some lipoprotein subclasses, with women showing a pattern of less increased VLDL-P and more increased HDL-P compared with men. These findings have support in the literature^(7,8,48,49) and one proposed mechanism is a higher lipoprotein lipase capacity in women, generating more

lipolysis⁽⁷⁾. This could explain both the partly lower VLDL-P responses and the partly higher HDL-P responses in women, as increased lipolysis stimulates the production of HDL particles by releasing more surface components to be incorporated into new HDL⁽⁴⁰⁾.

Our study has several strengths including a randomised controlled cross-over design with four dairy meals containing dairy products with different matrices. The meals were equal in fat content and not adjusted for differing nutrient contents since we were interested in the effect of the fat from dairy products as whole foods. This also means that we did not adjust the amount of fat based on body mass, and differences in responses due to variations in the amount of fat ingested can therefore be ruled out. The limitations of the study are what we cannot ensure absolute standardisation of the evening meal and amount of physical activity the day before each test day, even though the participants received guidelines and reminders before each visit. The male group had higher BMI than the female group; however, the median was within normal range for both groups, and BMI was adjusted for in the analysis. The Bonferroni method was applied to the meal and meal $\times$ sex comparisons to reduce the risk of false-positive findings. Since this was an exploratory sub-study, the number of parameters was not adjusted for.

In conclusion, the present study shows that intake of meals with the same amount of fat from different dairy products induces different postprandial effects on lipoprotein subclass concentrations, with sour cream potentially being the most healthy option. We also show that women and men respond differently to high-fat dairy meals in general, but also to certain dairy products in particular, and that women seem to respond more beneficially to high-fat dairy meals than men.

Acknowledgements

We would like to thank Navida Akhter Sheikh for help with blood sampling and all logistics, and Anne Randi Enget for help with blood sampling. We would also like to thank all the participants in the study for their time and efforts.

The present study was funded by the Research Council of Norway (IPN244633), University of Oslo, and the Throne-Holst Foundation for Nutrition Research, Oslo, Norway. TINE AS provided the dairy products included in the test meals, but was not involved in the study design, statistical analysis or interpretation of findings.

P. H., K. B. H., L. K. L. Ø., H. K. B., M. T. and S. M. U. designed research; P. H., K. B. H. and S. M. U. conducted research; P. H., L. K. L. Ø. and M. T. performed statistical analyses; G. O. G. provided essential material; P. H., K. B. H., L. K. L. Ø., H. K. B., G. O. G., M. T. and S. M. U. wrote the paper; P. H., K. B. H. and S. M. U. had primary responsibility for the final content of the paper. All authors read and approved the final manuscript.

S. M. U. has received research grants from Mills DA and Olympic Seafood, none of which are related to the content of this manuscript. K. B. H. has received research grants and/or personal fees from Mills DA, Olympic Seafood, Kaneka, Amgen, Sanofi and Pronova, none of which are related to the content of this manuscript. TINE AS partially funded the study via the

Research Council of Norway. S. M. U and K. B. H. have received funding from TINE AS, and G. O. G. is employed at TINE. She owns no stocks in the company. The other authors have no financial relationships relevant to disclose. P. H., K. B. H., L. K. L. Ø., H. K. B., M. T. and S. M. U. have no conflicts of interest.

Supplementary material

For supplementary materials referred to in this article, please visit <https://doi.org/10.1017/S0007114519001429>

References

- Nordestgaard BG (2016) Triglyceride-rich lipoproteins and atherosclerotic cardiovascular disease: new insights from epidemiology, genetics, and biology. *Circulation Res* **118**, 547–563.
- Nordestgaard BG, Benn M, Schnohr P, *et al.* (2007) Nonfasting triglycerides and risk of myocardial infarction, ischemic heart disease, and death in men and women. *JAMA* **298**, 299–308.
- Langsted A, Freiberg JJ, Tybjaerg-Hansen A, *et al.* (2011) Nonfasting cholesterol and triglycerides and association with risk of myocardial infarction and total mortality: the Copenhagen City Heart Study with 31 years of follow-up. *J Intern Med* **270**, 65–75.
- Langsted A, Freiberg JJ & Nordestgaard BG (2008) Fasting and nonfasting lipid levels: influence of normal food intake on lipids, lipoproteins, apolipoproteins, and cardiovascular risk prediction. *Circulation* **118**, 2047–2056.
- Kolovou GD, Mikhailidis DP, Kovar J, *et al.* (2011) Assessment and clinical relevance of non-fasting and postprandial triglycerides: an expert panel statement. *Curr Vasc Pharmacol* **9**, 258–270.
- Tot PH (2016) Triglyceride-rich lipoproteins as a causal factor for cardiovascular disease. *Vasc Health Risk Manage* **12**, 171–183.
- Lairon D, Lopez-Miranda J & Williams C (2007) Methodology for studying postprandial lipid metabolism. *Eur J Clin Nutr* **61**, 1145–1161.
- Wojczynski MK, Glasser SP, Oberman A, *et al.* (2011) High-fat meal effect on LDL, HDL, and VLDL particle size and number in the Genetics of Lipid-Lowering Drugs and Diet Network (GOLDN): an interventional study. *Lipids Health Dis* **10**, 181.
- Bots SH, Peters SAE & Woodward M (2017) Sex differences in coronary heart disease and stroke mortality: a global assessment of the effect of ageing between 1980 and 2010. *BMJ Global Health* **2**, e000298.
- Leening MJ, Ferket BS, Steyerberg EW, *et al.* (2014) Sex differences in lifetime risk and first manifestation of cardiovascular disease: prospective population based cohort study. *BMJ* **349**, g5992.
- Pirillo A, Norata GD & Catapano AL (2014) Postprandial lipemia as a cardiometabolic risk factor. *Curr Med Res Opin* **30**, 1489–1503.
- Nakajima K, Nakano T, Tokita Y, *et al.* (2011) Postprandial lipoprotein metabolism: VLDL vs chylomicrons. *Clin Chim Acta* **412**, 1306–1318.
- Wurtz P, Havulinna AS, Soininen P, *et al.* (2015) Metabolite profiling and cardiovascular event risk: a prospective study of 3 population-based cohorts. *Circulation* **131**, 774–785.
- Holmes MV, Millwood IY, Kartsonaki C, *et al.* (2018) Lipids, lipoproteins, and metabolites and risk of myocardial infarction and stroke. *J Am Coll Cardiol* **71**, 620–632.
- Lawler PR, Akinkuolie AO, Harada P, *et al.* (2017) Residual risk of atherosclerotic cardiovascular events in relation to reductions in very-low-density lipoproteins. *J Am Heart Assoc* **6**, e007402.
- Lawler PR, Akinkuolie AO, Chu AY, *et al.* (2017) Atherogenic lipoprotein determinants of cardiovascular disease and residual risk among individuals with low low-density lipoprotein cholesterol. *J Am Heart Assoc* **6**, e005549.
- Fischer K, Kettunen J, Wurtz P, *et al.* (2014) Biomarker profiling by nuclear magnetic resonance spectroscopy for the prediction of all-cause mortality: an observational study of 17, 345 persons. *PLoS Med* **11**, e1001606.
- Ference BA, Ginsberg HN, Graham I, *et al.* (2017) Low-density lipoproteins cause atherosclerotic cardiovascular disease. 1. Evidence from genetic, epidemiologic, and clinical studies. A consensus statement from the European Atherosclerosis Society Consensus Panel. *Eur Heart J* **38**, 2459–2472.
- Mora S, Caulfield MP, Wohlgemuth J, *et al.* (2015) Atherogenic lipoprotein subfractions determined by ion mobility and first cardiovascular events after random allocation to high-intensity statin or placebo: the Justification for the Use of Statins in Prevention: an Intervention Trial Evaluating Rosuvastatin (JUPITER) trial. *Circulation* **132**, 2220–2229.
- Bansal S, Buring JE, Rifai N, *et al.* (2007) Fasting compared with nonfasting triglycerides and risk of cardiovascular events in women. *Jama* **298**, 309–316.
- Madsen CM, Varbo A, Tybjaerg-Hansen A, *et al.* (2018) U-shaped relationship of HDL and risk of infectious disease: two prospective population-based cohort studies. *Eur Heart J* **39**, 1181–1190.
- Gordon DJ, Probstfield JL, Garrison RJ, *et al.* (1989) High-density lipoprotein cholesterol and cardiovascular disease. Four prospective American studies. *Circulation* **79**, 8–15.
- Di Angelantonio E, Sarwar N, Perry P, *et al.* (2009) Major lipids, apolipoproteins, and risk of vascular disease. *JAMA* **302**, 1993–2000.
- Koutsari C, Zagana A, Tzoras I, *et al.* (2004) Gender influence on plasma triacylglycerol response to meals with different monounsaturated and saturated fatty acid content. *Eur J Clin Nutr* **58**, 495–502.
- Mora S, Glynn RJ & Ridker PM (2013) High-density lipoprotein cholesterol, size, particle number, and residual vascular risk after potent statin therapy. *Circulation* **128**, 1189–1197.
- McGarrah RW, Craig DM, Haynes C, *et al.* (2016) High-density lipoprotein subclass measurements improve mortality risk prediction, discrimination and reclassification in a cardiac catheterization cohort. *Atherosclerosis* **246**, 229–235.
- Wu Y, Fan Z, Tian Y, *et al.* (2018) Relation between high density lipoprotein particles concentration and cardiovascular events: a meta-analysis. *Lipids Health Dis* **17**, 142.
- de Goede J, Geleijnse JM, Ding EL, *et al.* (2015) Effect of cheese consumption on blood lipids: a systematic review and meta-analysis of randomized controlled trials. *Nutr Rev* **73**, 259–275.
- Guo J, Astrup A, Lovegrove JA, *et al.* (2017) Milk and dairy consumption and risk of cardiovascular diseases and all-cause mortality: dose-response meta-analysis of prospective cohort studies. *Eur J Epidemiol* **32**, 269–287.
- Tognon G, Nilsson LM, Shungin D, *et al.* (2017) Nonfermented milk and other dairy products: associations with all-cause mortality. *Am J Clin Nutr* **105**, 1502–1511.
- Hansson P, Holven KB, Øyri LKL, *et al.* (2019) Meals with similar fat content from different dairy products induce different postprandial triglyceride responses in healthy adults: a randomized controlled cross-over trial. *J Nutr* **149**, 422–431.

32. Oyri LKL, Hansson P, Bogsrud MP, *et al.* (2018) Delayed postprandial TAG peak after intake of SFA compared with PUFA in subjects with and without familial hypercholesterolaemia: a randomised controlled trial. *Br J Nutr* **119**, 1142–1150.
33. Jackson KG, Walden CM, Murray P, *et al.* (2012) A sequential two meal challenge reveals abnormalities in postprandial TAG but not glucose in men with increasing numbers of metabolic syndrome components. *Atherosclerosis* **220**, 237–243.
34. Soininen P, Kangas AJ, Wurtz P, *et al.* (2015) Quantitative serum nuclear magnetic resonance metabolomics in cardiovascular epidemiology and genetics. *Circulation Cardiovasc Genet* **8**, 192–206.
35. Wurtz P, Kangas AJ, Soininen P, *et al.* (2017) Quantitative serum nuclear magnetic resonance metabolomics in large-scale epidemiology: a primer on -omic technologies. *Am J Epidemiol* **186**, 1084–1096.
36. Carstensen M, Thomsen C & Hermansen K (2003) Incremental area under response curve more accurately describes the triglyceride response to an oral fat load in both healthy and type 2 diabetic subjects. *Metab Clin Exp* **52**, 1034–1037.
37. Matthews JN, Altman DG, Campbell MJ, *et al.* (1990) Analysis of serial measurements in medical research. *BMJ* **300**, 230–235.
38. Liang L, Qi C, Wang X, *et al.* (2017) Influence of homogenization and thermal processing on the gastrointestinal fate of bovine milk fat: in vitro digestion study. *J Agric Food Chem* **65**, 11109–11117.
39. Islam MA, Devle H, Comi I, *et al.* (2017) Ex vivo digestion of raw, pasteurised and homogenised milk– Effects on lipolysis and proteolysis. *Int Dairy J* **65**, 14–19.
40. Camont L, Chapman MJ & Kontush A (2011) Biological activities of HDL subpopulations and their relevance to cardiovascular disease. *Trends Mol Med* **17**, 594–603.
41. Colhoun HM, Otvos JD, Rubens MB, *et al.* (2002) Lipoprotein subclasses and particle sizes and their relationship with coronary artery calcification in men and women with and without type 1 diabetes. *Diabetes* **51**, 1949–1956.
42. Vors C, Pineau G, Gabert L, *et al.* (2013) Modulating absorption and postprandial handling of dietary fatty acids by structuring fat in the meal: a randomized crossover clinical trial. *Am J Clin Nutr* **97**, 23–36.
43. Carmena R, Duriez P & Fruchart JC (2004) Atherogenic lipoprotein particles in atherosclerosis. *Circulation* **109**, Iii2–Iii7.
44. Diffenderfer MR & Schaefer EJ (2014) The composition and metabolism of large and small LDL. *Curr Opin Lipidol* **25**, 221–226.
45. Cohn JS (2006) Postprandial lipemia and remnant lipoproteins. *Clin Lab Med* **26**, 773–786.
46. Cohn JS, McNamara JR, Cohn SD, *et al.* (1988) Postprandial plasma lipoprotein changes in human subjects of different ages. *J Lipid Res* **29**, 469–479.
47. Bjorkegren J, Packard CJ, Hamsten A, *et al.* (1996) Accumulation of large very low density lipoprotein in plasma during intravenous infusion of a chylomicron-like triglyceride emulsion reflects competition for a common lipolytic pathway. *J Lipid Res* **37**, 76–86.
48. Teng KT, Chang CY, Kanthimathi MS, *et al.* (2015) Effects of amount and type of dietary fats on postprandial lipemia and thrombogenic markers in individuals with metabolic syndrome. *Atherosclerosis* **242**, 281–287.
49. Freedman DS, Otvos JD, Jeyarajah EJ, *et al.* (2004) Sex and age differences in lipoprotein subclasses measured by nuclear magnetic resonance spectroscopy: the Framingham Study. *Clin Chem* **50**, 1189–1200.