Chapitre 19 Impact différentiel de la matrice fromagère sur la réponse lipidique postprandiale : une étude contrôlée, randomisée, en chassé-croisé

Jean-Philippe Drouin-Chartier, André J. Tremblay, Julie Maltais-Giguère, Amélie Charest, Léa Guinot, Laurie-Eve Rioux, Steve Labrie, Michel Britten, Benoît Lamarche, Sylvie L Turgeon, Patrick Couture

L'article présenté dans ce chapitre s'intitule :

Differential impact of the cheese matrix on the postprandial lipid response: a randomized, crossover, controlled trial

Cet article est publié dans la revue :

The American Journal of Clinical Nutrition 2017;106(6);1358-1365.

Résumé

Contexte : Dans un environnement gastro-intestinal simulé, la matrice fromagère module la vitesse de digestion du gras laitier. À notre connaissance, l'impact de la matrice fromagère sur la lipémie postprandiale n'a jamais été évaluée chez des humains.

Objectif : Nous avons comparé l'impact de la consommation de gras laitiers via un fromage ferme, un fromage crémeux et du beurre sur la réponse postprandiale des TG 4 heures après le repas et sur l'aire sous la courbe incrémentale (iAUC) des concentrations de TG plasmatiques chez des sujets en santé.

Design : Un total de 43 sujets en santé ont été recrutés pour cette étude contrôlée, randomisée, en chassé-croisé. Dans un ordre aléatoire, à intervalle de 14 jours et après un jeûne de 12 h, les sujets devaient consommer 33 g de gras provenant d'un fromage ferme (cheddar jeune), d'un fromage crémeux (fromage à la crème) ou de beurre, incorporés dans des repas standardisés avec des compositions nutritionnelles équivalentes. Les concentrations plasmatiques de TG étaient mesurées avant le repas et 2, 4, 6, et 8 h après ce dernier.

Résultats : Le fromage cheddar, le fromage à la crème et le beurre ont induit des augmentations similaires dans les concentrations de TG 4 h après le repas (changements vs T=0 h : +59%, +59% et +62% respectivement ; *P*=0,9). Aucune différence n'a été observée dans l'iAUC des TG entre les 3 repas (*P*=0,9). Cependant, 2 h après le repas, la réponse des TG induite par la consommation de fromage à la crème (changements vs T=0 h : +44%) était significativement plus importante que celle induite par le beurre (changements vs T=0 h: +24% ; *P*=0,002) et le fromage cheddar (changements vs T=0 h: +16% ; *P*=0,0004). Six heures après le repas, la réponse des TG induite par le fromage à la crème était significativement atténuée comparativement à celle induite par le fromage cheddar (changements vs T=0 h : +14 vs + 44% ; *P*=0,0004).

Conclusion : Cette étude démontre que la matrice fromagère module l'impact du gras laitier sur la lipémie postprandiale chez des sujets sains.

Title page

Differential impact of the cheese matrix on the postprandial lipid response: A randomized, crossover, controlled trial

Jean-Philippe Drouin-Chartier[1], André J. Tremblay[1], Julie Maltais-Giguère[1], Amélie Charest[1], Léa Guinot[1-2], Laurie-Eve Rioux[1-2], Steve Labrie[1-2], Michel Britten[3], Benoît Lamarche[1], Sylvie L Turgeon[1-2], Patrick Couture[1-4]

Affiliations

1- Institute of Nutrition and Functional Foods, Laval University, Quebec City, Quebec, Canada

2- STELA Dairy Research Center, Laval University, Quebec City, Canada

3- Agriculture and Agri-Food Canada, Saint-Hyacinthe, Canada

4- Lipid Research Center, CHU de Québec-Laval University, Quebec City, Canada

Running head

Impact of cheese matrix on postprandial lipemia

Corresponding author

Patrick Couture, MD, FRCP(C), PhD
Institute of Nutrition and Functional Foods (INAF)
2440, Hochelaga Blvd
Pavillon des Services
Laval University
Quebec City, Canada
G1V 0A6
Phone: 418-654-2106
Fax: 418-317-1320
E-mail: patrick.couture@crchul.ulaval.ca

Sources of support

This study was funded by the Dairy Farmers of Canada. The Dairy Farmers of Canada had no role in the study design, analysis and interpretation of the results.

This trial was registered at clinicaltrials.gov as NCT02623790.

Abbreviations

apo: apolipoprotein;

AUC: area under the curve;

BMI: body mass index;

CVD: cardiovascular disease;

FFA: free fatty acid;

FFQ: food frequency questionnaire;

h: hour;

iAUC: incremental area under the curve;

m.-f: milk-fat;

MUFA: monounsaturated fatty acid;

OLTT: oral lipid tolerance test;

PUFA: polyunsaturated fatty acid;

SD: standard deviation;

SEM: standard error of the mean;

SFA: saturated fatty acid;

TG: triglyceride.

Abstract

Background: In a simulated gastrointestinal environment, the cheese matrix modulates dairy fat digestion. However, to our knowledge, the impact of the cheese matrix on postprandial lipemia in humans has not yet been evaluated.

Objective: In healthy subjects, we compared the impact of dairy fat provided from firm cheese, soft cream cheese, and butter on the postprandial response at 4 h and on the incremental area under the curve (iAUC) of plasma triglycerides.

Design: Forty-three healthy subjects were recruited to this randomized, crossover, controlled trial. In random order at intervals of 14 d and after a 12-h fast, subjects ingested 33 g fat from a firm cheese (young cheddar), a soft cream cheese (cream cheese), or butter (control) incorporated into standardized meals that were matched for macronutrient content. Plasma concentrations of triglycerides were measured immediately before the meal and 2, 4, 6 and 8 h after the meal.

Results: Cheddar cheese, cream cheese, and butter induced similar increases in triglyceride concentrations at 4 h (change from baseline: +59%, +59%, +62%, respectively; P = 0.9). No difference in the triglyceride $iAUC_{0\text{-}8h}$ (P-meal = 0.9) was observed between the 3 meals. However, at 2 h, the triglyceride response caused by the cream cheese (change from baseline: +44%) was significantly greater than that induced by butter (change from baseline: +24%, P = 0.002) and cheddar cheese (change from baseline: +16%, P = 0.0004). At 6 h, the triglyceride response induced by cream cheese was significantly attenuated compared with that induced by cheddar cheese (change from baseline: +14% compared with +42%; P = 0.0004).

Conclusion: This study demonstrates that the cheese matrix modulates the impact of dairy fat on postprandial lipemia in healthy subjects. This trial was registered at clinicaltrials.gov as NCT02623790.

Keywords: cheese, food matrix, postprandial, triglyceride, apolipoprotein B-48

Introduction

Postprandial lipoproteins play an important role in the pathogenesis of cardiovascular disease (CVD).[1] Remnants of intestinally-derived apolipoprotein B-48 (apoB-48)-containing lipoproteins (chylomicrons) are sufficiently small to penetrate the sub-endothelial space, where they contribute to the formation of foam cells, a hallmark of early atherosclerosis.[2] In addition, nonfasting triglyceride concentrations are associated with the risk of CVD.[3-5] Indeed, Bansal et al. previously observed among a large prospective cohort of women that postprandial triglyceride concentrations measured at 4 h had the strongest association with CVD risk.[4] Postprandial lipemia is modulated by the composition of food in fatty acids, which encompasses the degree of saturation and the length of the chain of the fatty acid,[6, 7] in carbohydrates[8, 9] and in proteins.[10-12] In addition, to macronutrient composition, the food texture,[13] fat globule organization,[14, 15] protein quality,[16] micronutrient composition,[17] and bacterial content[18] may influence the kinetics of food disintegration and dietary lipid bioaccessibility. Therefore, it is hypothesized that, independent of macronutrient composition, the food matrix per se modulates dietary lipid intestinal absorption and the postprandial lipid response.

Although cheese consumption is a major contributor to sodium and saturated fat intake in Western diets,[19, 20] it is not associated with an increased risk of CVD, hypertension or stroke.[21] Several clinical studies report that the cholesterol-raising effect of dairy fat was attenuated when provided from cheese rather than butter.[22, 23] These observations suggest that the cheese matrix modulates the effect of dairy fat on cardiovascular health. Moreover, cheeses with different hardnesses or cohesivenesses are digested differently and have varied lipid release rates within a simulated gastrointestinal environment.[24] In this context, the cheese matrix, compared with butter, has the potential to delay dairy fat digestion and reduce the postprandial lipid response. To our knowledge, however, the impact of dairy fat provided from different cheese matrices and from butter on postprandial lipid metabolism has not been thoroughly evaluated in humans.[25]

The objective of this study was to investigate the impact of the cheese matrix on the postprandial lipid response. We aimed to compare the impact of dairy fat from cheese and from butter on postprandial lipid concentrations in healthy subjects. The impact of dairy fat provided from firm cheese (cheddar) and from soft cream cheese (cream cheese) on postprandial lipid concentrations was also compared. We hypothesized that compared with butter, the matrix of both cheddar and cream cheeses would induce a slower lipid release and would decrease the postprandial levels of circulating triglycerides after a mixed meal. We also hypothesized that the firm cheddar matrix would induce a slower lipid response than the soft cream cheese matrix.

Subjects and Methods

The Laval University Medical Center ethical review committee approved the research protocol. Written consent was obtained from all participants. This trial was registered at clinicaltrials.gov as

NCT02623790. The study was conducted between February 2016 and June 2016 at the Laval University Institute of Nutrition and Functional Food in Québec City, Canada.

Study subjects

Forty-three healthy adults (19 men and 24 women) were recruited. The study inclusion criteria were as follows: age 18-65 y, stable body weight for > 3 mo before the screening, BMI (in kg/m^2) <35, fasting triglyceride concentrations <2.5 mmol/L, and blood pressure <150/90 mm Hg. Exclusion criteria were as follows: smoking status (>1 cigarette/d); alcohol abuse; illicit drug consumption; extreme dyslipidemias (e.g., familial hypercholesterolemia), type 2 diabetes, perimenopause, history of CVD or cancer, dairy product intolerance, vegetarianism, non-standard eating habits, or acute hepatic, gastrointestinal, or renal disease or any other condition that interfered with study participation. The use of antidepressants, levothyroxine or hormonal therapy was allowed only if the dose had been stable for >3 months prior to the study. Any other drug or therapy was considered cause for exclusion. Study subjects who typically used dietary supplements or natural health products were not allowed to use these products during the study. Participants notified the study coordinators if they had to initiate or modify any medications during the course of the study.

Study design

This study was designed as a single-center, randomized, crossover controlled trial. Participants who met the eligibility criteria first had to complete a validated, web-based, self-administered food frequency questionnaire (FFQ), which assessed participants' diet from the preceding 4 wk.[26] Subjects subsequently received dietary advice from a registered dietitian to adapt their diet to a prudent dietary pattern. Participants had a 2-wk run-in period to familiarize themselves with the dietary recommendations and to complete a validated 3-d physical activity diary.[27] At the end of the run-in period, participants were randomly assigned to 1 of 3 test sequences: cheddar-butter-cream cheese, butter-cream cheese-cheddar, and cream cheese-cheddar-butter. Three test sequences were used because of the unlikelihood of a carryover effect between the tests. The study coordinators (J-P Drouin-Chartier and J Maltais-Giguère, Institute of Nutrition and Functional Foods) used a computer-assisted program to perform the randomization, in which 8 blocks of 6 subjects were allocated to each sequence of tests.

The intervention consisted of 3 oral lipid tolerance tests (OLTTs), with each containing the same amount of dairy fat provided from cheddar cheese, cream cheese, or butter (control) and consumed at intervals of 14 d in the allocated test sequence. The nutritional composition of the test meals was equivalent and is detailed below. The 3 OLTTs were conducted in the morning after a 12-h fast. At 0700, an intravenous catheter was inserted into a forearm vein, and a fasting blood sample was collected. Immediately afterward, the participant ate the test meal containing the cheddar, cream cheese or butter. The 3 OLTTs were conducted under carefully controlled isocaloric conditions to

provide one-third of the daily energy requirements for each participant. Daily energy requirements were estimated using Harris-Benedict's formula and the assessment of participants' caloric intakes using the FFQ completed during the run-in period.[26] The time required to eat was monitored, limited to 20 min and had to be similar for the 3 meals for the same subject. Blood samples were drawn every 2 h over the 8-h postprandial period (2, 4, 6 and 8 h). For the duration of the OLTTs, subjects were not allowed to eat but had access to 500 mL of water.

On the evening before each OLTT, a standardized dinner was provided to the participants. The composition of the dinner was designed to provide one-third of the daily energy requirements of each participant. The dinner had to be ingested before 1900 to ensure a 12-h overnight fast. Forty-eight hours before the OLTTs, alcohol consumption and intense physical activity were prohibited. During the last week of the study, subjects had to complete a second physical activity diary. Subjects also had to complete a FFQ to assess their diet over the course of the study.

During the study, participants and coordinators were not blinded because of the tangibility of the study food. However, laboratory and statistical analyses were conducted with investigators blinded to the study foods until final analyses were completed.

Composition of the test meals

The cream cheese used in this study was a commercially available, 31% milkfat, 55% moisture, unripened, homogenized, fresh cheese. The cheddar cheese was a firm, uncooked, 32% milkfat, 37% moisture, young cheddar without a rind. The cream cheese and the cheddar cheese were selected from other commercially available cheeses because of their markedly different textural properties (24). The butter was a commercially available, salted butter. The test foods were accompanied by bread covered with icing (topping) and a fruit juice. The cheddar cheese was served in pieces beside the bread, whereas the cream cheese and butter were incorporated into the icing. The icing was prepared without heating and was spread on top of the bread to preserve the matrix of the cream cheese and the butter. The 3 test meals were designed using the Nutrition Data System for Research software (University of Minnesota, MN, USA) to provide 33 g of fat from cheddar cheese, cream cheese, or butter per 1000 kcal (**Table 1**). The composition in macronutrients (protein, lipids and carbohydrates), cholesterol, fiber and sodium of the test meals was matched to isolate the impact of the cheese matrix from the impact of macronutrient composition on postprandial lipemia. Sodium caseinate was added to the bread that accompanied the cream cheese and the butter to match the total protein content of the meals. Dietetic technicians prepared the test meals throughout the study. Each food or ingredient was weighed to a precision of ±0.1 g.

Biochemical measurements

Serum cholesterol and triglyceride concentrations were determined with a Roche/Hitachi MODULAR analyzer (Roche Diagnostics, Indianapolis, IN, USA) using the appropriated reagents. Blood glucose concentrations were measured by colorimetry and insulin concentrations by electrochemiluminescence (Roche Diagnostics, Indianapolis, IN, USA). Commercial ELISA kits were used to measure apoB-48 plasma concentrations (Shibayagi) and free fatty acids (FFAs) (ZenBio).

Study outcomes, sample size estimate and statistical analyses

The primary study outcome was changes in triglyceride concentrations from baseline to 4 h after the meal was consumed between the cheddar cheese and butter and between the cream cheese and butter. The triglyceride response at 4 h was selected as the primary outcome because of the association between postprandial triglyceride concentrations at 4 h and CVD risk.[4] Changes in the incremental area under the curve (iAUC) over the 8 h of the test of triglycerides and FFAs were secondary study outcomes. The assessment of the postprandial response of apoB-48 was not a predesignated endpoint and should be considered as exploratory.

The study was designed to have adequate power to test the main hypothesis that the cheese matrix would modulate the postprandial triglyceride response at 4 h in healthy subjects. The estimation of sample size was based on previous studies from our group that showed that the within-patient SD for triglyceride concentrations represents 40% of the mean value in a population.[28] A total of 40 patients completing the study would allow us to detect a 20% difference between the cheeses and the butter, with a power of 80% at a 2-sided, 5% significance level. Forty-three subjects were recruited on the basis of an anticipated drop-out rate of 5-10%.

Statistical analyses were conducted using mixed models for repeated measures with changes from baseline in triglyceride, FFA, or apoB-48 concentrations as dependent variables. The interaction between meals (cream cheese, cheddar, butter) and time (2, 4, 6, 8 h) was treated as the main fixed effect. Subjects were treated as a random effect. Potential covariates (baseline concentrations, sex, age, BMI, sequence, time required to eat the test meal, water consumed during the OLTTs, caloric content of the test meals) were added into the models as fixed effects. Only covariates with significant effects were maintained in the models. The adaptive Holm-Bonferroni method was used to adjust *P* values for multiple comparisons.[29] For the multiple comparison adjustment, only comparisons between the 3 test meals at the same time point were kept. Data were analyzed using SAS software (Studio University Edition, version 3.5, SAS Institute Inc.).

Results

The study flow chart is presented in **Figure 1**. No subjects dropped out during the research project. Baseline demographic, anthropometric and fasting biochemical characteristics of the 43 subjects

included in the study are presented in **Table 2**. Women represented 56% of the study subjects. The weights, lipid concentrations and surrogate markers of insulin-sensitivity for the study participants were all within the normal range. During the study, the mean daily caloric intake was significantly lower than the participants' usual intake (**Table 3**). No changes were observed in diet composition as macro- and micronutrient distribution remained constant. Energy expenditure, estimated from the 3-d physical activity diary, remained constant during the study. Finally, on the morning of the 3 test meals, fasting concentrations of triglycerides (P = 0.07), apoB-48 (P = 0.2) and FFAs (P = 0.5) were similar (data not shown). The time required to eat each meal (P = 0.1) and the volume of water consumed during the OLTTs (P = 0.4) were also similar between the 3 test meals (data not shown).

Postprandial triglyceride response

As presented in **Figure 2A**, the cream cheese, cheddar cheese and butter induced similar increases in triglyceride concentrations at 4 h (primary outcome) compared with baseline values (P =0.9*)*. No difference was observed in the triglyceride $AUC_{0\text{-}8h}$ (P-meal = 0.1) and the $iAUC_{0\text{-}8h}$ (P-meal = 0.9) (secondary outcome) between the cream cheese, cheddar cheese and butter (**Figure 2B**). However, the postprandial triglyceride response between the sources of dairy fat differed (P-meal X time < 0.0001). At 2 h, the cream cheese induced a greater increase in triglyceride concentrations than the butter (P = 0.002) and the cheddar cheese (P = 0.0004), while the cheddar and the butter induced a similar triglyceride response (P = 0.9). At 6 h, there was no difference in the triglyceride response between the cream cheese and the butter (P = 0.09) or the cheddar and the butter (P = 0.3), but triglyceride response of cream cheese was lower than that of cheddar (P = 0.0004). At 8 h, no difference in triglyceride response was observed between the 3 meals (0.07 < P < 0.9).

Postprandial apoB-48 response

The cheese matrix modulated the apoB-48 concentrations during the OLTT (P-meal X time = 0.004) as presented in **Figure 3A**. The apoB-48 response from baseline induced by cream cheese peaked at 2 h, whereas the response induced by the butter and the cheddar cheese peaked at 4 h. At 2 h, no difference between the 3 meals was observed (0.1 < P < 0.7). At 4 h and 6 h, the apoB-48 response from baseline with the cream cheese tended to be lower compared with the butter (P-4 h = 0.1; P-6 h = 0.07). No difference was observed between the cheddar and the butter at 4 h and 6 h (P-4 h = 0.9; P-6 h = 0.1). However, the cream cheese led to a lower apoB-48 response at 4 h (P = 0.02) and 6 h (P = 0.0002) compared with the cheddar cheese. There was no difference in the apoB-48 response at 8 h between the 3 meals (0.1 < P < 0.9). The cheese matrix had also an impact on the apoB-48 $iAUC_{0\text{-}8h}$ (P = 0.01, **Figure 3B**). No difference was observed in the apoB-48 $iAUC_{0\text{-}8h}$ between the two cheeses and the butter (0.08 < P<0.7), but the cream cheese induced a lower apoB-48 $iAUC_{0\text{-}8h}$ than the cheddar cheese (P = 0.01).

Postprandial FFA response

Significant effect of the 3 sources of dairy fat in the FFA response was observed (*P*-meal X time = 0.03, **Figure 4A**). At 2 h, the reduction in circulating FFA concentrations with the cream cheese was significantly lower compared with the cheddar cheese (*P* = 0.04). No other difference was observed in the FFA response between the 3 meals. As presented in **Figure 4B**, the cheese matrix had no impact on either the FFA $AUC_{0\text{-}8h}$ (*P* = 0.1) or the $iAUC_{0\text{-}8h}$ (*P* = 0.8).

Discussion

In this study, the impact of dairy fat provided from cheese and butter on postprandial lipemia was compared in healthy women and men. The firm cheddar matrix, the soft cream cheese matrix and the butter induced similar elevations in postprandial triglyceride concentrations at 4 h (primary outcome). No differences were observed in the triglyceride, FFA and apoB-48 iAUC0-8h between the cheeses and the butter, but the cream cheese attenuated the apoB-48 iAUC0-8h compared with the cheddar cheese. In addition, the dairy fat provided from the soft cream cheese matrix induced a greater increase in triglyceride concentrations at 2 h than the butter and the firm cheddar matrix. These data demonstrate for the first time, to our knowledge, that the cheese matrix modulates the postprandial release of triglycerides and apoB-48 but does not differentially modulate the impact of dairy fat on the magnitude of the postprandial peak and the response of triglycerides compared with butter in healthy subjects.

In this study, the lack of difference in the triglyceride $iAUC_{0\text{-}8h}$ reflects the similarity in fatty acid composition and fat content of the 3 meals.[30] Differences in the timing of the triglyceride response and in the timing and the magnitude ($iAUC_{0\text{-}8h}$) of the apoB-48 response between the cream cheese and the cheddar cheese or butter provide evidence regarding the differential impact of the cheese matrix on the postprandial lipid response. These results suggest that dairy fat provided from a cream cheese matrix, as characterized by small homogenized lipid droplets enclosed in a soft, semi-solid protein gel,[31] was more rapidly digested and absorbed than dairy fat provided from the cheddar matrix, which is characterized by fat globules of varying size surrounded by a firm, dense and solid protein network,[32] or from butter, a water-in-oil emulsion with a very low protein content.[33, 34] In addition, the reduced apoB-48 $iAUC_{0\text{-}8h}$ with cream cheese suggests that this particular cheese matrix induces the secretion of fewer and larger chylomicrons than those induced by cheddar cheese.

Among other factors, the protein curd plays an important role in defining the cheese matrix and may have contributed significantly to the modulation of the postprandial lipid response. In miniature pigs, the ingestion of a solid rennet curd, used for cheddar manufacturing, rather than a soft acid curd, used for cream cheese manufacturing, delayed digestion of the food matrix.[35] Indeed, in a gastric environment, the rennet curd forms firm aggregates that delay gastric emptying and matrix disintegration.[35] In addition, cheddar cheese curds exhibit lower moisture and higher hardness, which

confers the cheddar matrix a higher resistance to digestive enzymes compared with the cream cheese curds.[24, 36] The cheddaring and pressing processes also enhance the resistance to digestive enzymes of the cheddar curds by increasing the density and the stability of the protein gel compared with the cream cheese.[14, 32] Given the role of proteins in gastric emptying and gastrointestinal transport,[37] one cannot exclude that matching the total protein content of the mixed meals in the present study attenuated the differential effect of cheese matrix on postprandial lipemia by delaying the digestion of the cream cheese or the butter. On the other hand, matching the total protein content of the test meals isolated the effect of the protein network per se on cheese disintegration and dairy fat release from the effect of proteins on gastrointestinal transit. The differences observed in the triglyceride response between 0 and 2 h most likely reflect the delay in dairy fat release induced by the higher resistance of cheddar curds to digestive enzymes compared with the cream cheese.

Another factor that may have modulated the postprandial lipid response is the fat globule organization within cheese matrices. Homogenization of the cream cheese increases the number and stability and decreases the size of lipid droplets.[31, 38] For comparison, the diameter of a lipid droplet from cream cheese is ~0.5 µm, approximately one-sixth the size of a cheddar cheese lipid droplet (~3.0 µm).[39, 40] Smaller lipid droplets have a larger contact surface for lipases, which enhances digestion and absorption.[15, 41-44] Several studies have shown that dietary lipid droplet size is inversely associated with the postprandial triglyceride response.[15, 41-44] Therefore, the rapid postprandial increase in triglyceride concentrations in the first 2 hours following the consumption of cream cheese is consistent with the effect of lipid droplet size on dairy fat digestibility.

The lack of difference in the postprandial lipid response induced by the cheddar cheese and the butter could be interpreted as a limited effect of the cheese matrix on the bioaccessibility of dairy fat in the intestine. However, despite similarities in the postprandial lipid response, the gastrointestinal behavior of dairy fat from cheddar cheese versus butter is different.[45] Butter is a water-in-oil emulsion composed of large heterogeneous fat granules.[33] In this form, butter fat is not an adequate substrate for water-soluble digestive emulsifiers and lipases.[15, 46] In fact, butter fat probably separated into a layer on top of the aqueous phase in the stomach, which delayed the transit of dietary fats from the stomach to the intestine and induced a slow increase in triglycerides in the early postprandial phase.[46-48] Further investigation evaluating gastric emptying following the ingestion of cheese and butter is warranted to confirm this hypothesis.

The reduction in energy intake observed during the study period compared with the usual intake was unexpected, but is not unusual in the context of studies. This reduction in caloric intake, however, had no impact on the subjects' weight and is unlikely to have impacted postprandial lipemia. A full control of the diet between test days should be considered in future studies. Major strengths of this study include the crossover design and the use of a standardized dinner on the evening before the

study. These 2 elements limited the intra- and inter-individual variability in the postprandial lipid response. Additionally, while the incorporation of carbohydrate-rich foods in the test meals may have partly attenuated the effect of dairy fat per se on postprandial lipemia by enhancing triglyceride secretion, it allowed for the demonstration that, within a real-life mixed meal, the cheese matrix modulated postprandial lipemia. Finally, the absence of an extensive tracer analysis or data on postprandial concentraitons of insulin, glucose and glucagon-like peptide-1 limited the interpretation of the results on the impact of the cheese matrix on TG and chylomicron secretion and clearance rates.

In conclusion, this study showed that the cheese matrix modulates the postprandial release of triglycerides and apoB-48 in healthy subjects. Dairy fat provided from the soft cream cheese matrix was likely digested more rapidly than when provided from the butter or the firm cheddar matrix, inducing a more important triglyceride response in the early postprandial phase. However, the cheese matrix per se does not differentially impact the magnitude of the postprandial peak and the overall triglyceride response compared with butter. Results of this study are consistent with the neutral associations observed between both cheese and butter consumption and CVD risk.[21, 49] In sum, this study highlights that the food source of dairy fat modulates its effect on postprandial lipemia.

Acknowledgments

We thank Steeve Larouche and the staff of the metabolic kitchen of the Institute of Nutrition and Functional Food for their dedicated work.

Authors' contributions

The authors' responsibilities were as follows—PC, SLT and BL: designed the study; J-PD-C, JM-G, AJT, LG, L-ER, AC: conducted the research; J-PD-C, AJT and PC: analyzed the data; J-PD-C, AJT, LG, MB, SL, SLT, BL and PC: wrote the manuscript; PC: had primary responsibility for the final content of the manuscript; and all authors: read and approved the final manuscript.

Disclosures

AJT, JM-G, AC, LG, and L-ER had no conflicts of interest. J-PD-C is the recipient of doctoral scholarships from the Canadian Institute of Health Research and the Fonds de Recherche du Québec–Santé. He received speaker honoria from the Dairy Farmers of Canada (DFC) in 2016. SL received funding in the last 5 y from the National Research Council of Canada, Fonds de Recherche du Québec—Nature et Technologies, Ministère de l'Agriculture des Pêcheries et de l'Alimentation du Québec, Quebec Consortium for Industrial Bioprocess Research and Innovation, Genome Canada, Génome Québec, Ministère du Développement Économique de l'Innovation et de l'Exportation du Québec, Canadian Foundation for Innovation, DFC, Agropur Cooperative, Kraft Canada, Parmalat,

Saputo, and Novalait Inc. SL is a technical advisor for the Agropur Dairy Cooperative and was invited to be a guest speaker at 2 technical symposia. SL is also the director of the innovation support services for the Institute of Nutrition and Functional Foods. MB received funding in the last 5 y from Agriculture and Agri-Food Canada (Science Technology Branch Annual Call, A-Base Priority Agri-innovation Program, Dairy Research Cluster supported by DFC), Novalait Inc., Agropur Dairy Cooperative, and Rheolution Instruments. BL is chair of nutrition at Laval University, which is supported by endowments from Pfizer, Royal Bank of Canada, and Provigo-Loblaws. BL received funding in the past 5 y from the Canadian Institutes of Health Research, Natural Sciences and Engineering Research Council of Canada, Agriculture and Agri-Food Canada (Growing Forward program supported by the DFC, Canola Council of Canada, Flax Council of Canada, and Dow AgroSciences), Dairy Research Institute, Dairy Australia, Danone Institute, Merck Frosst, Pfizer, and Atrium Innovations. BL serves as the chair of the peer-review Expert Scientific Advisory Council of the DFC. He is also an advisory board member of the Canadian Nutrition Society and the Food Progress Initiatives Council and has served as an advisory expert for the Heart and Stroke Foundation of Canada saturated fat panel. BL has also received speaker honoraria from the International Chair on Cardiometabolic Risk, DFC, and World Dairy Platform. SLT received funding in the past 5 y from the Natural Sciences and Engineering Research Council of Canada, Fonds de Recherche du Québec— Nature et Technologies, Ministère du Développement Économique de l'Innovation et de l'Exportation du Québec PSRV2, Ministère de l'Agriculture des Pêcheries et de l'Alimentation du Québec, Agriculture and Agri-Food Canada (Growing Forward program supported by the DFC, Canadian Institutes of Health Research, Novalait Inc., Canadian Foundation for Innovation, Quebec Consortium for Industrial Bioprocess Research and Innovation, Agropur, Kraft Foods, Parmalat Canada, Saputo, and General Mills/Yoplait). SLT is the director of the Institute on Nutrition and Functional Foods, a Canadian representative on the standing committee on dairy science and technology for the International Dairy Federation (IDF), a member of IDF Canada, and cochair of the Committee on Science, Nutrition, and Health for IDF Canada. PC received funding in the past 5 y from the Canadian Institutes of Health Research, Agriculture and Agri-Food Canada (Growing Forward program supported by the DFC, Canola Council of Canada, Flax Council of Canada, and Dow AgroSciences), Dairy Research Institute, Dairy Australia, Danone Institute, Merck Frosst, Pfizer, Amgen, Sanofi, Kaneka Corporation, and Atrium Innovations. The Dairy Farmers of Canada had no role in the study design, analysis, or interpretation of the results.

References

1. Kolovou GD, Mikhailidis DP, Kovar J, Lairon D, Nordestgaard BG, Ooi TC, Perez-Martinez P, Bilianou H, Anagnostopoulou K, Panotopoulos G. Assessment and clinical relevance of non-fasting and postprandial triglycerides: an expert panel statement. *Curr Vasc Pharmacol* 2011;9(3):258-70.

2. Pal S, Semorine K, Watts GF, Mamo J. Identification of lipoproteins of intestinal origin in human atherosclerotic plaque. *Clin Chem Lab Med* 2003;41(6):792-5.

3. Nordestgaard BG, Benn M, Schnohr P, Tybjaerg-Hansen A. Nonfasting triglycerides and risk of myocardial infarction, ischemic heart disease, and death in men and women. *JAMA* 2007;298(3):299-308.

4. Bansal S, Buring JE, Rifai N, Mora S, Sacks FM, Ridker PM. Fasting compared with nonfasting triglycerides and risk of cardiovascular events in women. *JAMA* 2007;298(3):309-16.

5. Freiberg JJ, Tybjærg-Hansen A, Jensen J, Nordestgaard BG. Nonfasting triglycerides and risk of ischemic stroke in the general population. *JAMA* 2008;300(18):2142-52.

6. Karupaiah T, Sundram K. Modulation of human postprandial lipemia by changing ratios of polyunsaturated to saturated (P/S) fatty acid content of blended dietary fats: a cross-over design with repeated measures. *Nutr J* 2013;12:122.

7. Karupaiah T, Tan CH, Chinna K, Sundram K. The chain length of dietary saturated fatty acids affects human postprandial lipemia. *J Am Coll Nutr* 2011;30(6):511-21.

8. Jeppesen J, Chen YD, Zhou MY, Wang T, Reaven GM. Effect of variations in oral fat and carbohydrate load on postprandial lipemia. *Am J Clin Nutr* 1995;62(6):1201-5.

9. Westphal S, Leodolter A, Kahl S, Dierkes J, Malfertheiner P, Luley C. Addition of glucose to a fatty meal delays chylomicrons and suppresses VLDL in healthy subjects. *Eur J Clin Invest* 2002;32(5):322-7.

10. Westphal S, Kastner S, Taneva E, Leodolter A, Dierkes J, Luley C. Postprandial lipid and carbohydrate responses after the ingestion of a casein-enriched mixed meal. *Am J Clin Nutr* 2004;80(2):284-90.

11. Mamo JC, James AP, Soares MJ, Griffiths DG, Purcell K, Schwenke JL. A low-protein diet exacerbates postprandial chylomicron concentration in moderately dyslipidaemic subjects in comparison to a lean red meat protein-enriched diet. *Eur J Clin Nutr* 2005;59(10):1142-8.

12. Burn-Murdoch RA, Fisher MA, Hunt JN. The slowing of gastric emptying by proteins in test meals. *J Physiol* 1978;274:477-85.

13. Kong F, Singh RP. Disintegration of solid foods in human stomach. *J Food Sci* 2008;73(5):R67-80.

14. Lamothe S, Corbeil MM, Turgeon SL, Britten M. Influence of cheese matrix on lipid digestion in a simulated gastro-intestinal environment. *Food Func* 2012;3(7):724-31.

15. Vors C, Pineau G, Gabert L, Drai J, Louche-Pelissier C, Defoort C, Lairon D, Desage M, Danthine S, Lambert-Porcheron S, et al. Modulating absorption and postprandial handling of dietary fatty acids by structuring fat in the meal: a randomized crossover clinical trial. *Am J Clin Nutr* 2013;97(1):23-36.

16. Holmer-Jensen J, Mortensen LS, Astrup A, de Vrese M, Holst JJ, Thomsen C, Hermansen K. Acute differential effects of dietary protein quality on postprandial lipemia in obese non-diabetic subjects. *Nutr Res* 2013;33(1):34-40.

17. Lorenzen JK, Astrup A. Dairy calcium intake modifies responsiveness of fat metabolism and blood lipids to a high-fat diet. *Br J Nutr* 2011;105(12):1823-31.

18. Collins YF, McSweeney PLH, Wilkinson MG. Lipolysis and free fatty acid catabolism in cheese: a review of current knowledge. *Int Dairy J* 2003;13(11):841-66.

19. Weinberg LG, Berner LA, Groves JE. Nutrient contributions of dairy foods in the United States, Continuing Survey of Food Intakes by Individuals, 1994-1996, 1998. *J Am Diet Assoc* 2004;104(6):895-902.

20. Drewnowski A, Rehm CD. Sodium intakes of US children and adults from foods and beverages by location of origin and by specific food source. *Nutrients* 2013;5(6):1840-55.

21. Drouin-Chartier JP, Brassard D, Tessier-Grenier M, Côté JA, Labonté M-È, Desroches S, Couture P, Lamarche B. Systematic Review of the Association between Dairy Product Consumption and Risk of Cardiovascular-Related Clinical Outcomes. *Adv Nutr* 2016;7(6):1026-40.

22. de Goede J, Geleijnse JM, Ding EL, Soedamah-Muthu SS. Effect of cheese consumption on blood lipids: a systematic review and meta-analysis of randomized controlled trials. *Nutr Rev* 2015;73(5):259-75.

23. Brassard D, Tessier-Grenier M, Allaire J, Rajendiran E, She Y, Ramprasath V, Gigleux I, Talbot D, Levy E, Tremblay A, et al. Comparison of the impact of SFAs from cheese and butter on cardiometabolic risk factors: a randomized controlled trial. *Am J Clin Nutr* 2017;105(4):800-809.

24. Fang X, Rioux L-E, Labrie S, Turgeon SL. Commercial cheeses with different texture have different disintegration and protein/peptide release rates during simulated in vitro digestion. *Int Dairy J* 2016;56:169-78.

25. Drouin-Chartier JP, Côté JA, Labonté M-È, Brassard D, Tessier-Grenier M, Desroches S, Couture P, Lamarche B. Comprehensive Review of the Impact of Dairy Foods and Dairy Fat on Cardiometabolic Risk. *Adv Nutr* 2016;7(6):1041-51.

26. Labonte ME, Cyr A, Baril-Gravel L, Royer MM, Lamarche B. Validity and reproducibility of a web-based, self-administered food frequency questionnaire. *Eur J Clin Nutr* 2012;66(2):166-73.

27. Bouchard C, Tremblay A, Leblanc C, Lortie G, Savard R, Theriault G. A method to assess energy expenditure in children and adults. *Am J Clin Nutr* 1983;37(3):461-7.

28. Tremblay AJ, Lamarche B, Labonte ME, Lepine MC, Lemelin V, Couture P. Dietary medium-chain triglyceride supplementation has no effect on apolipoprotein B-48 and apolipoprotein B-100 kinetics in insulin-resistant men. *Am J Clin Nutr* 2014;99(1):54-61.

29. Holm S. A Simple Sequentially Rejective Multiple Test Procedure. *Scan J Stat* 1979;6(2):65-70.

30. Dubois C, Beaumier G, Juhel C, Armand M, Portugal H, Pauli AM, Borel P, Latge C, Lairon D. Effects of graded amounts (0-50 g) of dietary fat on postprandial lipemia and lipoproteins in normolipidemic adults. *Am J Clin Nutr* 1998;67(1):31-8.

31. Brighenti M, Govindasamy-Lucey S, Lim K, Nelson K, Lucey JA. Characterization of the rheological, textural, and sensory properties of samples of commercial US cream cheese with different fat contents. *J Dairy Sci* 2008;91(12):4501-17.

32. Lawrence RC, Gilles J, Creamer LK, Crow VL, Heap HA, Honoré CG, Johnston KA, Samal PK. Cheddar cheese and related dry-salted cheese varieties. In: Patrick F. Fox, Paul L.H. McSweeney, Timothy M. Cogan, Timothy P. Guinee, ed. Cheese: Chemistry, Physics and Microbiology: Academic Press, 2004:71-102.

33. Mortensen BK. Butter and other milk fat products | The Product and Its Manufacture A2 In: Fuquay, John W. ed. Encyclopedia of Dairy Sciences (Second Edition). San Diego: Academic Press, 2011:492-9.

34. Heaney RP. Factors influencing the measurement of bioavailability, taking calcium as a model. *J Nutr* 2001;131(4 Suppl):1344s-8s.

35. Barbé F, Ménard O, Gouar YL, Buffière C, Famelart M-H, Laroche B, Feunteun SL, Rémond D, Dupont D. Acid and rennet gels exhibit strong differences in the kinetics of milk protein digestion and amino acid bioavailability. *Food Chem* 2014;143:1-8.

36. Bornhorst GM, Ferrua MJ, Singh RP. A Proposed Food Breakdown Classification System to Predict Food Behavior during Gastric Digestion. *J Food Sci* 2015;80(5):R924-R34.

37. Jahan-Mihan A, Luhovyy BL, Khoury DE, Anderson GH. Dietary Proteins as Determinants of Metabolic and Physiologic Functions of the Gastrointestinal Tract. *Nutrients* 2011;3(5):574-603.

38. Michalski M-C, Januel C. Does homogenization affect the human health properties of cow's milk? *Trends Food Sci Technol* 2006;17(8):423-37.

39. Sainani MR, Vyas HK, Tong PS. Characterization of Particles in Cream Cheese. *J Dairy Sci* 2004;87(9):2854-63.

40. Gunasekaran S, Ding K. Three-Dimensional Characteristics of Fat Globules in Cheddar Cheese. *J Dairy Sci* 1999;82(9):1890-6.

41. Garaiova I, Guschina IA, Plummer SF, Tang J, Wang D, Plummer NT. A randomised cross-over trial in healthy adults indicating improved absorption of omega-3 fatty acids by pre-emulsification. *Nutr J* 2007;6:4.

42. Seimon RV, Wooster T, Otto B, Golding M, Day L, Little TJ, Horowitz M, Clifton PM, Feinle-Bisset C. The droplet size of intraduodenal fat emulsions influences antropyloroduodenal motility, hormone release, and appetite in healthy males. *Am J Clin Nutr* 2009;89(6):1729-36.

43. Tan KW, Sun LJ, Goh KK, Henry CJ. Lipid droplet size and emulsification on postprandial glycemia, insulinemia and lipidemia. *Food Func* 2016;7(10):4278-84.

44. Keogh JB, Wooster TJ, Golding M, Day L, Otto B, Clifton PM. Slowly and rapidly digested fat emulsions are equally satiating but their triglycerides are differentially absorbed and metabolized in humans. *J Nutr* 2011;141(5):809-15.

45. Mekki N, Charbonnier M, Borel P, Leonardi J, Juhel C, Portugal H, Lairon D. Butter differs from olive oil and sunflower oil in its effects on postprandial lipemia and triacylglycerol-rich lipoproteins after single mixed meals in healthy young men. *J Nutr* 2002;132(12):3642-9.

46. Marciani L, Faulks R, Wickham MS, Bush D, Pick B, Wright J, Cox EF, Fillery-Travis A, Gowland PA, Spiller RC. Effect of intragastric acid stability of fat emulsions on gastric emptying, plasma lipid profile and postprandial satiety. *Br J Nutr* 2009;101(6):919-28.

47. Marciani L, Wickham M, Singh G, Bush D, Pick B, Cox E, Fillery-Travis A, Faulks R, Marsden C, Gowland PA, et al. Enhancement of intragastric acid stability of a fat emulsion meal delays gastric emptying and increases cholecystokinin release and gallbladder contraction. *Am J Physiol Gastroinstest Liver Physiol* 2007;292(6):G1607-13.

48. Armand M, Borel P, Pasquier B, Dubois C, Senft M, Andre M, Peyrot J, Salducci J, Lairon D. Physicochemical characteristics of emulsions during fat digestion in human stomach and duodenum. *Am J Physiol* 1996;271(1 Pt 1):G172-83.

49. Pimpin L, Wu JH, Haskelberg H, Del Gobbo L, Mozaffarian D. Is Butter Back? A Systematic Review and Meta-Analysis of Butter Consumption and Risk of Cardiovascular Disease, Diabetes, and Total Mortality. *PLoS One* 2016;11(6):e0158118.

Tables

Table 19-1 Dietary composition of the test meals for a 1000-kcal serving[1]

	Butter	Cream cheese	Cheddar
Energy (kcal)	1000	1000	1000
Total protein (g)	33.4	33.4	33.5
Protein from butter/cheese (g)	0.4	9.5	24.9
Total fat (g)	41.5	41.5	41.5
Fat from butter/cheese (g)	33.2	33.2	33.1
Dietary cholesterol (mg)	180	180	180
Carbohydrates (g)	130	129	127
Fiber (g)	4.6	4.6	4.6
Calcium (mg)	311	390	589
Sodium (mg)	900	902	905

[1] The 3 test meals were designed to provide 33 g of fat from cheese or butter per 1000 kcal. The serving size was adjusted according to the participants' energy requirements.

Table 19-2 Anthropometric and fasting biochemical characteristics of the subjects at screening (n=43)

Characteristics	
Women (n)	24
Men (n)	19
Age (y)	36.9 ± 14.7[1]
Weight (kg)	70.7 ± 13.2
BMI (kg/m^2)	24.7 ± 3.9
Waist circumference (cm)	84.4 ± 11.5
Systolic blood pressure (mmHg)	112 ± 11
Diastolic blood pressure (mmHg)	71 ± 13
Total-cholesterol (mmol/L)	4.99 ± 0.91
Triglycerides (mmol/L)	0.94 ± 0.39
HDL-cholesterol (mmol/L)	1.80 ± 0.42
LDL-cholesterol (mmol/L)	2.76 ± 0.70
Total-cholesterol/HDL-cholesterol	2.88 ± 0.68
Insulin (pmol/L)	65 ± 22
Glucose (mmol/L)	5.0 ± 0.4

[1] Mean ± SD (all such values).

Table 19-3 Differences between dietary intakes, weight and energy expenditure before and during the study[1]

	Pre-study[2]	Study period	%Δ[3]	*P*[4]
Energy (kcal)	2420 ± 912	2079 ± 798	-14 ± 5[3]	0.01
Alcohol (%)	2.9 ± 2.8	2.8 ± 2.5	-2 ± 11	0.8
Lipids (%)	36.7 ± 5.5	36.3 ± 5.5	-1 ± 2	0.6
SFA (%)	12.1 ± 2.6	11.9 ± 2.4	-2 ± 8	0.6
MUFA (%)	15.2 ± 3.2	15.0 ± 3.0	-1 ± 2	0.7
PUFA (%)	6.6 ± 1.8	6.6 ± 1.7	0 ± 3	0.9
Protein (%)	16.3 ± 2.0	16.5 ± 2.1	1 ± 2	0.6
Carbohydrates (%)	47.2 ± 6.0	47.6 ± 6.1	1 ± 1	0.7
Diet cholesterol (mg)	299 ± 146	258 ± 143	14 ± 7	0.7
Fiber (g)	29.7 ± 12.2	26.1 ± 10.1	12 ± 5	0.7
Sodium (mg)	3200 ± 1323	2797 ± 1158	-13 ± 8	0.5
Weight (kg)	70.7 ± 13.2	70.6 ± 13.2	0 ± 4	0.6
Energy expenditure (kcal)	2970 ± 593	2929 ± 616	1 ± 4	0.2

[1] In all study subjects (n=43).

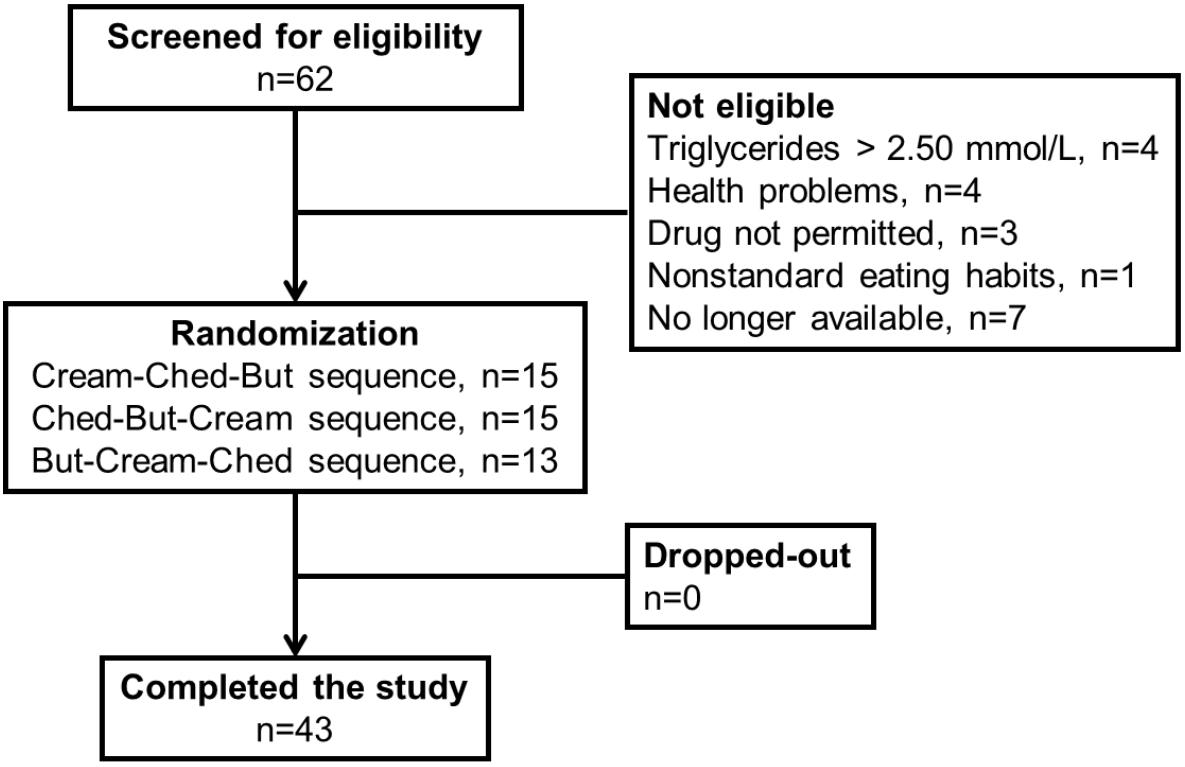
[2] Prestudy and study period intakes are presented as means ± SDs.

[3] Differences between prestudy and study period are presented as mean ± SEMs.

[4] *P* values were calculated with mixed procedures for repeated measures with subjects as random effect. *P* values for diet cholesterol, fiber and sodium intakes were adjusted for energy intake.

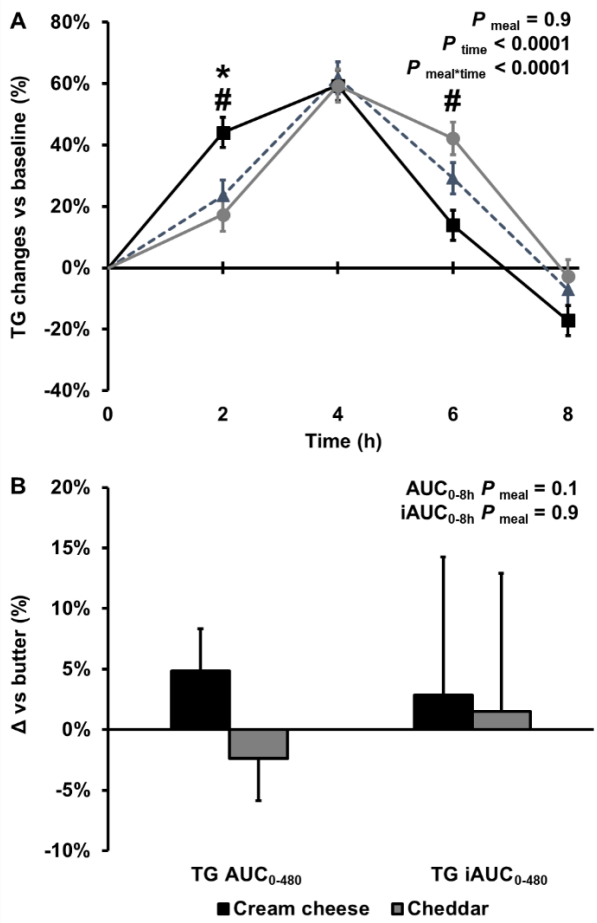
Figures

Figure 19-1 Flow chart of the study participants.



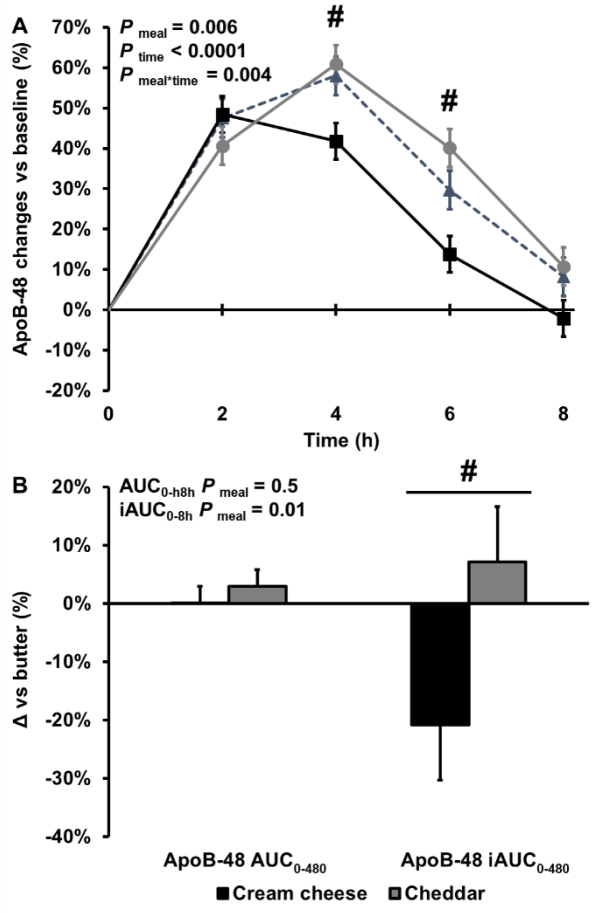
Of the 62 subjects screened for eligibility, 19 did not met eligibility criteria. Eligible subjects (n=43) were randomized to 1 of 3 test sequences. No subjects dropped-out of the study. But: butter; Ched: cheddar; Cream: cream cheese.

Figure 19-2 Differential impact of cheese matrix on postprandial TG response



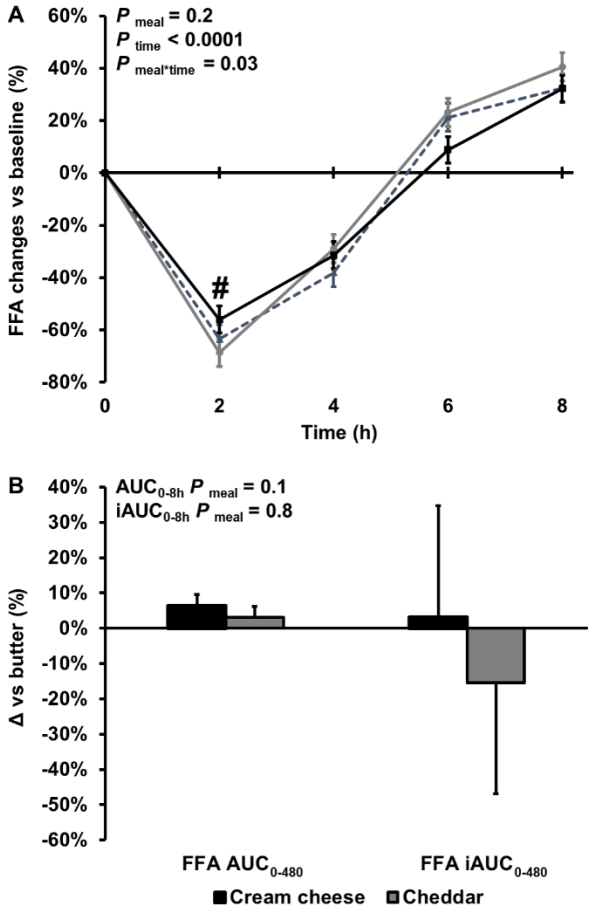
(A) Percentage of change in TG concentrations from baseline. Data are presented as the mean changes from baseline (expressed as %) ± SEMs. Triangles indicate butter (control), squares indicate cream cheese, and circles indicate cheddar. Statistical analyses were conducted with mixed models for repeated measures with changes from baseline as a dependent variable. The interaction between meals and time was treated as the main fixed effect. Subjects (n = 43) were treated as a random effect. Only covariates with significant effects (i.e., sex and caloric content of the test meals) were maintained in the model as fixed, independent factors. The adaptive Holm-Bonferroni method was used to correct *P* values for multiple comparisons. (B) Percentage of the difference in the TG AUC_{0-8h} and the $iAUC_{0-8h}$ of each cheese compared with butter. Data are presented as the mean differences ± SEMs. Statistical analyses were conducted with mixed models for repeated measures with the TG AUC_{0-8h} or $iAUC_{0-8h}$ as a dependent variable. The test meal (cream cheese, cheddar, butter) was the independent, fixed effect. Subjects (n = 43) were treated as a random effect. *$P < 0.05$ (cream cheese compared with butter); #$P < 0.05$ (cheddar compared with cream cheese). AUC_{0-8h}, area under the curve for the 8 hours of the test; $iAUC_{0-8h}$, incremental area under the curve for the 8 hours of the test; TG, triglyceride.

Figure 19-3 Differential impact of the cheese matrix on postprandial apoB-48 response



(A) Percentage of change in apoB-48 concentrations from baseline. Data are presented as the mean changes from baseline (expressed as %) ± SEMs. Triangles indicate butter, squares indicate cream cheese, and circles indicate cheddar. Statistical analyses were conducted with mixed models for repeated measures with changes from baseline as the dependent variable. The interaction between meals and time was treated as the main fixed effect. Subjects (n = 43) were treated as a random effect. Only covariates with significant effects (i.e., baseline apoB-48 concentrations, sex, and BMI) were maintained in the model as fixed, independent factors. The adaptive Holm-Bonferroni method was used to correct *P* values for multiple comparisons. (B) Percentage of the difference in the apoB-48 $AUC_{0–8\ h}$ and $iAUC_{0–8\ h}$ of each cheese compared with butter. Data are presented as the mean differences ± SEMs. Statistical analyses were conducted with mixed models for repeated measures with the apoB-48 $AUC_{0–8\ h}$ or $iAUC_{0–8\ h}$ as a dependent variable. The test meal (cream cheese, cheddar, or butter) was the independent, fixed effect. Subjects (n = 43) were treated as a random effect. $^{\#}P < 0.05$ (cheddar compared with cream cheese). apoB-48, apolipoprotein B-48; $AUC_{0–8\ h}$, AUC for the 8 h of the test; $iAUC_{0–8\ h}$, incremental AUC for the 8 h of the test.

Figure 19-4 Differential impact of the cheese matrix on postprandial FFA response



(A) Percentage of change in FFA concentrations from baseline. Data are presented as the mean changes (expressed as %) ± SEMs. Triangles indicate butter (control), squares indicate cream cheese, and circles indicate cheddar. Statistical analyses were conducted with mixed models for repeated measures with changes compared with baseline as the dependent variable. The interaction between meals and time was treated as the main fixed effect. Subjects (n = 43) were treated as a random effect. Only the covariate with a significant effect (i.e., sex) was maintained in the model as a fixed factor. The adaptive Holm-Bonferroni method was used to correct *P* values for multiple comparisons. (B) Percentage of difference in the FFA $AUC_{0–8\ h}$ and $iAUC_{0–8\ h}$ of each cheese compared with butter. Data are presented as the mean differences ± SEMs. Statistical analyses were conducted with mixed models for repeated measures with the FFA $AUC_{0–8\ h}$ or $iAUC_{0–8\ h}$ ratio as a dependent variable. The test meal (cream cheese, cheddar, or butter) was the independent, fixed effect. Only the covariate with a significant effect (i.e., the test meal sequence) was maintained in the model as a fixed, independent factor. Subjects (n = 43) were treated as a random effect. $^{\#}P$, 0.05 (cheddar compared with cream cheese). $AUC_{0–8\ h}$, AUC for the 8 h of the test; FFA, free fatty acid; $iAUC_{0–8\ h}$, incremental AUC for the 8 h of the test.