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Intake of Fermented Dairy Products Induces a Less Pro-Inflammatory Postprandial Peripheral Blood Mononuclear Cell Gene Expression Response than Non-Fermented Dairy Products: A Randomized Controlled Cross-Over Trial

Amanda Rundblad, Kirsten B. Holven, Linn K. L. Øyri, Patrik Hansson, Ingvild H. Ivan, Gyrd O. Gjevestad, Magne Thoresen, and Stine M. Ulven*

Scope: It is aimed to investigate how intake of high-fat meals composed of different dairy products with a similar fat content affects postprandial peripheral blood mononuclear cell (PBMC) expression of inflammation-related genes, as well as circulating inflammatory markers and metabolites.
Methods and results: Healthy subjects ($n = 47$) consume four different high-fat meals composed of either butter, cheese, whipped cream, or sour cream in a randomized controlled cross-over study. Fasting and postprandial PBMC gene expression, plasma metabolites, and circulating inflammatory markers are measured. Using a linear mixed model, it is found that expression of genes related to lymphocyte activation, cytokine signaling, chemokine signaling, and cell adhesion is differentially altered between the four meals. In general, intake of the fermented products cheese and sour cream reduces, while intake of the non-fermented products butter and whipped cream increases, expression of these genes. Plasma amino acid concentrations increase after intake of cheese compared to the other meals, and the amino acid changes correlate with several of the differentially altered genes.
Conclusion: Intake of fermented dairy products, especially cheese, induces a less inflammatory postprandial PBMC gene expression response than non-fermented dairy products. These findings may partly explain inconsistent findings in studies on health effects of dairy products.

1. Introduction

Cardiovascular disease (CVD) is the main cause of death worldwide, and atherosclerosis is the underlying cause of most CVDs.[1] The development of atherosclerosis is characterized by accumulation of cholesterol and other lipids in the intimal space of the arteries, and a low-grade inflammatory response.[2] Intake of saturated fatty acids (SFA), especially C12:0-C16:0, raises LDL-C in serum and may trigger an inflammatory response, partly by promoting LPS translocation to the bloodstream that activates toll-like receptor (TLR) 4.[3]

Dairy fat consists of about 65% SFA. However, the effect of different dairy products on LDL-C seems to vary, with butter inducing a larger increase in LDL-C than cheese.[4–7] Additionally, we previously showed that intake of different dairy products with the same amount of fat and fatty acid composition altered the postprandial triglyceride (TG) profile

Dr. A. Rundblad, Prof. K. B. Holven, L. K. L. Øyri, I. H. Ivan, Prof. S. M. Ulven
Department of Nutrition, Institute of Basic Medical Sciences
University of Oslo
Boks 1072 Blindern, Oslo 0316, Norway
E-mail: amanda.rundblad@medisin.uio.no

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Prof. K. B. Holven
Norwegian National Advisory Unit on Familial Hypercholesterolemia,
Department of Endocrinology, Morbid Obesity and Preventive Medicine
Oslo University Hospital
Postboks 4950 Nydalen, Oslo 0424, Norway

Dr. P. Hansson
Department of Clinical Medicine, Faculty of Health Sciences
UiT The Arctic University of Norway
Postboks 6050 Langnes, Tromsø 9037, Norway

Dr. G. O. Gjevestad
Marketing Department
TINE SA
Postboks 7, Oslo 0901, Norway

Prof. M. Thoresen
Oslo Center for Biostatistics and Epidemiology
Department of Biostatistics, Institute of Basic Medical Sciences
University of Oslo
Boks 1072 Blindern, Oslo 0316, Norway

differentially.[8] Controversies exist regarding health effects of dairy intake.[9,10] However, high-fat and non-fermented dairy may be associated with increased all-cause mortality and coronary heart disease risk, while low-fat and fermented dairy show the opposite effect.[11–14]

Different dairy products differ in food matrix and fermentation. In addition, they contain different amounts of nutrients such as fat, protein, and calcium, with cheese having a particularly high protein and calcium content. In turn, this may influence fat absorption, gut microbiota, as well as gene expression and blood metabolomics, which may affect different dairy products' effects on lipid metabolism and inflammation.[15,16] A systematic review of randomized controlled trials concluded that dairy intake was anti-inflammatory; however, the majority of the studies investigated the effect of low-fat dairy.[17] In addition, intake of fermented dairy products seems to have a more beneficial effect on circulating inflammatory markers than non-fermented dairy products,[17] although conflicting results exist.[18] To our knowledge, the effect of different high-fat dairy products on inflammation-related gene expression is less studied.

Peripheral blood mononuclear cells (PBMCs) consist of lymphocytes, natural killer cells, and monocytes and are cells of both the innate and adaptive immune system being important in the inflammatory process of atherosclerosis.[2,19] PBMCs are exposed to nutrients, metabolites, and peripheral tissues and are easily isolated from a blood sample. In addition, PBMCs reflect white adipose tissue postprandial inflammation-related gene expression, and liver fasting and cholesterol metabolism related gene expression.[20–22] Thus, PBMCs may be used as a surrogate tissue to study intervention effects on lipid metabolism and inflammation-related gene expression.

The aim of this study was to investigate how intake of four high-fat meals composed of different dairy products affects postprandial inflammation and lipid metabolism related PBMC gene expression and circulating inflammatory markers and metabolites. We hypothesized that the fermented dairy products would alter PBMC gene expression in a less pro-inflammatory manner than the non-fermented dairy products as previous studies have indicated a favorable effect of fermented dairy products.

2. Experimental Section

2.1. Subjects and Study Design

Healthy males and females aged 18–70 years were recruited through social media, local newspapers, and posters at universities and hospitals in the Oslo area, Norway, between June 2016 and January 2017. Inclusion criteria were BMI > 18.5 kg m^{-2}, a stable weight (±5%) the past three months, and willingness to eat the test meals. Exclusion criteria included elevated fasting blood glucose (≥7 mmol L^{-1}), C-reactive protein (CRP, > 10 mg L^{-1}), total cholesterol (>6.1 mmol L^{-1} aged 18–29 years, >6.9 mmol L^{-1} aged 30–49 years, and >7.8 mmol L^{-1} aged ≥50 years), or blood pressure (>160/100 mm Hg), and severe chronic disease, CVD, or cancer the past six months. Further exclusion criteria included pregnant or lactating females, allergy or intolerance against gluten, lactose or milk protein, smoking, use of lipid-lowering or anti-inflammatory medications, and unwillingness to stop use of omega-3 supplements four weeks prior to and during the study. Subjects willing to participate were contacted by telephone and eligible subjects were invited to a screening visit at the Center of Clinical Nutrition, Department of Nutrition at the University of Oslo, Norway.

Table 1. Nutritional composition of the study meals.

	Meals rich in fat from			
	Butter	Cheese	Whipped cream	Sour cream
Energy [kJ]	2614	2981	2712	2726
Energy [kcal]	629	715	652	655
Carbohydrate [g]	45.3	45.1	48.4	48.6
Carbohydrate [E%]	30.5	26.6	31.3	31.3
Protein [g]	8.8	30	10.9	11.2
Protein [E%]	5.7	17.2	6.9	7
Fat [g]	44.8	45.1	45.1	45.2
Fat [E%]	63.7	56.2	61.8	61.6
Calcium [mg]	9	678	85	94

E%, percent of energy; kcal, kilocalorie; kJ, kilo Joule.

In this randomized cross-over study, subjects were served four high-fat meals with four different dairy products containing the same amount of fat, as previously described in detail.[8] Each meal contained 45 g fat that corresponded to about 60 energy% (E%), an amount of fat that would induce a postprandial TG response,[23] as TG was the primary end point in this study. Furthermore, a meal containing 50 g of butter is sufficient to trigger an inflammatory response,[24] and was considered an edible amount for the participants. In short, each meal consisted of three slices of toasted white bread (84 g), raspberry jam (20 g), and either butter (52 g), cheese (113 g), whipped cream (113 g), or sour cream (113 g). The nutritional composition of the meals is shown in **Table 1** and the fatty acid composition of the meals was identical.[8] The subjects were not blinded, however, the meals looked similar and the subjects were blinded to the allocated meal order. Participants were randomized by the principal investigator to one of four fixed meal orders using block randomization in Microsoft Excel's random generator; 1: butter, cheese, whipped cream, and sour cream, 2: cheese, whipped cream, sour cream, and butter, 3: whipped cream, sour cream, butter, and cheese, and 4: sour cream, butter, cheese, and whipped cream. Between each meal, there was a washout period of three to five weeks for premenopausal females not using contraceptives and minimum two weeks for other participants. Subjects were instructed not to change their habitual diet and physical activity level during the study. Before each visit, subjects were instructed to fast for 12 h, avoid strenuous physical activity and alcohol for 24 h, and to not eat any fatty food after 6 pm the night before. At each test day, a fasting blood sample was collected before each participant got 20 min to eat the test meal together with water. A maximum of 1 L water was allowed during each visit. Postprandial blood samples were drawn 4 and 6 h after eating the meal.

This 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 laid down in

the Declaration of Helsinki. All subjects gave their written informed consent to participate. The study was registered at www.clinicaltrials.gov (NCT02836106).

2.2. Blood Sampling and Biochemical Routine Measures

Whole blood was collected in EDTA tubes that were kept at room temperature for maximum 48 h until white blood cell count was performed at a routine laboratory (Fürst Medical Laboratory, Oslo, Norway). Plasma was collected in EDTA tubes (Becton Dickenson Vacutainer Systems) that were kept on ice for <15 min before centrifugation (2000 × *g*, 15 min, 4 °C). Serum was collected in silica gel tubes (Becton Dickenson Vacutainer Systems) that were kept at room temperature for 30–60 min to allow for complete coagulation before centrifugation (1500 × *g*, 15 min) and storage at −80 °C. Standard biochemical measures, including total cholesterol, LDL-C, HDL-C, apolipoprotein A1 and B, glucose, insulin, and high-sensitivity CRP (hsCRP) were measured in fasting serum samples drawn at the first visit at an accredited medical laboratory (Fürst Medical Laboratory, Oslo, Norway).

2.3. Isolation of Peripheral Blood Mononuclear Cells and RNA

PBMCs from blood samples drawn fasting and 4 h postprandially were isolated with BD Vacutainer Cell Preparation tubes (Becton, Dickinson San Jose, CA, USA) which is a well-documented PBMC isolation method with more than 90% purity. PBMCs were isolated according to the manufacturer's instructions and were stored at −80 °C until RNA isolation. Total RNA was isolated using the RNeasy kit with QIAshredder homogenization of the cell lysates and DNase treatment using the automated protocol for the QIAcube according to the manufacturer's instructions (Qiagen, Valensia, CA, USA). The quantity and quality of the isolated RNA were analyzed with the NanoDrop ND-1000 Spectrophotometer (Thermo Fisher Scientific, Gothenburg, Sweden) and the Agilent RNA 6000 Nano kit using Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA).

2.4. Gene Expression Analyses

RNA expression analysis was performed using the NanoString nCounter system and the nCounter XT Human Immunology V2 Panel (Nanostring Technologies). This immunology panel contains code-sets that cover about 600 immune-related mRNAs, 15 reference genes, as well as positive and negative controls. All gene name abbreviations are listed in Supporting Information. In addition, the panel was customized by adding 30 additional code-sets covering lipid metabolism related mRNAs (Table S1, Supporting Information). The procedure was performed according to the manufacturer's instructions for nCounter Panel-Plus with an RNA input of 75 ng per sample.

Samples with an imaging quality control > 75%, binding density between 0.1 and 1.8, positive control linearity > 0.95, and a lowest positive control (0.5 fM) count higher than 2 SD above the negative controls were included. Samples were normalized to remove both technical and biological variation. The background threshold was set to be the geometric mean of the negative controls. Each sample was normalized to the mean of its positive controls relative to the geometric mean of positive controls in all samples. Finally, all samples were normalized to the 15 reference genes included in the panel (*ABCF1*, *ALAS1*, *EEF1G*, *G6PD*, *GAPDH*, *GUSB*, *HPRT1*, *OAZ1*, *POLR1B*, *POLR2A*, *PPIA*, *SDHA*, *TBP*, *TUBB*, and *RPL19*). The quality control and the technical and biological normalization was performed in the nSolver analysis software version 4.0 (NanoString Technologies). The stability of the 15 reference genes was assessed using NormFinder.[25]

2.5. ELISA

Circulating inflammatory markers in serum samples drawn fasting and 6 h postprandially were measured using ELISA. The concentration of circulating inflammatory markers was analyzed at 6 h, rather than 4 h, to be able to capture changes that could occur as a result of gene expression changes. Concentrations of vascular cell adhesion molecule-1 (VCAM-1) and intracellular adhesion molecule-1 (ICAM-1) were measured with DuoSet ELISA (R&D Systems, Minneapolis, MN, USA), while E-selectin, IL-6, tumor necrosis factor alpha (TNF-α), and TNF receptor 1 and 2 (TNFR1 and TNFR2) were measured with Quantikine kits (R&D Systems). All assays were performed according to the manufacturer's instructions.

2.6. Nuclear Magnetic Resonance Spectroscopy Analysis of Plasma Metabolites

The EDTA plasma concentration of metabolic biomarkers at 0 and 4 h postprandially was quantified with a commercial high-throughput ^{1}H nuclear magnetic resonance (NMR) spectroscopy platform (Nightingale Health, www.nightingalehealth.com). This platform quantifies the concentration of glycolysis-related metabolites (glucose, lactate, and citrate), ketone bodies (acetate, acetoacetate, and 3-hydroxybutyrate), amino acids (alanine, glutamine, histidine, isoleucine, leucine, valine, phenylalanine, and tyrosine), creatinine and glycoprotein acetyls, as well as the signal area for albumin. Details of the NMR metabolomics platform have previously been published.[26]

2.7. Statistical Analyses

Sample size was based on the expected response of the primary endpoint, TG, from previous intervention studies with high-fat meals, and a post hoc power calculation has previously been reported.[8] All statistical analyses were performed in R version 3.5.1.[27] normalized counts of the 609 genes were $\log_2$-transformed and the change from fasting to 4 h postprandially (log-ratio) was calculated. The change in serum concentrations of inflammatory markers from fasting to 6 h postprandially and the change in plasma concentrations of metabolites analyzed with NMR spectroscopy from fasting to 4 h postprandially were calculated directly from non-transformed concentrations.

The difference in gene expression, inflammatory marker, and metabolite changes between the four meals were analyzed with a linear mixed model (*lmer* function) with meal,

visit, sex, age, and BMI as fixed effects and subject ID as a random effect using the lme4 package.[28] The genes with a significant ($p < 0.05$) overall meal effect were manually grouped by function based on information provided at NCBI (https://www.ncbi.nlm.nih.gov/pubmed/), GeneCards (https://www.genecards.org), Uniprot (https://www.uniprot.org) as well as gene annotations provided with the NanoString Human Immunology Panel (https://www.nanostring.com/products/gene-expression-panels/ncounter-immunology-panels). Genes, inflammatory markers, and metabolites with a significant meal effect were further analyzed for pairwise comparisons using *emmeans::lsmeans*.[29] *p*-values from the six pairwise comparisons within a variable were adjusted using the Tukey method. Between meal comparisons were considered significant with an adjusted *p*-value < 0.05. The correlations between significant gene expression changes and changes in metabolite concentrations were analyzed with Spearman's correlation using the rcorr function.

Gene expression changes within a meal were analyzed by comparing $\log_2$-transformed counts fasting and 4 h postprandially, while changes in inflammatory markers within a meal were analyzed by comparing fasting and 6 h postprandial concentrations, using a paired *t*-test. Within-meal comparisons were considered significant with an FDR *q*-value < 0.1.

The difference between the meals in change of lymphocyte and monocyte composition relative to the total white blood cell pool from fasting to 4 h was analyzed with mixed models with subject ID as random effect.

Analyses of changes in PBMC mRNA expression of inflammation and lipid metabolism related genes, changes in circulating inflammatory markers, and changes in blood metabolites were predeclared secondary outcomes (www.clinicaltrials.gov, NCT02836106), while the analysis of metabolite-gene expression correlations were exploratory.

3. Results

3.1. Characteristics of the Study Population

In total 47 participants consumed at least one of the four test meals and were included in this study. A flow-chart showing participant losses and exclusions from assessment of eligibility to completion of the study has previously been published.[8] Participants were 70% female, on average (SD) 35 (12) years and had a BMI of 24.1 (3.9) kg m^{-2}. Fasting TG, total cholesterol, LDL-C, HDL-C, apolipoprotein A1 and B, glucose, insulin, hsCRP, and blood pressure were within the normal ranges (**Table 2**). The BMI of the participants did not change during the study, as previously reported.[8] The habitual dietary intake of macronutrients among the participants was 35.0 E% total fat, 12.6 E% SFA, 13.1 E% MUFA, 6.1 E% PUFA, 17.7 E% protein, and 41.5 E% carbohydrates.[8]

Table 2. Baseline characteristics of the participants.

	Mean/*n*	SD
Participants [*n*]	47	
Sex [*n*, male/female]	14/33	
Age [y]	35	12
BMI [kg m^{-2}]	24.1	3.9
Triglycerides [mmol L^{-1}]	0.93	0.37
Total cholesterol [mmol L^{-1}]	4.9	0.8
HDL-C [mmol L^{-1}]	1.7	0.4
LDL-C [mmol L^{-1}]	3.0	0.8
ApoA1 [g L^{-1}]	1.6	0.2
ApoB [g L^{-1}]	0.9	0.2
Glucose [mmol L^{-1}]	4.9	0.5
Insulin [pmol L^{-1}]	51	32
hsCRP [mg L^{-1}]	1.3	1.4
Systolic BP [mm Hg]	114	11
Diastolic BP [mm Hg]	67	8

ApoA1, apolipoprotein A1; ApoB, apolipoprotein B; BP, blood pressure; hsCRP, high-sensitivity C-reactive protein; SD, standard deviation.

3.2. Gene Expression Differences

In total 290 PBMC samples were available from the 0 and 4 h time points. Because of a too low RNA quality for NanoString analyses, 24 samples were excluded (RNA integrity number < 5.5), and 12 samples were excluded after quality control. Hence, gene expression analyses were performed with 254 samples (butter $n = 60$, cheese $n = 60$, whipped cream $n = 70$, and sour cream $n = 64$, **Figure 1**). The change in the proportion of lymphocytes and monocytes in whole blood from 0 to 4 h did not differ between the four meals ($p = 0.95$ and $p = 0.92$, respectively; data not shown). Of the 609 genes analyzed, 35 genes were differentially altered between the meals ($p < 0.05$, Table S2, Supporting Information). These 35 genes were grouped by function, and the largest gene groups were lymphocyte activation, cytokine signaling, antigen presentation, and chemokine signaling (**Figure 2**). In addition, genes related to cell adhesion, complement system, immune signaling, TLR and TNF signaling, lipid metabolism, host–virus interactions, and apoptosis were differentially altered between the meals. Pairwise comparisons between meals revealed that gene expression changes after intake of cheese and butter differed most from the other meals (Figure 2; Table S3, Supporting Information). Gene expression changes after intake of the non-fermented products, butter and whipped cream, were only significantly different from the two fermented dairy products, cheese and sour cream. Hence, there were no significant differences between the two non-fermented products after pairwise comparisons. Moreover, the gene expression changes after intake of cheese were mostly significantly different from the changes after intake of the non-fermented products butter and whipped cream, although there were some significant differences between cheese and sour cream. Strikingly, expression of many of the genes related to lymphocyte activation, cytokine signaling, and chemokine signaling were reduced after intake of cheese and increased after intake of butter. Finally, PBMC expression of antigen presentation related genes increased after intake of cheese and decreased after intake of butter and whipped cream (Figure 2).

Within each meal from fasting to 4 h postprandially, the expression of 35, 22, 25, and 34 genes was altered after intake

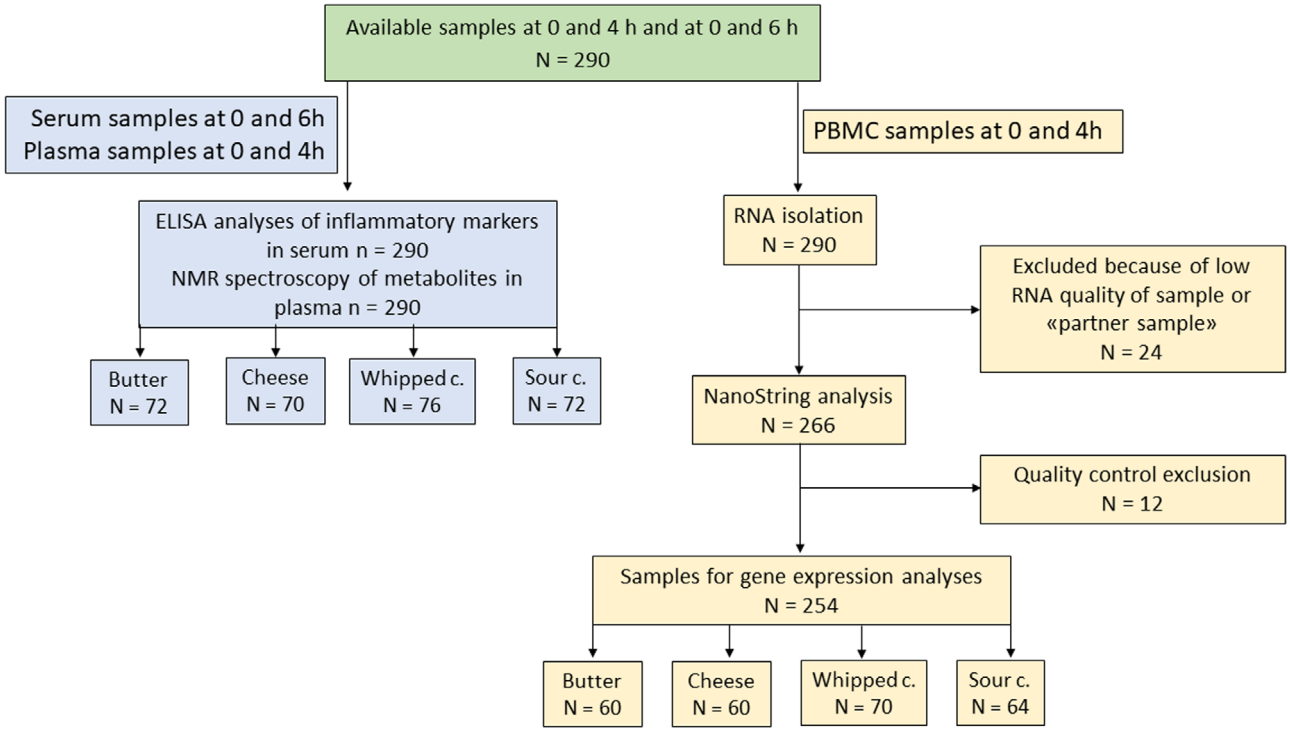

Figure 1. Flow chart of the samples included in the gene expression analyses (right) and the analyses of circulating inflammatory markers and metabolites (left). NMR, nuclear magnetic resonance, PBMC, peripheral blood mononuclear cell, Sour c., sour cream, whipped c., whipped cream.

of butter, cheese, whipped cream, and sour cream, respectively (FDR < 10%, **Figure 3**). Among the gene transcripts that were altered within each meal, there were overlaps between the meals (Figure 3). All the genes with a significantly altered expression within more than one meal were altered in the same direction. Results of the within meal analyses for all 609 genes are shown in Table S4, Supporting Information.

3.3. Circulating Inflammatory Markers

In total, 290 serum samples were available for ELISA analyses from the 0 and 6 h time points (butter $n = 72$, cheese $n = 70$, whipped cream $n = 76$, and sour cream $n = 72$, Figure 1). There were no significant differences between the meals in the change of circulating ICAM-1, VCAM-1, IL-6, hsCRP, TNFα, TNFR1, or TNFR2 (Table S5, Supporting Information). However, the change in serum concentrations of E-selectin from fasting to 6 h postprandially was significantly different between the four meals ($p = 0.03$, **Figure 4**). The concentration of E-selectin decreased after intake of cheese and increased after intake of whipped cream, although this difference was not significant in the pairwise meal comparisons ($p = 0.05$, Table S6, Supporting Information). In the within-meal analyses, the concentration of adhesion molecules increased after intake of the non-fermented products (Table S7, Supporting Information); Intake of butter increased ICAM-1 and VCAM-1 concentrations, while whipped cream increased E-selectin concentrations. The concentration of adhesion molecules did not change after intake of the fermented dairy products. Finally, intake of butter and whipped cream reduced TNFα concentrations and intake of cheese and whipped cream reduced TNFR2 concentrations (Table S7, Supporting Information).

3.4. Metabolite Changes and Correlations with Gene Expression Changes

In total 290 plasma samples were available for NMR metabolomic analyses of metabolites from the 0 and 4 h time points (butter $n = 72$, cheese $n = 70$, whipped cream $n = 76$, and sour cream $n = 72$, Figure 1). The plasma concentrations of alanine, histidine, isoleucine, leucine, valine, phenylalanine, and tyrosine increased from fasting to 4 h postprandially after intake of cheese, while the concentration of these amino acids decreased after intake of the three other dairy products (**Figure 5**). The overall meal effect was significant for these amino acids, and pairwise meal comparisons revealed that the increase after intake of cheese was significantly different from the reductions after intake of all the three other meals (Tables S8 and S9, Supporting Information). The plasma concentration of glutamine increased after of all four meals, and pairwise comparisons showed that the increase was greater after intake of cheese than butter and whipped cream (Figure 5). In addition, the overall meal effect for lactate and 3-hydroxybutyrate was significant (Table S8, Supporting Information). The lactate concentration increased significantly more after intake of butter compared to cheese, and the concentration of 3-hydroxybutyrate increased significantly more after intake of butter and whipped cream compared to cheese and sour cream.

We correlated the change in expression of genes with a significant overall meal effect with the changes in all metabolites analyzed with NMR spectroscopy (**Figure 6**; Table S10,

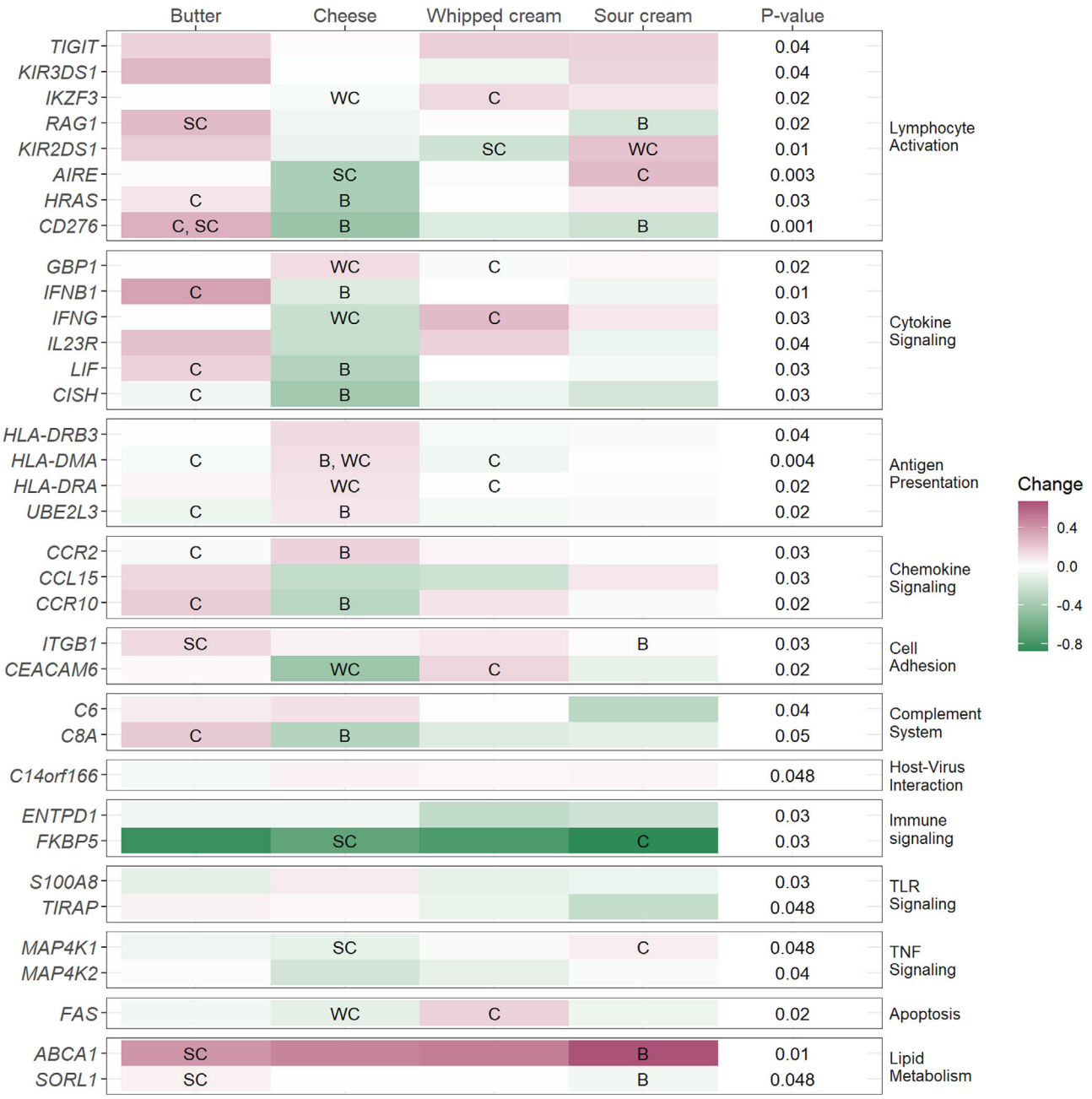

Figure 2. Genes with a significantly different change ($p < 0.05$) across the four meals, grouped by function. The gene expression heatmap shows the estimates of change within each meal; log-ratio adjusted for sex, age, BMI, and visit as fixed effects and subject ID as a random effect. *p*-values of the overall meal effect from the mixed model analyses are shown to the right. Significant differences ($p < 0.05$) in the pairwise meal comparisons, analyzed with least-squares means, are indicated with letters; B, significantly different compared to butter, C, significantly different compared to cheese, WC, significantly different compared to whipped cream and SC, significantly different compared to sour cream.

Supporting Information). In general, the changes in expression of genes related to lymphocyte activation and cytokine signaling were negatively correlated, while genes related to antigen presentation were positively correlated with the changes in amino acids. Branched-chain and aromatic amino acids had the strongest correlations with gene expression changes. Several of the genes with a reduced expression after intake of cheese compared to the other meals were negatively correlated with the changes in amino acid concentrations. Conversely, several of the genes with an increased expression after intake of cheese were positively correlated with the changes in amino acid concentrations (Figures 2 and 6).

4. Discussion

In this study, we have shown that intake of a high-fat meal composed of fermented dairy products, and especially cheese, has a less pro-inflammatory effect than intake of the non-fermented

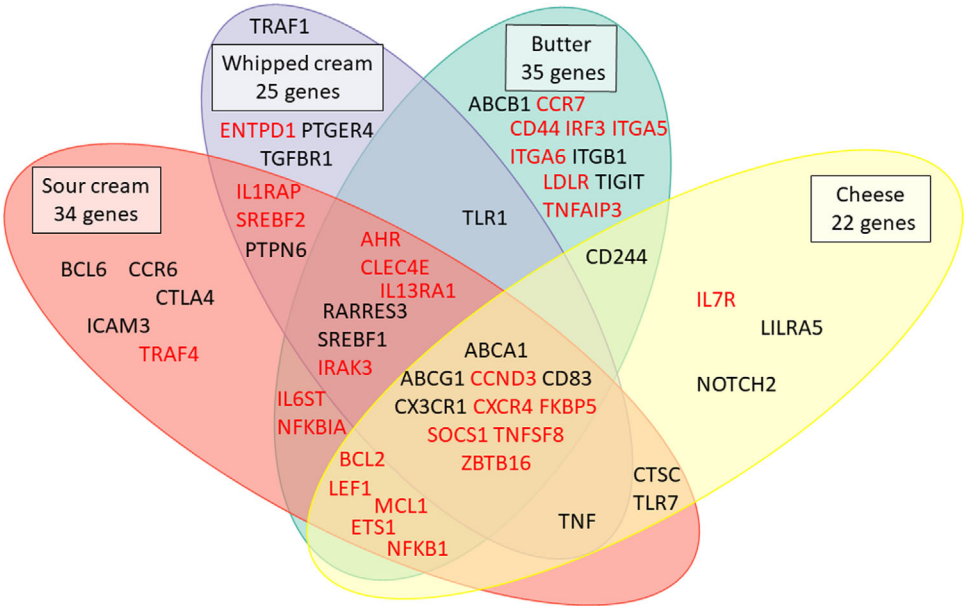

Figure 3. Genes that were altered from fasting to 4 h postprandially within each meal (FDR < 10%). Genes with a reduced expression are shown in red and genes with an increased expression are shown in black. Log_2-transformed counts at 0 and 4 h postprandially within each meal were analyzed with a paired *t*-test.

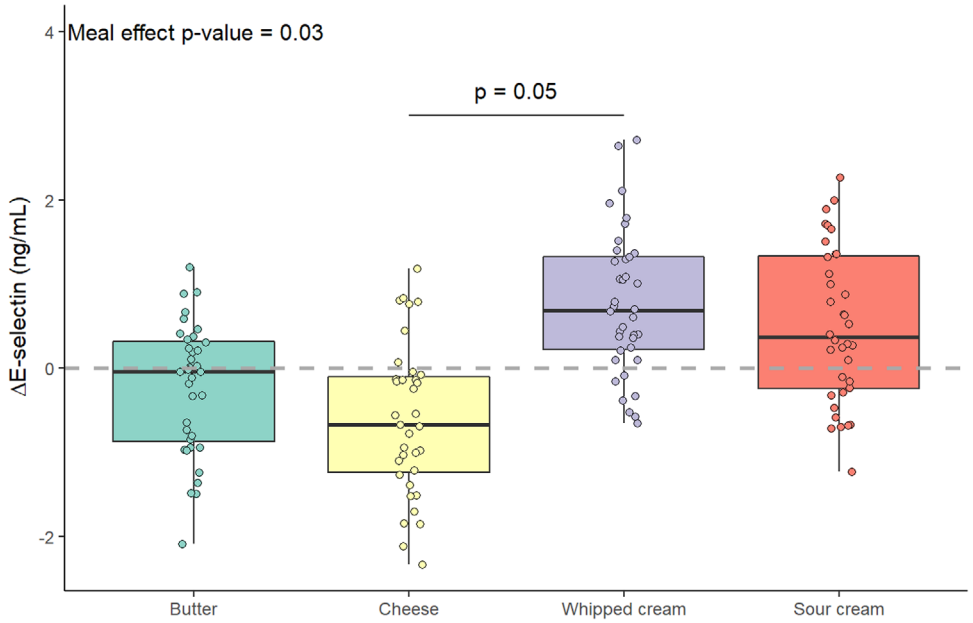

Figure 4. Changes in serum E-selectin concentrations (ng mL^{-1}), adjusted for sex, age, BMI, and visit as fixed effects and subject ID as a random effect, after intake of meals containing butter, cheese, whipped cream, and sour cream. The overall difference in change between the four meals was tested with a linear mixed model and the pairwise meal comparisons were analyzed with least square means.

dairy products butter and whipped cream. This was demonstrated by altered PBMC expression of genes related to lymphocyte activation, cytokine and chemokine signaling, as well as cell adhesion between the four meals we investigated. Pairwise comparisons revealed that the changes after intake of the non-fermented products were only significantly different from the fermented products. Additionally, only the non-fermented dairy products increased circulating concentrations of adhesion molecules.

Intake of high-fat meals rich in SFA may induce a postprandial inflammatory response,[30] in agreement with the results we observed after intake of butter and whipped cream. In a systematic review of observational studies, intake of SFA was associated with elevated concentrations of cytokines and

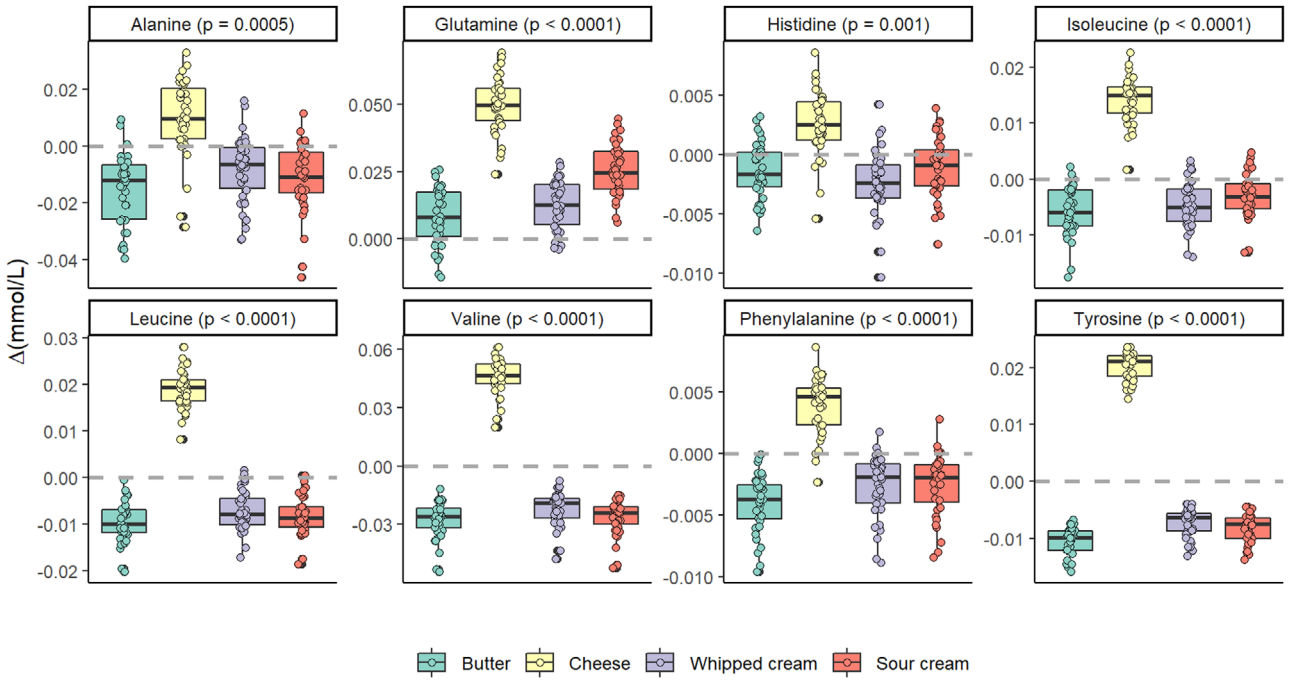

Figure 5. Changes in plasma concentrations (mmol L^{-1}) of the amino acids alanine, glutamine, histidine, isoleucine, leucine, valine, and phenylalanine adjusted for sex, age, BMI, and visit as fixed effects and subject ID as a random effect, after intake of meals containing butter, cheese, whipped cream, and sour cream. The overall difference in change between the four meals was tested with a linear mixed model.

adhesion molecules.[31] In line with this, we found increased concentrations of ICAM-1, VCAM-1, and E-selectin, and increased expression of cell adhesion related genes after intake of butter and whipped cream. Although intake of high-fat dairy has been shown to increase postprandial CRP concentrations,[32] we did not find that any of the high-fat dairy meals increased CRP. Several mechanisms may be involved in the pro-inflammatory response we observed after intake of butter and whipped cream. High-fat meals may promote an inflammatory response through LPS-TLR4 binding that activates nuclear factor kappa B and expression of inflammation-related genes.[33] SFA may also modify the gut microbiota to overproduce LPS, hence worsening the inflammatory response of a high-fat meal.[3,34]

The meals containing cheese and sour cream induced a less pro-inflammatory response than the meals containing butter and whipped cream, although they contained the same amount of fat and fatty acid composition. The response after intake of cheese differed the most from the non-fermented products. Cheese contains more protein than the three other meals in this study, and we found that plasma concentrations of amino acids increased after intake of cheese compared to the other meals. Similarly, the concentration of glutamine, isoleucine, leucine, valine, and tyrosine increased more after intake of cheese compared to milk in a study that aimed to find intake biomarkers for milk and cheese.[16] We found that changes in amino acids, especially the branched-chain amino acids (BCAA) and tyrosine, correlated with gene expression, suggesting that the amino acids are involved in mediating the less pro-inflammatory PBMC gene expression response observed after intake of cheese. Although little is known about effects of dairy protein and amino acid intake on gene expression, it has been found that intake of dipeptides of tryptophan and tyrosine found in cheese and other fermented dairy products prevented LPS induced inflammation in microglial cells in an Alzheimer's mouse model.[35] Moreover, an in vitro study showed that physiological concentrations of BCAA reduced inflammation-related gene expression in human endothelial cells, and BCAA supplementation improved immune function in humans and reduced inflammatory gene expression in liver and adipose tissue of obese mice.[36–38] However, we have previously reported minor effects on circulating inflammatory markers after intake of a protein-enriched milk in older adults,[39] although the same protein-enriched milk altered PBMC expression of genes related to immune function.[40] Cheese is also rich in calcium, which has been shown to suppress inflammatory stress.[41] This may explain why cheese that contained seven times more calcium than sour cream induced a more anti-inflammatory PBMC gene expression response. The gene expression changes after intake of the non-fermented products were only significantly different from the fermented products in pairwise meal comparisons, indicating that there is an effect of the fermentation. A systematic review of clinical trials investigating inflammatory markers reported that only studies of intake of fermented, and not non-fermented, dairy products demonstrated an anti-inflammatory activity, which is in line with our findings.[42] It has been shown that compared to milk (non-fermented), intake of yoghurt (fermented) caused a smaller increase of blood concentrations of indole metabolites produced by microbiota. These metabolites can activate the aryl hydrocarbon receptor, which is involved in immune regulation.[43] In the same study, PBMC expression of inflammation-related genes was more reduced after intake of yoghurt compared to milk.[44] Intake of whipped cream, a high-fat non-fermented dairy

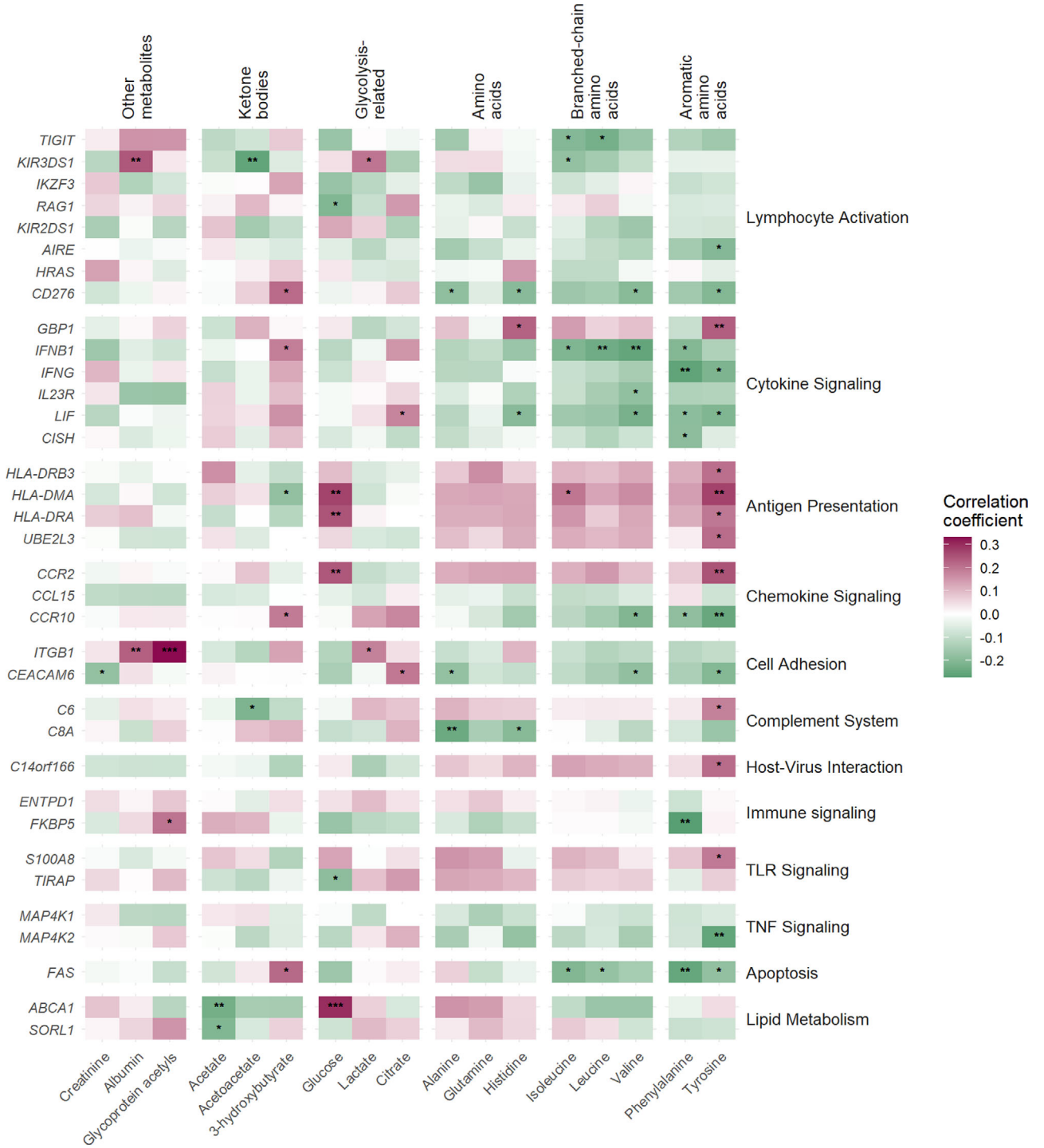

Figure 6. Correlation heatmap of genes with a significantly different change between the four dairy meals, and changes in plasma concentrations of metabolites analyzed with Spearman's correlation. Genes are grouped by function and metabolites are grouped by type. Significant correlations are indicated with symbols: ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$.

product, has been shown to increase concentrations of circulating inflammatory markers.[45] Furthermore, intake of high-fat fermented dairy products, including cheese, for two weeks reduced IL-6 concentrations compared to the high-fat non-fermented products butter and ice cream.[46] These findings concur with our results and suggest that fermented dairy products induce a less inflammatory response than non-fermented dairy products. Although the underlying mechanisms of the anti-inflammatory effect of fermented dairy products is poorly understood, fermentation products of lactic acid bacteria may

modulate immune responses.[47,48] Furthermore, intake of fermented dairy products alter the composition of the gut microbiota, which has been hypothesized to mediate their beneficial effects on inflammation.[49,50] Finally, the dairy matrix may modify the interactions between nutrients and may therefor explain differences in health effects of intake of different dairy products.[15]

Unexpectedly, PBMC expression of genes related to antigen presentation increased after intake of cheese, while it decreased following intake of butter and whipped cream. In line with this, PBMC expression of antigen presentation genes in rats decreased after a 4-week high-fat diet, and increased after a high-protein diet.[51] Hence, the three times higher content of protein in the meal containing cheese compared to the other meals in the current study may explain the differential effects on PBMC expression of antigen presentation related genes.

In addition to investigating effects on genes related to inflammation, we analyzed expression of 30 genes related to lipid metabolism. We found that postprandial expression of *ABCA1* and *ABCG1* increased within all four meals, which is in line with studies investigating intake of SFA compared to MUFA and PUFA.[52–54] Upregulation of these genes may indicate that there is an increased cholesterol efflux and reverse cholesterol transport via HDL, however, in this study, HDL-C increased postprandially only after intake of sour cream.[8] Furthermore, we found that *SREBF1* expression increased after intake of butter, sour cream, and whipped cream. This may lead to an increased expression of fatty acids synthesis genes and increased de novo lipogenesis that may have contributed to the postprandial TG increase in this study, as we previously reported.[8] On the other hand, expression of *SREBF2* decreased after intake of all four meals, but only significantly after intake of whipped cream and sour cream. Other studies have also found that expression of *SREBF1* increases and *SREBF2* decreases after intake of SFA compared to PUFA and MUFA.[52,53] A reduced *SREBF2* expression could lead to a lower expression of its target gene *LDLR*, encoding the LDL receptor. In this study, mRNA expression of *LDLR* decreased after intake of all four meals, however, only significantly after intake of butter. Correspondingly, it has been shown that intake of SFA reduces PBMC expression of *LDLR* compared to PUFA and MUFA, which has been suggested as a potential mechanism for the LDL-C increasing effect of SFA in the long term.[53–55]

This study is strengthened by the randomized cross-over design with meals composed of four different dairy products, but with the same content of fat and SFA. Because we aimed to test the effect of intake of whole products, and not specific nutrients, we cannot dissect whether the fermentation status, the protein or calcium content or other factors causes the observed effects. Furthermore, as we measured the postprandial response to intake of different high-fat dairy products, we cannot draw conclusions about health effects of long-term consumption. In Western countries today, most people are in the postprandial phase during the entire day and postprandial responses may therefore be key to understanding long-term effects. The lack of significant findings on the majority of circulating inflammatory markers may be due to the relatively small sample size, or that changes occur after the last blood sampling 6 h postprandially. In line with our findings, a postprandial study that compared intake of milk, butter, cream, yoghurt, and cheese found no differences in inflammatory biomarkers.[18] Moreover, we only measured PBMC gene expression at fasting and 4 h postprandially; nonetheless, according to a study by Sagaya et al., the change from fasting to 4 h may capture the main postprandial response.[56] This study is also limited by the lack of analyses of the PBMC transcripts' protein products. However, other postprandial studies of dairy products have found effects on gene expression, and not protein levels, indicating that it may be appropriate to study gene expression in postprandial studies of dairy intake.[44,50] Finally, as the mixed model analyses are not adjusted for multiple testing, the results needs to be interpreted with some caution.

To conclude, we have shown that intake of fermented dairy products, and especially cheese, induces a less inflammatory postprandial PBMC gene expression response than non-fermented dairy products. The high protein and calcium content of the cheese may explain some of these differences. Our findings may partly explain why studies on health effects of intake of dairy products are inconsistent, and highlight the need to categorize dairy products into specific food groups. We also showed that all the high-fat meals rich in SFA altered PBMC expression of lipid metabolism related genes in the same direction, which may contribute to an understanding of SFA's adverse effects on blood lipids.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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Conflict of Interest

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Author Contributions

A.R., K.B.H., L.K.L.Ø., P.H., M.T., and S.M.U. designed the research. K.B.H., P.H., and S.M.U. conducted the intervention study. G.O.G. provided essential material. A.R. and I.I.H. performed laboratory analyses. A.R. performed statistical analyses. L.K.L.Ø. and M.T. contributed to statistical analyses. A.R., K.B.H., L.K.L.Ø., and S.M.U. interpreted the data. A.R., K.B.H., L.K.L.Ø., P.H., I.I.H., G.O.G., M.T., and S.M.U. drafted the manuscript. A.R., K.B.H., and S.M.U. had primary responsibility for the final content of the manuscript. All authors read and approved the final manuscript.

Keywords

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