The effect of heat treatments and homogenization of cow's milk on gastrointestinal symptoms, inflammation markers and postprandial lipid metabolism

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Abstract

Dairy products are often reported as a source of stomach discomfort, and processing of cow's milk has been claimed to be one reason for that. To investigate the role of milk processing on adverse gastrointestinal symptoms, a cross-over, double blind clinical trial with fourteen milk sensitive subjects was set up. Pasteurized, pasteurized and homogenized, and ultra-high temperature-treated and homogenized milk, representing products from the mildest and hardest processing, were used as study meals. The amount, severity or duration of the reported symptoms or postprandial lipemia did not differ while significant differences were seen in the postprandial fatty acid composition of plasma between the milk types. The 92 inflammation markers measured in plasma did not differ between the subjects who consumed different types of milk. The results of the present study do not support the hypothesis that cow's milk processing could induce gastrointestinal symptoms in milk sensitive but lactose tolerant subjects.

1 Introduction

Gastrointestinal (GI) symptoms and inflammatory responses induced by cow's milk have interested researchers for nearly two decades as some people claim to tolerate raw cow's milk better than the commercial, homogenized and pasteurized cow's milk. The surface of fat globules in homogenized milk are mainly covered with denatured proteins, which have more surface exposed antigenic determinants and thus, in theory, could be more allergenic compared to intact proteins in raw milk (Pelto, et al., 2000). Consumption of raw milk has been linked to reductions in childhood asthma and allergies (Brick, et al., 2016; Loss, et al., 2011). In fact, significant reductions in the levels of heat sensitive proteins, like lactoferrin and lactoperoxidase, were seen when raw milk was heated to high temperatures suggesting that these proteins, abundant in raw milk but absent in high temperature treated milk, could potentially have a role in the protection of asthma and allergies (Brick, et al., 2017). Milk fat globule membrane (MFGM) in non-homogenized milk on the other hand has been reported to have anti-inflammatory properties (Chatterton, et al., 2013; Snow, et al., 2011) and a meal rich in MFGM has been shown to reduce postprandial inflammation markers in overweight and obese individuals compared to meal high in saturated fatty acids (Demmer, et al., 2016). Several research groups (Mummah, et al., 2014; Nuora, et al., 2018; Paajanen, et al., 2003; Pelto, et al., 1998; Pelto, et al., 2000) have investigated differences in symptom severities and levels of inflammation markers as response to raw milk and differently processed milk samples. However, significant differences have not been found, partly due to the often undersized groups of volunteers.

While dairy products are often reported as a source of GI discomfort, there seems to be a lack of evidence of what causes the symptoms (Michalski and Januel, 2006). One hypothesis has been that in milk hypersensitive persons processed milk could increase the levels of circulating inflammation markers and thus induce chronic inflammation in the GI tract leading to

discomfort. However, conflicting results have been reported on the matter (Pelto, et al., 1998; Pelto, et al., 1999; Pelto, et al., 2000). Some raw milk defenders claim that consumption of raw milk is associated with reduction of lactose intolerance symptoms. No scientific evidence can be linked to the claim as no difference has been found in the symptom severities between raw milk and processed milk in lactose intolerant subjects (Mummah, et al., 2014). Milk processing, especially homogenization, influences the droplet size in milk fat, making the droplets smaller than in raw milk. It has been speculated that the smaller size and new interface could induce GI symptoms. This phenomenon has been investigated also by our group (Nuora, et al., 2018) by serving raw and homogenized and pasteurized milk to milk-sensitive subjects. No statistically significant differences were found in the symptom severity, duration and quality between the two milk types. However, the P-values were between 0.05 and 0.15 even with a small subject group (N = 11), encouraging us to further investigate the role of milk processing in GI symptoms.

In this double-blind cross-over clinical trial with milk sensitized volunteers we investigated whether different milk processing methods affect the severity and duration of subjective symptoms, and if the degree of processing has an effect. Raw milk is an approved food in Finland, but as raw milk does possess a risk of foodborne pathogens we chose to use thermally treated milk samples in this study. Three common milk processing methods were used: pasteurization, homogenization and ultra-high temperature treatment (UHT). In addition to GI symptoms, we investigated whether the differently processed milk samples induced different responses in low grade inflammation markers, hyperlipidemia, hyperinsulinemia, and hyperglycaemia.

2 Materials and Methods

2.1 Milk

Pasteurized (PM), homogenized and pasteurized (HPM), and homogenized and UHT-treated (UHTM) milk samples were used as study drinks. The milk samples were obtained from the research dairy farm of the Natural Resources Institute Finland, LUKE. Thus, all milk samples originated from the same herd of cows and the same milk batches. Heat treatments and homogenization were carried out with an industrial scale equipment according to manufacturer's instruction at the LUKE Pilot Dairy Plant in Jokioinen, Finland. The temperature of pasteurization was set to 73 °C and that of UHT to 135 °C. The PM was heated for 15 seconds. The HPM was first homogenized with a two-stage homogenizer at 16 MPa and then heated for 15 seconds. The UHTM was first homogenized at 16 MPa and then heated for 3 seconds. All milk samples were processed and aseptically packed the day before the study day and stored below 6 °C. Milk was served at the latest two days after milking.

2.2 Clinical Trial

Six healthy male and eight healthy female volunteers (age 20 – 45; BMI 19 – 29 kg/m^2) were recruited to participate in a randomized, cross-over clinical trial to consume all three milk types, PM, HPM, UHTM on three separate occasions with "wash-out" periods of at least two weeks between occasions. This trial was limited to healthy subjects with normal liver and kidney functions, who reported abdominal pain, cramping, bloating or watery feces after drinking homogenized and pasteurized milk but who did not report having lactose intolerance. The exclusion criteria were: history of cardiovascular disease, diabetes or any GI conditions or GI surgery within the past three months, dysphagia, celiac disease, Crohn's disease or diverticulitis, regular medication, regular smoking or participation in intervention within two months prior to this study. Healthy subjects were recruited, as diseases and medication may give confounded results, and gastrointestinal conditions may increase the risk of erroneous reporting of GI symptoms. The trial was conducted according to the Declaration of Helsinki. The ethics approval was obtained from the Ethics Committee, Hospital District of Southwest

Finland. All subjects provided a written informed consent. The trial was registered prospectively to the U.S. National Institute of Health ClinicalTrials.gov registry (NCT03010904).

The subjects were on a non-dairy diet for five days prior to their study visit. They were asked to keep a diary of their symptoms during the day of visit and the following day, and mark down the type (flatulence, abdominal pain or cramping, bloating, watery feces, constipation), duration, and the severity of symptoms in a scale of 1 to 3 where 1 is mild and 3 is an unbearable pain. The diary was divided into time slots, shorter slots during the study visit and longer slots on the following day. In each time slot, the subjects marked whether they had symptoms or not and the type of the symptom.

In the morning of each study visit, following an overnight fast, a catheter was inserted into an antecubital vein and a baseline blood sample was obtained. The study meal was then served to the subjects. Each meal consisted of 4 dL of one of the study milk types and 24 grams of rice cakes, 85 grams of turkey cold cuts and 50 grams of cucumber. The entire meal contained 460 kcal of energy, 29 g of protein, 20 g of fat, and 38 g of carbohydrate. Milk was served cold from paper cups covered with a lid and aluminum foil and shaken before serving. It was drunk with a straw to make the mouth feel as similar as possible for all of the milk samples.

Blood samples were drawn at 20, 40, 60, 90, 120, 180, 240 and 300 min after ingestion of the meal for investigation of changes in the levels of blood glucose, insulin, triacylglycerols (TAGs) and inflammation markers. The subjects were asked to restrain from eating or drinking for five hours after the ingestion of the test meal. A standardized lunch was offered to subjects five hours after ingestion of the test meal. The subjects were required to avoid dairy products 48 hours after the test meal.

2.3 Analysis of Plasma Insulin, Glucose, Lipid and Inflammation Markers

Blood samples were collected in Li-heparin and EDTA blood collection tubes (Vacuette®, Greiner Bio-One) and centrifuged at 1500 x g for 15 minutes for plasma separation. Plasma insulin was analyzed with electro-chemiluminescence immunoassay. Plasma glucose was analyzed enzymatically with hexokinase assay. Plasma TAGs were analyzed enzymatically with colorimetric method. Insulin, glucose and TAGs were analyzed by Tykslab, Turku University Hospital, Finland and measured with a Cobas 8000 analyzer (Roche Diagnostics, Basel, Switzerland).

Incremental areas under the curve (iAUC) were calculated for glycemia, insulinemia and lipemia after all the three milk types. The baselines were subtracted from the plasma TAG samples as they varied between persons.

From the baseline and 300 min postprandial plasma samples, 92 biomarkers of inflammation were analyzed using cDNA-based multiplex immunoassay and the values are presented as normalized protein expression which is an arbitrary unit on Log2 scale. The analyses were done by Olink Proteomics, Uppsala, Sweden. Leukocytes were counted from baseline and 180 and 300 min postprandial blood samples with automatic cell count with Sysmex XN-9000 by Tykslab, Turku University Hospital, Finland.

For fatty acid (FA) analysis, lipids were extracted from the plasma samples with a modified Folch's method (Folch, et al., 1957). In short, lipids were extracted with chloroform-methanol (2:1), and TAGs were isolated from lipid extracts by solid phase extraction using Sep-Pak Vac 1cc (100 mg) Silica Cartridges (Waters, Dublin, Ireland) (Hamilton and Comai, 1988). The fatty acid methyl esters (FAME) were prepared with a sodium methoxide method (Christie, 1982) for gas chromatographic analysis.

The FAMEs were analyzed with a Shimadzu GC-2010 gas chromatograph equipped with a flame ionization detector (Shimadzu Corporation, Kyoto, Japan). A wall-coated open tubular

DB-23 column was used (60 m, i.d. 0.25 mm, liquid film 0.25 µm, Agilent technologies, J.W. Scientific, Santa Clara, CA). Supelco 37 Component FAME Mix (Supelco, St. Louis, MO) and 68D (Nu-Chek-Prep, Elysian, MN) were used as external reference compounds.

2.4 Lactose Malabsorption Genotyping

The ability to digest lactose is associated with the SNP rs4988235 located in the *MCM6* gene in European Caucasian populations (Enattah, et al., 2002). Sanger sequencing was used to genotype this SNP from the blood samples in order to investigate adult-type hypolactasia in the subjects. For genotyping, Qiagen's blood and tissue kit was used to extract DNA from blood samples. Altogether 400bp around rs4988235 was amplified using the primer pair 5'-ACCCCCTTTTCAAAGACGAC and 5'-TGCTCATACGACCATGGAAT. Amplified DNA fragment was sequenced and individual genotypes were determined from the chromatograms.

2.5 Statistical Analysis

Statistical analyses were performed with SPSS 23.0 software (SPPS Inc, Chicago, IL). Normal distribution of data was tested with Shapiro-Wilkin test. Depending on the normality of data, paired samples t-test or Wilcoxon matched-pairs signed ranks test was used to compare the measured responses. Statistical significance was indicated with $P < 0.05$. For the multiplex immunoassay $P < 0.001$ was treated as significant. Data are presented as means ± SD, presented in units as indicated.

3 Results

3.1 Symptom Diary

The diary of symptoms was divided to time slots and the different GI symptoms reported during those slots were calculated. The sum of reported GI symptoms: flatulence, abdominal pain or cramping, bloating, watery feces or constipation was 100 after ingestion of UHTM, 97 after

ingestion of PM and 83 after ingestion of HPM. No significant differences were seen between the milk types (UHTM–HPM P = 0.408; UHTM–PM P = 0.972; HPM–PM P = 0.949). Five subjects reported the highest number of adverse symptoms after UHTM, three subjects after HPM and four after PM. The adverse symptoms were divided into three categories according to their severity, 1 being mild, 2 being moderate and 3 being unbearable pain. Two subjects reported exquisite adverse symptoms after UHTM and one after PM. Most of the symptoms reported were categorized as mild. The most common symptoms after all three milk types were flatulence 35.7 %, bloating 22.1 % and abdominal pain 13.6 %. No significant difference was seen in the duration of symptoms between milk types (UHTM–HPM P = 0.401; UHTM–PM P = 0.646; HPM–PM P = 0.192).

3.2 Plasma Insulin, Glucose and Triacylglycerols

No significant differences were found in the plasma insulin, glucose and TAG concentrations measured as iAUC between PM, HPM and UHTM (data not shown). However, 90 minutes after UHTM ingestion, the TAG concentration was significantly higher compared to the TAG concentration after HPM (P = 0.04). No such difference was seen between UHTM and PM or HPM and PM (Figure 1).

3.3 Plasma Fatty Acid Composition

The most abundant FAs were myristic (14:0), palmitic (16:0), stearic (18:0), oleic (18:1) and linoleic (18:2) acids (Figure 2). After ingestion of UHTM at two hour time point there were significantly more myristic (P = 0.016), palmitic (P = 0.001), oleic (P = 0.012) and linoleic (P = 0.019) acids compared to HPM. After ingestion of PM at two hour time point there were significantly more myristic (P = 0.048), palmitic (P = 0.008), oleic (P = 0.018) and linoleic (P = 0.03) acids compared to HPM. There were no significant difference between UHTM and PM at two hour time point. At four hour time point only linoleic acid differed significantly between

UHTM and HPM (P = 0.001), between UHTM and PM (P = 0.001) as well as between HPM and PM (P = 0.035).

3.4 Lactose Malabsorption Genotypes

Three out of 14 subjects had the C/C genotype which is associated with a low lactase enzyme activity. Two subjects were carriers of T/T genotype and the remaining nine had the C/T genotype. Both T/T and C/T are linked to lactase persistency (Jarvela, 2005). According to Tolonen et al. (Tolonen, et al., 2011) 17 % of Finns are carriers of the C/C genotype. From the three C/C genotype carriers two reported more adverse GI symptoms after PM compared to UHTM and HPM and one had more adverse GI symptoms after UHTM compared to HPM and PM.

3.5 Inflammation Markers

No significant differences were seen in the 92 inflammation markers between the milk types at baseline or 300 minutes postprandially. However, the levels of a few biomarkers changed significantly between baseline and 300 minutes after UHTM, HPM and PM challenges. After UHTM the concentration of interleukin-6 (IL6) increased significantly and the concentration of fibroblast growth factor 21 (FGF21) decreased significantly (Table 1). After HPM the concentrations of IL6 increased significantly and the concentrations of interleukin-8, FGF21 and Fms related tyrosine kinase 3 ligand (Flt3L) decreased significantly (Table 1). Also, after PM the concentrations of FGF21 and TNF-related activation-induced cytokine (TRANCE) decreased significantly (Table 1). No significant differences were seen in the leukocyte levels between milk types at baseline or 180 and 300 minutes postprandially, and all leukocyte levels were between reference values for healthy adults.

4 Discussion

This trial was designed to determine whether milk homogenization or heat treatment would influence the occurrence and severity of GI symptoms and whether these symptoms would influence low grade inflammation in sensitized subjects. These hypotheses were not supported by the results. No significant differences were seen in the symptom severity and occurrence between UHTM, HPM and PM. Overall this 3-arm trial provided little evidence of the role of milk homogenization or severe heat treatment on enhancement of GI symptoms compared to only pasteurized milk. Notably, none of the subjects had a previously diagnosed lactose-intolerance by a physician and only three subjects had the genotype C/C which has been linked to lactose malabsorption. However, there were 19 grams of lactose in the 4 dL of each study milk and reported symptoms (flatulence, bloating and abdominal pain) were similar to those observed in lactose-intolerance. Thus, the role of lactose in the milk cannot be excluded. Previous studies have not found differences between milk processing methods or between processed milk and raw milk in the occurrence of GI symptoms or inflammation marker levels (Mummah, et al., 2014; Nuora, et al., 2018; Paajanen, et al., 2003; Pelto, et al., 1998; Pelto, et al., 2000). Mummah et al. (Mummah, et al., 2014) even investigated whether raw milk could reduce the symptoms of lactose intolerance compared to PM but found no difference in the symptoms severities.

Leukocyte levels remained comparable with reference values as expected for healthy subjects. The three milk types did not differ in terms of responses in the 92 inflammation markers measured. Possibly one milk dose is not enough to induce inflammatory reactions and several consecutive doses are needed even in sensitive subjects. Dencker et al. (Dencker, Bjorgell, et al., 2017; Dencker, Gardinger, et al., 2017) investigated whether a single meal intake would have an effect on 92 biomarkers for cardiovascular disease or on 92 neurological biomarkers. They measured the levels of the biomarkers from 22 healthy subjects 30 minutes and 120 minutes after food intake. The results showed very modest effect of the meals on those 92

biomarkers. However, it is worth noticing that a single meal containing UHTM or HPM increased the IL6 in plasma compared to the baseline, and the FGF21 level was decreased after consumption of meals containing each type of milk. The nutritional and clinical significance of these changes remains to be investigated in future studies.

This trial also aimed to investigate whether mechanical processing, resulting in different lipid droplet size, and mild or severe heating temperatures could possibly have an effect on plasma levels of lipids, insulin and glucose. Homogenization results smaller lipid droplet size (0.03 – 2 µm) compared to non-homogenized milk (3 – 5 µm) (WALSTRA, 1975) and heat treatment denaturizes proteins, which may improve their digestibility (Wada and Loennerdal, 2014). No significant differences were seen in the plasma insulin and glucose levels between the milk samples. The plasma TAG concentration after UHTM was significantly higher compared to the TAG concentration of HPM at 90 minute time point ($P = 0.04$) and UHTM caused also (non-significantly) highest insulin and glucose values at this point. The FA composition analyses showed significantly more myristic, palmitic, oleic and linoleic acids at two hour time point after UHTM and PM compared HPM. It is not clear why the FA composition of UHTM and PM differed from HPM at this one time point.

Previously our group (Nuora, et al., 2018) investigated differences in GI symptoms between raw milk and HPM. Although, more adverse GI symptoms were reported after HPM compared to raw milk the difference was not statistically significant. This is in line with the present study. In both studies no significant differences were seen in the postprandial lipemia, measured as iAUC, between milk types, suggesting that the difference in lipid droplet size does not influence the intestinal absorption of TAGs from the lipid droplets. Significant differences in the FA composition were found in both studies. However, in the first study significantly more saturated FAs were found after HPM compared to raw milk four hours after the study meal. In contrast, significantly more FAs, mainly saturated, were found at two hour time point after

UTHM and PM compared to HPM in the present study. Our previous study used SmartPill capsule, ingestible pressure measuring probe, during the milk challenge to investigate whether the pressure in the GI tract could be linked to adverse GI symptoms or if the GI transit time differed between the milk types. No differences were seen in the pressure or in the GI transit time between HPM and raw milk which led to the decision not to use the SmartPill capsule in the present study.

There were several strengths in the design of this study, including each subject serving as their own control in the cross-over design, the use of both objective (inflammation markers, postprandial glycemic and lipemic parametres of blood samples) and subjective (symptom diaries) outcome measures, the fact that the milk samples came from the same herd of cows and same milk batches and were processed and packed at same facilities as well as the volunteers reporting to be sensitized to milk. However, this study also included limitations. Fourteen subjects was relatively small group, the previous evening meal was not fully standardized and the raw milk was not included as one of the milk samples.

5 Conclusion

Our results do not support the hypothesis that common milk processing would induce GI symptoms or that the harder processing would create more symptoms than milder processes. Homogenization and heat treatments did not influence intestinal absorption of TAGs from milk fat globules despite differing droplet sizes. Significant difference in one time point is evidently not of importance in a context of mixed meal. These findings support previous studies where no statistical differences were found between processed and raw milk in the amount or severity of GI symptoms.

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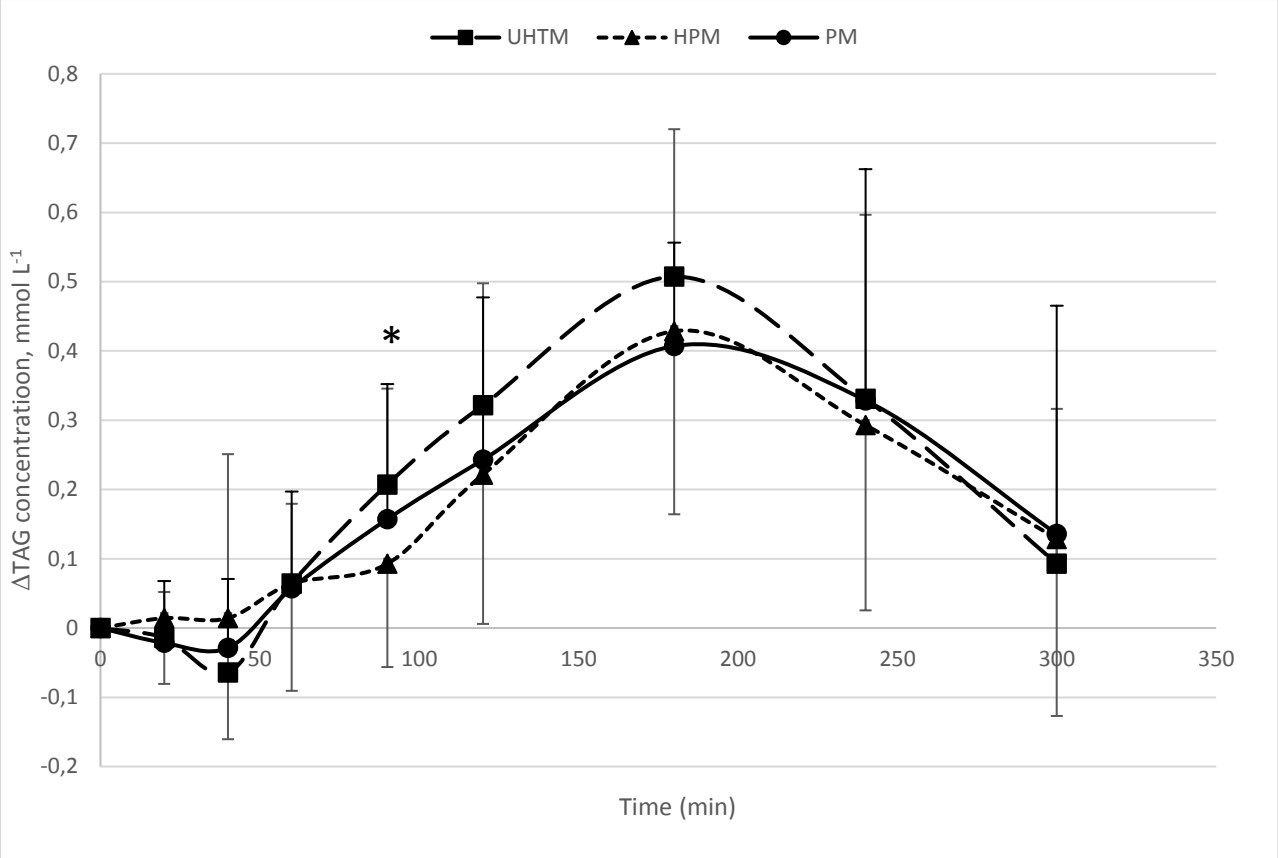
Figure captions

Figure 1. Plasma triacylglycerol (TAG) concentrations (Δ=deviation from baseline) after UHT homogenized milk (UHTM, black longer dashes), homogenized, pasteurized milk (HPM, black short dashes) and pasteurized milk (PM, black line). The TAG concentrations of UHTM is significantly higher at 90 min time point compared HPM ($P = 0.04$), marked with *. $N = 14$, values are mean with SD.

Figure 2. Major fatty acids of postprandial plasma at 2 and 4 hour time points after consumption of UHT homogenized milk (UHTM, black and diagonal striped bars), pasteurized milk (PM, horizontal striped and grey bars) and homogenized, pasteurized milk (HPM, light grey and dark grey bars). $N = 14$, values are mean with SD. Significant differences ($P < 0.05$) are marked with different letters with in each fatty acid.



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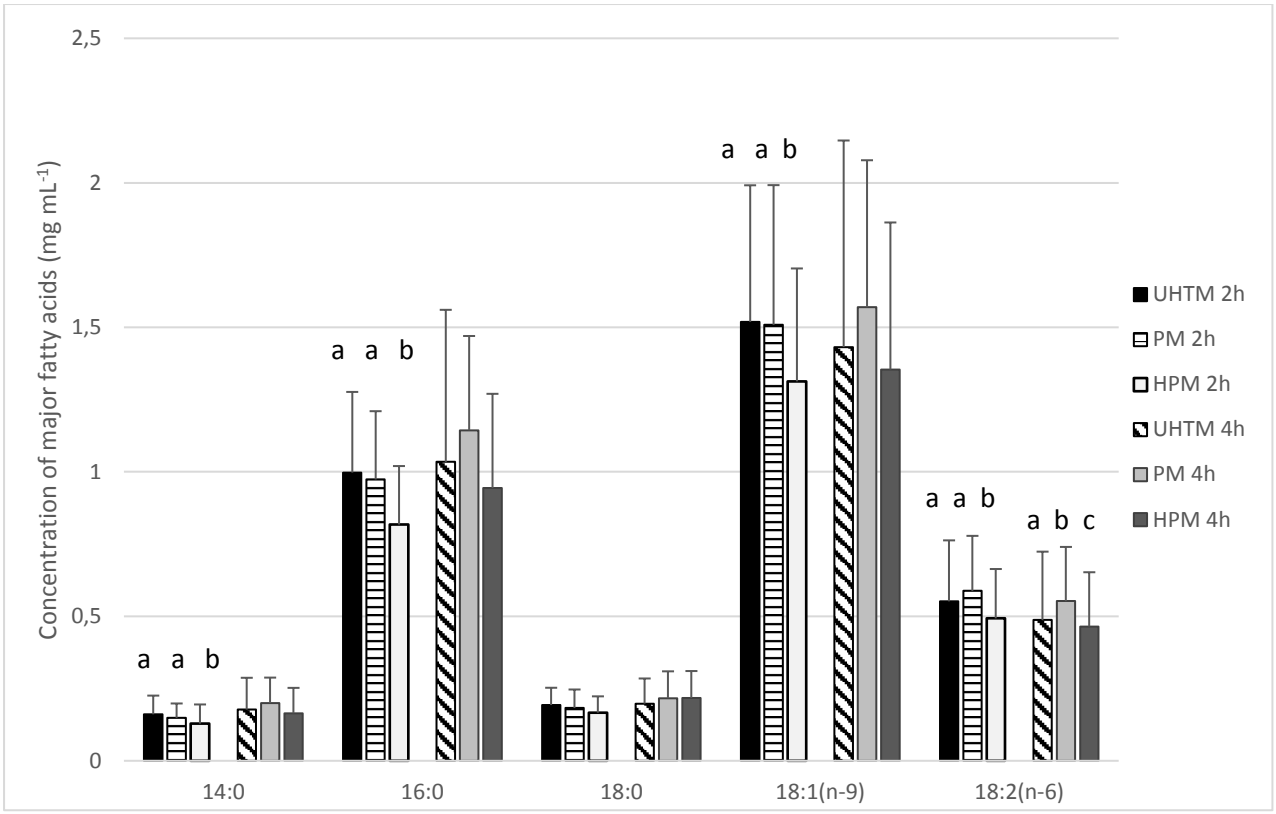

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Table 1. Baseline and end of study (300 min) measurements of 92 inflammation biomarkers with Olink Proteomics inflammation panel cDNA-based proximity extension multiplex system. Concentration values are log2. P-values are marked for significant differences between the values of baseline and 300 min within milk type. No significant differences were detected between milk types at baseline or at 300 min after the meal.

	UHT-treated homogenized milk					Homogenized pasteurized milk					Pasteurized milk				
	Baseline		300 min		P-value	Baseline		300 min		P-value	Baseline		300 min		P-value
	Mean	±SD	Mean	±SD		Mean	±SD	Mean	±SD		mean	±SD	Mean	±SD	
Interleukin-8	5,00	0,46	4,93	0,53		5,03	0,39	4,81	0,31	< 0.001	5,00	0,40	4,84	0,29	< 0.001
Vascular endothelial growth factor A	8,00	0,34	8,02	0,30		8,02	0,29	7,85	0,44		7,99	0,35	7,86	0,26	
Monocyte chemotactic protein 3	1,87	0,49	1,97	0,40		1,88	0,49	1,81	0,37		1,93	0,42	1,94	0,55	< 0.001
Glial cell line-derived neurotrophic factor	0,58	0,03	0,59	0,06		0,57	0,02	0,58	0,04		0,57	0,02	0,57	0,00	
CUB domain-containing protein 1	1,46	0,39	1,53	0,38		1,59	0,34	1,40	0,51		1,44	0,29	1,30	0,33	
Natural killer cell receptor 2B4	5,27	0,33	5,38	0,42		5,41	0,44	5,23	0,57		5,29	0,31	5,18	0,30	
Interleukin-7	1,73	0,32	1,83	0,38		1,82	0,33	1,84	0,51		1,81	0,37	1,78	0,43	
Osteoprotegerin	9,47	0,40	9,57	0,37		9,65	0,36	9,43	0,58		9,51	0,30	9,38	0,40	
Latency-associated peptide transforming growth factor beta-1	6,33	0,41	6,46	0,42		6,43	0,32	6,48	0,44		6,34	0,39	6,34	0,24	
Urokinase-type plasminogen activator	9,80	0,33	9,81	0,38		9,91	0,34	9,69	0,52		9,82	0,28	9,63	0,35	
Interleukin-6	2,19	0,63	4,34	0,72	< 0.001	2,24	0,55	3,56	0,89	< 0.001	2,55	1,07	3,98	1,03	
Interleukin-17C	0,66	0,10	0,61	0,02		0,64	0,09	0,67	0,13		0,67	0,19	0,70	0,21	
Monocyte chemotactic protein 1	9,88	0,44	9,67	0,51		9,95	0,43	9,56	0,51		9,91	0,45	9,56	0,33	
Interleukin-17A	0,30	0,04	0,34	0,15		0,32	0,07	0,34	0,14		0,35	0,19	0,33	0,13	
C-X-C motif chemokine	10,91	0,76	10,93	0,72		10,59	0,55	10,54	0,48		10,54	0,37	10,52	0,43	
Axin-1	1,48	0,63	1,69	0,49		1,63	0,79	1,46	0,61		1,38	0,58	1,70	0,28	
TNF-related apoptosis-inducing ligand	7,63	0,48	7,68	0,40		7,76	0,48	7,50	0,46		7,66	0,38	7,45	0,40	
Interleukin-20RA	0,31	0,08	0,29	0,00		0,29	0,00	0,29	0,00		0,29	0,00	0,29	0,00	
C-X-C motif chemokine 9	6,92	0,88	6,79	0,80		6,63	0,42	6,32	0,57		6,50	0,34	6,20	0,44	
Cystatin D	5,04	0,50	5,08	0,53		5,18	0,43	5,04	0,51		5,09	0,46	5,00	0,47	
Interleukin-2RB	0,80	0,06	0,79	0,00		0,79	0,00	0,79	0,00		0,79	0,00	0,79	0,00	
Oncostatin-M	1,01	0,73	1,03	0,83		0,82	0,60	0,83	0,49		1,01	0,74	1,04	0,43	

Interleukin-2	0,49	0,00	0,49	0,00		0,49	0,00	0,49	0,00		0,49	0,00	0,49	0,00	
C-X-C motif chemokine 1	7,50	0,53	7,74	0,49		7,51	0,33	7,51	0,46		7,48	0,48	7,72	0,44	
Thymic stromal lymphopoietin	0,67	0,00	0,67	0,00		0,67	0,00	0,67	0,00		0,67	0,00	0,67	0,00	
C-C motif chemikine 4	5,62	0,57	5,52	0,68		5,58	0,60	5,32	0,63		5,56	0,62	5,35	0,46	
T cell surface glycoprotein CD6 isoform	3,58	0,44	3,62	0,39		3,80	0,67	3,46	0,72		3,70	0,38	3,43	0,49	
Stem cell factor	9,39	0,44	9,49	0,32		9,54	0,28	9,39	0,50		9,48	0,35	9,38	0,42	
Interleukin-18	6,63	0,66	6,80	0,65		6,81	0,55	6,61	0,57		6,64	0,58	6,55	0,54	
Signaling lymphocytic activation molecule	0,74	0,30	0,74	0,28		0,68	0,32	0,72	0,29		0,64	0,23	0,69	0,28	
Transforming growth factor alpha	1,97	0,38	1,90	0,31		2,02	0,30	1,76	0,48		1,94	0,34	1,79	0,28	
Monocyte chemotactic protein 4	5,12	0,68	5,15	0,90		5,20	0,76	5,04	0,62		5,15	0,76	5,03	0,69	
Eotaxin	8,08	0,31	8,25	0,32		8,18	0,25	8,14	0,24		8,11	0,29	8,13	0,29	
Tumor necrosis factor ligand superfamily member 14	3,30	0,54	3,44	0,56		3,32	0,35	3,31	0,47		3,29	0,30	3,43	0,40	
Fibroblast growth factor 23	1,75	0,61	1,57	0,46		1,65	0,57	1,37	0,62		1,56	0,43	1,41	0,73	
Interleukin-10RA	0,49	0,50	0,43	0,52		0,42	0,40	0,45	0,37		0,57	0,71	0,53	0,63	
Fibroblast growth factor 5	0,32	0,13	0,30	0,05		0,29	0,00	0,29	0,03		0,29	0,00	0,29	0,00	
Matrix metalloproteinase-1	10,27	0,72	10,34	0,89		10,24	0,90	10,28	0,87		10,28	0,88	10,34	0,75	
Leukemia inhibitory factor receptor	2,08	0,37	2,16	0,34		2,22	0,33	2,07	0,54		2,15	0,34	1,94	0,43	
Fibroblast growth factor 21	4,08	1,15	2,20	0,77	< 0.001	3,82	1,03	1,88	0,54	< 0.001	3,92	1,32	1,90	0,90	
C-C motif chemikine 19	7,95	0,61	8,16	0,66		7,97	0,56	7,87	0,63		7,94	0,55	7,91	0,54	< 0.001
Interleukine-15 receptor subunit alpha	0,23	0,02	0,22	0,00		0,22	0,00	0,22	0,00		0,22	0,00	0,22	0,00	
Interleukin-10 receptor subunit beta	6,24	0,40	6,32	0,51		6,42	0,43	6,14	0,62		6,33	0,40	6,10	0,39	
Interleukin-22 receptor subunit alpha-1	1,13	0,00	1,13	0,00		1,13	0,00	1,13	0,00		1,13	0,00	1,13	0,00	
Interleukine-18 receptor 1	5,85	0,59	5,96	0,55		5,98	0,49	5,79	0,57		5,88	0,54	5,75	0,41	
Programmed cell death 1 ligand	3,33	0,69	3,29	0,70		3,45	0,56	3,15	0,49		3,35	0,58	3,10	0,51	
Beta-nerve growth factor	1,23	0,29	1,13	0,33		1,21	0,22	1,13	0,40		1,15	0,30	1,02	0,29	
C-X-X motif chemokine 5	9,76	0,89	10,01	0,96		9,84	0,94	9,89	0,94		9,82	0,96	10,09	0,84	
TNF-related activation-induced cytokine	4,25	0,78	3,96	0,73		4,39	0,74	3,86	0,74		4,26	0,47	3,71	0,58	
Hepatocyte growth factor	6,55	0,42	6,63	0,37		6,60	0,34	6,47	0,48		6,56	0,27	6,41	0,38	< 0.001
Interleukin-12 beta	3,17	0,39	3,23	0,44		3,22	0,48	3,07	0,67		3,14	0,55	3,04	0,54	
Interleukine-24	1,08	0,00	1,08	0,00		1,08	0,00	1,08	0,00		1,08	0,00	1,08	0,00	

Interleukine-13	0,46	0,01	0,46	0,00	0,46	0,00	0,46	0,00		0,46	0,00	0,46	0,00
Artemin	0,12	0,00	0,12	0,00	0,12	0,00	0,12	0,00		0,12	0,00	0,12	0,00
Matrix metalloproteinase-10	5,10	0,57	5,08	0,47	5,36	0,51	5,07	0,66		5,18	0,38	4,88	0,45
Interleukine-10	1,56	0,53	1,66	0,53	1,74	0,47	1,45	0,50		1,65	0,49	1,42	0,46
Tumor necrosis factor	0,44	0,00	0,43	0,00	0,43	0,00	0,43	0,00		0,43	0,00	0,43	0,00
C-C motif chemokine 23	9,13	0,56	9,35	0,56	9,33	0,52	9,20	0,85		9,19	0,42	9,17	0,49
T-cell surface glycoprotein CD5	4,29	0,36	4,32	0,45	4,46	0,44	4,23	0,64		4,38	0,44	4,17	0,46
C-C motif chemokine 3	3,88	0,43	3,75	0,54	3,83	0,46	3,56	0,53		3,81	0,47	3,62	0,42
Fms-related tyrosine kinase 3 ligand	7,75	0,61	7,53	0,51	7,98	0,51	7,29	0,71	< 0.001	7,82	0,37	7,29	0,45
C-X-C motif chemokine 6	8,18	0,65	8,30	0,68	8,25	0,73	8,16	0,70		8,21	0,68	8,20	0,53
C-X-C motif chemokine 10	7,92	1,03	7,84	1,02	7,77	0,88	7,36	0,88		7,95	1,04	7,59	1,00
Eukaryotic translation initiation factor 4E-binding protein	4,96	1,50	5,10	1,07	4,18	1,69	4,75	1,08		4,39	1,60	4,69	1,23
Interleukine-20	0,47	0,06	0,46	0,00	0,46	0,00	0,46	0,00		0,46	0,00	0,46	0,00
SIR2-like protein 2	1,38	0,43	1,63	0,46	1,47	0,46	1,34	0,39		1,24	0,26	1,40	0,40
C-C motif chemokine 28	0,31	0,07	0,33	0,08	0,29	0,01	0,31	0,08		0,30	0,02	0,29	0,02
Delta and Notch-like epidermal growth factor-related receptor	7,55	0,38	7,66	0,35	7,72	0,32	7,50	0,48		7,65	0,35	7,50	0,38
Protein S100-A12	1,95	0,63	2,27	0,88	2,16	0,67	2,44	0,76		2,07	0,76	2,16	0,58
CD40L receptor	8,93	0,41	9,05	0,41	9,08	0,44	8,88	0,48		8,86	0,30	8,93	0,36
Interleukine-33	0,81	0,00	0,81	0,00	0,81	0,00	0,81	0,00		0,81	0,00	0,81	0,00
Interferon gamma	0,45	0,00	0,45	0,00	0,45	0,00	0,45	0,00		0,45	0,00	0,45	0,00
Fibroblast growth factor 19	7,15	0,82	7,77	0,60	7,04	1,05	7,47	0,49		6,90	1,10	8,02	0,51
Interleukine-4	0,51	0,04	0,49	0,00	0,49	0,01	0,49	0,02		0,49	0,00	0,49	0,01
Leukemia inhibitory factor	0,55	0,28	0,49	0,07	0,51	0,15	0,48	0,04		0,50	0,07	0,47	0,00
Neurturin	0,53	0,76	0,47	0,62	0,54	0,90	0,52	0,83		0,50	0,76	0,53	0,86
Monocyte chemotactic protein 2	7,49	0,64	7,53	0,66	7,42	0,46	7,30	0,39		7,35	0,57	7,32	0,49
Caspase-8	0,95	0,28	1,05	0,48	1,04	0,44	1,01	0,56		0,94	0,28	1,01	0,34
C-C motif chemokine 25	5,28	0,54	5,30	0,48	5,41	0,46	5,05	0,73		5,26	0,54	5,03	0,51
Fractalkine	4,75	0,58	4,82	0,50	5,19	0,33	4,62	0,69		5,06	0,55	4,55	0,53
Tumor necrosis factor receptor superfamily member 9	5,55	0,44	5,36	0,45	5,58	0,56	5,17	0,73		5,55	0,53	5,14	0,51
Neurotrophin-3	0,92	0,40	1,16	0,52	0,91	0,61	1,05	0,56		0,95	0,53	0,93	0,31

Tumor necrosis factor (Ligand) superfamily,member 12	8,49	0,57	8,63	0,54	8,64	0,49	8,47	0,58	8,56	0,34	8,41	0,39
C-C motif chemokine 20	4,72	0,86	4,36	0,60	4,63	0,86	4,25	0,87	4,72	0,86	4,16	0,70
Sulfotransferase 1A1	2,86	0,81	3,59	0,71	3,13	0,66	3,29	1,02	2,79	0,81	3,80	0,68
STAM-binding protein	3,18	0,59	3,55	0,65	3,32	0,69	3,27	0,62	3,10	0,36	3,51	0,37
Interleukine-5	1,14	1,03	1,08	0,78	1,10	0,98	1,20	1,07	1,15	0,99	1,13	0,89
Adenosine Deaminase	3,15	0,39	3,27	0,33	3,23	0,40	3,14	0,42	3,19	0,26	3,13	0,30
TNF-beta	3,30	0,57	3,36	0,46	3,50	0,36	3,27	0,55	3,40	0,34	3,20	0,34
Magrophage colony-stimulating factor 1	7,01	0,41	7,09	0,32	7,09	0,29	6,84	0,47	7,05	0,37	6,93	0,29