J. Dairy Sci. 101:10636–10648
https://doi.org/10.3168/jds.2018-14782
© American Dairy Science Association®, 2018.

Metabolic effects in mice of cream processing: Direct ultra-high-temperature process lowers high-fat-induced adipose tissue inflammation

M. Milard,* F. Laugerette,* S. Bugeat,† P. Plaisancié,* M. Létisse,* E. Meugnier,‡ E. Loizon,‡ A. Durand,* C. Buisson,* A. Géloën,* S. Serieye,† and M.-C. Michalski*[1]
*Univ Lyon, CarMeN Laboratory, INSERM, INRA, Université Claude Bernard Lyon-1, INSA Lyon, 69621 Villeurbanne, France
†Sodiaal International Research and Innovation Department, Paris, France
‡Univ Lyon, CarMeN Laboratory, INSERM, INRA, Université Claude Bernard Lyon-1, INSA Lyon, 69600 Oullins, France

ABSTRACT

Although UHT heat treatment is being optimized to improve the stability and functional properties of dairy products, its metabolic effects remain scarcely known. As such, we studied the effect of the type of UHT process on lipid metabolism, intestinal barrier, and inflammation in mice. Nine-week-old male C57Bl/6J mice were fed a diet composed of nonlipidic powder mixed with different UHT dairy creams (final: 13% milkfat) for 1 or 4 wk. All creams contained 0.02% of thickener (carrageenan) and were treated via either (1) classical indirect heating process (Th), (2) indirect process at higher temperature (Th+), or (3) direct process by steam injection (ThD). Plasma, epididymal adipose tissue (EAT), and intestine were analyzed. Multivariate principal component analyses were used to identify differential effects of processes. Th+ differed by a globally higher liver damage score compared with that of the other creams. After 4 wk, the duodenal expression of lipid absorption genes fatty acid binding protein 4 (*Fatp4*) and microsomal triglycerides transfer protein (*Mttp*) was lower in the Th+ versus Th group. Expression in the colon of tight junction protein zonula occludens 1 (*Zo1*) and of some endoplasmic reticulum stress markers was lower in both Th+ and ThD versus the Th group. In EAT, ThD had lower gene expression of several inflammatory markers after 4 wk. Some differential effects may be related to heat-induced physicochemical changes of creams. The type of cream UHT process differentially affected metabolic parameters in mice after a 4-wk fat-rich diet, partly due to cream structure. Altogether, direct steam injection process induced the lowest early markers of high-fat-induced metabolic inflammation in EAT.

Key words: nutrition, dairy matrix, cow milk, food process

INTRODUCTION

Milk and dairy products (yogurts, dairy desserts, cheese, butter, and cream) represent a major food group of the Western diet and occupy a central role in the food pyramid graphics of many countries. According to estimates (IDF, 2013), world production of bovine milk reached 636.7 million tonnes in 2012 (33% in Europe, 28% in Asia, 18% in North and Central America). According to nutritional and public health recommendations, dairy products are recommended as part of a healthy diet. They are in effect associated with lower risk of cardiovascular disease (Huth and Park, 2012) and obesity (Astrup et al., 2014). Furthermore, recent works suggest an inverse dose-response relationship between dairy product consumption and risk of metabolic syndrome (Chen et al., 2015; Thorning et al., 2017). However, no work has been reported regarding the effect of dairy process type on metabolic function. As such, the differential metabolic effect of dairy products subjected to various treatment processes remains to be elucidated, which is a timely topic as highlighted in recent reviews (Michalski et al., 2013; Thorning et al., 2017).

Mammalian milk, being the only food available during the first stages of life, contains a great amount of various nutrients. As milk can rapidly become spoiled by microorganisms, the food industry has developed preservation processes to increase shelf-life and therefore ensure access to this dairy pool of nutrients for the largest possible population and for the longest preservation time. In this respect, a challenge for the dairy industry is to optimize the preservation of nutritional and organoleptic qualities of milk on the one hand, while avoiding its microbial degradation on the other. For milk and cream, food safety is achieved by heat treatment at UHT and stability during storage by ho-

Received March 20, 2018.
Accepted August 21, 2018.
[1]Corresponding author: marie-caroline.michalski@insa-lyon.fr

mogenization processes. These treatments have complementary effects on milk components, such as proteins, milk fat globule membrane, lipids, and minerals. The interface of native milk fat globules, for example, is stabilized by a complex membrane composed by polar lipids, glycoproteins, glycolipids, cholesterol, and some proteins. After heat and homogenization treatments, the interface is totally modified and is mostly composed of casein and whey proteins, hence affecting its nutritional, functional, and biological properties (Bolling et al., 2005; Michalski and Januel, 2006).

To date, several studies have shown that milk digestion varies depending on the way it is processed to reduce or destroy bacteria (Tunick et al., 2016). Lacroix et al. (2008) examined the effect of different treatments (microfiltration, pasteurization, and sterilization) on postprandial utilization of milk nitrogen in humans. This study suggests a modification of the protein metabolism due to a structural modification of the casein micelles under UHT treatment.

Modifications of certain functionalities of dairy products after heat treatment are thus likely to possess metabolic effects, which, up until now, have been little studied. Indeed, heating temperature affects the configuration of the different milk components (notably proteins, milk fat globule membrane). Therefore, manufacturers continually develop new processes aimed at optimizing milk and cream heating to limit its alteration. In this context, a direct type of UHT treatment by steam injection has been developed, which makes it possible to heat the product from 80 to 140°C (and cool from 140 to 80°C) for less than a second. In comparison, the most current indirect process used (indirect heating) requires approximately 100 s so as to achieve the same thermal efficiency. This shorter time at high temperature (for the same holding time at the sterilization temperature) allows reduction of protein denaturation and Maillard reaction development. A recent study has shown the influence of sterilization by indirect heating versus direct steam injection on skim and 2% fat milks. Indirect heating of milks had higher sweet aromatic, sweet taste, and serum protein denaturation compared with direct steam injection (Lee et al., 2017).

Until now, studies of the health effects of dairy products consist mainly in epidemiological studies or in randomized controlled trial comparing the consumption of different dairy matrixes (e.g., cheese vs. butter, calcium content; Lamarche, 2008; Astrup et al., 2014), but data on the metabolic effect of UHT heat treatments of milk and cream are still lacking. Our study hence focuses on the short-term effect of UHT process type (direct and indirect heat treatments of cream) on metabolic parameters in mice. Because the size of the milk fat droplet properties can be changed by processing and modifying lipemia (Armand et al., 1996; Bourlieu et al., 2015; Bourlieu and Michalski, 2015), we studied effects of cream on the gene expression of proteins involved in intestinal lipid absorption and fatty acid fecal excretion. Nutrients, but also a high-fat diet, can modify intestinal barrier and induce an increased inflammation of the distal colon (Gulhane et al., 2016). Therefore, we studied the effect of cream on proteins implicated in paracellular transport (tight junction protein) and endoplasmic reticulum (**ER**) stress in colon. Importantly, adipose tissue inflammation is associated with metabolic disorders (Hotamisligil et al., 1993; Hofmann et al., 1994; Hotamisligil and Spiegelman, 1994; Kern et al., 2001; Pietilainen et al., 2006). We consequently measured the gene expression of inflammatory markers in epididymal adipose tissue (**EAT**). Altogether, the aim of this study was to determine which type of UHT heat treatment results in the least metabolic disorders in mice, by comparing classic indirect UHT treatment (**Th**), indirect treatment UHT with a heat charge 4.5 times higher (**Th+**), and a direct UHT treatment by steam injection (**ThD**) with the same heat charge as Th.

MATERIALS AND METHODS

Animals and Experimental Protocols

Male C57BL/6J mice (19–25 g, 9 wk) from Janvier SA (Saint Berthevin, France) were housed 5 per cage in a temperature-controlled room (24 ± 1°C, 12-h daylight cycle; ±SD) with free access to food and water. After a week of acclimatization, they were randomly divided into 10 groups (n = 10). Five groups (corresponding to 5 creams) were fed for 7 d to characterize the early intestinal response, or 5 other groups for 4 wk to show the long-term effects of diets on metabolic parameters. Body weight and food intake were measured twice per week. The BW of each animal after 4 wk was recorded. Body weight gain was calculated as follows: BW gain (g) = final weight − initial weight.

At the end of the experimental period, food was withheld for the night. Mice were anesthetized with intraperitoneal injection of urethane. After laparotomy, blood was collected by cardiac puncture with heparinized syringes. Plasma was collected by centrifugation (2,000 × *g*, 5 min, 4°C). The duodenum and colon were collected after a wash of the intestine lumen with PBS. Liver and white adipose epididymal fat, were harvested, weighed, and processed. Samples were immediately frozen in liquid nitrogen and stored at −80°C until analysis. All the animal experiments were carried out in accordance with the Guiding Principles for the Care and Use of Vertebrate Animals in Research and

Training and the French Ministère de l'Agriculture (no. 87–848 completed by no. 2001–464) and EU Council Directive for the Care and Use of Laboratory Animals (no. 2010/63/EU). The experimental protocol was approved by the Animal Ethics Committee of INSA-Lyon.

Diets

Three UHT creams (35% wt fat including 0.02% carrageenans) were studied. All creams (Sodiaal-Candia, La Talaudière, France) were prepared from the same milk batch, therefore only differing by their processing (2 indirect treatments differing in heat charge intensity, one direct treatment; Table 1).

Of note, the Th group is the same as in our companion study on the effect of cream additives (Milard et al., 2018). We designed the diets to (1) provide mice a complete moderately high-fat diet in which all lipids are those from cream, (2) while not altering cream structure that would be induced by a classical solid pellets production, but (3) by avoiding a daily gavage with creams for several days that would stress mice and induce bias in studies metabolic outcomes. To this aim, as previously recommended to preserve the lipid structure (Oosting et al., 2014), diets were not pelletized but given to the mice as dough (creams mixed with nonlipidic powder). The lipid content of each diet was 13.2%. The alipidic powder was purchased from SAFE (Augy, France).

Table 1. Composition of the experimental diets[1]

Cream processing	Th	Th+	ThD
Type of treatment	Indirect	Indirect	Direct
Heat charge (F)[1]	33.8	150	33
Ingredient, g/100 g of diet			
Dairy cream	37.8	37.8	37.8
Total dry extract	15.1	15.1	15.1
Lipids	13.2	13.2	13.2
Defatted DM	1.9	1.9	1.9
Water	22.7	22.7	22.7
Additive			
κ-CGN	0.008	0.008	0.008
Alipidic power	62.2	62.2	62.2
Dextrose	28.0	28.0	28.0
Cellulose	5.6	5.6	5.6
Vitamin mixture	6.5	6.5	6.5
Mineral mixture	0.9	0.9	0.9
Alipidic casein	21.2	21.2	21.2

[1]F = the sterilization force, which is the microbial lethality expressed in terms of equivalent time in minutes at a temperature of 121.1°C. For the F calculation, the Z-value (thermal inactivation of selected bacteria) is taken at 7.5. Th = UHT cream treated by an indirect process; Th+ = UHT cream treated with an indirect process with higher temperature; ThD = UHT cream treated by a direct process. κ-CGN = κ-carrageenan.

Biochemical Analyses of Plasma

Different plasma measurements in mice (fasted overnight) were assessed. Concentrations of glucose were performed after tail vein incision and measured by MyLife PuraX blood glucose meter (MyLife, New Delhi, India). For mice that were fed diets for 1 wk, we measured insulinemia with the Mouse Ultrasensitive Insulin ELISA (Eurobio, Alpco, Les Ulis, France) and for mice fed for 4 wk, using the Ultrasensitive Mouse Insulin ELISA kit (Crystal Chem, Zaandam, the Netherlands). Plasma triglyceride (**TG**) concentration was determined by colorimetric method (Triglyceride PAP 150 kit, bioMérieux, Marcy l'Etoile, France). Plasma nonesterified fatty acid (**NEFA**) concentration was measured using NEFA-C kit (Wako Chemicals, Richmond, VA).

Markers of Inflammation in Plasma

Endotoxemia (LPS) in plasma was determined using the Limulus Amoebocyte Lysate assay in kinetic chromogenic conditions (Associates of Cape Cod, East Falmouth, MA; Laugerette et al., 2015), using simple-use nonpyrogenic supplies [i.e., PS tubes (Becton Dickinson, Franklin Lakes, NJ), MAXYmum Recovery tubes (Axygen, VWR, Pessac, France), and pyrogen-free pipet tips (Eppendorf, VWR)]. Lipopolysaccharide-binding protein (**LBP**) concentration was measured in plasma samples via ELISA kit according to manufacturer's recommendations (Hycult Biotech, Uden, the Netherlands). Soluble cluster of differentiation 14 (**sCD14**) was assayed using a sandwich ELISA kit (Elabscience, Hubei, China). Plasma monocyte chemoattractant protein 1 (**MCP1**) levels were measured using a Mouse MCP1 ELISA kit (Ray Biotech, Norcross, GA) following the manufacturer's instructions.

Histological Examination of Liver Damage

Imaging experiments were performed on the Cell Station of CellImaP platform (IFR100, Dijon, France). Liver section of mice were immediately fixed in 4.5% formalin containing fixation solution (4.5% Roti Histofix, Carl Roth, Karlsruhe, Germany). After 48 h of fixation in Histofix, samples were transferred in 70% ethanol until paraffin inclusion. Liver samples were embedded in paraffin and cut in 4-μm sections. Sections were then stained with hematoxylin and eosin. Liver histology was examined using a light microscope at 40× magnification (VWR). Scoring was realized using the scoring system consisting of 4 histological features, which were evaluated semiquantitatively: steatosis (0–4), lobular inflammation (0–2), hepatocellular ballooning (0–2), and fibrosis (0–3).

Tissue RNA Extraction and Quantitative Real-Time PCR

Total RNA was extracted from whole intestine segments (duodenum and colon) and EAT of mice with TRI Reageant (Sigma, Saint-Quentin-Fallavier, France). The RNA concentration was measured with Nanodrop-ND1000 (Labtech, Healthfield, UK) and RNA samples with A260/240 ration between 1.7 and 2.1 were considered of good quality. One microgram of RNA was used for target gene expression using quantitative real-time PCR. TATA-box-binding protein (**TBP**) expressions were used as the internal standard for normalization of target mRNA expression. The list of the PCR primers used is shown in Supplemental Table S1 (https://doi.org/10.3168/jds.2018-14782).

Measurement of Fecal Fatty Acid Content

Feces of mice were collected at the end of the experiment in the cage. Feces were cleaned and dried for 72 h at 60°C. Ten-milligram aliquots were extracted by using the method of Folch et al. (1957) for the extraction of total lipids. Feces fatty acid profiles were analyzed by GC.

Physicochemical Parameters of UHT Cream

Globule size distribution was analyzed by a laser particle size analyzer (Malvern Mastersizer 2000, Worcestershire, UK); to inhibit flocculation, 19 mL of a 10 g/L SDS solution was added to 1 mL of cream. Obscuration was set between 5 and 7% and the recorded value was the volume-weighted average diameter $d_{4,3}$. The flocculation index was calculated by dividing the $d_{4,3}$ of the cream diluted with water by the $d_{4,3}$ of the cream with the addition of SDS. Interfacial and aqueous phase proteins were estimated as the proportion of total cream proteins being present around the fat droplet interface versus in the aqueous phase by dosing proteins in floating layer versus in the pellet after ultracentrifugation.

Statistical Analysis

In this study, we performed a parallel-group study testing the type of cream processing type, whereby mice were randomly assigned to one of the treatments. Correlation, principal component analysis, normality test were performed using the computing environment R (R Development Core Team, R Foundation for Statistical Computing, Vienna, Austria) and principal component analysis was performed on normalized data. Normality of data was examined using Shapiro-Wilk test with the computing environment R (R Development Core Team). Variance homogeneity was examined by using Bartlett's test and Brown-Forsythe. One-way ANOVA were performed on normal data using GraphPad Prism software (GraphPad, San Diego, CA) followed by a Tukey post-hoc multiple comparisons test. For nonnormal data or a small number of observations, a Kruskal-Wallis test was performed followed by a Dunn post hoc test. Differences were considered significant at the $P < 0.05$ level, whereas differences in which $0.05 < P < 0.1$ were considered tendencies. Different letters (a–c) indicate significant differences ($P < 0.05$) after 1 wk of diet and (x–z) after 4 wk of diet. All data are presented as means ± standard error of the mean.

RESULTS

Principal Component Analyses

In Figure 1, the principal component analysis loading plot (first 2 components, 39.3% of the data variation) reveals different effects between groups according to heat treatment. We observed less inflammatory effects in EAT with ThD group.

Effect of Diet on the Morphological Parameters

Intake of different diets as well as BW throughout the 1 and 4 wk feeding period, respectively, are summarized in Table 2. Body weight, tissue weight, and daily food intake did not differ among groups. The response of fasting glucose and insulinemia was not significantly influenced by the treatment. No significant group effect was observed for plasma concentrations of NEFA and TG. As shown in Figure 2, the liver damage score appeared higher in Th+ group but not significantly because of limited group size.

Effect on Lipid Metabolism-Related Proteins in the Intestine

The effect of heat treatment may affect the configuration of the proteinaceous interface of milk fat droplets, which may affect lipid digestion and metabolism. Thus, we studied the expression of genes involved in intestinal lipid absorption in the duodenum (Figure 3). We focused on genes playing a role in the transport of long-chain fatty acids (fatty acid transport protein 4, *Fatp4*, and fatty acid binding protein 2, *Fabp2*) and in lipoprotein secretion (microsomal TG transfer protein, *Mttp*). After 1 wk of diet, gene expression of *Fabp2* was higher when the cream was treated by a direct sterilization process (ThD) or a higher indirect thermal intensity (Th+) in contrast to the conventional indirect heat treatment (Th; $P < 0.05$). A tendency for a higher expression of *Fatp4* was observed for mice

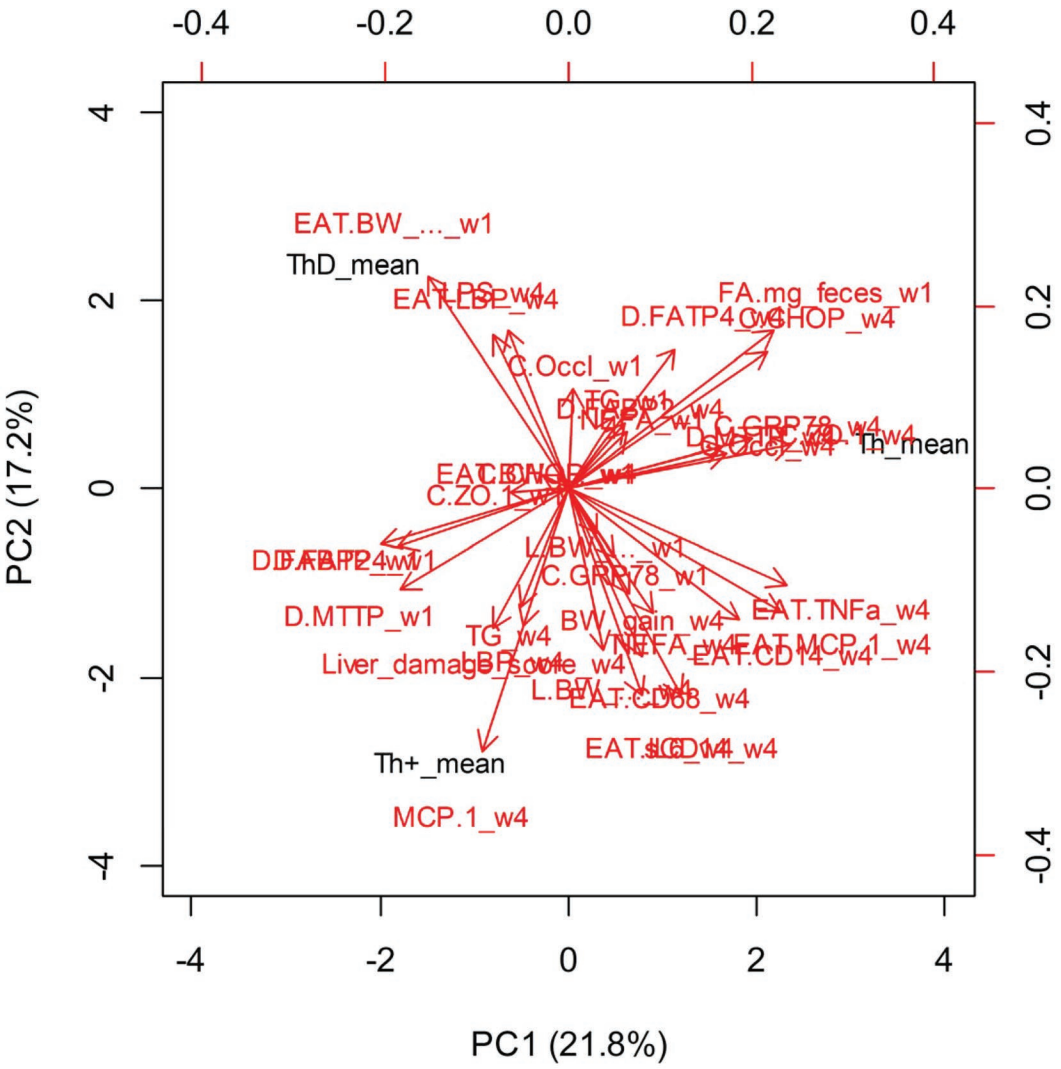

Figure 1. Principal component (PC) analysis of the various outcomes (notably in plasma, intestine, liver, white adipose tissue) measured in male C57BL/6J mice fed UHT creams (35% wt fat) reconstituted with nonlipidic powder (final milkfat 13% wt) for 1 or 4 wk (the first 2 components representing 39.3% of the data are shown). BW = body weight gain; C.Occl = occludin in the colon; C.GRP78 = *Grp78* in the colon; C.CHOP = *Chop* in the colon; C.ZO-1 = zonula-occludens-1 in the colon; D.mMuc2 = mucin-2 in the duodenum; D.Mttp = microsomal TG transfer protein in the duodenum; D.FABP2 = fatty acid binding protein 2 in the duodenum; D.FATP4 = fatty acid transport protein 4 in the duodenum; EAT BW = epididymal adipose tissue weight to BW ratio; EAT.LBP = LPS binding protein in EAT; EAT.CD14 = *Cd14* in the EAT; EAT.TNFa = *Tnfα* in the EAT; EAT.CD68 = *Cd68* in the EAT; EAT.MCP1 = *Mcp1* in the EAT; EAT.IL6 = *Il6* in the EAT; FA.mg_feces = fecal lipid excretion; L.BW = liver weight to BW ratio; LBP = lipopolysaccharide binding protein; sCD14 = soluble cluster of differentiation 14; MCP-1 = monocyte chemoattractant protein 1; NEFA = nonesterified fatty acids; TG = triglycerides; Th = UHT cream treated by an indirect process; Th+ = UHT cream treated with an indirect process with higher temperature; ThD = UHT cream treated by a direct process. Color version available online.

fed with ThD cream versus Th group ($P = 0.07$), and we observed a tendency for a higher expression of *Mttp* for Th+ group versus Th group ($P = 0.05$). After 1 wk, the gene expression of *Fatp4* was correlated with *Fabp2* (r = 0.72, $P < 0.001$), as well as *Fatp4* with *Mttp* (r = 0.80, $P < 0.001$) and *Fabp2* with *Mttp* (r = 0.71, $P < 0.001$; Supplemental Table S2; https://doi.org/10.3168/jds.2018-14782). However after 4 wk of diet, expression profile of intestinal lipid transporters among groups was modified, revealing a possible adaptation process along time. Indeed, expression of *Fabp2* and *Fatp4* in ThD group after 4 wk was similar to the Th and Th+ groups. However, a weaker expression of *Fatp4* was observed for Th+ compared with the Th group ($P < 0.05$). The *Mttp* expression was lower for both ThD and Th+ mice than for Th mice ($P < 0.05$). Fatty acid excretion in the feces of mice was similar among groups after 1 wk, but after 4 wk it was higher

Table 2. Biometric data and plasma concentration of glucose, insulin, triglycerides (TG), and nonesterified fatty acids (NEFA) in male C57Bl/6J mice fed different UHT creams, which differed by heat treatment for 1 or 4 wk[1]

	Diet group					
	1 wk			4 wk		
Item	Th	Th+	ThD	Th	Th+	ThD
Biometric data						
Initial BW, g (n = 10)	21.2 ± 0.5	21.5 ± 0.5	22.2 ± 0.3	23.0 ± 0.5	24.1 ± 0.5	23.0 ± 0.5
BW gain, g (n = 7)	—	—	—	5.5 ± 1.2	5.6 ± 1.3	4.1 ± 1.8
EAT to BW, % (n = 10)	—	—	—	1.4 ± 0.1	1.5 ± 0.3	1.5 ± 0.1
Liver to BW, % (n = 10)	4.43 ± 0.1	4.44 ± 0.1	4.27 ± 0.1	4.44 ± 0.1	4.61 ± 0.2	4.16 ± 0.1
Food intake, g/d	4.5 ± 0.3	4.1 ± 0.2	4.7 ± 0.4	4.4 ± 0.1	4.5 ± 0.1	4.3 ± 0.1
Plasma concentration[2]						
Glucose, mg/dL (n = 10)	123 ± 10.8	150 ± 6.9	141 ± 9.4	138 ± 13	167 ± 8	151 ± 13
Insulin, mg/dL	0.66 ± 0.19	0.78 ± 0.2	0.62 ± 0.18	0.35 ± 0.06	0.35 ± 0.11	0.28 ± 0.1
TG, mg/mL	0.53 ± 0.04	0.48 ± 0.03	0.53 ± 0.03	0.70 ± 0.09	0.69 ± 0.1	0.55 ± 0.02
NEFA, mg/dL (n = 10)	19.2 ± 1.5	17.0 ± 0.8	17.1 ± 1.2	22.5 ± 1.1	22.8 ± 1.0	19.9 ± 0.7

[1]Each values represent the mean ± SEM. The numbers in parentheses represent the number of mice. For insulin, after 1 wk of diet n = 6 for Th, n = 8 for Th+ and n = 9 for ThD group. After 4 wk, n = 6 for Th and Th+, n = 5 for ThD. For TG, after 1 wk of diet n = 10. After 4 wk, n = 10 for Th, n = 7 for Th+ and n = 6 for ThD. EAT = epididymal adipose tissue. Th = UHT cream treated by an indirect process; Th+ = UHT cream treated with an indirect process with higher temperature; ThD = UHT cream treated by a direct process.

[2]Measurements were performed on fasting plasma.

for the cream treated by a higher temperature (Th+, $P < 0.01$ vs. Th). However, no differences were observed in fecal fatty acid profile (data not shown).

Effect of Process on Plasma Markers of Metabolic Endotoxemia after 4 wk

To determine whether the different diets could induce alterations of intestinal barrier function, markers of the translocation of bacterial LPS were measured, namely endotoxemia (e.g., LPS activity in plasma) and plasma concentrations of the LPS transporter, LBP, and soluble LPS receptor, sCD14 (Figure 4A and 4B). After 4 wk, the endotoxemia of both Th+ and ThD groups was not different from Th group. However, ThD group exhibited higher endotoxemia than Th+ group ($P < 0.05$), though with greater intragroup variability. Diets had no significant effects on plasma LBP concentration. Lower plasma concentration of sCD14 was observed for ThD group versus Th+ ($P < 0.05$; Figure 4C). No difference was observed among groups for the plasma concentration of MCP1 (Figure 4D), but from a kinetics point of view regarding the onset of metabolic effects, we note that both LBP and MCP1 appeared to

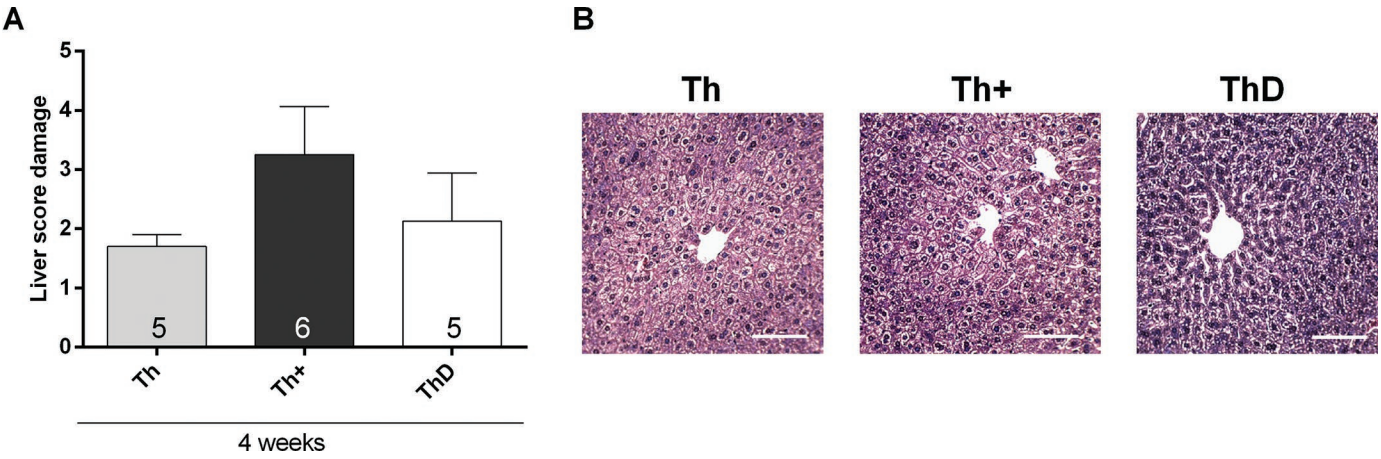

Figure 2. Effects of 4 wk consumption of UHT creams differing by the heating process (Th = indirect treatment; Th+ = indirect treatment with higher temperature; ThD = direct treatment) on the liver of C57Bl/6J mice. (A) Change in liver score damage. Values are means ± SEM. The number of mice in each group is indicated within the bars. (B) Representative photomicrographs of liver sections [hematoxylin and eosin staining (hematoxylin and eosin); original magnifications: 40×] of Th (left), Th+ (middle), and ThD (right). Scale bars = 100 μm. Color version available online.

begin to increase in Th+ group at 4 wk compared with Th and ThD.

Effect of Heating Process on Tight Junction-Associated Proteins and ER Stress in the Colon and on Adipose Tissue Inflammation

To assess the effect of cream on intestinal barrier parameters, we measured the gene expression of tight junction protein zonula-occludens 1 (*Zo1*) in the colon (Figure 5A) and the expression of 2 markers of ER stress, glucose-related protein precursor 78 (*Grp78*) and CCAAT-enhancer-binding protein homologous protein (*Chop*; Figure 5B, 5C). After 1 wk, no significant difference was observed. After 4 wk, ThD and Th+ had lower expression of *Zo1* in the colon ($P < 0.01$ vs. Th). Gene expression of occludin in the colon (data not shown) did not differ, but was correlated with *Zo1* (r

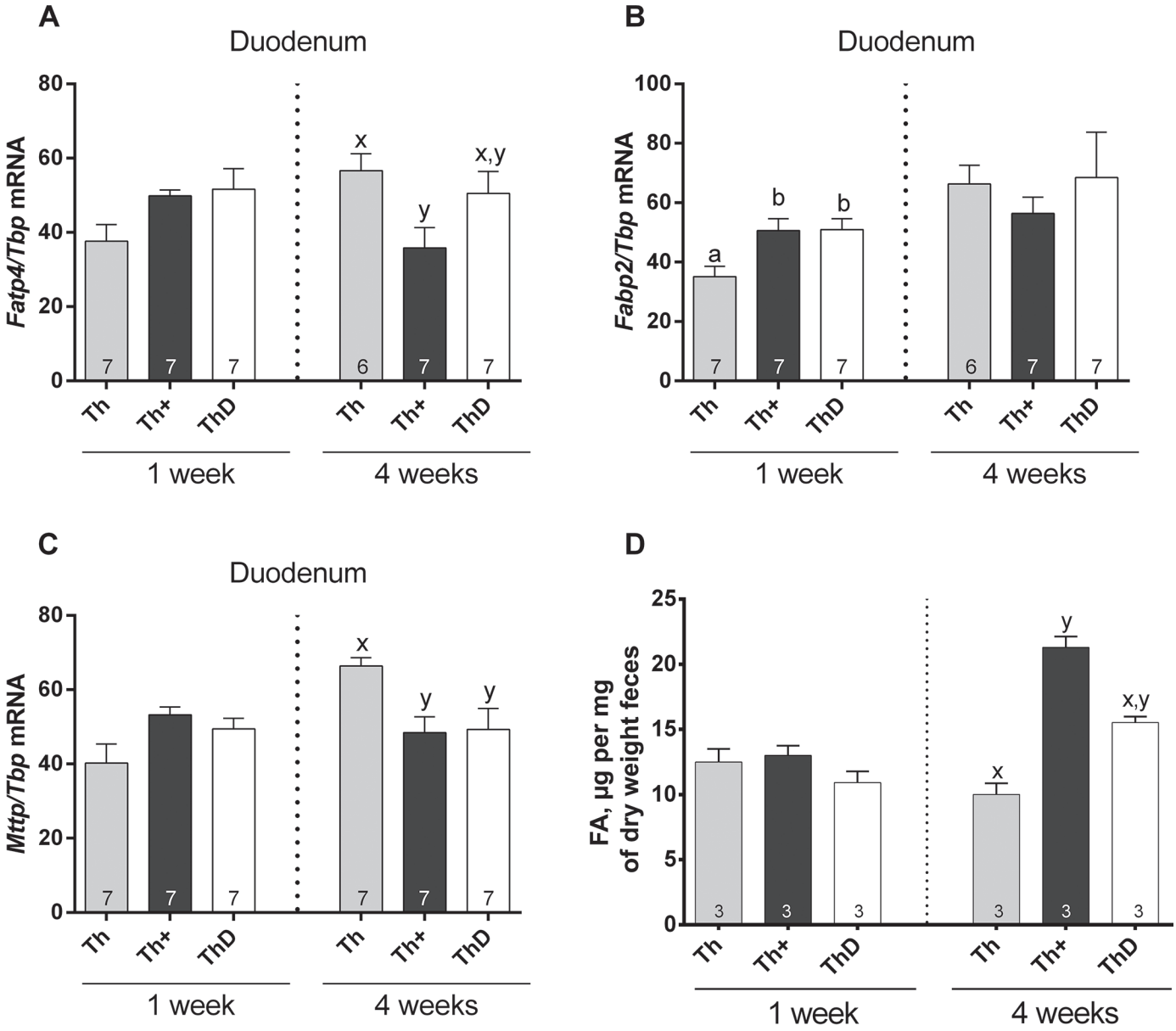

Figure 3. Expression of genes involved lipid metabolism in the duodenum and fecal lipid excretion of mice after 1 or 4 wk of diet with UHT creams differing by the type of process. (A) Fatty acid binding protein 2 (*Fabp2*), (B) fatty acid transport protein 4 (*Fatp4*), and (C) microsomal triglyceride transfer protein (*Mttp*) were quantified by quantitative real-time PCR. Values are normalized to the levels of TATA-binding protein (*Tbp*) mRNA. (D) Fecal lipids of mice after 1 or 4 wk of diet were obtained by GC. Number of mice in each group is indicated within the bars. Bars represent means ± SEM. Means without a common letter differ ($P < 0.05$): (a–c) after 1 wk of diet; (x–z) after 4 wk of diet. FA = fatty acid; Th = UHT cream treated by an indirect process; Th+ = UHT cream treated with an indirect process with higher temperature; ThD = UHT cream treated by a direct process.

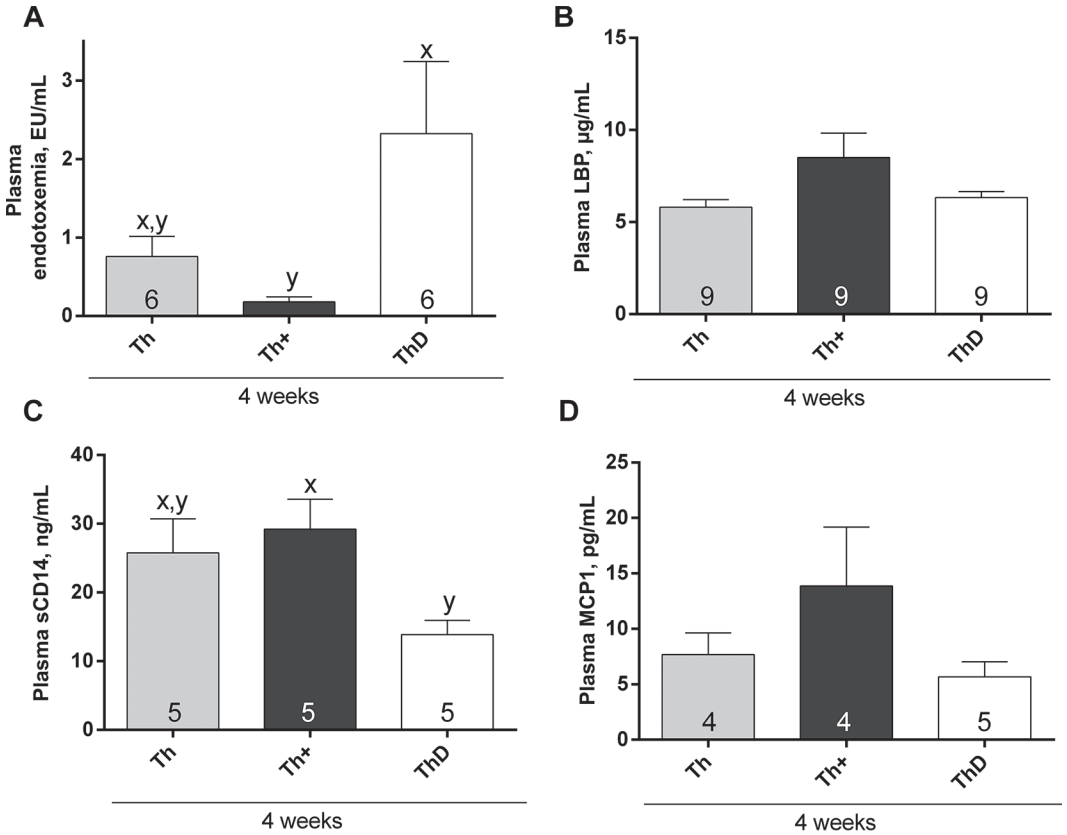

Figure 4. Assessment of intestine-derived inducers of inflammation through different plasma markers after 4 wk of diet with UHT creams differing by the type of process. (A) Plasma endotoxemia (LPS activity) expressed in endotoxin units (EU/mL); plasma concentrations of (B) lipopolysaccharide binding protein (LBP), (C) soluble cluster of differentiation 14 (sCD14), and (D) monocyte chemoattractant protein 1 (MCP1). Number of mice in each group is indicated within the bars (for LPS; Th+, n = 6). Bars represent means ± SEM. Means without a common letter differ ($P < 0.05$): (x–z) after 4 wk of diet. Th = UHT cream treated by an indirect process; Th+ = UHT cream treated with an indirect process with higher temperature; ThD = UHT cream treated by a direct process.

= 0.73, $P < 0.001$; Supplemental Table S2; https://doi.org/10.3168/jds.2018-14782). Regarding markers of cell stress in the colon, *Grp78* was lower with Th+ and ThD ($P < 0.05$ vs. Th) after 4 wk. *Chop* was also lower for ThD ($P < 0.01$ vs. Th) and for the Th+ group ($P < 0.0001$ vs. Th). Furthermore, gene expression of *Chop* was correlated with *Grp78* (r = 0.74, $P < 0.001$), *Zo1* (r = 0.71, $P < 0.001$), and occludin (r = 0.55, $P < 0.01$) and gene expression of *Grp78* was correlated with *Zo1* (r = 0.61, $P < 0.01$). To evaluate if the differences observed of ER and cell stress in colon could be associated with differences in EAT inflammation, we analyzed the expression of some markers of inflammation and macrophage infiltration in EAT after 4 wk of diet (Figure 5D–5I). No difference was observed for the gene expression of *Lbp* in EAT. The ThD mice showed lower expression of genes encoding macrophage markers cluster of differentiation 68, *Cd68* ($P < 0.05$ vs. Th and $P = 0.06$ vs. Th+) and *Cd14* ($P < 0.05$ vs. Th). We observed lower expression of pro-inflammatory genes for ThD such as tumor necrosis factor, *Tnfa* ($P < 0.01$ vs. Th), and *Mcp1* ($P < 0.01$ vs. Th). In EAT, several correlations were observed between mRNA *Cd14* and *Tnfa* (r = 0.79, $P < 0.001$), between gene expression of *Cd14* and *Mcp1* in EAT (r = 0.71, $P < 0.01$), and between *Cd68* and *Mcp1* (r = 0.76, $P < 0.01$). We observed a lower expression of *Il6* in ThD group versus Th+ ($P < 0.05$). The *Il6* and *Mcp1* gene expressions were also positively correlated (r = 0.71, $P < 0.01$) in EAT (Supplemental Table S2; https://doi.org/10.3168/jds.2018-14782).

Correlations Between Cream Properties and Metabolic Responses

In an attempt to state hypotheses for the differential effect of creams according to heating process, we performed correlation analyses between metabolic outcomes and some cream properties listed in Table 3. A correlation was observed between *Chop* and *Grp78*

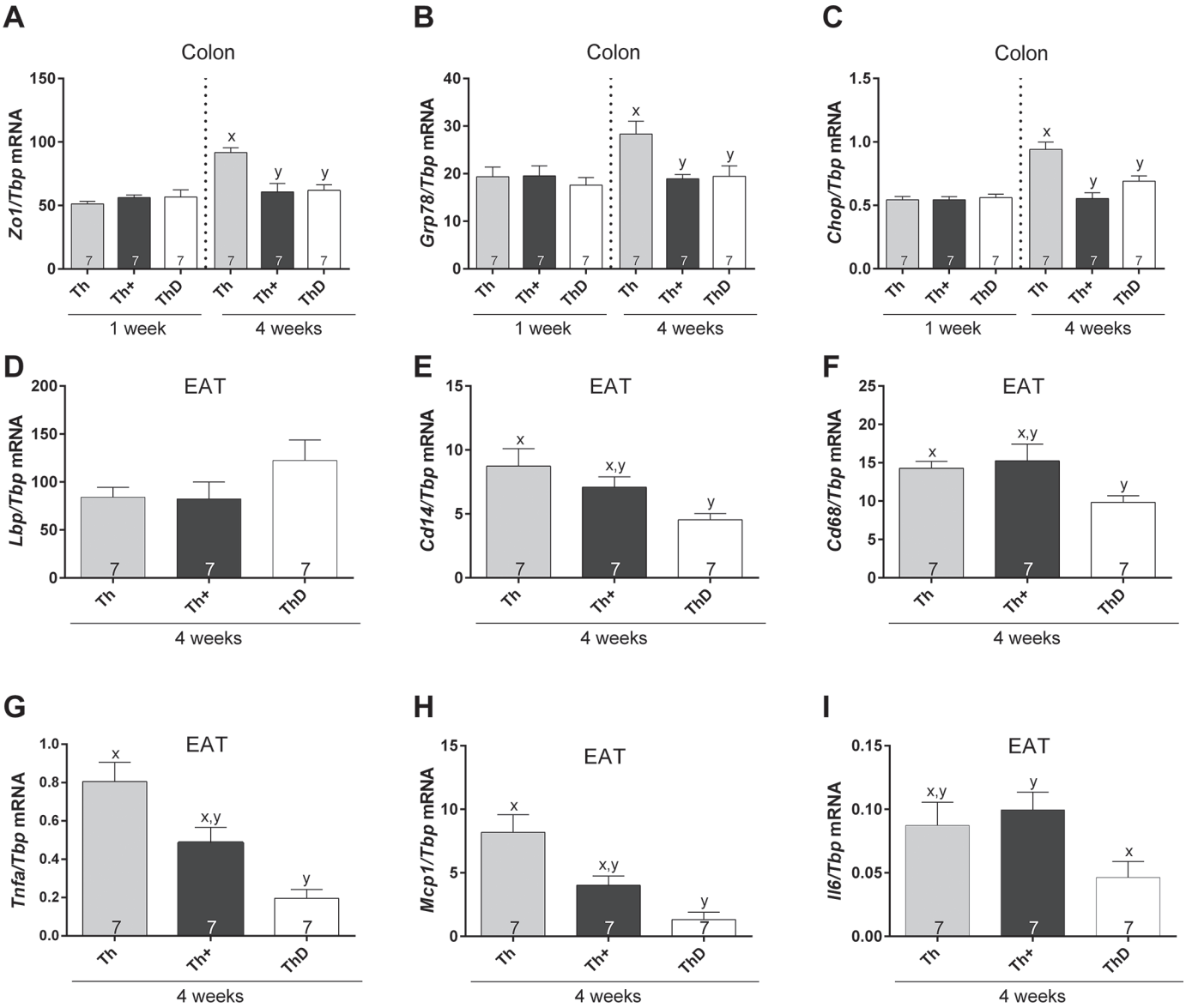

Figure 5. Gene expressions in the colon (A–C) of Zo-1, a tight junction protein, and on endoplasmic reticulum (ER) stress makers and in epididymal adipose tissue (EAT; D–I) of inflammation markers. Real-time quantitative (q)PCR assay of mRNA levels of (A) zonula occludens 1 (*Zo1*), (B) glucose-related protein precursor 78 (*Grp78*), and (C) CCAAT-enhancer-binding protein homologous protein (*Chop*) in the colon. qPCR assay of mRNA levels of (D) LPS-binding protein (*Lbp*), (E) cluster of differentiation 14 (*Cd14*), (F) cluster of differentiation 68 (*Cd68*), (G) tumor necrosis factor (*Tnf*), (H) monocyte chemoattractant protein 1 (*Mcp1*), and (I) *Il6* in EAT. Number of mice in each group is indicated within the bars. Bars represent means ± SEM. Means without a common letter differ ($P < 0.05$): (a–c) after 1 wk of diet, and (x–z) after 4 wk of diet. Values are normalized to the levels of TATA box-binding protein (*Tbp*) mRNA. Th = UHT cream treated by an indirect process; Th+ = UHT cream treated with an indirect process with higher temperature; ThD = UHT cream treated by a direct process.

expression in the colon after 4 wk of diet and cream particle/fat droplet size (namely $d_{4,3}$ water; respectively, r = 0.67, $P < 0.001$, and r = 0.62, $P < 0.01$). The gene expression of *Tnfa* in EAT was negatively correlated with soluble β-LG content of cream (r = −0.67, $P < 0.001$), which was also observed for *Mcp1* (r = −0.79, $P < 0.001$) and *Il6* (r = −0.51, $P < 0.05$). α-Lactalbumin content of cream was negatively correlated with *Tnfa* (r = −0.55, $P < 0.01$), *Mcp1* (r = −0.79, $P < 0.001$), and *Il6* (r = −0.52, $P < 0.05$) in EAT. Markers involved in the EAT inflammation (*Tnfa* and *Mcp1*) were correlated with aqueous phase proteins (respectively r = −0.61, $P < 0.01$ and r = −0.79, $P < 0.001$) and interface proteins (r = 0.78, $P < 0.0001$ and r = 0.79, $P < 0.001$; Supplemental Table S3; https://doi.org/10.3168/jds.2018-14782).

Table 3. Main physico-chemical parameters of creams modified by the type of UHT treatment

Physico-chemical parameter[1,2]	Type of UHT treatment[3]		
	Th	Th+	ThD
$d_{4,3}$ water, μm	5.6	4.8	4.5
Flocculation index (−)	2.15	1.78	1.67
Interfacial proteins, %	75	68	59
Aqueous phase-proteins, %	11	16	15
Soluble α-LA, g/kg	0.28	0.19	0.55
Soluble β-LG, g/kg	0.07	0.06	0.66

[1]Uncertainty values of physico-chemical parameters: volume-weighted diameter ($d_{4,3}$) in water, 5%; flocculation index, 5%; interfacial proteins, 10%; aqueous phase proteins, 2.5%; soluble α-LA, 3%; soluble β-LG, 3%.

[2]For other physicochemical parameters, see Supplemental Table S4 (https://doi.org/10.3168/jds.2018-14782).

[3]Type of UHT treatment: Th = UHT cream treated by an indirect process; Th+ = UHT cream treated with an indirect process with higher temperature; ThD = UHT cream treated by a direct process.

DISCUSSION

The dairy industry is interested in optimizing heat treatments to improve product organoleptic properties such as color (browning observed when the cream is excessively heated), taste (avoid the so-called cooked taste), and avoid emulsion destabilization. In this context, the advantage of using the direct heating process is due to a more rapid temperature rise and cooling of the product than with indirect heat treatment. The problem of overheating is thereby limited. The performance of different types of sterilization treatments have been studied on the physicochemical properties and on milk flavor (Bolling et al., 2005; Morin et al., 2007; Lee et al., 2017). Temperature can also affect fat particle size and consequently cream physical stability. When emulsions containing proteins, such as creams, are heated (especially by pasteurization or sterilization), proteins generally tend to aggregate and cause flocculation of the droplets (Leal-Calderon et al., 2007). Moreover, an increase in the temperature of the cream intensifies the thermal agitation, which then multiplies the collisions and therefore increases the likelihood of coalescence, as observed here where Th+ cream had a higher flocculation index than Th and ThD.

In this study we investigated, to our knowledge for the first time, the effects of different types of cream UHT heating processes on metabolic parameters in mice. Indeed, until now, few data reported the metabolic effects of different qualities of dairy products (e.g., according to cow diet), but not about products modified by the processing. We were also interested in how metabolic differences may be associated with changes in the physicochemical properties of the creams.

After 1 wk of diet, we showed that heating process modulated the expression of genes involved in lipid absorption in the duodenum. The ThD and Th+ groups had higher gene expression of *Fabp2* compared with the Th group. After 4 wk of diet, we observed a lower gene expression of *Mttp* in the duodenum for Th+ and ThD group in comparison to Th. *Fatp4* was less expressed for the Th+ group versus Th group and we observed a consistently higher excretion of fatty acid in feces. We had previously demonstrated that a diet with pasteurized pasture dairy cream resulted in higher expression of *Fatp4*, fatty acid absorption (*Fabp2*), diacylglycerol acyltransferase 1 (*Dgat1*), chylomicron formation (*Mttp*), and *Sar1b* than a standard cream (Benoit et al., 2014). The present results show, to our knowledge for the first time, that intestinal lipid absorption metabolism can also be modulated by heat processing of cream.

However, we did not observe differences in plasma TG and NEFA concentrations. Different physicochemical properties of dairy products such as viscosity, particle size distribution, and also protein content can influence the digestion and absorption of fat and thereby affect the lipemic response (Michalski, 2009; Michalski et al., 2013). The present study investigated short-term adaptation effects of the different creams. Because different gene expressions were observed, longer-term effects on lipemia parameters cannot be ruled out and should now be investigated.

A high-fat diet is also reported to increase inflammation of distal colon and is associated with low-grade inflammation that is accompanied by cellular stress (Gulhane et al., 2016). We studied the effects of the heating process on tight junction and ER stress in the colon. The ER is an organelle important for lipid metabolism and protein synthesis. The expression of *Grp78* was reported to increase during chronic inflammation in the intestine, inducing Crohn's disease and ulcerative colitis (Shkoda et al., 2007). Chop is a protein involved in the ER stress-mediated apoptosis pathway. The gene expressions of markers in colon such as *Zo1*, *Grp78*, and *Chop* was lower for Th+ and ThD. *Grp78* and *Chop* were more expressed in the Th-fed mice. The differences of droplet flocculation between creams could be indirectly involved in modification of ER stress. Indeed the size of milk fat droplet, and possibly their aggregation, can affect the kinetics of digestion (Armand et al., 1996; Bourlieu et al., 2015; Bourlieu and Michalski, 2015). Larger particle surface area increases the gastric hydrolysis rate and affects TG lipolysis in rat (Borel et al., 1994) and in human (Armand et al., 1999). Here, individual droplet sizes were similar ($d_{4,3}$ SDS) but their aggregates present in cream ($d_{4,3}$ water) were larger in Th group than for Th+ and ThD, and

smallest for ThD. Moreover, a correlation was observed between *Chop* and the diameter of the aggregates. Distal intestine ER stress could thus be affected by different amounts or kinetics of appearance of residual lipids due to differential fat aggregate sizes.

Importantly from a metabolic standpoint, we reveal that ThD group had lower expression in EAT after 4 wk of markers of inflammation and macrophage inflammation (Hotamisligil et al., 1993; Hofmann et al., 1994; Hotamisligil and Spiegelman, 1994; Kern et al., 2001; Pietilainen et al., 2006), namely *Cd14*, *Cd68*, *Il6*, and *Tnfa*. This suggests a lower inflammatory tone with direct heat treatment than with creams submitted to indirect heat treatment, which would be a beneficial metabolic effect of the ThD treatment in the present study that deserves further longer-term explorations. We observed a decreased *Mcp1* gene expression in the adipose tissue with ThD cream compared with Th and Th+ creams. This could be beneficial because a higher expression of *Tnfa* and *Mcp1* is associated with larger adipocytes (Skurk et al., 2007; Lecomte et al., 2016). The frequency distribution of adipocyte size in the EAT of mice fed the different creams would deserve investigation in a follow-up study to further understand the effects of UHT creams.

In the frame of high-fat diets, endotoxemia in plasma can be caused by LPS coabsorption with lipids, by an alteration of colon barrier due to the presence of residual dietary lipids in the distal intestine, or both (Lam et al., 2015). Such metabolic endotoxemia can participate in EAT inflammation (Kim et al., 2012; Laugerette et al., 2012; Lecomte et al., 2016). It is now well known that LBP and sCD14 (respectively, the transporter and soluble receptor of endotoxins in plasma) are good clinical markers that reflect longer term plasma exposures to endotoxin (Sun et al., 2010; Laugerette et al., 2012). In this study, the Th+ group had the lowest endotoxemia but the highest LBP and MCP-1 concentration in plasma and the highest liver score contrary to the ThD group, as best shown by the principal component analysis. This is consistent with the previously observed higher plasma LBP and EAT inflammation in mice fed a high-palm-oil diet, which also presented the lowest plasma endotoxemia versus diets with other oils (Laugerette et al., 2012). Notably, it has been suggested that a high hepatic uptake of LPS results in lower plasma endotoxemia but higher accumulation in the liver; this could explain why we observed here an onset of higher liver score in Th together with lower endotoxemia. In contrast, the lower EAT inflammation in ThD group paralleled with a lower plasma concentration of sCD14 after 4 wk. Therefore, it seems that different components of the LPS signaling cascade and consequences in both liver and EAT could be differentially affected by the cream heat processing, which deserves further investigation with longer term studies.

Regarding possible associations between cream composition and structure, the gene expression of *Tnfa* in EAT was negatively correlated with soluble whey protein contents (β-LG and α-LA), these proteins in their soluble form (unaggregated) being highest in ThD cream. A study showed that UHT treatment of milk, but not pasteurization, affects the postprandial kinetics of milk proteins in humans (Lacroix et al., 2008). Moreover, both β-LG and α-LA are prone to thermal denaturation (Huppertz et al., 2004), which induces slower intestinal transport as reported notably for β-LG (Mohan Reddy et al., 1988; Villamiel et al., 1997). Here, denaturation of β-LG and α-LA occurred due to the UHT process but was lowest for ThD cream as revealed by its highest soluble β-LG and α-LA content (i.e., their lower aggregation). Moreover, we observed a correlation between EAT inflammation markers and several markers of apparent protein alteration, including heat-induced protein interfacial absorption. Therefore, a further effect on EAT inflammation via a differential absorption of bioactive peptides cannot be ruled out and should now be elucidated. Moreover, β-LG denaturation can cause higher viscosity of cream (Jeurnink and De Kruif, 1993), which is observed here as Th+ had the highest viscosity and ThD cream the lowest. As viscosity can reduce gastric emptying time (Fruekilde and Hoy, 2004; Sanggaard et al., 2004), we can wonder whether excessive-heating-induced delayed transit time could contribute to global undesirable effects in the gut, liver, and adipose tissue, including by affecting the gut microbiota.

We revealed that direct UHT treatment, an innovative process to improve quality of cream and milk, resulted in less inflammation of adipose tissue, whereas indirect treatment at higher temperature tended toward more deleterious effects. However, despite the potential beneficial effects of direct heat treatment revealed here, such a process uses devices that are more complex and costly than indirect treatment (clean steam use, water removal from the product, no heating energy recuperation). Therefore, longer-term metabolic studies should be performed to support the beneficial effects and provide incentives of such investment for the dairy industry. In the current context of highlighting the dairy matrix effect and the need to increase knowledge on the health effects of dairy processing (Michalski and Januel, 2006; Thorning et al., 2017), this newly acquired knowledge can contribute to further enhance the nutritional quality of dairy products.

CONCLUSIONS

Our short-term in vivo study in mice shows, for the first time to our knowledge, that different types of UHT processes can modulate the early effects of a dairy-cream-enriched diet on the gut, liver, and adipose tissue. We also could relate some metabolic outcomes with heat-induced changes in cream composition or structure (or both). Regardless of the thermal processes, dairy creams can also contain different additives such as stabilizer and emulsifier: it is therefore also important to study their metabolic effect, which is the subject of the companion study (Milard et al., 2018) in this issue. This first study in a dietary rodent model fed a moderate high-fat diet may be completed by testing the effect of different fat contents and diet durations according to heating process in mice, and to test the concept in humans to verify the relevance of the findings for human nutrition.

ACKNOWLEDGMENTS

This study was funded by Sodiaal-Candia R&I (Research & Innovation Department, Paris, France). The authors thank CellImaP (Plateforme d'imagerie cellulaire, Federative Structure for Research 100, Dijon, France) for facilities in histological technics and Dominique Martel (SAFE, Augy, France) for his assistance in diet formulation. We thank Armelle Penhoat (CarMeN Laboratory, Villeurbanne, France) for fruitful discussions, Véronique Pierre (CarMeN Laboratory) for assistance in diet preparation, and the Functional Lipidomics Plaform of IMBL (Institut Multidisciplinaire de Biochimie des Lipides, Villeurbanne, France) for assistance in fatty acid analyses. We thank Gaëlle Pineau (CarMeN Laboratory) and Alexandre Debain (INSA, Villeurbanne, France) for their help with the animal study. Chloé Robert (MSci) is acknowledged for English language editing. S. Serieye and S. Bugeat are employees of Sodiaal-Candia R&I. M. C. Michalski (CarMeN Laboratory) received other research funding from Sodiaal-Candia R&I, CNIEL (the French Dairy Interbranch Organization, Paris, France), and Nutricia Research (Utrecht, the Netherlands) and has consulting activities for food and dairy companies. These activities had no link with the present study. Other authors declared no conflict of interest.

REFERENCES

Armand, M., P. Borel, B. Pasquier, C. Dubois, M. Senft, M. Andre, J. Peyrot, J. Salducci, and D. Lairon. 1996. Physicochemical characteristics of emulsions during fat digestion in human stomach and duodenum. Am. J. Physiol. 271:G172–G183.

Armand, M., B. Pasquier, M. Andre, P. Borel, M. Senft, J. Peyrot, J. Salducci, H. Portugal, V. Jaussan, and D. Lairon. 1999. Digestion and absorption of 2 fat emulsions with different droplet sizes in the human digestive tract. Am. J. Clin. Nutr. 70:1096–1106.

Astrup, A., M. L. Larsen, S. Stender, and J. Dyerberg. 2014. Effect of fats on cardiovascular disease prevention in Denmark. Ugeskr. Laeger 176.

Benoit, B., P. Plaisancie, A. Geloen, M. Estienne, C. Debard, E. Meugnier, E. Loizon, P. Daira, J. Bodennec, O. Cousin, H. Vidal, F. Laugerette, and M. C. Michalski. 2014. Pasture v. standard dairy cream in high-fat diet-fed mice: Improved metabolic outcomes and stronger intestinal barrier. Br. J. Nutr. 112:520–535.

Bolling, J. C., S. E. Duncan, W. N. Eigel, and K. M. Waterman. 2005. Processing effects on physicochemical properties of creams formulated with modified milk fat. J. Dairy Sci. 88:1342–1351.

Borel, P., M. Armand, B. Pasquier, M. Senft, G. Dutot, C. Melin, H. Lafont, and D. Lairon. 1994. Digestion and absorption of tube-feeding emulsions with different droplet sizes and compositions in the rat. JPEN J. Parenter. Enteral Nutr. 18:534–543.

Bourlieu, C., K. Bouzerzour, S. Ferret-Bernard, C. Le Bourgot, S. Chever, O. Ménard, A. Deglaire, I. Cuinet, P. Le Ruyet, C. Bonjhomme, D. Dupont, and Le-Huërou-Luron.. 2015. Infant formula interface and fat source impact on neonatal digestion and gut microbiota. Eur. J. Lipid Sci. Technol. 117:1500–1512.

Bourlieu, C., and M. C. Michalski. 2015. Structure-function relationship of the milk fat globule. Curr. Opin. Clin. Nutr. Metab. Care 18:118–127.

Chen, G. C., I. M. Szeto, L. H. Chen, S. F. Han, Y. J. Li, R. van Hekezen, and L. Q. Qin. 2015. Dairy products consumption and metabolic syndrome in adults: Systematic review and meta-analysis of observational studies. Sci. Rep. 5:14606.

Folch, J., M. Lees, and G. H. Sloane Stanley. 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226:497–509.

Fruekilde, M. B., and C. E. Hoy. 2004. Lymphatic fat absorption varies among rats administered dairy products differing in physiochemical properties. J. Nutr. 134:1110–1113.

Gulhane, M., L. Murray, R. Lourie, H. Tong, Y. H. Sheng, R. Wang, A. Kang, V. Schreiber, K. Y. Wong, G. Magor, S. Denman, J. Begun, T. H. Florin, A. Perkins, P. O. Cuiv, M. A. McGuckin, and S. Z. Hasnain. 2016. High fat diets induce colonic epithelial cell stress and inflammation that is reversed by IL-22. Sci. Rep. 6:28990.

Hofmann, C., K. Lorenz, S. S. Braithwaite, J. R. Colca, B. J. Palazuk, G. S. Hotamisligil, and B. M. Spiegelman. 1994. Altered gene expression for tumor necrosis factor-alpha and its receptors during drug and dietary modulation of insulin resistance. Endocrinology 134:264–270.

Hotamisligil, G. S., N. S. Shargill, and B. M. Spiegelman. 1993. Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. Science 259:87–91.

Hotamisligil, G. S., and B. M. Spiegelman. 1994. Tumor necrosis factor alpha: A key component of the obesity-diabetes link. Diabetes 43:1271–1278.

Huppertz, T., P. F. Fox, and A. L. Kelly. 2004. High pressure-induced denaturation of alpha-lactalbumin and beta-lactoglobulin in bovine milk and whey: A possible mechanism. J. Dairy Res. 71:489–495.

Huth, P. J., and K. M. Park. 2012. Influence of dairy product and milk fat consumption on cardiovascular disease risk: A review of the evidence. Adv. Nutr. 3:266–285.

International Dairy Federation (IDF). 2013. Bulletin of the IDF No. 470/ 2013: The World Dairy Situation 2013. IDF, Brussels, Belgium.

Jeurnink, T., and K. De Kruif. 1993. Changes in milk on heating: Viscosity measurements. J. Dairy Res. 60:139–150.

Kern, P. A., S. Ranganathan, C. Li, L. Wood, and G. Ranganathan. 2001. Adipose tissue tumor necrosis factor and interleukin-6 expression in human obesity and insulin resistance. Am. J. Physiol. Endocrinol. Metab. 280:E745–E751.

Kim, K. A., W. Gu, I. A. Lee, E. H. Joh, and D. H. Kim. 2012. High fat diet-induced gut microbiota exacerbates inflammation and obesity in mice via the TLR4 signaling pathway. PLoS One 7:e47713.

Lacroix, M., C. Bon, C. Bos, J. Leonil, R. Benamouzig, C. Luengo, J. Fauquant, D. Tome, and C. Gaudichon. 2008. Ultra high temperature treatment, but not pasteurization, affects the postprandial kinetics of milk proteins in humans. J. Nutr. 138:2342–2347.

Lam, Y. Y., C. W. Ha, J. M. Hoffmann, J. Oscarsson, A. Dinudom, T. J. Mather, D. I. Cook, N. H. Hunt, I. D. Caterson, A. J. Holmes, and L. H. Storlien. 2015. Effects of dietary fat profile on gut permeability and microbiota and their relationships with metabolic changes in mice. Obesity (Silver Spring) 23:1429–1439.

Lamarche, B. 2008. Review of the effect of dairy products on non-lipid risk factors for cardiovascular disease. J. Am. Coll. Nutr. 27:741S–746S.

Laugerette, F., J. P. Furet, C. Debard, P. Daira, E. Loizon, A. Geloen, C. O. Soulage, C. Simonet, J. Lefils-Lacourtablaise, N. Bernoud-Hubac, J. Bodennec, N. Peretti, H. Vidal, and M. C. Michalski. 2012. Oil composition of high-fat diet affects metabolic inflammation differently in connection with endotoxin receptors in mice. Am. J. Physiol. Endocrinol. Metab. 302:E374–E386.

Laugerette, F. P. G., C. Vors, and M. C. Michalski. 2015. Endotoxemia analysis by the Limulus Amoebocyte Lysate Assay in different mammal species used in metabolic studies. J. Anal. Bioanal. Tech. 6:251.

Leal-Calderon, F., V. Schmitt, and J. Bibette. 2007. Emulsion Science. Basic Principles. 2nd ed. Springer-Verlag, New York, NY.

Lecomte, M., L. Couedelo, E. Meugnier, P. Plaisancie, M. Letisse, B. Benoit, L. Gabert, A. Penhoat, A. Durand, G. Pineau, F. Joffre, A. Geloen, C. Vaysse, F. Laugerette, and M. C. Michalski. 2016. Dietary emulsifiers from milk and soybean differently impact adiposity and inflammation in association with modulation of colonic goblet cells in high-fat fed mice. Mol. Nutr. Food Res. 60:609–620.

Lee, A. P., D. M. Barbano, and M. A. Drake. 2017. The influence of ultra-pasteurization by indirect heating versus direct steam injection on skim and 2% fat milks. J. Dairy Sci. 100:1688–1701.

Michalski, M. C. 2009. Specific molecular and colloidal structures of milk fat affecting lipolysis, absorption and postprandial lipemia. Eur. J. Lipid Sci. Technol. 111:413–431.

Michalski, M. C., C. Genot, C. Gayet, C. Lopez, F. Fine, F. Joffre, J. L. Vendeuvre, J. Bouvier, J. M. Chardigny, and K. Raynal-Ljutovac. 2013. Multiscale structures of lipids in foods as parameters affecting fatty acid bioavailability and lipid metabolism. Prog. Lipid Res. 52:354–373.

Michalski, M.-C., and C. Januel. 2006. Does homogenization affect the human health properties of cow's milk? Trends Food Sci. Technol. 17:423–437.

Milard, M., F. Laugerette, S. Bugeat, P. Plaisancié, M. Létisse, E. Meugnier, E. Loizon, A. Durand, C. Buisson, A. Géloën, S. Serieye, and M.-C. Michalski. 2018. Metabolic effects in mice of cream formulation: Addition of both thickener and emulsifier does not alter lipid metabolism but modulates mucus cells and intestinal endoplasmic reticulum stress. J. Dairy Sci. 101:10649–10663. https://doi.org/10.3168/jds.2018-14783.

Mohan Reddy, I., N. Kella, and J. Kinsella. 1988. Structural and conformational basis of the resistance of 13-lactoglobulinto peptic and chymotryptic digestion. J. Agric. Food Chem. 36:737–741.

Morin, P., R. Jimenez-Flores, and Y. Pouliot. 2007. Effect of processing on the composition and microstructure of buttermilk and its milk fat globule membranes. Int. Dairy J. 17:1179–1187.

Oosting, A., N. van Vlies, D. Kegler, L. Schipper, M. Abrahamse-Berkeveld, S. Ringler, H. J. Verkade, and E. M. van der Beek. 2014. Effect of dietary lipid structure in early postnatal life on mouse adipose tissue development and function in adulthood. Br. J. Nutr. 111:215–226.

Pietilainen, K. H., K. Kannisto, E. Korsheninnikova, A. Rissanen, J. Kaprio, E. Ehrenborg, A. Hamsten, and H. Yki-Jarvinen. 2006. Acquired obesity increases CD68 and tumor necrosis factor-alpha and decreases adiponectin gene expression in adipose tissue: A study in monozygotic twins. J. Clin. Endocrinol. Metab. 91:2776–2781.

Sanggaard, K. M., J. J. Holst, J. F. Rehfeld, B. Sandstrom, A. Raben, and T. Tholstrup. 2004. Different effects of whole milk and a fermented milk with the same fat and lactose content on gastric emptying and postprandial lipaemia, but not on glycaemic response and appetite. Br. J. Nutr. 92:447–459.

Shkoda, A., P. A. Ruiz, H. Daniel, S. C. Kim, G. Rogler, R. B. Sartor, and D. Haller. 2007. Interleukin-10 blocked endoplasmic reticulum stress in intestinal epithelial cells: Impact on chronic inflammation. Gastroenterology 132:190–207.

Skurk, T., C. Alberti-Huber, C. Herder, and H. Hauner. 2007. Relationship between adipocyte size and adipokine expression and secretion. J. Clin. Endocrinol. Metab. 92:1023–1033.

Sun, L., Z. Yu, X. Ye, S. Zou, H. Li, D. Yu, H. Wu, Y. Chan, J. Dore, K. Clément, F. Hu, and X. Lin. 2010. SA marker of endotoxemia is associated with obesity and related metabolic disorders in apparently healthy Chinese. Diabetes Care 33:1925–1932.

Thorning, T. K., H. C. Bertram, J. P. Bonjour, L. de Groot, D. Dupont, E. Feeney, R. Ipsen, J. M. Lecerf, A. Mackie, M. C. McKinley, M. C. Michalski, D. Remond, U. Riserus, S. S. Soedamah-Muthu, T. Tholstrup, C. Weaver, A. Astrup, and I. Givens. 2017. Whole dairy matrix or single nutrients in assessment of health effects: Current evidence and knowledge gaps. Am. J. Clin. Nutr. 105:1033–1045.

Tunick, M. H., D. X. Ren, D. L. Van Hekken, L. Bonnaillie, M. Paul, R. Kwoczak, and P. M. Tomasula. 2016. Effect of heat and homogenization on in vitro digestion of milk. J. Dairy Sci. 99:4124–4139.

Villamiel, M., R. Lopez-Fandino, N. Corzo, and A. Olano. 1997. Denaturation of beta-lactoglobulin and native enzymes in the plate exchanger and holding tube section during continuous flow pasteurization of milk. Food Chem. 58:49–52.