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ORIGINAL CONTRIBUTION

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The supramolecular structure of milk fat influences plasma triacylglycerols and fatty acid profile in the rat

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Summary *Background* The digestion fate of milk fat depending on its supramolecular structure for a given dairy product composition has rarely been studied. *Aim of the study* To highlight differences of lipid digestion, we measured (i) the plasma triacylglycerol and cholesterol concentrations and (ii) the total plasma fatty acid profile of fasted rats force-fed with different dairy preparations; the three creams and the unemulsified preparation had a similar composition with different and controlled fat suprastructures. *Methods* All preparations, manufactured in the laboratory from a given milk batch, contained 205 ± 3 g·kg^{-1} fat that was either fed (i) unemulsified consecutively to the skim milk phase, or as a cream with the following fat globule structures: (ii) native milk fat globules of ~4 µm covered with the native milk fat globule membrane (MFGM), (iii) small native milk fat globules of ~2 µm selected from the latter by microfiltration and covered by the MFGM, or (iv) fine homogenized fat droplets of ~1 µm covered mainly with caseins. *Results* The plasma triacylglycerol appearance was delayed for the creams compared with the rapid onset for the unemulsified preparation. At 90 and 180 min after feeding, the plasma triacylglycerol enrichment was significantly lower for the homogenized cream than for the unemulsified preparation. At 120 min after feeding, triacylglycerol enrichment was significantly lower for each cream than for the unemulsified preparation. At 180 min after feeding, the plasma relative enrichment in C12, C14, C15, C16 and C18:1 *n*-9 fatty acids was significantly lower for the homogenized cream than for unemulsified fat and regular cream. *Conclusions* Global lipid digestion based on plasma triacylglycerol enrichment and relative enrichments in some fatty acids was decreased with small homogenized milk fat droplets compared to unemulsified milk fat. These data show that dairy products with the same composition but varying in fat supramolecular structure result in different kinetics of lipid digestion, which could be of health concern.

Key words milk fat – cream – droplet size – digestion – plasma triacylglycerol – plasma fatty acid

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Abbreviations

AMF	Anhydrous milk fat
d_{32}	Sauter volume-surface average diameter
d_{43}	Volumic average diameter
F	Fat surface area
FA	Fatty acid
HC	Homogenized milk fat droplet-Cream
MFGM	Milk fat globule membrane
ρ	Milk fat density
RC	Regular native milk fat globule-Cream
S	Specific surface area
SC	Small native milk fat globule-Cream
TAG	Triacylglycerol
UP	Unemulsified preparation composed of skim milk and AMF

Introduction

Milk fat has received much attention as a major source of lipids in the Western diet but also for its controversial role in contribution to human health and disease [1]. Triacylglycerols (TAG) represent 98 % of total milk fat [1, 2]; their fatty acid (FA) composition is complex, with over 400 distinct FA detected [1]. The nutritional properties of butterfat were found to be affected by its TAG and FA composition, which can be modulated by fractionation technology [3]. Moreover, the TAG structure can affect the bioavailability of FA, whether they are esterified on the *sn*-1, 2 or 3 position of the TAG glycerol backbone [4]. Regarding its supramolecular structure, fat is present in milk in the form of globules of ~4 μm surrounded by a native trilayered biological membrane composed mainly of phospholipids, glycoproteins and enzymes: the milk fat globule membrane (MFGM) [2]. This unique emulsified structure can be altered by dairy processes such as homogenization, leading to milk fat droplets of < 1 μm covered by caseins. Natural fat globules can also coalesce to form unemulsified fat inclusions in the proteinaceous matrix of some cheeses or to form the continuous fat phase in butter [2, 5–7].

Digestion of dietary fats begins in the stomach and is completed in the small intestine where final absorption occurs. Lingual (in rats), gastric (in humans) and pancreatic lipases (in rats and humans) act on emulsified substrates [8, 9]. Therefore, the specific surface area of the lipid droplets, which is directly related to the droplet size of dietary emulsions, plays an essential role in lipolysis [8]. In addition, droplet stability and lipase adsorption depend on the nature of the surfactant at the droplet interface.

The impact of milk fat composition and TAG structure on its complex profiles of gastrointestinal digestion and absorption has been widely studied [10–12]. The specific short- and medium-chain FA of milk fat, predominantly located at the *sn*-3 position, are subjected to gastric lipolysis [10]. Butter was shown to result in a lower postprandial lipemia and chylomicron accumulation in the circulation of humans than vegetable oils after consumption of a mixed meal [12]. However, only few studies to date about the nutritional properties of milk fat take the structure of the dairy food product into account. If butter is clearly hypercholesterolemic as a unique source of dietary fat [13], questions remain about the digestion of milk fat under different suprastructures as appear in a mixed diet. Two studies recently suggest that hard cheese may increase cholesterol less than butter [14], while the consumption of dairy products would lead to a more favorable LDL size distribution [15, 16]. We recently performed a $^{13}CO_2$ breath test study in the rat with artificial milk fat emulsions of controlled composition and structure [17]. This study supports the concept that dairy products with different fat suprastructures are digested and metabolized differently, as indicated by overall triglyceride metabolism. However, there is still no information about the lipid assimilation from real and accurately characterized dairy products, in which the supramolecular structure of milk fat varies.

In this study, we show the effect of the supramolecular structure of milk fat on its digestion kinetics in rats. Our objective was to characterize differences in the time course of TAG, cholesterol and FA enrichment in plasma of rats force-fed dairy preparations containing the same milk fat but with various physico-chemical structures. The unique feature of our approach is the controlled manufacture of dairy creams with the same composition but different types of lipid droplets, together with the corresponding unemulsified milk fat. The rat model was used since the steps involved in fat digestion are basically comparable with those in humans [18].

Materials and methods

Materials

Raw whole milk was purchased in September at Compagnie Laitière Européenne (Montauban-de-Bretagne, France). Tritridecanoin (99 %) was from Sigma (Saint-Quentin-Fallavier, France). A stock solution of tritridecanoin at 0.54 $\mu g \cdot \mu L^{-1}$ in chloroform was kept at –20 °C. Boron trifluoride solution (BF_3, 14 % in methanol) was from Fluka, solvents from Carlo Erba (Val-de-Reuil, France) and K_2CO_3 from Prolabo (Fontenay-sous-bois, France).

Dairy preparations containing milk fat with different supramolecular structures

Four different dairy preparations were produced, with the same composition but varying in milk fat supramolecular structure: (i) unemulsified fat as appear in butter; fat globules of (ii) ~2 µm or (iii) ~4 µm coated with the MFGM as appear in raw milk and some cheeses [5, 19]; (iv) fat droplets of < 1 µm coated with caseins as appear in homogenized milk, yoghurts and some cheeses [2, 5]. The preparations were manufactured from a single milk batch using different technological steps as described in Fig. 1. Milk was creamed to obtain a stock cream and skim milk. The latter was used to adjust the fat content of the final creams and to feed rats with an unemulsified preparation (see below). Stock cream was diluted to obtain the regular native fat globule cream (RC) at 20.5 ± 0.3 wt% fat (Table 1). Part of this cream was homogenized in order to obtain a homogenized fat droplet cream (HC). Part of the initial milk was microfiltered [19, 20] to select the small sized globules in the permeate that was creamed and whose fat content was adjusted to obtain the small native fat globule cream (SC) at 20.5 ± 0.3 wt% fat. Finally, in order to feed rats with an unemulsified fat product, anhydrous milk fat (AMF) was prepared (Fig. 1): part of the concentrated stock regular cream was churned, the butter was collected, washed, melted and centrifuged in order to obtain AMF as the supernatant. In the case of the unemulsified preparation (UP), skim milk was fed to the rats first, followed by AMF, to ensure the same composition as the creams. Products contained 0.6 mL fat per given food portion of 2.5 mL. They were pasteurized and kept at 4 °C. Pasteurization may result in adsorption of some whey proteins around the fat globule interface [21]; however, we checked that the droplet size did not vary (results not shown).

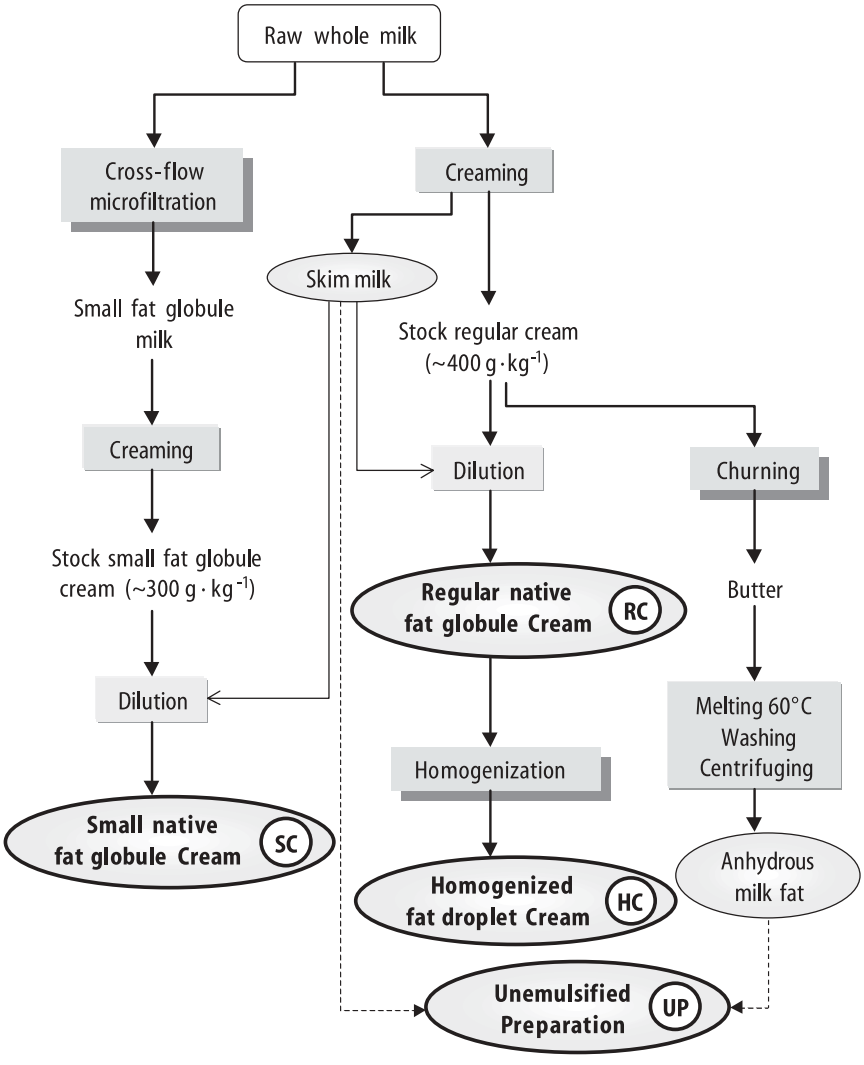

Fig. 1 Technological scheme describing the method used to obtain dairy products with various milk fat suprastructures from a single milk batch: regular native fat globule cream (RC), small native fat globule cream (SC), homogenized fat droplet cream (HC) and unemulsified preparation (UP) composed of anhydrous milk fat and skim milk. See text for further explanation

Table 1 Bulk composition (g per 100 g product) and total fatty acid profile (mg per 100 mg fatty acid) of the dairy products fed to rats

	Mean ± SEM	
Total nitrogen matter	2.85 ± 0.03	
Non-casein nitrogen	0.56 ± 0.04	
Nonprotein nitrogen	0.17 ± 0.00	
Dry matter	27.4 ± 0.3	
Fat	20.5 ± 0.3	
Lactose	3.52 ± 0.30	
Calcium		
Total	0.081 ± 0.002	
Soluble	0.031 ± 0.003	
Fatty acid	**Mean ± SEM**	**Literature value***
8:0	1.0 ± 0.1	1.6 ± 0.2
10:0	2.4 ± 0.1	4.9 ± 0.2
12:0	3.9 ± 0.4	5.0 ± 0.2
14:0	14.2 ± 0.3	13.6 ± 0.2
14:1	1.9 ± 0.1	0.5 ± 0.0
15:0	1.4 ± 0.0	1.5 ± 0.1
16:0	37.4 ± 0.7	33.1 ± 0.6
16:1 *n*-9	0.1 ± 0.1	0.2 ± 0.0
16:1 *n*-7	2.2 ± 0.2	2.1 ± 0.1
17:0	0.6 ± 0.1	0.8 ± 0.1
18:0	8.4 ± 0.6	8.8 ± 0.3
18:1 *n*-9 *cis*	23.6 ± 0.3	19.2 ± 0.4
18:1 *n*-7	0.3 ± 0.2	0.6 ± 0.1
18:2 *n*-6 *cis*	1.4 ± 0.1	1.8 ± 0.2
18:3 *n*-3	0.4 ± 0.0	0.3 ± 0.0
20:1 *n*-9	0.5 ± 0.1	0.1 ± 0.0
21:0	0.1 ± 0.0	–
22:1 *n*-9	0.1 ± 0.1	–

* Milk fat composition, adapted from Jensen [39]

Particle size measurements

The method for particle size analysis by laser light scattering using a Mastersizer2000 (Malvern, UK) has been described previously [22]. The software calculated the following: the volumic average diameter d_{43}, the volume-surface average diameter d_{32} and the specific surface area $S = 6 \cdot \rho^{-1} \cdot d_{32}^{-1}$ (ρ = milk fat density). We could thus calculate the fat surface area contained in 2.5 mL of cream: $F = 0.546 \cdot S$. Details of the calculations are given elsewhere [23].

Experimental animals

Male Wistar rats (260–300 g; Harlan, Gannat, France) were housed in a thermostatized room (24 ± 1°C) with a 12-h light cycle and had free access to food (AO3, UAR, 3.2 kcal · g^{-1}) and water. Experiments were carried out according to the ethical principles laid down by the EU Council Directives for care and use of laboratory animals (N 02889). A polyethylene catheter (EO 3403, Biotrol, Villeron, France), to be used for blood sampling, was inserted into the left carotid under halothane anesthesia. It was exteriorized through an incision in the nape of the neck and filled with citrate saline. Experiment was conducted at least four to five days after surgery. Animals were deprived of food the night before the experiment.

Each experimental day, the rats assigned to the given treatment were fed the same preparation to be sure that they were fed with the same structure (aging and tempering steps would have destabilized creams). RC (least stable preparation) was fed first and UP was fed last. The number of rats assigned to each treatment was: (i) 8 rats for HC, (ii) 4 rats for SC due to the unfortunate loss of part of this cream before the experiment, (iii) 7 rats for RC due to a catheter problem with 1 rat during the experiment, and (iv) 10 rats for UP since we could use 2 additional rats left. All the experiments started at the same time in the morning and were performed on awake and unrestrained animals. Blood samples (250 µL) were collected at time –10 min before forced feeding as basal value. At time 0, rats were force-fed with a single preparation intragastrically using a syringe equipped with a curved canula. For RC, SC and HC, a single 2.5 mL dose was force-fed at 40 °C. The UP was force-fed as follows: 0.6 mL of melted AMF at 40 °C, followed by 1.9 mL of skim milk at 40 °C. Thus, rats received 546 mg of fat.

Immediately after the force-feeding, rats were contained inside individual glass chambers through which air was pumped at a constant flow rate. Blood samples of ~250 µL were taken at time 60, 180 and 360 min for plasma TAG, cholesterol and FA analyses. Blood samples of ~120 µL were collected at time 30, 90, 120 and 270 min for plasma TAG and cholesterol measurements only. Blood samples were collected on heparin-containing tubes and centrifuged at 1000 rpm, 1 min. Aliquots of plasma were promptly frozen and stored at –80 °C.

Plasma triacylglycerol and cholesterol measurements

Plasma TAG were measured with the Triglyceride PAP Kit (Biomérieux, Marcy l'Etoile, France) using culture plates (Corning Costar 3599, 96 well cell culture cluster sterile). The Biomerieux standard solution and plasma samples were diluted 5-fold and 10 µL of water was added to 40 µL of diluted plasma. The reaction was initiated by adding 200 µL of Biomérieux reagent. The plate was maintained on a slow-agitating table for 30 min at room temperature. The optical density (OD) was read simultaneously at 505 nm using a spectrophotometer PowerWave X (Biotek Instruments; KC4 software). The

free glycerol in plasma was measured with the Glycerol UV-method (R-Biopharm/Boehringer, Mannheim, Germany), with 50 µL glycerol samples and solutions 2 and 3 from the kit diluted 10-fold; OD was read at 340 nm as described above. The real plasma TAG concentration was calculated as: Plasma Triacylglycerol = Triglyceride PAP result – Glycerol result. Plasma total cholesterol was measured using the Cholesterol RTU Kit (Biomérieux). To 20 µL of plasma diluted 5-fold, 30 µL water was added. Subsequent steps were the same as with the Triglyceride PAP Kit.

Fatty acid measurements

A total of 60 µL of tritridecanoin internal standard solution was added to 60 µL of plasma, or 60 µL of cream diluted 100-fold, or 13 mg of AMF. Then, 500 µL of toluene: methanol (2:3) was added. Tubes were kept at 4 °C under N_2 overnight. Then, 500 µL of BF_3 solution was added before heating at 95 °C, 2 h in a dry bath. Tubes were cooled on ice; 1.5 mL K_2CO_3 (10 %) and 1.8 mL isooctane were added [24]. After centrifugation, upper phases were concentrated under N_2. Fatty acid methyl esters were measured on a Hewlett Packard gas chromatograph (HP 6890, Agilent Technologies, Les Ullis, France) with a flame ionization detector, a programmed temperature injector and a fused silica capillary column coated with stabilized poly-90 % bis-cyanopropyl/10 % cyanopropylphenyl siloxane (60 m × 0.25 mm; film thickness 0.25 µm, Supelco 24111-SP 2380, Supelco Inc., Bellefonte, USA). The initial temperature of the 1 µL-splitless injection was 230 °C. The oven temperature was 57 °C for 2 min, increased from 57 °C to 130 °C at 20 °C · min^{-1} and remained for 5 min, increased to 210 °C at 1.5 °C · min^{-1}, and finally to 250 °C at 10 °C · min^{-1}. The detector temperature was 270 °C, under hydrogen flux (30 mL · min^{-1}); the carrier gas was helium (160 kPa).

Statistical analysis

Results are expressed as means ± SEM. Since the variances were heterogeneous and data unbalanced, the non-parametric Kruskal-Wallis test was mandatory. All pairwise comparisons were performed using this test by the Conover-Inman procedure, using the StatsDirect software (Sale, UK). *P*-values of < 0.05 were considered to be significant.

Results

Table 1 shows that there was no difference in the composition of each dairy preparation. Fig. 2 presents the fat droplet size distribution of the creams (RC, SC, HC) and their particle size parameters. The globules in RC were larger than the droplets in HC, resulting in a 10-fold larger fat interface for HC. Moreover, the droplets were coated with different molecules: MFGM or casein + whey proteins [25]. The fatty acid profile of the fat contained in RC, SC, HC and UP is shown Table 1 and is consistent with the literature (variations appear due to seasonal feeding and breed [2]). Since RC, HC and UP were made from the same milk batch, they had the same FA composition. Only SC contained 2 % more C12, 0.3 % more C16, 2.5 % less C18 and 3.2 % less C18:1 than RC, in agreement with Briard et al. [26]. However, the rats fed with SC were not subjected to extensive plasma FA analysis due to few individuals (some of our SC samples were lost before the experiment).

Fig. 3 A shows the plasma TAG content after ingestion of the different preparations. Fig. 3 B presents the evolution of plasma total cholesterol. The TAG appearance was delayed for the creams compared with the rapid onset observed for UP. Besides, the UP curve presents a sharp peak, whereas most cream curves present a small peak with a hint of a second peak; this is consistent with our previous ^{13}C breath test study [17]. At 90 and 180 min after feeding, the difference of plasma TAG over baseline (so-called TAG enrichment) of rats fed with HC was significantly lower than that of rats fed with UP ($P < 0.05$). At 120 min, the plasma TAG enrichment of rats fed with either one of the creams was significantly lower than that of rats fed with UP ($P < 0.05$). The initial fat surface area F in the different dairy preparations is greater when fat droplets are smaller (F is propor-

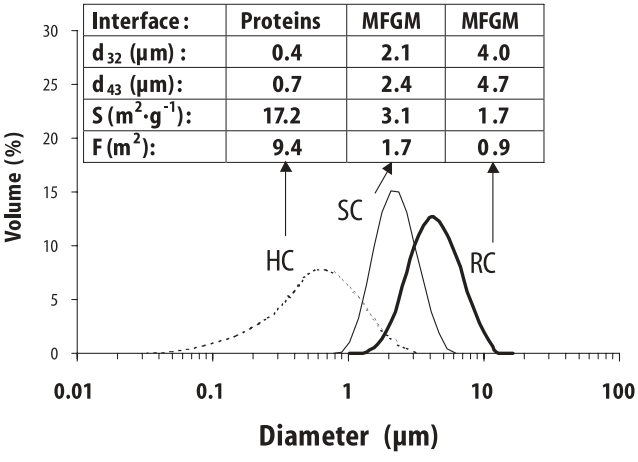

Fig. 2 Particle size distribution of the creams fed to rats, prepared with the process described in Fig. 1: regular native fat globule cream (RC, thick line), small native fat globule cream (SC, thin line), homogenized fat droplet cream (HC, dotted line). The corresponding interface composition (natural milk fat globule membrane, MFGM, or caseins + whey proteins [2, 25]) and particle size parameters are shown: volume-surface average diameter (d_{32}), volumic average diameter (d_{43}), specific surface area (S) and fat interface contained in 2.5 mL of cream fed to rats (F). Means in each row are significantly different ($P < 0.01$) and consistent with the literature [2, 5, 29]

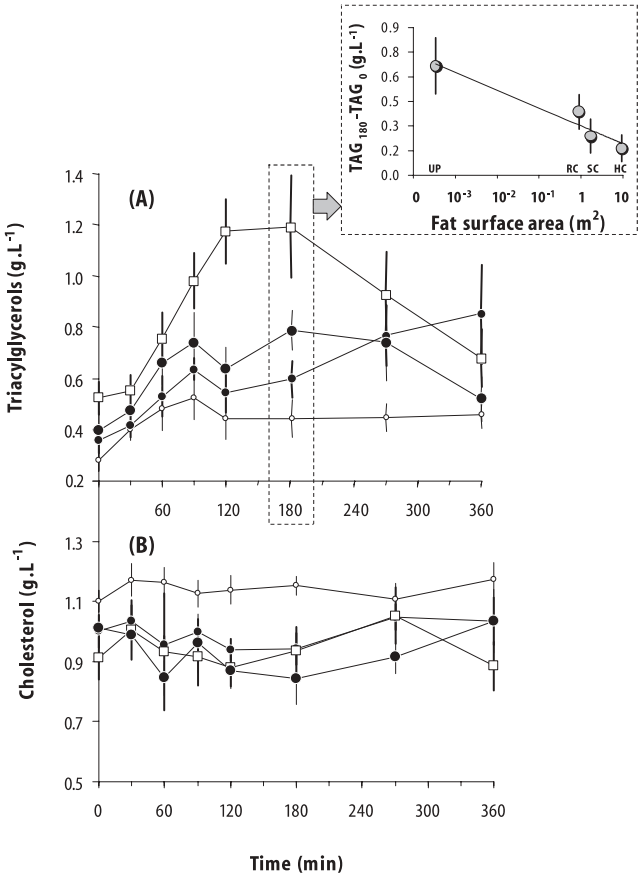

Fig. 3 **A** Plasma triacylglycerols (TAG); **B** Plasma total cholesterol of rats as a function of time after feeding one of the dairy preparations. (□) Unemulsified preparation (UP-anhydrous milk fat + skim milk-, $n = 10$); (●) regular native fat globule cream (RC, $n = 7$); (•) small native fat globule cream (SC, $n = 4$); (○) homogenized fat droplet cream (HC, $n = 8$). Insert shows the corresponding plasma TAG enrichment at 180 min after feeding as a function of the fat surface area in 2.5 mL of the given dairy preparation (a logarithmic trend curve is presented with $R^2 = 0.94$). Bars represent SEM

tional to $6/d_{32}$). The insert in Fig. 3A shows that the plasma TAG enrichment at 180 min decreased logarithmically as a function of F. No significant difference of plasma cholesterol was observed among preparations (Fig. 3B).

The change of total plasma fatty acid levels with time was in agreement with the TAG enrichment presented above (results not shown). Interesting is the different relative proportion of each FA (or FA profile, as mass percentage) among groups fed with different preparations. Table 2 shows the plasma total FA profile at 180 min and Fig. 4 presents the related difference over baseline (FA enrichment profile). At 180 min, the rats were at the peak of the absorptive state, so that we observed an enrichment in exogenous FA typical of milk fat (C12, C14, C15, C16, C18:1 *n*-9) and a decrease in endogenous FA (C18:2 *n*-6, C20:4 *n*-6, C22:6 *n*-3). The enrichment in C12, C14, C15, C16 and decrease in C22:6 *n*-3 was lower for HC than for UP and RC. For C18:1 *n*-9 and C18:2 *n*-6, the decrease was lower for HC than for UP.

Discussion

The aim of the present study was to show differences in lipid digestion among dairy preparations with different fat supramolecular structures but similar composition. Our objective was met (Table 1) and the observed differences in plasma TAG and FA profiles were likely to be due to the structure of the preparation. Since casein molecules coagulate at pH 4.6 [27], the aqueous phase of UP is likely to clot in the stomach [27] while the fat phase remains aside [28]. The native fat globules in RC and SC should be inert fillers in the pores of the gastric clot

Table 2 Plasma total fatty acid profile of rats, 180 min after feeding one of the dairy products (mg per 100 mg). Results are means ± SEM

Fatty acid	Unemulsified preparation (UP; $n = 10$)	Regular cream (RC; $n = 7$)	Small fat globule cream (SC; $n = 4$)	Homogenized cream (HC; $n = 8$)
8:0	0.2 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.2 ± 0.1^{a}
10:0	0.2 ± 0^{a}	0.2 ± 0^{a}	0.2 ± 0^{a}	0.1 ± 0^{a}
12:0	0.9 ± 0.1^{a}	0.9 ± 0.1^{a}	0.6 ± 0.1^{b}	0.6 ± 0.1^{b}
14:0	4.0 ± 0.4^{a}	3.8 ± 0.3^{a}	2.7 ± 0.3^{b}	2.4 ± 0.4^{b}
14:1	0.5 ± 0.1^{a}	0.5 ± 0^{a}	0.7 ± 0.1^{a}	0.3 ± 0.1^{b}
15:0	0.6 ± 0^{a}	0.5 ± 0^{a}	0.5 ± 0^{a}	0.4 ± 0^{b}
15:1	0.0 ± 0^{a}	0.0 ± 0^{a}	0.2 ± 0.2^{a}	0.0 ± 0^{a}
16:0	26.1 ± 0.5^{a}	25.8 ± 0.4^{a}	$24.2 \pm 1.1^{a,b}$	23.2 ± 0.6^{b}
16:1 *n*-9	0.2 ± 0^{a}	0.0 ± 0^{a}	0.2 ± 0^{a}	0.1 ± 0^{a}
16:1 *n*-7	1.6 ± 0.1^{a}	1.4 ± 0.1^{a}	1.5 ± 0.1^{a}	1.2 ± 0.1^{b}
17:0	0.4 ± 0^{a}	0.3 ± 0^{a}	0.7 ± 0.2^{b}	0.4 ± 0^{a}
18:0	9.5 ± 0.1^{a}	10.4 ± 0.3^{b}	13.3 ± 1.3^{b}	10.9 ± 0.2^{b}
18:1 *n*-9 *cis*	13.4 ± 0.7^{a}	11.2 ± 0.5^{b}	8.7 ± 0.7^{c}	$9.8 \pm 0.7^{b,c}$
18:1 *n*-7	2.1 ± 0.1^{a}	2.2 ± 0.2^{a}	0.5 ± 0.1^{b}	1.8 ± 0.1^{a}
CLA	0.2 ± 0^{a}	0.1 ± 0^{a}	0.0 ± 0^{a}	0.1 ± 0^{a}
18:2 *n*-6 *cis*	16.7 ± 0.7^{a}	15.1 ± 0.4^{b}	$15.5 \pm 0.5^{a,b}$	17.0 ± 0.7^{a}
18:3 *n*-6	0.2 ± 0^{a}	0.2 ± 0^{a}	0.2 ± 0^{a}	0.1 ± 0^{a}
18:3 *n*-3	0.5 ± 0^{a}	0.3 ± 0^{a}	0.4 ± 0^{a}	0.4 ± 0^{a}
20:1 *n*-9	0.3 ± 0^{a}	0.2 ± 0^{a}	0.5 ± 0.1^{a}	0.3 ± 0^{a}
21:0	0.1 ± 0^{a}	0.0 ± 0^{a}	0.0 ± 0^{a}	0.1 ± 0^{a}
20:2 *n*-6	0.1 ± 0^{a}	0.0 ± 0^{a}	0.0 ± 0^{a}	0.1 ± 0^{a}
20:3 *n*-9	0.1 ± 0^{a}	0.0 ± 0^{a}	0.1 ± 0^{a}	0.1 ± 0^{a}
20:3 *n*-6	0.2 ± 0^{a}	0.2 ± 0^{a}	0.2 ± 0^{a}	0.2 ± 0^{a}
22:0	0.0 ± 0^{a}	0.0 ± 0^{a}	0.1 ± 0^{a}	0.0 ± 0^{a}
20:4 *n*-6	17.0 ± 1^{a}	21.7 ± 0.7^{b}	21.6 ± 0.3^{b}	24.4 ± 1.3^{b}
22:1 *n*-9	0.3 ± 0.1^{a}	0.4 ± 0.1^{a}	0.7 ± 0.1^{a}	0.4 ± 0^{a}
22:2 *n*-6	0.6 ± 0.1^{a}	0.3 ± 0^{a}	0.7 ± 0.1^{a}	0.4 ± 0^{a}
24:0	0.4 ± 0^{a}	0.5 ± 0^{a}	0.8 ± 0.1^{a}	0.6 ± 0^{a}
22:4 *n*-6	0.1 ± 0.1^{a}	0.0 ± 0^{a}	0.0 ± 0^{a}	0.0 ± 0^{a}
24:1 *n*-9	0.6 ± 0.1^{a}	0.8 ± 0^{a}	1.9 ± 0.5^{a}	1.0 ± 0.1^{a}
22:5 *n*-6	0.2 ± 0^{a}	0.3 ± 0^{a}	0.1 ± 0.1^{a}	0.2 ± 0^{a}
22:5 *n*-3	0.4 ± 0^{a}	0.3 ± 0^{a}	0.4 ± 0^{a}	0.4 ± 0^{a}
22:6 *n*-3	2.5 ± 0.1^{a}	2.4 ± 0.1^{a}	2.8 ± 0.3^{a}	2.8 ± 0.1^{b}

Means in a row with different superscripts are significantly different ($P < 0.05$)

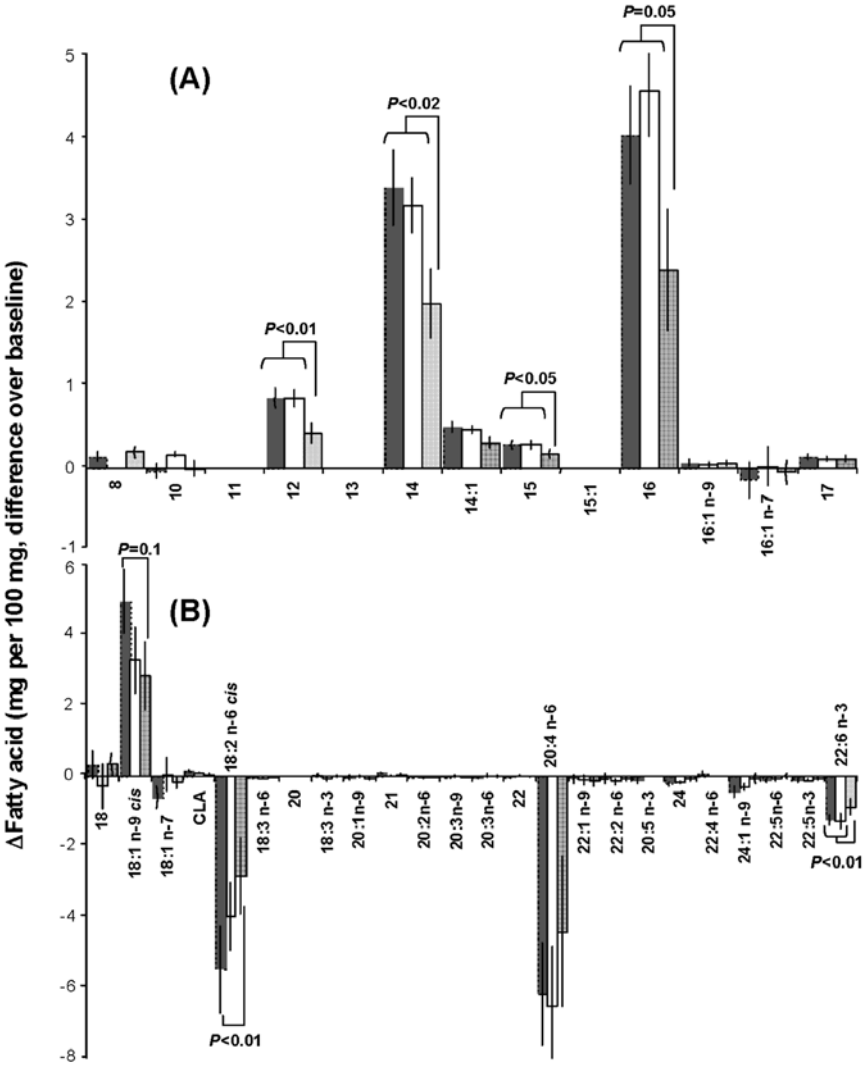

Fig. 4 Plasma total fatty acid enrichment profile (mass %, expressed as the difference over baseline) of rats 180 min after feeding one of the dairy preparations. **A** Short and medium chain fatty acids; **B** long chain fatty acids. (■) Unemulsified preparation (UP; anhydrous milk fat + skim milk); (□) regular native fat globules cream (RC); (▩) homogenized fat droplet cream (HC). Bars represent SEM. (Results are not presented for small native fat globule cream, SC, due to few samples turning the differences over baseline to be nonsignificant compared with other preparations)

while the droplets in HC interact with caseins to form a firmer and more finely dispersed gel [29]. Moreover, the globules in RC and SC are covered with the MFGM with a specific fat surface area (S) of 1.5–3 $m^2 \cdot g^{-1}$, while HC droplets are covered mainly by caseins and some whey proteins and present a much larger S of $> 15\ m^2 \cdot g^{-1}$ [5, 25]. Finally, the fat in UP has a smaller interface depending on the gastric mechanical work [30]; the interface can be covered by surface active molecules present in the stomach and in the food (phospholipids, proteins). Therefore, both the supramolecular structure of milk fat and the related structural modification of the dairy preparation in the stomach can affect the lipid digestive metabolism.

The present results of plasma TAG enrichment are consistent with the global lipid metabolism previously studied using a $^{13}CO_2$ breath test with rats fed with different labeled milk fat emulsions [17]. In the latter, we show that unemulsified fat or large droplets of 10 µm coated with lecithin result in a faster and sharper appearance of ^{13}C in expired air samples during digestion than homogenized casein-coated fat droplets of 1 µm. However, the relative action of digestive and oxidative processes is not known. The present study suggests that fat structure would influence more the digestive than the oxidation step.

In humans, Swiss cheese and butter were not found to result in any difference in plasma TAG [14]; however, studies were performed chronically with a test diet. Fruekilde and Høy [31] examined the lymphatic fat absorption among rats fed with different dairy products. One disadvantage is that the relative amount of other components than fat (proteins, carbohydrates, calcium) varied. Cream results in faster lymphatic fat absorption than the cream cheese and butter, and at 8 h the accumulated absorption of fat is significantly higher [31]. This discrepancy with our result should be explained by the different product composition. Since the composi-

tion of our products was constant, we highlight the importance of the supramolecular structure of dairy fat in its digestion.

Relative role of gastric and postgastric metabolisms on plasma triacylglycerols

The smallest fat droplets (SC and HC) are likely to be more lipolyzed due to their larger surface area. During flow in the duodenum, this can lead to an inhibition of gastric emptying via the generated fatty acids that stimulate cholecystokinin [8]. These phenomena can partly explain the lower peak of plasma TAG observed for creams compared with UP (Fig. 3A). However, if gastric emptying was the only factor explaining the different TAG curves, then only the appearance of their peak would be delayed for the most emulsified substrates. Fig. 3A shows that the shape of plasma TAG enrichment is also different between UP and the creams (RC, SC, HC). Therefore, factors other than gastric emptying should play a role. Borel et al. [8] observe that lipid emulsions fed intragastrically in rats undergo significant gastric lipolysis. Thus, the latter cannot be ruled out in the present study with milk fat [10], and the shape of TAG curves (Fig. 3A) can be related to gastric emptying and preduodenal + pancreatic lipase activities that are sensitive to the structure of the preparation. In type 2 diabetic patients, the consumption of butter, milk or mozzarella did not affect plasma TAG concentration and gastric emptying, and TAG peak was delayed for butter and cheese compared with milk [32]. This discrepancy with our study can be due to the test meal given with dairy products: bread and banana were probably important products governing gastric emptying.

The extent of gastric lipolysis and the decrease in the relative amount of TAG in the duodenum are higher with a fine vegetable oil emulsion than with a coarse emulsion in humans [33]. Lipases are present in excess amount in the digestive tract: the larger the lipid surface, the higher the number of lipase molecules able to bind at the interface. Therefore, the lipolysis should presently increase in the order UP < RC < SC < HC, conversely to the observed height of TAG peaks (Fig. 3A). The TAG peak is delayed for the smaller droplets, consistently with the literature [33]. This apparently conflicting observation (higher lipolysis vs delayed TAG peak) can be explained by slower gastric emptying for the smallest droplets [33]. Compared with our previous study [17], the present work confirms that absorption occurs slower with the most emulsified milk fat substrates. Further absorption and oxidation mechanisms remain to be elucidated, and the hypothesis of different absorption rates of lipid nutrients by the small intestinal mucosa depending on the supramolecular structure of milk fat should be investigated.

Importance of droplet size and surface coating on plasma triacylglycerols

Fat emulsification usually occurs in the stomach rather than in the duodenum [30]. Droplets from vegetable oil emulsions reach a steady state in the stomach of rats from 17 to 37 μm [8] while in humans, the mean diameter of fine emulsions (0.7 μm) increases up to ~2 to 6 μm in the stomach and remains stable in the duodenum [33]. Our HC droplets were of similar size and interface composition as the latter fine droplets; therefore, if part of these droplets coalesced due to gastric mechanical energy, they could be in a size range closer to that of RC and SC (Fig. 2). This might explain why the lower TAG enrichment for HC vs RC was not significant; however, the present gastric content was different from other reports [8, 33].

Globules in RC and SC were covered with the native MFGM, whereas droplets in HC were covered mainly with caseins. In the stomach, the solid and fat phases empty together after an initial lag time unlike the aqueous phase that empties promptly [28]. Thus, HC would be expected to empty more slowly due to droplet interactions with the coagulum [17, 29]. In a previous work, homogenized droplets seem to present a slower gastric emptying than large lecithin-coated droplets [17]. No such difference was observed here between HC droplets and MFGM-coated globules (RC, SC). However, UP results in a higher enrichment in plasma TAG compared with HC between 90 and 180 min: this can be explained by such different fat interactions. The unemulsified fat would drain promptly from the stomach while homogenized droplets drain more slowly with the coagulum phase. This is consistent with Borel et al. [8]: a complex fine emulsion (with casein-coated droplets) drains more slowly than a coarse emulsion [17].

Fractions of milk phospholipids reduce the level of human postprandial blood lipids after consumption of milk fat [34]. This is supposed to be due to the ability of milk phospholipids (mainly contained in the MFGM) to modify fat emulsion in the digestive tract. Due to their native structure, the creams tested in our study (RC, SC) contained more phospholipids than the AMF with skim milk (UP). Indeed, milk fat globules contain on average 0.5 % phospholipids, and ~40 % of total milk phospholipids are in the skim milk phase [2]. Thus, 2.5 mL of RC and SC contained ~3 mg phospholipids, 90 % of which were in the MFGM and 10 % in the aqueous phase. In turn, during the production of anhydrous milk fat (Fig. 1), part of the initial milk phospholipids are discarded in the buttermilk and washing water fractions. Therefore, UP contained mainly the phospholipids from the skim milk phase, i.e., about 300 μg per 2.5 mL. It should be investigated whether this 10-fold difference in phospholipid content can explain the greater plasma TAG enrichment at 180 min after feeding UP.

Effect of digestive steps and droplet interface on plasma fatty acids

Preduodenal lipases act mainly on the *sn*-3 position of the TAG, while pancreatic lipase acts on the *sn*-1 and *sn*-3 positions. Therefore, the *sn*-2 position remains essentially intact in the chylomicrons and the rate limiting step in the digestion of milk fat in the duodenum is the lipolysis of the *sn*-1 and *sn*-3 positions [35, 36]. However, milk fat has quite a high content of short- and medium-chain FA (C4-C12), which are mainly absorbed directly through the portal vein [37]. Short- and medium-chain FA are digested, absorbed, and transported rapidly and are a source of rapidly available energy [38]. Since rats were fasted before being force-fed, they were likely to metabolize quickly these FA from digested milk fat; apart from analytical aspects, this can explain why medium chain FA are hardly found in plasma (Table 2, [31]).

The plasma FA profile at 180 min differed for HC and for UP and RC (Table 2). Decreasing fat surface area leads to an increased proportion of C12, C14, C15, C16 and C18:1 *n*-9 in total plasma FA. In the literature, rat lymph collected 8 h after feeding cream cheese has higher contents of C10-C16 and a lower content of C18:2 *n*-6 than with cream or butter [31]. The authors conclude that the absorption of fat from cream cheese continues to occur. These results can hardly be compared with ours due to the different dairy product composition. Besides, the measured C10 content can be highly dependant on the analytical method used.

The TAG composition and structure in milk fat influence the action of lipases and thus, absorption [39]. In milk fat TAG: 23.7 % of C12 is located on *sn*-1 and 13.4 % on *sn*-3; 27.3 % of C14 is located on *sn*-1 and 7.1 % on *sn*-3; 44.1 % of C16 is located on *sn*-1 and 10.5 % on *sn*-3; 37.3 % of C18:1 is located on *sn*-1 and 41.5 % on *sn*-3 [39]. Therefore, 40–80 % of these FA are prone to preduodenal + pancreatic lipolysis; C18:1 being particularly sensitive to gastric lipolysis. Therefore, the lower proportion at 180 min of C12-C16 and C18:1 FA in rats fed HC (Table 2) might be partly explained by a faster gastric and duodenal lipolysis thanks to the larger interface (Fig. 2). Free FA levels with time should be analyzed to test this hypothesis and the long term consistency regarding the assimilation of lipid nutrients should be investigated.

Conclusion

In this study, we show that the composition of plasma lipids can be different if milk fat is consumed free or as native fat globules or homogenized droplets. Indeed, taking care to keep constant the composition of the different products, the enrichment in plasma TAG was lower with emulsified milk fat compared with anhydrous milk fat. Moreover, during digestion and absorption, the FA profile of plasma lipids was different for the homogenized cream than for the unemulsified fat. These data show that dairy products with the same composition but varying in fat suprastructure result in different kinetics of lipid digestion which could be of concern for health. Further studies will aim to characterize the human gastric and duodenal contents after ingestion of controlled dairy products, in order to understand to respective role of gastric and duodenal steps in the observed differences of lipid digestion.

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