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ORIGINAL CONTRIBUTION

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The dispersion state of milk fat influences triglyceride metabolism in the rat

A $^{13}CO_2$ breath test study

Summary *Background* Milk fat, which has different structures in the various dairy products, is a major and controversial lipid source in the Western diet. However, information about the digestion fate of milk fat depending on its supramolecular structure for a given composition is scarce. *Aim of the study* In this study, $^{13}CO_2$ breath tests were performed with fasted rats force-fed different dairy preparations of similar composition but differing in fat suprastructure in order to highlight differences of general lipid metabolism. *Methods* Each preparation consisted of a NaCl solution, anhydrous milk fat labelled with a ^{13}C mixed triacylglycerol, casein (as native phosphocaseinate powder with some lactose), and dipalmitoylphosphatidylcholine. Milk fat was either fed (i) unemulsified consecutively to the aqueous phase, or emulsified as (ii) coarse droplets of ~10 µm covered mainly with the phospholipid, or (iii–iv) fine droplets of ~1 µm covered mainly with casein, force-fed either in the liquid state or in a semi-crystallized state. ^{13}C abundance in expired air samples was measured by isotope ratio mass spectrometry; results were expressed as ^{13}C enrichment and were submitted to an ANOVA analysis. *Results* The $^{13}CO_2$ excretion curves of the unemulsified preparation and the coarse emulsion were similar and presented a sharp peak, both significantly different from the fine emulsion curves characterized by a nearly linear cumulative recovery. The crystalline state of the fine emulsion droplets and the viscosity of these emulsions did not affect significantly their excretion curves. The lipid metabolization (indicated by the ^{13}C recovery) was significantly slower for the fine droplets coated with casein than for the large droplets coated with the phospholipid and the unemulsified fat. For the latter, a single ^{13}C peak rapidly appeared, while for small droplets coated with caseins, ^{13}C excretion was continuous up to 6 h. *Conclusions* Global lipid metabolism based on oxidation to CO_2 was decreased with smaller compared to larger emulsified milk fat particles with different coatings. These data support the concept that dairy products with different fat suprastructures are digested and metabolized differently.

Key words ^{13}C MTG breath test – digestion – emulsion droplet size – interface – milk fat

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Abbreviations

CEP	coarse emulsion whose fat droplets are coated with phospholipid (dipalmitoyl-phosphatidylcholine)
δ	^{13}C abundance of the sample
δ_0	baseline ^{13}C abundance
d_{32}	Sauter volume-surface average diameter
d_{43}	volume averaged diameter
FEC	fine emulsion whose fat droplets are coated with casein
FFP	preparation consisting of free fat plus aqueous-proteinaceous phase force-fed consecutively
MTG	mixed triacylglycerol
S	specific surface area
Slope	slope of the cumulative $^{13}CO_2$ excretion curve (from measured values)
St-[1-^{13}C]Oct-St	2-octanoyl [1-^{13}C]-1.3 distearoyl glycerol
$t_{0.5}$	time of half $^{13}CO_2$ elimination compared to the maximum for free fat at 360 min (from measured values)

Introduction

Dairy products are of great economical and sensory importance in the diet and, in this respect, milk fat has received much attention as a major source of energy but also for its controversial role in human nutrition [1, 2]. Fat is present in milk in the form of globules of ~4 μm surrounded by a native biological membrane composed mainly of phospholipids, glycoproteins and enzymes organized as a trilayer [3]. The fat globule core is composed almost entirely of triacylglycerols, representing 98 % of total milk fat [1, 3]. Due to dairy processes, the structure of milk fat globules can be greatly modified. For example, tiny homogenized milk fat globules smaller than 1 μm and covered by caseins can be obtained to stabilize the milk emulsion for UHT milk, or native fat globules can also aggregate or coalesce to form so-called "free fat" inclusions in the casein matrix of some cheeses [4, 5]. Moreover, depending on the composition of their interface (native membrane, caseins or other proteins), milk fat globules interact differently with the cheese or yoghurt casein matrix in which they are entrapped [6].

The role of milk fat in contributing to health and disease is quite controversial [2]. The nutritional profile of butterfat is affected by its triacylglycerol and fatty acid composition, which can be modulated by fractionation [7]. The triacylglycerol structure can also affect the bioavailability of fatty acids [8]. Moreover, milk fat is known to present complex profiles of gastrointestinal digestion and absorption, presumably due to its particular physical properties, which are linked to the polymorphism and unique composition of triacylglycerol molecular species [9]. The impact of milk fat composition and triacylglycerol structure on its digestion properties has been widely studied [9–11]. 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 [11]. Butter also resulted in a lower accumulated absorption of fat in the rat than cream [12]. Lipolysis of dairy triacylglycerols is also one of the key steps involved in the assimilation of bioactive lipophilic molecules such as cholesterol, vitamins A and E, etc. However, as highlighted by Gurr [2], most studies to date regarding the nutritional properties of milk fat do not take the structure of the dairy food product into account, which can result in differences with pure milk fat tested in simplified experimental conditions.

There is thus limited scientific evidence that the structure of milk fat affects its digestibility and nutritional properties. Information on the importance of droplet size and composition on fat assimilation is scarce. The relationship between the physico-chemical properties of milk fat, digestion, and assimilation of lipid nutrients from this disparaged lipid source remains to be elucidated.

Stable isotope breath tests consist in measuring the ^{13}C enrichment in breath throughout digestion after ingestion of a ^{13}C-enriched meal [13]. They are presently used for the measurement of fat digestion, since the end product of oxidative metabolism is $^{13}CO_2$, which is expired in the breath and can be measured by isotope ratio mass spectrometry. Using a mixed triacylglycerol, the ^{13}C label is on the *sn*–2 medium chain fatty acid (octanoic acid, ~2 % of cow milk fatty acids), which is completely absorbed and rapidly oxidized. Converse to the free octanoic breath test that focuses on gastric emptying, the mixed triacylglycerol breath test allows a global characterization of the general lipid metabolization process.

In this study, we show the effect of the supramolecular milk fat structure on its digestion kinetics in rats, using model dairy preparations. Our objective was to characterize the time course of ^{13}C output in expired breath from a labeled digested triacylglycerol (mixed triacylglycerol, MTG) in rats force-fed lipid emulsions containing the same milk fat but with various physico-chemical structures similar to milk products. Two unique features of our experimental approach are (i) the controlled manufacture of dairy emulsions with the same composition but different types of lipid droplets and (ii) the examination of dynamic changes in breath output throughout the postprandial phase. The rat model was used since the gastric and intestinal steps involved in fat digestion are basically comparable with those in humans [14].

Materials and methods

Materials

Anhydrous milk fat was purchased from Lactalis (Bourgbarré, France) and NaCl from Merck (Darmstadt, Germany). dipalmitoyl-phosphatidylcholine (1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine, synthetic, 99 %) was from Sigma (St Louis). The mixed triacylglycerol 2-octanoyl[1-^{13}C]-1,3 distearoyl glycerol (St-[1-^{13}C]Oct-St) was from Euriso-Top (99 %; Saint-Aubin, France).

A native phosphocaseinate suspension was prepared by microfiltration and diafiltration (pore diameter: 0.1 µm) of microfiltered raw skim milk on MFS 19 equipment (Tetra Laval, Århus, Denmark; 4.6 m^2) at 50 °C, according to Pierre et al. [15]. The spray drying (GEA, Niro Atomizer, St Quentin en Yvelines, France) of concentrates was performed at Bionov (Rennes, France). The native phosphocaseinate powder contained 80.8 % total protein, 4.9 % noncasein proteins, 1.8 % lactose, and 8.3 % salts.

A NaCl saline solution was prepared at 80 mM (milk ionic strength). A Casein Premix was obtained by dissolving 30 g of native phosphocaseinate powder with 150 g of saline at 60 °C using a high-shear Polytron mixer. A Phospholipid Premix was prepared by sonicating (Vibracell, Bioblock Scientific, France; 20 kHz) 140 mg of dipalmitoyl-phosphatidylcholine in 13.86 g of saline.

Milk fat was primed by mixing 1.80 g of St-[1-^{13}C]Oct-St with 18.96 g of anhydrous milk fat (8.7 % w/w of ^{13}C-labeled triacylglycerol in anhydrous milk fat).

Dairy preparations containing milk fat in different dispersion states with a ^{13}C-labelled triacylglycerol

Four different dairy preparations were produced, with the same composition but varying in milk fat dispersion state (non-emulsified, or emulsified with two selectively chosen surface coating/droplet size combinations, and fat being either liquid or crystallized in one of the latter combinations – Table 1). The latter were simplified models to simulate different structures in dairy products: non-emulsified fat particles appear in butter or cheeses such as Emmental [5]; fat globules of ~3–10 µm coated with the phospholipid-containing milk fat globule membrane appear in raw milk and cheeses such as Camembert [16]; fat droplets of ~1 µm coated with caseins appear in homogenized milk, yoghurts and some cheeses [3].

A coarse emulsion with droplets coated mainly with phospholipids (CEP) was prepared by mixing anhydrous milk fat with dipalmitoyl-phosphatidylcholine and saline at 60 °C using a high-shear Polytron mixer, and refining by sonication for 9 min at 20 kHz (output 4; Vibracell). The Casein Premix was added after emulsification so that it remained mostly in the aqueous phase.

Two fine emulsions with droplets coated with casein (FEC) were prepared by mixing anhydrous milk fat with the Casein Premix at 60 °C using a high-shear mixer (Polytron), and refining by sonication for 6 min at 20 kHz (output 4). The Phospholipid Premix was added after emulsification so that it remained mostly in the aqueous phase.

An unemulsified fat product, the free fat consisting of ^{13}C-labeled anhydrous milk fat and a blend of Casein Premix & Phospholipid Premix was prepared.

All the products (CEP; FECs; free fat + separate aqueous phase containing proteins and phospholipid, FFP) had the following composition per preparation of 1.3 mL: 138.0 mg (10 % w/w) of native phosphocaseinate powder (including 104.7 mg = 7.6 % of casein and 2.5 mg = 0.2 % of lactose), 282.8 mg (20.2 %) of total fat (i. e., 256.0 mg = 18.3 % of anhydrous milk fat, 24.0 mg = 1.7 % of St-[1-^{13}C]Oct-St and 2.8 mg = 0.2 % of dipalmitoyl-phosphatidylcholine), and 960.0 mg (69.8 %) of NaCl solution 80 mM. Each product provided an energetic load of 0.72 kJ (3 kcal) per preparation. Final aqueous products were pasteurized and kept at 4 °C until use. The milk fat droplet size did not vary due to pasteurization.

Animals

Male Sprague-Dawley rats (n = 7; Harlan, Gannat, France), weighing 260–300 g were used following the

Table 1 Characteristics of the four different dairy preparations used in the experimental design: dispersion state, droplet volume-averaged diameter (d_{43}), droplet specific surface area (S), viscous state of the preparation and crystalline state of the fat within, and the dairy product for which the preparation is a model

Abbreviation*	Dispersion state	d_{43} (µm)	S ($m^2 \cdot g^{-1}$)	Viscous state	Simplified model for
CEP	Emulsified in phospholipid	11.2	0.62	Viscous with semi-crystallized fat	Raw milk, Camembert cheese
Viscous FEC	Emulsified in casein	0.78	23.5	Viscous with semi-crystallized fat	Homogenized milk, yoghourt
Liquid FEC	Emulsified in casein	0.68	24.5	Liquid with liquid fat	Homogenized milk
FFP	Not emulsified	–	–	Liquid with liquid fat	Butter + skim milk

* *FFP* free fat + aqueous phase preparation; *CEP* coarse emulsion with droplets coated with phospholipid; *FEC* fine emulsion with droplets coated with casein

official French regulation (87–848, 19 October 1987). They were housed in individual plastic cages under controlled conditions (12 h light:dark cycle, temperature 22 ± 1 °C, 50 % humidity) and fed a commercial diet (Harlan). They were fasted overnight before the experiment and were weighted in the morning before being force-fed.

The experimental design was unrandomized: each experimental day, all rats were fed the same preparation in order to be sure that each given preparation was fed to rats under the same conditions. Indeed, aging and tempering steps would have destabilized emulsions. After the end of each experiment, a 48 h stabilization with the commercial diet was respected before testing the next preparation. Each rat was its own reference. The CEP (least stable preparation) was fed first and the free fat preparation was fed last.

All the experiments were started around 9 o'clock in the morning to avoid nyctemeral variations. Two baseline expired-air samples were taken while rats were in the glass cages but before the application of the ^{13}C-preparations. At time 0, rats were force-fed one of the preparations in a single bolus intragastrically using a syringe equipped with a curved canula. For emulsified products, a single 1.3 mL dose was force-fed. CEP was given at room temperature (partially crystallized fat; viscous aqueous phase) while one FEC was given at room temperature (partially crystallized fat, viscous aqueous phase) and the other at 38 ± 1 °C (liquid). The unemulsified preparation was force-fed at 38 ± 1 °C: 0.3 mL of free fat, followed by 1 mL of aqueous phase (both liquid). Therefore, for each preparation, each rat received 35 µmol of ^{13}C. Force feeding did not last longer than 30 s for each rat.

Immediately after the force-feeding, rats were placed in individual air-tight glass cages equipped with a sealed tube for air extraction with a septum for sample collection. Air samples were collected at 30 min intervals for a total of 6 h by aspirating slowly (~within 1 min) exhaled air into a 60-mL syringe through the septum, while air-flow in the cage was ensured by a small hole (20 mm^2) in front of the rat head. The correct withdrawal of samples was achieved by a continuous suction of air (100 $L \cdot h^{-1}$), which maintained an equilibrium between ^{13}C and ^{12}C in the expired air [17]. Subsequently, the air from the syringe was transferred (via needle insertion along a rubber stopper) into a 10 mL vacuum test tube (Vacutainer, BD, Elvetec, France) and submitted for ^{13}C analysis.

It was checked that the endogenous ^{13}C content of milk fat and milk proteins was not detectable in $^{13}CO_2$ recovery by feeding each rat an unlabeled preparation.

^{13}C measurement and mathematical analysis

$^{13}CO_2/^{12}CO_2$ ratios were measured using an Isoprime Isotope Ratio Mass Spectrometer (GV instruments – Manchester UK) working in a continuous flow mode. Expired air samples were injected by the way of a Gilson autosampler filling a 50 µL loop. The CO_2 peak separated by gas chromatography was directed to the mass spectrometer by activating a switching valve, and was followed by a pulse of the laboratory reference CO_2 gas calibrated against a certified reference standard. Isotopic abundances were expressed as δ(‰) relative to the international standard of Vienna Pee Dee Belemnite (‰). Results were expressed as δ(‰) over baseline (δ-δ_0), further normalized by rat weight. The cumulative $^{13}CO_2$ excretion curves were calculated as the area under the (δ-δ_0) curves [18].

Statistical analysis

Results are expressed as means ± SEM. A one-way ANOVA was performed, either on data or on log-transformed data depending on whether variance was homogeneous or heterogeneous and data balanced or unbalanced [19]. *P*-values of < 0.05 were considered to be significant, *P*-values of < 0.01 were often obtained.

Results

Physico-chemical properties of the preparations

Typical fat droplet size distributions of the emulsions are shown Fig. 1. The mode diameter (diameter at the peak maximum) was 9.9 µm for CEP, 0.96 µm for viscous

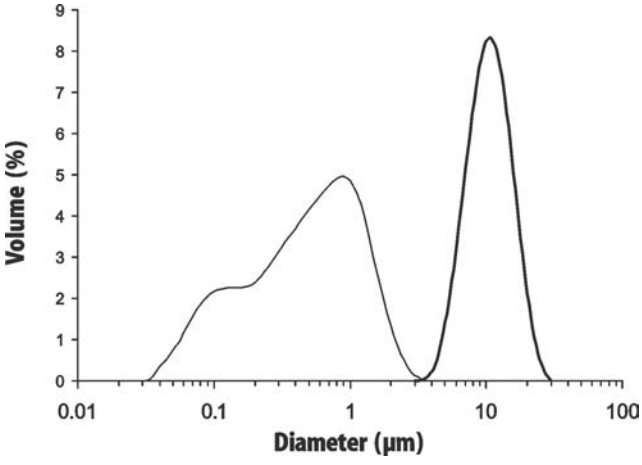

Fig. 1 Particle size distribution of the coarse emulsion with droplets coated with phospholipid (thick line) and of a fine emulsion with droplets coated with casein (thin line)

FEC and 0.94 µm for liquid FEC. The entire distribution corresponded to a d_{43} (volume averaged diameter) of 11.2 µm for CEP, 0.78 µm for viscous FEC and 0.68 µm for liquid FEC. The specific surface area of fat droplets was 0.62 $m^2 \cdot g^{-1}$, 23.5 $m^2 \cdot g^{-1}$ and 24.5 $m^2 \cdot g^{-1}$ for CEP, viscous FEC and liquid FEC, respectively. Therefore, CEP droplets were ~15 fold larger than FEC droplets. The characteristic diameters did not vary significantly from emulsion pasteurization to rat feeding. The order of magnitude of fat droplet size of the CEP is consistent with that of milk fat globules encountered in milk or cheese such as Camembert [16]. The fat droplet diameter of the FEC was consistent with that of homogenized milk [4] such as commercial UHT milk. Therefore, our objectives to simulate native and homogenized milk fat globules regarding fat droplet size were met. We should, however, indicate that a single dipalmitoyl-phosphatidylcholine layer coating emulsion droplets cannot fully represent the trilayered biological membrane that contains enzymes and naturally coats native milk fat globules. However, since no labeled triglyceride can be incorporated into native milk fat globules, this simplified model was chosen because it allows consistent milk fat droplet sizes with phospholipids at the interface to be achieved. Moreover, not only were the droplets in our different preparations coated with different molecules (phospholipid or casein), but the specific surface area of the fine emulsions was almost 40-fold that of the coarse emulsion. Because the milk fat content of the emulsions was 280 mg per preparation, this means that the CEP contained ~0.2 m^2 of fat/aqueous phase interface, whereas the FEC contained ~5.7 m^2 at the interface. Also, the casein molecules adsorbed to the surface of the fine emulsion droplets coagulate with the free casein molecules of the aqueous phase at pH 4.6, conversely to CEP droplets that should remain entrapped in coagulum pores as inert fillers [6].

$^{13}CO_2$ excretion

The $^{13}CO_2$ excretion curves in breath after ingestion of the different preparations are shown in Fig. 2A. Fig. 2B presents the corresponding cumulative excretion curves, calculated as the area under curve. A difference was observed in the pattern of the $^{13}CO_2$ recovery curves that represents the pattern of gastric emptying and subsequent lipolysis (overall lipid metabolism) of these preparations. The global metabolization of the two FEC, liquid or viscous, appeared to be delayed compared with the rapid ^{13}C recovery onset of the CEP and the FFP. After the peak excretion of $^{13}CO_2$ both CEP and FFP showed a rapid decay (Fig. 2A), while the cumulative recovery of FECs was nearly linear (Fig. 2B). Moreover, there was a hint of a second peak in $^{13}CO_2$ excretion (Fig. 2A; though not statistically significant) for both

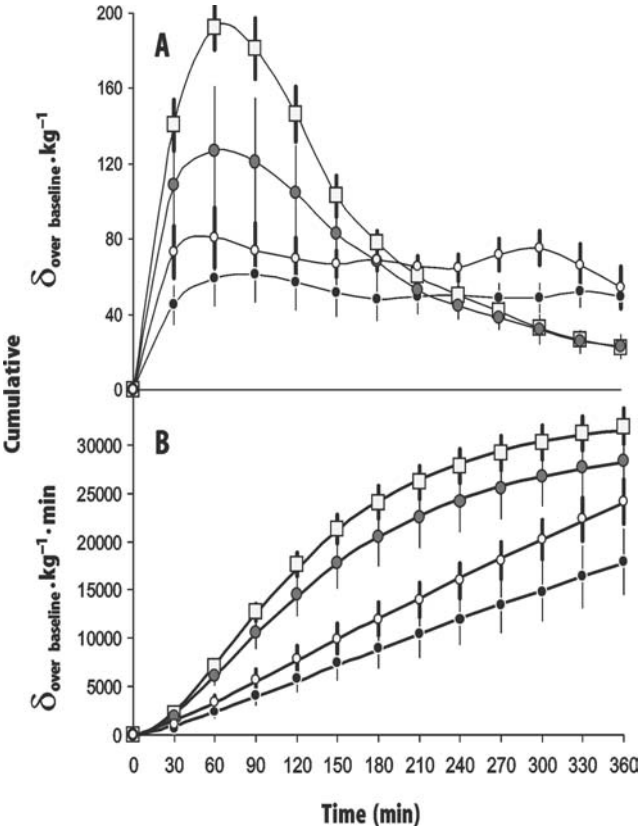

Fig. 2 **A** Average elimination curves; **B** Average cumulative elimination curves (area under **A** curves). □ unemulsified preparation –fat, proteinaceous phase, FFP–; ● coarse emulsion with droplets covered by phospholipids –CEP–; • viscous fine emulsion with droplets covered by caseins –viscous FEC–; ○ liquid fine emulsion with droplets covered by caseins –liquid FEC–. Bars represent SEM; n = 7 in each group

fine emulsions, but not for the coarse emulsion and free fat.

Table 2 shows kinetic parameters calculated from Fig. 2B: (i) the time of half ^{13}C elimination compared to the maximum obtained for free fat after 6 h ($t_{0.5}$) and (ii) the initial slope between 30 and 90 min. The characteristic time $t_{0.5}$ and initial slope were slightly higher for the CEP compared to the FFP preparation; however, the difference was not significant. Among FEC, the viscous preparation tended to present a slower recovery compared with the liquid preparation, though not significantly. However, the $t_{0.5}$ and slope of the two FEC were significantly lower than those of CEP and FFP.

Fig. 3 shows the impact of the initial specific surface area S of the different preparations (m^2 interface per g of fat) on the $t_{0.5}$ and initial slope of the cumulative excretion curve. S is greater when the fat droplets are smaller since $S \propto 6/d_{32}$. For $S < 1$ $m^2 \cdot g^{-1}$ (dairy products with natural or coalesced milk fat globules and free fat), S did not affect digestion process time significantly. However, for $1 < S < 10$ $m^2 \cdot g^{-1}$ and fat droplets covered with ca-

Table 2 Kinetic characteristics of the cumulative δ-over-baseline curve (Fig. 2-B) for the four different preparations varying in milk fat dispersion state: $t_{0.5}$ (time for half of the $^{13}CO_2$ to be eliminated compared to the maximum for free fat at 360 min calculated from values), and slope between 30 and 90 min (expressed as δ over baseline per kg per min; calculated from values between 30 and 90 min after fat ingestion)

	FFP		CEP Viscous		FEC Viscous		FEC Liquid	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
$t_{0.5}$ (min)	110[a]	4	139*[a, c]	26	223*[b]	17	215[b, c]	24
Slope	177[a]	12	144*[a]	21	56*[b]	13	77[b]	14

[a, b, c] Means (n = 4–7) in a row with different letters are significantly different at $P < 0.01$, except $P < 0.05$ between two values marked with *

FFP free fat + aqueous phase preparation; *CEP* coarse emulsion with droplets coated with phospholipid; *FEC* fine emulsion with droplets coated with casein

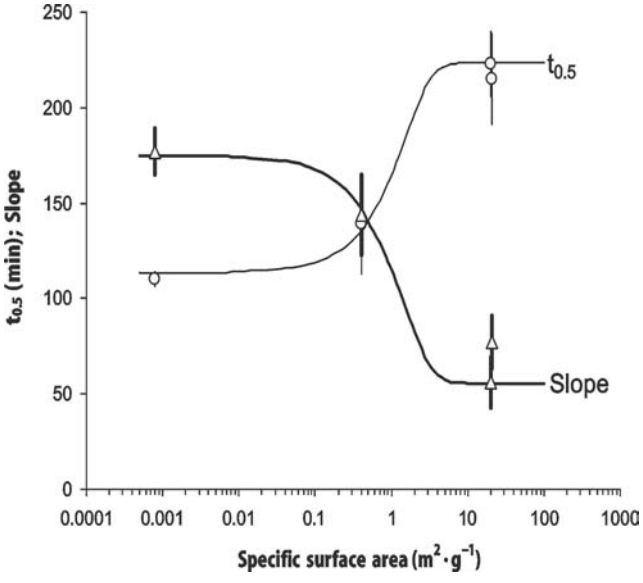

Fig. 3 Characteristic time $t_{0.5}$ (○) and slope (△) from the cumulative elimination curves (dots, Table 2) and fit (lines, in order to guide the eye) as a function of the specific surface area of fat at feeding. Fit was $y = (a + b.e^{-x})^{-1}$ for $t_{0.5}$ and $y = (a + b.e^{-x})^{1/2}$ for the slope ($R^2 > 0.98$)

seins (dairy products using homogenized milk), $t_{0.5}$ increased sharply and the initial slope of the cumulative excretion curve decreased. The slowing down of global lipid metabolization when the specific surface area of lipid droplets in the preparation increases is thus not a linear process.

Discussion

Relevance of the mixed triacylglycerol

The mixed triacylglycerol used in this study is well metabolized and the ^{13}C label is located at the *sn*–2 position that remains essentially intact in the chylomicrons [20, 21]. The rate limiting step in its digestion is hydrolysis of the *sn*–1,3 stearoyl groups. Since the preparation was force-fed, the lingual lipase could not act and the gastric lipase has a low activity in rodents [20]. The excretion curves were thus mostly related to gastric emptying and pancreatic lipase activity [13]. The fatty acid composition of milk fat triacylglycerols is complex, with over 400 distinct fatty acids detected [1]. Therefore, several thousand triacylglycerols are present in milk fat with only a few in amounts greater than 1 %. In cow milk, 43.5–55.2 % of octanoic acid is esterified at the *sn*–2 position (like in the mixed triacylglycerol) and 34.5–52.5 % at the *sn*–3 position [22]. Regarding stearic acid, 84 % is acylated at the *sn*–1 and *sn*–3 positions [22]. Therefore, even if the St-[1-^{13}C]Oct-St cannot be a true representative of the complex milk triglyceride composition (several thousands species are present in milk fat, most in traces), it is a reasonably good tracer. In order to be accessible to lipases, the mixed triacylglycerol tracer is bound to the global digestibility of the entire fat that it is dissolved in. Thus, it accounted for the general metabolism of other milk triglycerides present in each preparation.

Relative role of gastric and post-gastric metabolisms

Digestion of dietary fats begins in the stomach and is completed in the small intestine where final absorption occurs. In humans, gastric lipolysis can help to increase emulsification of fat in the stomach [23]. In the rat, gastric lipolysis is catalyzed by an enzyme acting similarly to human gastric lipase: the lingual lipase [24]. The action of pancreatic lipase could be powered by the prehydrolysis of fat catalyzed by preduodenal lipases. Lipases act on emulsified substrates; 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 [25]. Moreover, droplet stability and lipase adsorption are dependent on the nature of the surfactant at the droplet interface. *In vitro* studies [26, 27] have shown that the particle size distribution of lipid emulsion droplets governs the activity of gastric and pancreatic lipases, the activity being higher when droplets are smaller. *In vivo*, a fine oil emulsion drains more slowly from the stomach while the enterocytic uptake, blood transport and hepatic metabolism are faster with a coarse oil emulsion [23]. Drainage being slower, the absorption and distribution of lipidic nutrients to the different organs are thus delayed.

Borel et al. [25] suggested that the free fatty acids generated in greater amount by the lipolysis of fine emulsion droplets in the stomach led to an inhibition of gastric emptying due to cholecystokinin stimulation. Our results are consistent with the latter, since part of the delayed metabolization observed in the present study can be due to a slower gastric emptying. However, if this was the only factor, then the cumulative recovery of ^{13}C in breath CO_2 would be similar for all emulsions, but the peak would be later for the slower emptying products. The data presented in Fig. 2 indicate that if similar amounts of tracer were absorbed from the different emulsions, then the lipids from the fine emulsions could be digested, absorbed and/or oxidized more slowly than from CEP and FFP. Since the cumulative recovery at 6 h is lower for the fine emulsions, we may also speculate that maybe more of the tracer incorporated into the fine emulsions could be absorbed, but not oxidized, than for the coarse emulsion and free fat, which would deserve to be investigated further by measuring lipid metabolism after 6 h (assuming the tracer is absorbed and not excreted in stool, which is unlikely in healthy animals).

Thus, our data indicate that post-gastric metabolism of the mixed triglyceride is different for the different lipid preparations. Mekki et al. [11] have shown that butter resulted in a lower postprandial lipemia and chylomicron accumulation in the circulation of humans than vegetable oils after consumption of a single mixed meal. The authors hypothesized a possible delayed gastric emptying due to the unemulsified state of butterfat. Our work shows that in rats, non-emulsified fat rather results in faster gastric emptying and that the post-gastric process is more likely to be the cause of this difference.

Importance of droplet surface coating

Native milk fat globules covered by phospholipids are entrapped in serum pores of milk coagulum, whereas smaller, homogenized fat globules covered by caseins interact with the casein network, participating in the coagulum structure [6]. Since caseins coagulate in the stomach, this difference of fat globule interactions during gel formation can affect gastric emptying and lipase accessibility. Probable interactions of FEC droplets with the gastric casein coagulum are consistent with a slower gastric emptying, while CEP "inert" droplets would be more likely to drain together with the aqueous phase. Indeed, it has been shown in humans that the aqueous phase emptied promptly from the stomach, while the solid and fat phases emptied together, in parallel, after an initial lag time [28]. Moreover, part of the fat empties at the surface of the solid particles and the rest of it as an oil phase. These differences in surface coatings and interactions with the coagulum are also likely to affect the way lipases access the lipid interface and can adsorb to it. The delayed label recovery observed in our study for the FEC could be partly explained by the fact that the lipases reach triacylglycerols less easily when they are covered by large casein particles instead of phospholipids.

Borel et al. [25] fed fasted rats intragastrically with coarse (22 μm fat droplet diameter) or fine (0.6 μm diameter) triolein emulsions. In the stomach, the fat droplet size of the fine emulsions increased up to 24 μm, while the coarse emulsion was ~35 μm. However, converse to our study, both types of fat globules were probably covered with lecithin. In the present study, the large droplets covered by dipalmitoyl-phosphatidylcholine were more prone to coalesce than the more stable small droplets covered mainly by caseins [3], which may explain the differences between CEP and FEC and the similar excretion curves for free fat preparation and CEP (Fig. 2A). In the study by Borel et al., after 2 h, gastric triacylglycerol hydrolysis was significantly higher with the fine than with the coarse emulsion. Borel et al. also prepared a complex fine emulsion by adding casein, maltodextrin and minerals to the regular fine emulsion [25]. Gastric triacylglycerol hydrolysis was lower with their complex fine emulsion whose fat droplets were probably covered with casein. However, different interface compositions were not considered in their discussion and were not characterized. Gastric emptying increased from complex fine, fine to coarse emulsion in the study by Borel et al. [25]. The assimilation of the fine complex emulsion was also significantly delayed in the small intestine, while the fine and coarse "simple" emulsions were processed comparably [25], which is consistent with our results. Therefore, their result can be due to the modified interface composition compared to the "simple" emulsions. Compared with our results, this could be interpreted as a delayed action of pancreatic lipases due to the casein composition of the interface. Moreover, Borel et al. [25] suggest that the physico-chemical properties and the biochemical events taking place in the stomach contents are such that emulsion sizes of 20 to 40 μm can be generated in a steady-state situation in the stomach, roughly independently from the particle size of the ingested emulsion. This can explain why our unemulsified FFP preparation led to similar excretion curves as the CEP.

Importance of product composition and viscosity

Fruekilde and Høy [12] have shown that butter resulted in a lower accumulated fat absorption to the lymphatic system than cream cheese and cream in the rat. In the latter products, fat, protein, calcium contents, viscosity, particle size and microbiological cultures varied, which were not controlled by the authors and are possibly the

cause for the different absorption patterns [12]. Cream results in a more rapid absorption of fat [12]; the authors suppose this is due to a more rapid lipolysis in the stomach due to the emulsion nature of the cream (a larger initial surface area of the particle increases the hydrolysis rate in the stomach). Differences with cream cheese are explained by its higher viscosity that could affect the gastric emptying rate. In the present study, however, the two FEC emulsions of constant composition but varying viscosity did not present significantly different label excretion profiles.

Practical relevance of the findings

We have shown that at constant feed composition, the metabolization peak of lipids is more rapid for some ingestion structures than others, including gastric emptying, absorption and oxidation steps. Consequently, the following relevance can be hypothesized for future research: (i) milk fat structure could affect satiety and consequently the regulation of food intake (meal duration and inter-meal interval), (ii) in humans, the milk fat structure could affect blood cholesterol and cholesterol profile, (iii) since there is a link between the kinetics of appearance of plasma free fatty acid and the utilization speed of metabolic substrates, a greater oxidation of free fatty acid using less emulsified fat structures could be linked to a lower lipogenesis. For a given diet composition, some fat structures could be less lipogenic than others; this hypothesis should be studied.

Conclusion

The different kinetics of lipid metabolism observed in our study with milk fat, an important source of saturated fatty acids and cholesterol but also fat-soluble vitamins, suggest that their transport to plasma can be very different if milk fat is consumed free or as homogenized droplets. Global lipid metabolism based on oxidation to CO_2 was indeed decreased with smaller compared to larger emulsified milk fat particles with different coatings. These data support the concept that dairy emulsions with different droplet sizes and interface composition are digested and metabolized differently. However, we still need to understand the effect of milk fat globule size and membrane composition on the pancreatic lipase activity, implied in the present results. Further studies in our laboratory aim at characterizing the plasma cholesterol and triacylglycerol profiles after ingestion of milk fat in different physical structures. This way, the respective role of gastric processes and pancreatic lipase in the kinetic differences observed will be characterized. Moreover, it would be useful to synthetize ^{13}C-labeled triglycerides typical of milk fat for further breath test studies.

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