Imaging gastric structuring of lipid emulsions and its effect on gastrointestinal function: a randomized trial in healthy subjects1–5

Andreas Steingoetter, Tijana Radovic, Simon Buetikofer, Jelena Curcic, Dieter Menne, Michael Fried, Werner Schwizer, and Tim J Wooster

ABSTRACT

Background: Efficient fat digestion requires fat processing within the stomach and fat sensing in the intestine. Both processes also control gastric emptying and gastrointestinal secretions.

Objective: We aimed to visualize the influence of the intragastric stability of fat emulsions on their dynamics of gastric processing and structuring and to assess the effect this has on gastrointestinal motor and secretory functions.

Design: Eighteen healthy subjects with normal body mass index (BMI) were studied on 4 separate occasions in a double-blind, randomized, crossover design. Magnetic resonance imaging (MRI) data of the gastrointestinal tract and blood triglycerides were recorded before and for 240 min after the consumption of the following 4 different fat emulsions: lipid emulsion 1 (LE1; acid stable, 0.33 μm), lipid emulsion 2 (LE2; acid stable, 52 μm), lipid emulsion 3 (LE3; acid unstable, solid fat, 0.32 μm), and lipid emulsion 4 (LE4; acid unstable, liquid fat, 0.38 μm).

Results: Intragastric emulsion instability was associated with a change in gastric emptying. Acid-unstable emulsions exhibited biphasic and faster emptying profiles than did the 2 acid-stable emulsions (P ≤ 0.0001). When combined with solid fat (LE3), different dynamics of postprandial gallbladder volume were induced (P ≤ 0.0001). For acid-stable emulsions, a reduction of droplet size by 2 orders of magnitude [LE1 (0.33 μm) compared with LE2 (52 μm)] delayed gastric emptying by 38 min. Although acid-stable (LE1 and LE2) and redispersible (LE4) emulsions caused a constant increase in blood triglycerides, no increase was detectable for LE3 (P < 0.0001). For LE3, MRI confirmed the generation of large fat particles during gastric processing, which emptied into and progressed through the small intestine.

Conclusions: MRI allows the detailed characterization of the in vivo fate of lipid emulsions. The acute effects of lipid emulsions on gastric emptying, gallbladder volume, and triglyceride absorption are dependent on microstructural changes undergone during consumption. Gastric peristalsis and secretion were effective at redispersing pools of liquid fat in the stomach. This trial was registered at clinicaltrials.gov as NCT01253005. Am J Clin Nutr 2015;101:714–24.

Keywords: acid stability, fat digestion, gastrointestinal function, lipid emulsion, magnetic resonance imaging

INTRODUCTION

The increasing incidence of nutrition-related illnesses and their adverse impact on the quality of life has led to strong efforts to understand how food is sensed, processed, and digested within the gastrointestinal tract (1). The overconsumption of highly appealing energy dense foods has been seen as a key contributor to this issue, and the consumption of diets rich in lipids has often been cited as a leading cause of obesity (2). However, lipids are a good source of energy and essential fatty acids and play an important role in the absorption of micronutrients (vitamins and minerals), antinutrients (e.g., phytosterols), and class II (low solubility and high permeability) pharmaceuticals (3–8). Hence, there is keen interest in understanding how foods in general and lipids in particular are transformed during eating, digestion, and absorption (4, 9, 10).

The physical state of the dietary lipid affects the rate of fat digestion by regulating lipase access to its insoluble substrate and can be altered via interaction with the intragastric environment (11, 12). Despite this general understanding, which has been confirmed by extensive in vitro studies (5, 10, 11, 13–20), little is known about how different lipid structures behave and change within the human gastrointestinal tract (21–25). Studies by Marciani et al. (21–24) with the use of MRI showed that emulsions that were unstable to acidification form a layered structure within the stomach caused more-rapid gastric emptying but minimal or no change in fat absorption. With the combination of in vitro models and continuous blood sampling, Golding et al. (26–28) showed that the incorporation of solid fat into an acid-unstable emulsion could dramatically slow fat digestion, which the authors proposed was due to disruption of the stomach’s ability to process and emulsify fat. In addition, Armand et al. (29, 30) showed that the presence of viscous soluble fibers

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4Supplemental Table 1 and Supplemental Figures 1–5 are available from the “Supplemental data” link in the online posting of the article and from the same link in the online table of contents at http://ajcn.nutrition.org.

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only impacted fat digestion (in vitro) when there was a considerable increase in bolus viscosity (>10 mPa/s). A key gap in these studies was a lack of understanding about the dynamic interaction between the digestive tract and bolus structure and the effect this has on gastrointestinal function (e.g., gastric emptying).

One reason for our limited understanding of how different lipid structures behave within the digestive tract is that there are few techniques that allow the visualization and assessment of such information. MRI represents a valuable tool for the quantification of gastrointestinal function and characterization of luminal contents (31). MRI has only been used in a few studies to image gastrointestinal function and meal structural changes during digestion, likely because of the complexity of data analysis. The work of Marciani et al. (24) is a standout and highlights that some fat emulsions can separate into 2 layers within the stomach. Questions that arise from such observations as follows: 1) How common is gastric separation of lipid emulsions? 2) How extensive is such separation, and what is the role of trituration and gastric sieving on normalizing bolus structure? 3) How does such separation impact on the interpretation of measures of gastrointestinal function such as gastric emptying?

In the current study, we used MRI to probe the digestion of 4 emulsions that were designed to interact with the stomach in different ways. Our aims were to understand how the design of each emulsion (acid stable compared with acid unstable and resistant to redispersion compared with redispersible) altered their processing in the upper gastrointestinal tract and how this affected the interpretation of common measures of gastrointestinal function such as gastric emptying.

METHODS

This study was approved by the local ethics committee and registered at clinicaltrial.gov as NCT01253005. Written informed consent was obtained before inclusion.

Study design

This study was performed in a randomized, double-blinded, 4-armed, crossover study design. A total of 18 healthy subjects with normal BMI (in kg/m²) (≥18.5 to <25) and no history of gastrointestinal or metabolic disorders were recruited. On each of the 4 study days, after 8 h of fasting, subjects arrived in the morning or midday at the magnetic resonance (MR) center of the University Hospital Zurich. Subjects underwent MRI and blood sampling for a maximum of 4 h after receiving 1 of 4 different lipid emulsions. There was a minimum break of 5 d between visits. The sample size was determined on the basis of findings of a previous study that analyzed the intersubject and intrasubject variability of gastric liquid emptying in health and functional dyspepsia (32). We estimated that a total of 16 subjects would be needed to detect a difference of 13 min in the gastric half-emptying time between groups with a 2-tailed α of 0.05 and a (1-β) of 0.80. Author DM defined the randomized sequence for all subjects by computer generating 4 permuted blocks of n = 24. Assistant doctors and study nurses enrolled subjects and assigned lipid emulsions. To ensure the blinding of investigators and subjects, lipid emulsions were stored and administered in opaque bottles.

Lipid emulsions

Subjects consumed 400 mL of 1 of 4 emulsions [lipid emulsion 1 (LE1), lipid emulsion 2 (LE2), lipid emulsion 3 (LE3), and lipid emulsion 4 (LE4); 1.9 kcal/mL] on each study day, the composition and properties of which are outlined in Table 1. To reduce and optimize the number of subject visits, not all combinations of fat-particle size, emulsion stability, and fat physical form were examined. The selected 4 emulsions were prepared differently to obtain the desired particle sizes as follows.

LE1 was prepared by mixing 240 g rapeseed oil with 360 g of a 2.7-weight percent (wt%) polysorbate 80 (Palsgaard) solution by using a rotor stator homogenizer (MICCRA D 15, 25-mm rotor stator; MICCRA) at 10,500 × g and 50°C for 5 min. A fine emulsion was created by passing this mixture through a microfluidizer 3 times at 200 bar (model M110Y equipped with an H30Z 200-μm and F20Y 75-μm chamber; Microfluidics). This fine emulsion was diluted one to one with a solution containing 0.8-wt% xanthan to achieve the final composition.

LE2 was prepared by mixing 240 g of rapeseed oil with 360 g of a 0.02-wt% polysorbate 80, 0.1-wt% xanthan solution by using the rotor stator homogenizer at 10,500 × g and 70°C for 5 min. This coarse emulsion was diluted one to one with a solution containing 0.7-wt% xanthan to achieve the final composition.

LE3 and LE4 were prepared by mixing 243 g rapeseed oil and 3 g distilled monoglyceride (Dimodan HP-M; DuPont Danisco) with 957 g 1.25-wt% sodium caseinate (DMV International) solution by using the rotor stator homogenizer at 10,500 × g and 70°C for 5 min. For LE3, the oil phase consisted of 72 g powdered hydrogenated palm fat (Sett P55; BASF Personal Care and Nutrition GmbH) and 168 g rapeseed oil. For LE4, 240 g rapeseed oil was used. A fine emulsion was created by passing these mixtures through the microfluidizer twice at 300 bar.

The particle size of all emulsions was characterized by laser diffraction by using the particle size analyzer Mastersizer 3000 (Malvern), and particle-size distributions were obtained by fitting the laser diffraction pattern with the use of Mie theory by using a differential refractive index of 1.101 and an absorbance of 0.01. Particle-size distributions of all 4 emulsions are presented in Supplemental Figure 1.

MRI

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TABLE 1
Composition and physical properties of the 4 lipid emulsions consumed by participants

<table>
<thead>
<tr>
<th></th>
<th>LE1</th>
<th>LE2</th>
<th>LE3</th>
<th>LE4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fat content</td>
<td>20 wt% (liquid)</td>
<td>20 wt% (liquid)</td>
<td>20 wt% (solid/liquid)</td>
<td>20 wt% (liquid)</td>
</tr>
<tr>
<td>Emulsifier</td>
<td>0.8 wt%</td>
<td>0.00625 wt%</td>
<td>1 wt% NaCas</td>
<td>1 wt% NaCas</td>
</tr>
<tr>
<td>Thickener</td>
<td>Polysorbate 80</td>
<td>Polysorbate 80</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Mean particle size</td>
<td>0.33 μm</td>
<td>52 μm</td>
<td>0.32 μm</td>
<td>0.38 μm</td>
</tr>
<tr>
<td>Acid stable</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Redispersible</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
</tbody>
</table>

3Refer to Supplemental Figure 1 for particle-size distribution of all emulsions.

The timeline of MRI sessions is shown in Figure 1. MR image data of gastric contents were used to determine the gastric structuring and emptying of lipid emulsions. MR image data of gallbladder volume (gbv) and small bowel fluid content were used to derive information on the extent of intestinal secretion during emulsion digestion. The gastric content and gbv were imaged by using a T2/T1-weighted imaging sequence. Intraluminal and tissue water as well as visceral and subcutaneous fat appeared bright within these images, whereas liquid oil and solid fat appeared as signal voids (dark areas). To be able to differentiate the fat-induced signal voids from air, fat-selective imaging of the gastric content was subsequently performed. In these fat-selective images, signal intensity scales with the fat concentration in luminal content or tissue. Later in the study course and, therefore, performed in only 12 subjects, abdominal coronal T2-weighted scans with fat suppression were performed before meal intake and at time point $t = 190$ min. These image data were further acquired to gain preliminary information on the effect of lipid emulsions on the small-bowel fluid content.

Blood triglycerides

Blood triglycerides were assessed to have information on the metabolism of the lipid emulsion. Venous blood samples were taken by using a serum tube (Sarstedt) without any additives in a fasted state and, thereafter, at 0, 30, 60, 85, 135, 180, and 240 min after meal intake. The plasma and serum of samples were isolated by centrifugation (10 min, $10,389 \times g,$ $4^\circ C) \approx 60$ min of collection.

FIGURE 1 Timeline of the study protocol. Periods of continuous MRI scanning are indicated by gray blocks below the time axis. Sampling time points for whole blood triglycerides are indicated as crosses above the time axis.

Aliquots were stored at $-20^\circ C$ for later analysis. Plasma concentrations of triglycerides and free glycerol were determined with commercially available test kits (MODULAR Analytics (catalog no. 11 877 771 216; Roche Diagnostics) and Free Glycerol Reagent (product no. F6428; Sigma-Aldrich), respectively). The absorbance of the resulting metabolite was measured with a spectrophotometer (Cobas Mira auto analyzer; Hoffman La Roche). The true whole blood triglyceride concentration (in mmol/L) (cTG) was calculated by subtraction of the endogenous glycerol concentration from the total triglyceride concentration.

Visceral sensation rating

Before emulsion intake and after each MRI measurement, subjects rated their subjective sensations of hunger, fullness, nausea, bloating, and abdominal pain. A scale ranging from 0 and 10 ($0 =$ no symptoms; $10 =$ extremely prominent symptoms) was used for sensation ratings.

Data analysis

All quantitative image processing was performed under a blinded condition. A custom-made software tool written in MATLAB (version R2012b; The MathWorks) was used for a semi-automated image segmentation and quantification of gastric content volume and gbv according to previously reported procedures (33). The gastric content volume (gcv) at time $t = 0$ min ($gcv_0$) was approximated by summing the detected fasting volume and the 300-mL lipid-emulsion volume. Volumes were plotted over time to generate gastric emptying and gbv curves. For acid-stable LE1 and LE2, gastric emptying curves were analyzed by using the linear exponential (LinExp) gastric emptying fit given in Equation 1 as follows:

$$gcv(t) = gcv_0 \left(1 + \frac{t}{\text{temp}_{e}}\right) \exp\left(-\frac{t}{\text{temp}_{e}}\right)$$

Fit variables temp_e and $\kappa$ were estimated as $\text{temp}_{e} = (\text{temp}_{e} + \text{temp}_{c})$ and $\kappa = (\kappa_{i} + \kappa_{c})$, where temp_{e} and $\kappa_{i}$ are estimates for each individual (index i) and temp_{c} and $\kappa_{c}$ are estimated variations around temp_{e} and $\kappa_{i}$, respectively, induced by the lipid emulsions (index e). Variable temp_{e} = (temp_{e} + temp_{c}) in minutes is the emptying time constant and reflects the speed of gastric...
content emptying. Variable $\kappa = (\kappa_1 + \kappa_2) > 1$ quantifies an overshoot in gcv during the initial emptying process reflecting the accumulation of gastric secretion. Variable gcv0 was calculated per emptying curve. For acid-unstable LE3 and LE4, which exhibited biphasic gastric emptying dynamics, an exponentially extended version of the LinExp fit, named LinExpExp, given in the following equation 2 was applied:

$$
gcv(t) = \begin{cases} 
gcv_0 (1 + \frac{t}{\text{temp1}}) \exp(-\frac{t}{\text{temp1}}), & t \leq t_s 
gcv_1 \exp(-\frac{t}{\text{temp2}}), & t > t_s 
\end{cases}$$

Equation 2 is illustrated in Supplemental Figure 2. Fit variables temp1, temp2, and $\kappa$ were again estimated as temp1 = (temp1 + tempt1), temp2 = (temp2 + tempt2), and $\kappa = (\kappa_1 + \kappa_2)$. Variable $t_s$ represents the time point at which the emptying pattern started to deviate from the initial linear exponential behavior and was calculated per emulsion. Variable gcv1 in milliliters represents gcv($t = t_s$) and, like gcv0, was calculated per emptying curve.

ggb data, as one marker for intestinal secretion, were square-root transformed before plotting and statistical testing. For the reporting of interpretable physiologic results, ggb curves were normalized to their baseline values. The initial drop in gallbladder volume until 30 min (idgbv30) and average gallbladder volume between times 65 and 225 min (meanLategbv) were extracted and compared.

The change in small bowel fluid content after 190 min, as another marker for intestinal secretion, was qualitatively determined by visual inspection of the bright luminal fluid content before and after emulsion intake. Because of the limitation of 2 sampling time points for this variable, we refrained from a more sophisticated and quantitative analysis approach as described by Hoad et al. (34). No visual difference, a small difference, and a clear difference in luminal fluid were rated as 0, 1, and 2, respectively. This visual rating was independently performed by 3 assessors.

cTG was log transformed before plotting and statistical testing. For the reporting of interpretable physiologic results, the average $\delta$ over baseline triglyceride concentration for each subject and lipid emulsion was calculated.

Gastric emptying curves were fitted by using a hierarchical Bayesian Markov chain Monte Carlo sampling strategy. To ensure identifiability, weakly informative priors with a mean value of zero were applied to the distributions of temp1, tempt1, temp2, and $\kappa$. The R package interface for the Stan modeling language (RStan, version 1.3.0; http://mc-stan.org) and respective C++ library for Bayesian inference was used (35). The effect of the lipid emulsions on gastric emptying fit variables was determined by highest posterior density CIs. The shortest possible interval enclosing 95% of the posterior mass was selected. Differences in the variables are presented as means and highest posterior density 95% CIs.

The effect of lipid emulsions on the square-root transformed ggb and log-transformed cTG was tested by using a linear mixed effect model with function lme of R package nlme (version 3.1-113, 2013) with lipid emulsion and time as fixed effects and the subject as a random effect (26). In addition, function lme was used to test for the effect of emulsions on idgbv30 and meanLategbv. Statistical analyses were carried out with the R program (27). Estimated variables are presented as the estimate (±SEE).

The intraclass correlation coefficient between the 3 raters for visual ratings on changes in intestinal fluid content was determined by using function ICC of the R package psych (version 1.5.0) (36). The effect of the lipid emulsions on these changes in small bowel fluid content was tested by using a cumulative link mixed model with function clmm of R package ordinal with lipid emulsion as fixed effects and the subject as well as the interaction of subject and emulsion as random effects (28).

Because only sensations for hunger and fullness differed considerably from zero, sensations for nausea, bloating, and pain were converted to binary data (i.e., 0 and ≥1). Histograms were plotted to visually compare the distribution of ratings per sensation. Mean sensations over time were calculated per subject and emulsion. The effect of lipid emulsions on the different mean sensations was tested by cumulative link mixed models with lipid emulsion as fixed effects and the subject as a random effect.

RESULTS

MRI

Seventeen volunteers (7 men; age: 24.9 ± 6.9 y; BMI: 22 ± 1) successfully finished the study. The intake of all lipid emulsions was well tolerated by all subjects. Sensations of nausea were predominantly rated as zero for all lipid emulsions. One woman was withdrawn from the study after the second visit because of repeated protocol violations. Gastric content and gbv curves were successfully determined in all remaining volunteers. Two measurement time points were missing because of technical errors. For LE1, one subject was in a nonfasting condition at study start. These data were excluded from the statistical analysis.

Intragastric MR-signal dynamics of lipid emulsions

The 4 lipid emulsions showed clearly different intragastric MR-signal dynamics indicative of differences in structural stability. Acid-unstable emulsions LE3 and LE4 underwent clear structural and MR-signal changes during gastric emptying. The intragastric properties and dynamics of LE3 are depicted in Figure 2A. Within the first 60 min, the water and lipid phases of LE3 were separated continuously until only aggregates of liquid oil or lumps of fat stayed in the stomach. Fat-selective MR images identified intraluminal signal voids in the T2/T1-weighted images as liquid oil or fat particles in the stomach and duodenum. Liquid oil or fat particles were partly surrounded by gastric secretion of a bright MR signal in the T2/T2-weighted images. Subsequently, gastric secretion and mechanical processing allowed, to some degree, the re-emulsification of the remaining oil and fat as indicated by increased water and decreased fat signals in the respective MR images. In some volunteers, MRI visualized the presence of aggregates of fat also in the duodenal bulb, which strongly indicated passage through the pylorus (Figure 3A). Figure 3B shows an exemplary coronal image sequence of the stomach, duodenum, and parts of the small bowel post-ingestion. It was clear that the signal voids were transiting the small bowel over time, which indicated that these solid fat lumps appeared to have low digestibility and high resistance to redispersion during peristalsis. The intragastric properties and dynamics of LE4 are depicted in Figure 2B. Within the first 15–30 min, creaming occurred in LE4 [i.e., LE4 started to separate into a high-fat content emulsion
Intragastric dynamics of LE1 and LE2 are depicted in Figure 4. Acid-stable emulsions LE1 and LE2 largely maintained their MR-signal properties throughout the 225-min measurement period as recognized in the T2/T1-weighted MR images. Nevertheless, intragastric signal intensities in the fat-selective MR images were subject to continuous variations (i.e. by the appearance of bands of less intense fat signal, which was most likely caused by mixing and dilution with gastric secretion). Gastric secretion continuously mixed with the emulsions and decreased the intragastric fat signal intensity until the end of the measurement period.

Gastric emptying of lipid emulsions

Individual gastric emptying curves grouped per lipid emulsion are presented in Figure 5. The gastric stability of LE1 and LE2 resulted in a relatively homogeneous intragastric fat concentration and, hence, exhibited a single, steady gastric emptying pattern. Therefore, the content emptying time of acid-stable emulsions LE1 and LE2 was longer than for acid-unstable emulsions. The 2 acid-unstable lipid emulsions LE3 and LE4 showed a distinct biphasic emptying pattern, which clearly deviated from the more-common emptying dynamics reflected by the 2 acid-stable emulsions LE1 and LE2. During the fast content-emptying period between 15 and 65 min for LE3 and LE4, the gastric content of low (brighter signal in the fat selective image) and low–fat content emulsion due to the different droplet sizes. The high–fat content emulsion was sitting more proximal floating on top of the low–fat content emulsion that filled the distal stomach. After early emptying of the latter, areas of liquid oil partly appeared inside the remaining high–fat content emulsion. This phenomenon was clearly detectable in the fat-selective MR images at 30 min as pointed out by a white arrow. Areas of liquid oil appearing inside the remaining high–fat content emulsion (t = 80 min) were subsequently re-emulsified by mixing with the gastric secretion that accumulated in the distal stomach (t = 225 min). LE3, lipid emulsion 3; LE4, lipid emulsion 4; MR, magnetic resonance.
fat concentration and, thus, low energy density was rapidly emptied. Calculated variables of the LinExp gastric emptying fit applied to LE1 and LE2 are summarized in Supplemental Table 1. Individual gastric emptying curves and computed fits are presented in Supplemental Figure 3. The LinExp fit detected slower gastric emptying for LE1 (particle size: 0.33 μm) than LE2 (particle size: 52 μm). The difference, presented as mean and highest posterior density 95% CI, in the emptying time constant was $D_{\text{empt}_1} = 38 \text{ min} (10–64 \text{ min})$. No difference in $k$ (reflecting the accumulation of gastric secretion) was observed $[D_{k_e} = 0.03 (0.20–0.30)]$.

Calculated variables of the biphasic LinExpExp gastric emptying fit applied to LE3 and LE4 are summarized in Supplemental Table 1. Individual gastric emptying curves and computed fits are presented in Supplemental Figure 4. The LinExpExp fit detected slower gastric emptying in the first emptying phase for LE4 (redispersible; liquid oil) than LE3 (not redispersible; liquid oil plus solid fat). The difference in this early emptying time constant was $D_{\text{empt}_1} = 11 \text{ min} (7–15 \text{ min})$. In accordance, a later onset of the second emptying phase was detected for LE4 with $D_{t_2} = 10 \text{ min} (2–17 \text{ min})$. No difference in $\kappa$ (reflecting the accumulation of gastric secretion) was observed $[D_{\kappa_e} = -0.01 (-0.28 to 0.30)]$ or in the emptying time constant for the second emptying phase $[D_{\text{empt}_2} = -11 \text{ min} (-68 to 47 \text{ min})]$ was observed.

$gbv$

Square-root transformed individual $gbv$ curves grouped per lipid emulsion are presented in Figure 6. Within the first 60 min, there was a consistent decrease of $gbv$, which indicated an immediate sensing of fat in the proximal intestine in all individuals and for all lipid emulsions. This decrease was followed by highly variable, subject-specific $gbv$ dynamics. Theses dynamics in $gbv$ were similar for the 2 acid-stable emulsions LE1 and LE2 ($P = 0.95$) and LE4 ($P = 0.06$) but different for LE3 ($P\text{LE1 and LE2} < 0.001$). LE3 showed the maximum and LE1 the minimum initial decrease in $gbv$ (LE3: id$gbv_{30} = 14 \text{ mL};$ LE1: id$gbv_{30} = 9 \text{ mL};$ $P = 0.001$). LE3 showed the fastest recovery of $gbv$ during the measurement period as indicated by the highest value for meanLate$gbv$ ($9.9 \pm 1 \text{ mL}$), which was different than for all other emulsions (LE1/LE2/LE4: 5.1/6.6/6.2 ± 1 mL, respectively; all $P \# 0.001$).

Change in small-bowel fluid content

The ICC coefficient of visual ratings of the small-bowel fluid content was $0.73 (0.61–0.83)$, which indicating good agreement between the 3 assessors. LE1 had the highest increase, and LE3 had the lowest increase, in small-bowel fluid content with mean ($\pm$SD) ratings of $1.83 \pm 0.48$ and $0.67 \pm 0.64$, respectively. The probability of receiving the next higher rating of small-bowel fluid content was lowest for LE3 and different than for LE1 and LE2 ($P < 0.004$) and LE4 ($P = 0.005$). These results further supported the observation of LE3 being a less-effective stimulus of intestinal secretion for LE3. LE4 was only different compared with LE1 ($P = 0.02$) but not LE2 ($P = 0.08$).

Blood triglycerides

Log-transformed individual cTG profiles grouped per lipid emulsion are presented in Figure 7. Although LE1, LE2, and LE4 caused a constant increase in cTG, no increase was detectable for LE3 ($P < 0.001$). The estimated mean ($\pm$SD) of $\delta$ over baseline of cTG for LE1, LE2, LE3, and LE4 were...
This finding confirmed the observation in the MR-image data of LE3 that the majority of the ingested fat phase passed through the gastrointestinal tract as aggregated solid particles that underwent slow metabolism. The result was also in line with the observed reduced gallbladder contraction and intestinal secretion.

Visceral sensations

Histograms per visceral sensation and lipid emulsion together with estimated ORs and respective 95% CIs are displayed in Supplemental Figure 5. Mean sensation ratings of hunger were lowest for LE1 and different than for other emulsions ($P = 0.002$). LE3 showed the highest hunger ratings ($P = 0.008$). Vice versa, and consistent with these findings, ratings for fullness were highest for LE1 ($P = 0.01$) and lowest for LE3 ($P < 0.001$). Nausea, bloating, and pain were rarely rated higher than zero. LE1 showed highest ratings for nausea and pain, whereas LE4 had highest ratings for bloating.

DISCUSSION

Our study showed that the in vivo fate of lipid emulsions could be followed in detail in both the stomach and intestine by using water and fat-selective MR imaging. With the use of MRI, we quantified lipid-emulsion–dependent changes in the gastric emptying behavior and detected differences in the intragastric structuring of fat particles and food boluses. Stable lipid emulsions (LE1 and LE2) exhibited similar steady gastric emptying patterns, a continuous increase in blood triglycerides, and lowest hunger ratings. Unstable lipid emulsions LE3 and LE4 both exhibited biphasic emptying patterns and, therefore, gastric emptying mathematical models needed refinement. For LE3, intestinal MRI showed that large fat particles exited the pylorus and transited through the small bowel. The flat triglyceride-absorption profile and highest hunger and lowest fullness ratings of LE3 indicated that these solid fat particles underwent very slow metabolic modification. In contrast, LE4, which had liquid fat, underwent a similar metabolic transformation as for LE1 and LE2 despite extensive intragastric fat layering. This result suggested the intragastric re-emulsification of LE4 by antral contractile activity. After similar initial changes in gbv, which indicated similar immediate intestinal fat sensing for all lipid emulsions, LE3 exhibited a steady rise in gbv at later time points. This rise coincided with low intestinal secretion, which confirmed the slow metabolic modification of fat particles.

MRI data revealed that the deliberate design of emulsions, which were acid unstable and resistant to redispersion within the...
intragastric environment, can have a dramatic effect on the food-
bolus structure, distribution, and emptying. Previous studies by
Golding et al. (11, 37) showed that similar emulsions underwent
extensive structural transformations in vitro that lead to slow and
delayed triglyceride absorption. Compared with previous studies
(12, 21–24), the frequent imaging of gastric content and gbv,
especially during the first 1.5 h, allowed a more-detailed de-
scription of the temporal changes in the gastric emptying and
structuring of unstable and stable fat emulsions in the stomach.
The 2 stable emulsions (LE1 and LE2) did not undergo any
relevant structural change in the stomach, and the fat content
constantly decreased over time, which indicated dilution by
gastric secretions. The 2 unstable emulsions underwent 3 dis-
tinguishable phases of dramatic structural evolution as follows:
1) initially evenly well dispersed; 2) 15–70-min structure for-
mation with the appearance of layers and large lumps of fat and

![Figure 5](https://academic.oup.com/ajcn/article-abstract/101/4/714/4564528)

**FIGURE 5** Gastric content volume data. Individual ($n = 17$) gastric emptying curves are grouped by lipid emulsion. The different biphasic emptying pattern of the unstable lipid emulsion was very distinct and consistent for all individuals. Slower gastric emptying was detected for LE1 (particle size: 0.33 μm) than for LE2 (particle size: 52 μm). The difference in the emptying time constant was $\Delta t_{empt1} = 38\text{ min (10–64 min)}$. LE4 (redispersible; liquid oil) showed slower gastric emptying in the first emptying phase than did LE3 (not redispersible; liquid oil plus solid fat). The difference was $\Delta t_{empt1} = 11\text{ min (7–15 min)}$. A later onset of the second emptying phase was detected for LE4 with $\Delta t_2 = 10\text{ min (2–17 min)}$. No difference in the emptying time constant for the second emptying phase ($\Delta t_{empt2} = -11\text{ min (68 to 47 min)}$) was observed. Differences were analyzed by using highest posterior-density CIs of Markov chain Monte Carlo chains. LE1, lipid emulsion 1; LE2, lipid emulsion 2; LE3, lipid emulsion 3; LE4, lipid emulsion 4.

![Figure 6](https://academic.oup.com/ajcn/article-abstract/101/4/714/4564528)

**FIGURE 6** sqrt(gbv) data. Individual ($n = 17$) curves (black solid lines and dots) are grouped by lipid emulsion. The mean value at each time point (thick black line and dots) is overlaid to improve readability. Dynamics in the gbv were similar for the 2 acid-stable emulsions LE1 and LE2 ($P = 0.95$) and LE4 ($P = 0.06$) but different for LE3 ($P$-LE1 and LE2 < 0.001). LE3 showed the maximum, and LE1 showed the minimum, initial decrease in gbv (LE3: idgbv30 = \(-14 \pm 2\text{ mL},\) LE1: idgbv30 = \(-9 \pm 2\text{ mL},\) $P = 0.001$). LE3 showed fastest gbv recovery and thus highest meanLategbv = 9.9 ± 1 mL compared with all other emulsions (LE1 / LE2 / LE4: 5.1 / 6.6 / 6.2 ± 1 mL, all $P \leq 0.001$). Differences were analyzed by using linear mixed-effect modeling. gbv, gallbladder volume; idgbv30, initial drop in gallbladder volume until 30 min; LE1, lipid emulsion 1; LE2, lipid emulsion 2; LE3, lipid emulsion 3; LE4, lipid emulsion 4; meanLategbv, average gallbladder volume between times 65 and 225 min; sqrt(gbv), square-root transformed gallbladder volume.
liquid-oil pools (dark voids); 3) at later times, varying degrees of erosion, degradation, and re-emulsification of the large fat pools and lumps formed in the stomach. The nature of the fat in the 2 acid-unstable emulsions appeared to have an impact on the efficiency of redispersion during passage through the antrum into the duodenum. The acid-unstable emulsion with liquid fat (LE4) had a similar triglyceride absorption profile to that of LE1 and LE2, despite extensive intragastric fat layering. This result suggested that fat droplets present within the intestine had a size comparable to that of droplets of LE1 and LE2 and, hence, implied the intragastric re-emulsification of LE4 by antral contractile activity. In contrast, the large solid-fat aggregates of LE3 did not appear to be extensively restructured within the stomach but were gradually eroded, and fat particles <1.35 cm were able to exit the pylorus. The detection of large solid-fat particles in the small bowel indicated that the forces present in the distal stomach were not strong enough to re-emulsify. This finding might contradict our current understanding of the role of the pylorus as a gatekeeper to prevent large food particles existing during digestion (5, 38). However, it appears that these fat particles are deformable, and this feature may be the reason that they can exit the pylorus, whereas more-firmer particles, such as those of Marciani et al. (39), do not.

LE1 and LE2 showed similar, commonly observed gastric emptying profiles. In contrast, LE3 and LE4 revealed distinct and consistent biphasic emptying profiles as a result of their structuring. These profiles could not be described by standard curve-fitting methods. Therefore a hierarchical Bayesian Markov chain Monte Carlo nonlinear fit was applied to extract gastric emptying variables. The hierarchical model and weakly informative priors enforced constraints on the emptying curves. With the borrowing of strength from other curves in the population, stable estimates could be derived for curves with ambiguous parameterization. The results showed that a smaller lipid-droplet size (LE1: 0.33 µm) delayed gastric emptying (LE2: 52 µm). This observation was consistent with data on the intraduodenal infusion of different sized emulsions (40); however, to our knowledge, the current study is the first demonstration of oral feeding of different sized emulsions. Biphasic emptying patterns of LE3 and LE4 were similar to those obtained by Marciani et al. (22) for a different acid-unstable formulation; it would be interesting to see the similarity in gastric emptying variables.

As generally understood, lipid digestion requires the rapid sensing of fat in the duodenum and immediate secretion of bile salts and pancreatic enzymes (41). Marciani et al. (42) recently highlighted that highly emulsified fat is a potent stimulator of gallbladder emptying. In the current study, the gbv and intestinal fluid content were used to indirectly track the need for such secretions to facilitate digestion. The gbv decreased and remained stable for LE1, LE2, and LE4, which indicated a steady state in bile outflow into the intestine. In comparison, the gbv of LE3 initially decreased and then increased at later time points, which indicated the accumulation of bile in the gallbladder. The fact that all 4 emulsions had similar initial decreases in gbv indicated similar fat sensing in the duodenum. Despite slower gastric emptying, an increased intestinal volume was observed for the stable lipid emulsions (LE1 and LE2), which correlated with the low gbv indicated steady bile outflow. In contrast, the accumulation of bile in the gallbladder and unaltered intestinal fluid content seen for LE3 indicated a reduced demand for intestinal secretions, and this occurred despite the presence of lipid in the intestinal lumen. This result supported the finding that the fat was not detected by the intestinal lumen and was, therefore, less digestible. Because fat is sensed by the release of fatty acids in the lumen, this lower digestibility should be reflected in triglyceride profiles. Indeed, this effect was confirmed by the markedly different triglyceride blood profiles seen with LE3. These findings are supported by the recent work of Marciani et al. (42) who concluded that the amount of fat and its degree of emulsification impact gallbladder secretory function.

The differences in gastric emptying and fat digestibility of each lipid emulsion were paralleled by reported visceral sensations. Similar to other studies, the smaller fat droplet size and
slower gastric emptying of LE1 than LE2 resulted in increased fullness and decreased hunger sensations (40, 43, 44). The faster gastric emptying, reduced intestinal secretion, and lower fat digestibility of LE3 resulted in the highest and lowest ratings of hunger and fullness sensations, respectively. This result is in line with the study of Keogh et al. (37), who reported increased hunger and decreased satiety for a sodium-caseinate–stabilized emulsion containing hydrogenated rapeseed oil.

It might be argued that the emulsions in this study were of highly artificial fat compositions in the absence of significant amounts of protein or carbohydrates and, hence, not representative of the Western mixed diet. However, the lipid ingredients of these emulsions are commonly used in the fabrication of foods (i.e., solid fats are used for the structuring of chocolate, hydrogenated fats are used in cooking shortenings, and fat-particle aggregation via partial coalescence is key to the stabilization of whipped foams in dairy creams and ice cream). The absence of significant amounts of protein or carbohydrates will affect the rheology and texture of the gastric contents and likely slow down or limit the degree of fat droplet aggregation. One of the key findings of this study was that MR imaging detected the transit of large solid-fat particles through the intestine. The implications of this detection on the overall nutrient transit and fat content in stool are unknown. Previous studies reported that the transit of indigestible fat through the intestine was associated with abnormal bowel events or delayed gastric emptying triggered by ileal brake (45–47). Neither of these effects was observed in the current study, suggesting that the majority of fat underwent metabolic modification during intestinal transit. The imaging of the stomach and intestine was performed by using sequential MRI. Although such sequential recordings provided insights, these could have led to minor artifacts in the analysis because of evolution in bolus structure and gastrointestinal secretion volume over time. Hence, the additional development of methods that can simultaneously acquire detailed anatomical and functional MRI images of the entire intestinal tract is needed.

In conclusion, semiquantitative MRI allowed the in vivo fate of lipid emulsions to be followed in detail in both the stomach and intestine by using water and fat-selective MR imaging. This technique not only revealed information of the complexity of food structural transformation as a result of iteration within the digestive tract but also gave insight into the mechanical breakdown and re-emulsification of lipid droplets via trituration and peristaltic activity. In the future, quantitative MRI methods such as fat-fraction measurement and T1/T2 relaxometry will enable us to assess the separate distribution and emptying of different liquid and solid components of gastric content. This ability will provide greater understanding of the 2-way interaction between food and the digestion physiology and initiate new methods to tailor nutritional interventions for diseases such as cystic fibrosis and nutritional disease states such as diabetes and obesity.

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