Intragastric layering of lipids delays lipid absorption and increases plasma CCK but has minor effects on gastric emptying and appetite

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1Unilever R&D, Vlaardingen; 2Division of Gastroenterology-Hepatology, Department of Internal Medicine, University Hospital Maastricht, Maastricht; 3Department of Gastroenterology-Hepatology, Leiden University Medical Centre, Leiden; and 4Center for Liver, Digestive and Metabolic Diseases, University Medical Center Groningen, Groningen, The Netherlands

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Foltz M, Maljaars J, Schuring EA, van der Wal RJ, Boer T, Duchateau GS, Peters HP, Stellaard F, Mascele AA. Intragastric layering of lipids delays lipid absorption and increases plasma CCK but has minor effects on gastric emptying and appetite. Am J Physiol Gastrointest Liver Physiol 296: G982–G991, 2009. First published March 26, 2009; doi:10.1152/ajpgi.90579.2008.—Intestinal intubation studies have demonstrated that the layering of lipids in the stomach delays lipid absorption and increases fullness. However, it is unknown whether this effect is specific to the stomach and remains unchanged when a second meal is administered. Here, we present an explorative study testing whether intragastric layering of lipids delays lipid absorption and increases plasma CCK in healthy men. Intragastric infusion of 40 g of high-oleic-acid rapeseed oil (325 ml, 181 kcal) was infused as a control. In OT and EM a second LM was infused. In OT rapeseed oil was incorporated into the LM (325 ml, 181 kcal); 4.6 ml rapeseed oil (4.6 ml, 36 kcal) labeled with 77 mg glyceryl-[13C]trioleate (145 kcal) followed by intragastric infusion of 40 g of high-oleic-acid rapeseed oil (4.6 ml, 36 kcal) labeled with 77 mg glyceryl-[13C]trioleate. In the emulsion treatment (EM, control), 4.6 ml of labeled rapeseed oil was incorporated into the LM (325 ml, 181 kcal); 4.6 ml of saline was infused as a control. In OT and EM a second LM was consumed at time t = 270 min. Plasma [13C]-C18:1, CCK and satiety were measured over 480 min. GE was determined by the paracetamol absorption test. OT delayed oleic acid absorption shown by an increased lag time of absorption (EM: 37 ± 7 min; OT: 75 ± 10 min; P < 0.01) and time at maximum concentration (EM: 162 ± 18 min; OT: 280 ± 33 min; P = 0.01). OT released more CCK than EM (P = 0.03), including increased CCK after the second meal. OT accelerated initial GE until 30 min postprandial. OT showed a tendency (P = 0.06) to suppress hunger and increase satiety and fullness 120–270 min postprandially. The results demonstrate that low amounts of lipids, when separated from the aqueous phase of a meal, delay lipid absorption and increase CCK. An escalating-dose study should determine whether this could have implications for the development of weight-control foods.

hunger; satiety; oleic acid; paracetamol absorption test; liquid meal

LONG-TERM DIETARY INTERVENTIONS using low-energy liquid meals (LM) have clearly demonstrated effectiveness in achieving and maintaining weight loss in addition to improving metabolic biomarkers of comorbid diseases in clinical trials (3, 13). Despite the proven success of LM in clinical trials, it remains a challenge to achieve and maintain weight loss in all users because of perceived hunger, which is a significant predictor of failure to lose weight (44). This suggests that delaying the return of hunger and increasing satiety following consumption of LM would help to improve compliance and would aid in achieving weight loss and weight control (39).

Food stimulates satiety and inhibits food intake by complex and interdependent mechanisms in which signals arising from the gastrointestinal tract play an important role (10). Gastrointestinal satiety consists of two interacting components: distention of the stomach (mechanical) and release of gastrointestinal peptides by the small intestine (nutritive) (10). In the intestine, lipids are sensed in the wall of the intestine by specialized receptors, induce inter alia release of cholecystokinin (CCK) from enteromedocrine cells, and activate extrinsic vagal afferent nerve terminals. Together, this leads to a multitude of physiological processes including an inhibition of gastric emptying (GE) and finally to a reduction in hunger and food intake (25, 40).

Underlying mechanisms of satiety signaling are often investigated by using small intestinal intubation studies. In these studies, nutrients are infused at constant rates into specific small intestinal regions. In general, infusion rates (0.1–2 kcal/min) and concentrations (14–80 mmol) that are suggested to mimic the postprandial physiological concentrations entering the small intestine after consumption of a meal (2, 5, 22) were used. It has been proven that these mechanisms are associated with satiety, food intake, and satiety-related physiological parameters such as GE (16, 17, 27, 28).

However, such mechanistic studies neglect that after intake of complex meals, nutrients may arrive in nonuniform patterns to the duodenal and jejunal region (34) and not at constant rates. Furthermore, effects on satiety and physiological parameters related to satiety (GE and gut hormone secretion) that are observed after infusion of low-lipid doses may be overruled in complex meals by the effects of other meal components (proteins and/or carbohydrates) (11, 18). Therefore, in our view, compared with “real meal”-situations, small intestinal infusion studies neglect the effects of 1) intragastric distribution of lipids and the subsequent effects on lipid delivery to the small intestine and 2) complex and interacting effects of macronutrients on satiety-inducing mechanisms under fed conditions. Support for the former hypothesis, i.e., sequential lipid delivery from the gastric lumen to the gut, originates from early studies using gamma scintigraphy. These studies show that fat empties with the meal from the stomach when it is an integral part of the food (14). However, when the fat is free and liquid, phase separation occurs, i.e., the fat forms a layer on top of the meal (“creaming”), resulting in slower GE (14, 23). Recent magnetic resonance imaging (MRI) studies including echo-planar imaging have demonstrated that the rate of emptying of lipids from the gastric lumen influences emptying rates of the total meal.
and is dependent on the physical state of the lipid (whether the lipid is part of the meal or free), the ingestion order, and the posture of the subject (6, 7, 24, 30–33).

Most processed foods contain lipids in the form of emulsions. By varying the emulsifier type and emulsification process these emulsions can be tailored toward their stability under gastric conditions; i.e., emulsions that stay intact at low pH (gastric stable) or emulsions that release free lipid due to emulsifier breakdown under gastric conditions (gastric unstable). Lipids in gastric-stable emulsions have been shown to empty with the aqueous phase. Compared with gastric-unstable emulsions, in which the oil forms a layer on top of the aqueous phase, they increased the forward and backward antral flow in the stomach, increased CCK secretion and increased gallbladder contraction (7, 30, 32). However, in these studies, pure oil in water emulsions at very high lipid doses (75–100 g) were used. It is unclear whether gastric-unstable emulsions will continue to yield the same results when these emulsions, at physiological concentrations, are incorporated into a real food matrix.

On the basis of previous work on intragastric lipid distribution in the stomach, intraduodenal lipid sensing and triggering of mediators of satiety, we questioned whether a modulation is also possible under more physiological conditions. The aim of this explorative, randomized, placebo-controlled, crossover study was to investigate whether it would be possible to modulate release of lipids from the stomach, plasma CCK levels, and GE in healthy volunteers by using low lipid loads layered in the stomach on an orally consumed LM. Possible translation of these changes, if present, into concomitant changes in perceptive satiety was measured as additional outcomes. In healthy men, nasogastrically infused 4 g of high-oleic-acid rapeseed oil labeled with $^{13}$C-C18:1 (oleic acid given in the form of a triacylglyceride) on top of an orally consumed LM. In the control treatment 4 g of labeled rapeseed oil was emulsified in the LM, and saline was infused as a control. The effect of emptying of lipids from the stomach measured by plasma $^{13}$C-C18:1 appearance and CCK profiles (primary outcomes) as well as GE of the aqueous phase of the LM and satiety perception (secondary outcomes) were determined.

**MATERIALS AND METHODS**

**Subjects**

Male subjects aged 18–45 yr were recruited from the campus population of the Leiden University Medical Center. Subjects were screened to be generally healthy as assessed by means of a screening questionnaire, BMI assessment (19–25 kg/m$^2$), and standard hematology. Smokers, restrained eaters [assessed with the Dutch Eating Behavior Questionnaire (43) with a score of restriction H11022>2.5], subjects with hemoglobin concentrations <8.4 mmol/l or who had donated blood in the last 4 wk, and subjects who reported dieting or who were on a medically prescribed diet in the last 6 mo before the start of the study were excluded from participation. Subjects on medication that may influence appetite and sensory functioning or subjects who reported a metabolic or endocrine disease or gastrointestinal disorders were not included. Six subjects (age 22.0 ± 1.4 yr; BMI 23.8 ± 1.1 kg/m$^2$) were included in the study, and all subjects finished the study. Participants were informed about the study and all subjects signed an informed consent form before participation. The Medical Ethics Committee of the Leiden University Medical Center (Leiden, The Netherlands) approved the study.

**Study Design**

The study was an explorative, randomized, double-blind, placebo-controlled, crossover study performed at the University of Leiden Medical Center and was conducted following good clinical practice guidelines. The study design is presented in Fig. 1. The study used a within- and between-subject design with two treatments (OT and EM) that each subject underwent twice, leading to four interventions per subject. Thus, the study consisted of 4 intervention days separated by...
washedout periods lasting 1 wk each. This study design led to six possible sequences in receiving the treatments. Each subject was randomly allocated to one of the six possible treatment sequences. The study had a staggered start, with two subjects starting per day. The study hypothesis was that intragastric lipid layering leads to delayed lipid absorption compared with emulsified lipids when incorporated into a LM. The delayed lipid absorption in turn enhances postprandial CCK concentrations and modulates GE. Primary outcomes of the study were changes in plasma $^{13}$C-C18:1 concentrations [maximum concentration ($C_{\text{max}}$), time at $C_{\text{max}}$ ($t_{\text{max}}$), and lag time ($t_{\text{lag}}$)] and CCK concentrations [area under the time-plasma concentration curve (AUC)]. Secondary outcomes were changes in GE (GE half-time, $T_{50}$) as well as satiety and hunger perception (area under the time-appetite curve).

Sample Size Estimate

The present study was designed as an explorative study because no data on either the variance of the difference between treatments nor on the variance within subjects for the primary outcome parameters (oleic acid kinetics) was available. Previous studies showed significant differences in fat absorption kinetics with six to nine subjects (1). Our own studies showed a within-subject variance in the incremental AUC of CCK of 0.64 pmol·1$^{-1}$·min$^{-1}$. On the basis of an estimated physiological meaningful effect of at least 10% needed for CCK, ten observations would be sufficient for CCK (2-sided test, power 0.8, α 0.05). For satiety, we found in earlier studies a within-subject variance of ~85 (AUC). On the basis of a two-sided test, power of 0.8 and α of 0.05, 12 observations would be sufficient to detect significant differences in satiety of at least 12%. We decided in this crossover study to use six subjects but to repeat each treatment twice in each subject. By repetition of both treatments in each individual, the treatment effect could be estimated with the same precision compared with a trial with 12 individuals and one observation per treatment. The advantage of this design is that it allows for a statistical analysis in which both interindividual as well as between individual variations are estimated. In crossover trials the within-individual variation is the most important variation because the treatment difference is estimated within each person. Thus the present design improves the precision of the determination of the within-subject variation. The model used in the analysis of covariance (ANCOVA) analysis (see Data and Statistical Analysis) took the repetition of the treatments per individual into account.

Treatments

Two treatments were tested. In the “on-top” treatment (OT), subjects received orally a fat-free LM (325 ml, 145 kcal, 0.4% intrinsic fat) followed by a nasogastric infusion of 4.06 g high-oleic-acid rapeseed oil (4.6 ml, 36 kcal) labeled with 77 mg $[^{13}$C]$\text{C}$triolein (glyceryl-trioleate with a uniformly labeled $^{13}$C-C18:1 on the sn-1 position, isotopic enrichment 99%; Buchem, The Netherlands). In the second treatment, “emulsion” (EM), subjects received orally a LM (325 ml, 181 kcal, 1.6% fat) containing 4.06 g high-oleic-acid rapeseed oil (4.6 ml) labeled with 77 mg $[^{13}$C]$\text{C}$triolein as a fine emulsion; 4.6 ml of saline was infused via the nasogastric tube as a control. All nasogastric infusions were given as bolus infusion within 1 min. In both conditions, 1.5 g of paracetamol was dissolved in the LM immediately and not later than 10 min prior to consumption.

Experimental Protocol

Two days prior to the intervention subjects were asked to refrain from foods rich in $^{13}$C (list provided to participants). In every period the day prior to the intervention the subjects received the same standardized dinner. After dinner, subjects stayed absent from food and caloric beverages and came on each intervention day to the study facility in a fasted state. Subjects arrived at the study facility at least 1.5 h before their scheduled intervention. Prior to all interventions the subjects were taken to a room equipped with a hospital bed, where a nasogastric tube (Flocare Bengmark, Nutricia, Zoetermeer, The Netherlands) was inserted. To avoid detection of differences between the two types of treatments by the subject as well as investigator, the tube was obscured with opaque tape. A cannula was inserted into the antecubital vein of each subject’s forearm, and a fasting blood sample was taken ($t = -15$ min). Subjects then completed a validated electronic visual analog scale (EVAS) questionnaire to assess satiety related parameters and nausea (see Measurements). Subsequently subjects consumed the LM using a straw and were asked to finish the beverage in 5 min. The moment in which the last gulp was swallowed was considered to be $t = 0$ min. Immediately after consumption of the LM, the intragastric infusion (bolus infusion within 1 min) of rapeseed oil (condition OT) or saline (condition EM) was performed manually by use of an obscured syringe and taking into account the void volume of the nasogastric tube. At $t = 270$ min subjects consumed within 5 min a standardized, commercially available LM (325 ml, 176 kcal). Blood sampling and EVAS measurements schedules were as outlined in Fig. 1. At $t = 480$ min, the nasogastric tube and the cannula were removed and subjects departed from the clinic after receiving a macaroni meal.

Preparation of Test Products

For this study two LM formulations were produced, referred here as “fat-free” and “fat emulsion-containing” LM. Both formulations were processed in the pilot plant of Unilever R&D (Vlaardingen, The Netherlands) by ultra high temperature by a two-stage homogenization process (100 and 40 bar) on a sterilization line connected to an isolator (La Calhene, Vendôme, France) for aseptic filling. All ingredients used were food grade. The $^{13}$C-labeled high-oleic-acid rapeseed oil was prepared by homogenously mixing 6 g of $[^{13}$C]$\text{C}$triolein (glyceryl-trioleate, with uniformly labeled $^{13}$C-C18:1 on sn-1 position) with 317.5 g high-oleic-acid rapeseed oil (Clear Valley 75 high oleic canola oil, Cargill) by using a high shear mixer (Silverson L4RT). Fifteen aliquots of 10 ml were filled into syringes for later administration via the nasogastric tube. The remaining amount was used to produce the fat emulsion-containing LM. Emulsification of the $^{13}$C-labeled oil was achieved during processing. The fat-free formulation was produced on the same line in an identical process, but without adding high-oleic-acid rapeseed oil to the premix. Oleic acid content of both LM was confirmed by gas chromatography (GC) coupled to a flame ionization detector (FID; see Analytical Measurements). Both LM formulations were filled into 330 ml polyethylene terephthalate (PET) bottles (Amcor PET Packaging; The Netherlands). The fat-free and fat emulsion-containing LM were spiked with paracetamol (acetaminophen, European pharmaceutical grade, Pharmachemie, Haarlem, The Netherlands) at the intervention day by manually dissolving 1.5 g paracetamol into 325 ml of LM until

Table 1. Nutritional composition of the LM

<table>
<thead>
<tr>
<th>Nutritional Facts</th>
<th>EM</th>
<th>OT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serving size</td>
<td>1 bottle (325 ml)</td>
<td></td>
</tr>
<tr>
<td>Amount per serving</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Energy, kcal*</td>
<td>181</td>
<td>145</td>
</tr>
<tr>
<td>Fat, g</td>
<td>5.4</td>
<td>1.35</td>
</tr>
<tr>
<td>Rapeseed oil, g</td>
<td>4.06</td>
<td></td>
</tr>
<tr>
<td>Glyceryl-trioleate ($^{13}$C-C18:1 in sn-1), mg</td>
<td>77</td>
<td></td>
</tr>
<tr>
<td>Carbohydrates, g</td>
<td>15.2</td>
<td>15.2</td>
</tr>
<tr>
<td>Fructose</td>
<td>8.1</td>
<td>8.1</td>
</tr>
<tr>
<td>Sucrose</td>
<td>7.1</td>
<td>7.1</td>
</tr>
<tr>
<td>Fiber</td>
<td>5.0</td>
<td>5.0</td>
</tr>
<tr>
<td>Protein, g*</td>
<td>15.6</td>
<td>15.6</td>
</tr>
</tbody>
</table>

LM, liquid meal; EM, emulsion treatment; OT, on-top treatment. *Values are calculated based on weighted ingredients.
homogeneity. The nutritional composition of both LM is given in Table 1.

Analytical Measurements

**Blood sampling.** Venous blood samples (10 ml) were collected into ice-chilled EDTA-treated tubes containing 400 kU aprotinin/ml (TrasyloL, Bayer, Leverkusen, Germany). Plasma was separated by centrifugation at 3,200 rpm for 15 min at 4°C within 30 min after collection and stored in aliquots at −70°C until analyzed.

**Paracetamol absorption test for determining GE of the aqueous phase of the meal.** Postprandial plasma paracetamol concentrations were used as an indirect marker for the rate of GE of the aqueous phase of the LM (37). Plasma paracetamol (acetaminophen) concentrations were analyzed with a clinical chemistry analyzer (Roche Hitachi 911 Chemistry Analyzer, Hitachi, Japan) by using a clinical chemistry paracetamol test kit (product no. 03255379, Roche Diagnostics, Mannheim, Germany) according to the supplier’s instructions. The limit of quantification was 1.2 μg/ml and the assay was linear up to a concentration of 600 μg/ml (7.9–3,972 μmol/l) as determined with a commercially available linearity set (Roche Diagnostics).

**Plasma CCK concentrations.** Plasma CCK concentrations (pmol/l) were determined using an established radioimmunoassay as described previously (26) with the following modifications. A rabbit antibody binding to all circulating CCK peptides, including sulfated cholecystokinin octapeptide, but not with gastrin, was used.125Iodine labeling was performed with Bolton-Hunter reagent (PerkinElmer Life Sciences, Wellesley, MA) and human sulfated CCK-33 (Bachem Holding, Bubendorf, Switzerland). Measurement of CCK in plasma requires extraction with ethanol. Separation of bound and unbound label was done by decanting suspension anti-rabbit IgG Sac-Cel (IDS, Boldon, UK). The detection limit of the assay was 0.2 pmol/l. The intra-assay variation ranges from 3.9 to 9.1% and the interassay variation from 10.4 to 19.9%.

**Plasma oleic acid analysis.** Oleic acid plasma concentrations (μmol/l) were performed by applying routine techniques by GC analysis (Agilent HP6890 GC equipped with a HP7683 autosampler) coupled to a FID (Agilent, Amstelveen, The Netherlands). The method used was a modification of the boron-trifluoride procedure originally described by Morrison and Smith (36). Data processing was performed by use of the Totalchrom multiclient data system (Perkin Elmer, Norwalk, CT).

The 13C/12C ratio of oleic acid (C18:1) in plasma was determined using GC-combustion isotope ratio mass spectrometry (GC-C-IRMS) with a Delta plus/Agilent 6890 instrument (Thermo Fisher Scientific, Bremen, Germany) as described previously (38) with the exception that a 60 mm × 0.32 mm DB-23 column with a film thickness of 0.25 μm (J&W Scientific, Folsom, CA) was used. The GC temperature programming conditions were optimized to obtain baseline separation between oleic acid methyl ester and other fatty acid methyl esters. C17:0 was added as an internal standard for fatty acid quantification and was used to check the variation of measurements. Calibration samples consisting of mixtures of 13C-C18:1 and unlabeled C18:1 were used to convert measured enrichment values to molar enrichments. The molar enrichment was expressed as concentration of 13C-C18:1 by multiplying molar percent 13C-C18:1 enrichment by the endogenous C18:1 plasma concentration (μmol/l) determined by GC-FID. This resulted in the actual 13C-C18:1 concentration expressed in μmol/l.

**Appetite perception.** Self-assessed ratings of appetite were monitored by a validated questionnaire on satiety feelings (appetite for a meal, prospective food consumption, fullness, hunger, appetite for a snack, thirst, satiety) immediately prior to and every 30 min after start of the intervention till 480 min. Ratings were made on a handheld computer (iPAQ, Hewlett Packard), using EVAS (42) anchored at the low end with the most negative or lowest intensity feelings (e.g., extremely unpleasant, not at all), and with opposing terms at the high end (e.g., extremely pleasant, very high, extreme). Volunteers were asked to indicate on a line scale what score on the scale best reflects their feeling at that moment.

**Data and Statistical Analysis**

Results are graphically and numerically presented for six subjects with the duplicate measures per treatment included for paracetamol, CCK, and appetite perception data. Plasma samples for 13C-C18:1 were analyzed per individual for the first intervention per treatment only, i.e., replicate measures per treatment were not available. Thus data for 13C-C18:1 are graphically and numerically presented for six subjects for the first intervention per treatment.

**Kinetic analysis.** Individual plasma concentrations vs. time data for 13C-C18:1 and paracetamol were analyzed in WinNonlin version 5.1 (Pharsight, Mountain View, CA) by noncompartmental analysis with lag time included. The following parameters were derived from this analysis: Tlag, Cmax, Tmax, AUC, and mean residence time (MRT). Modeling of the paracetamol data using a one- or two-compartmental model was not possible in two of the six subjects, since a high variability was observed for the absorption phase of paracetamol. Therefore, to determine the half-life of absorption (T1/2 absorption), a population-based pharmacokinetic analysis using a mixed-effects model was performed by using the software R with the package ‘nlme’ (R core team, 2007).

CCK plasma concentrations were plotted against time and the AUC was calculated by using the trapezoidal equation. Cmax and Tmax of CCK were directly derived from the nonmodeled data set.

**Statistical analysis.** The statistical analysis was performed by use of SAS (version 9.1, SAS Institute, Cary, NC). Descriptive analysis consisting of distribution statistics (number of available observations, mean, standard error of the mean, and 95% confidence intervals) is presented for continuous data. The data were further analyzed by ANCOVA with subjects and treatments as main effect. Baseline measurements were used as covariate to correct for individual CCK and satiety levels. The statistical model used took the repetition of the treatments per individual into account. Differences between the treatment group (OT) and the control group (EM) were established on the basis of adjusted means by using Fisher’s least significance difference (two-tailed). We did not adjust for multiplicity because we tested multiple (secondary) variables. We expected the secondary analyses to support the primary results. A P value of less than or equal to 0.05 was considered to be statistically significant. Descriptive data are expressed as means ± SE, and group analysis data of 13C-C18:1, CCK, satiety, and paracetamol are expressed as adjusted least square means (LSmeans) ± SE.

**RESULTS**

**Experimental Procedures**

The study protocol was well tolerated and completed by all six subjects. None of the subjects enrolled withdrew from the study. The nasogastric tube was well positioned in the proximal stomach as controlled by length and auscultation.

**Plasma 13C-C18:1 Concentration**

13C-C18:1 was quantified in total plasma using GC-C-IRMS. The measured maximum enrichments obtained were all <0.1 atom % excess. Time vs. 13C-C18:1 plasma concentrations were analyzed with a clinical chemistry analyzer (Roche Hitachi 911 Chemistry Analyzer, Hitachi, Japan) by using a clinical chemistry paracetamol test kit (product no. 03255379, Roche Diagnostics, Mannheim, Germany) according to the supplier’s instructions. The limit of quantification was 1.2 μg/ml and the assay was linear up to a concentration of 600 μg/ml (7.9–3,972 μmol/l) as determined with a commercially available linearity set (Roche Diagnostics).

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**Plasma 13C-C18:1 Concentration**

13C-C18:1 was quantified in total plasma using GC-C-IRMS. The measured maximum enrichments obtained were all <0.1 atom % excess. Time vs. 13C-C18:1 plasma concentrations for OT and EM are shown in Fig. 2A (individual data available in Supplemental Fig. S1 in the online supporting material at the American Journal of Physiology Gastrointestinal and Liver Physiology website). Baseline 13C-C18:1 concentration in plasma did not differ from zero in both treatments. The Tlag (time between 13C-C18:1 administration and the time point

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GASTRIC LIPID LAYERING DELAYS LIPID ABSORPTION AND INCREASES CCK

Plasma CCK Concentrations

Baseline plasma CCK concentrations were not significantly different between both treatments (EM: 0.25 ± 0.08 pmol/l, OT: 0.40 ± 0.13 pmol/l; P > 0.05). Baseline concentrations had no significant effect on the AUC for plasma CCK concentrations (P > 0.05). CCK concentrations increased very rapidly and peaked ~15 min after intake of the LM (Fig. 3A) with no difference in Cmax-1 and Tmax between EM and OT (Table 3). CCK plasma concentrations were numerically higher in OT compared with EM between t = 50 and 480 min after ingestion of the LM and were significantly (P < 0.05) elevated at the time points t = 50 and t = 300 min. The total integrated area under the CCK curve (AUC_{0-480}) was significantly higher for OT than for EM (P = 0.03), in particular this effect was more pronounced when integrated for the area between 240 and 480 min (AUC_{240-480} = 0.01) (Table 3). Plasma CCK levels returned to baseline within 60–90 min after consumption of the LM. CCK concentrations increased after intake of the second LM with a significant difference in Cmax-2 between EM and OT (Fig. 3A and Table 3).

Gastric Emptying

GE of the aqueous phase was estimated by the paracetamol absorption test; pharmacokinetic parameters were modeled by noncompartmental analysis of the individual data using population-based model. In EM and OT, plasma paracetamol concentrations increased very rapidly (Fig. 3B) with no significant differences in Cmax and AUC_{0-480} values between EM and OT (P > 0.05). No significant differences in Tmax were noted between EM and OT (P > 0.05). CCK concentrations increased very rapidly (Fig. 3B) with no significant differences in Tmax between EM and OT (P > 0.05). There was no difference in the

Table 2. \(^{13}\)C-C18:1 kinetic parameters in healthy male subjects after consumption of a LM containing 4 g rapeseed oil as an emulsion and after consumption of a fat-free LM and nasogastric application of 4 g rapeseed oil

<table>
<thead>
<tr>
<th>Treatment</th>
<th>EM</th>
<th>OT</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tlag, min</td>
<td>37 ± 7 (16–62)</td>
<td>75 ± 10 (53–112)</td>
<td>0.01</td>
</tr>
<tr>
<td>Tmax, min</td>
<td>163 ± 18 (105–240)</td>
<td>280 ± 34 (180–360)</td>
<td>0.01</td>
</tr>
<tr>
<td>Cmax, pmol/l</td>
<td>1.97 ± 0.24 (1.34–2.81)</td>
<td>2.12 ± 0.20 (1.51–2.88)</td>
<td>0.5</td>
</tr>
<tr>
<td>AUC_{0-480}, (\mu)mol-min^{-1}</td>
<td>572 ± 53 (431–697)</td>
<td>494 ± 65 (304–703)</td>
<td>0.2</td>
</tr>
<tr>
<td>MRT, min</td>
<td>266 ± 9 (244–298)</td>
<td>304 ± 13 (261–349)</td>
<td>0.003</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SE (range). Tlag, lag time; Cmax, maximum concentration; Tmax, time at Cmax; AUC_{0-480}, integrated area under the CCK curve between 0 and 480 min; MRT, mean residence time.
effect of the treatments on other pharmacokinetic parameters such as half-life of elimination, $\text{AUC}_{0-240}$, and MRT for plasma paracetamol concentrations (Table 4). Total GE, determined as GE half-time, was not significantly different between EM and OT as derived from the cumulative fraction paracetamol absorbed (data not shown). Modeling of the paracetamol concentrations in a population-based analysis revealed that the treatment affected only $T_{1/2}$ absorption. The effect of OT on $T_{1/2}$ absorption was significant compared with EM ($P = 0.001$) resulting in a 2.1 times faster absorption of paracetamol in OT ($T_{1/2}$ absorption: OT: 6 ± 4 min; EM: 12 ± 7 min) in the absorption phase, i.e., 30 min postprandial.

**Dispersive Perceptions**

Overall, the effects of the treatments on appetite scores were minor (Fig. 4 and 5). Satiety was significantly decreased ($P = 0.01$) in OT when integrated over the whole day ($AUC_{0-480}$) and in particular in the time period after receiving the second LM ($AUC_{240-480}$) (Fig. 5). Self-rated scores of hunger and fullness were not different between OT and EM when integrated over the whole day and the period after the second meal. Although not statistically significant, in the period $AUC_{150-270}$ there was a trend for increased perception of satiety ($P = 0.08$) and fullness ($P = 0.07$), and lower perceptive hunger ($P = 0.06$) in OT compared with EM.

**Discussion**

Structuring of emulsions has been suggested to be useful for weight management purposes (30–32). Postprandial sequential release of lipids from the stomach into the small intestine achieved by food structuring could modulate secretion of

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**Table 3. CCK plasma concentrations in healthy male subjects after consumption of a LM containing 4 g rapeseed oil as an emulsion and after consumption of a fat-free LM and nasogastric application of 4 g rapeseed oil.**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>EM</th>
<th>OT</th>
<th>$P$ Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_{\text{max}}$, pmol/l</td>
<td>1.56±0.40 (0.5–3.2)</td>
<td>1.35±0.32 (0.6–2.6)</td>
<td>0.5</td>
</tr>
<tr>
<td>$T_{\text{max}}$, min</td>
<td>12±2 (5–20)</td>
<td>11±2 (5–15)</td>
<td>0.7</td>
</tr>
<tr>
<td>$C_{\text{max}}$, pmol/l</td>
<td>0.87±0.16 (0.5–1.4)</td>
<td>1.28±0.26 (0.6–2.3)</td>
<td>0.03</td>
</tr>
<tr>
<td>$AUC_{0-120}$, pmol·min$^{-1}$</td>
<td>402±114</td>
<td>519±114</td>
<td>0.03</td>
</tr>
<tr>
<td>$AUC_{240-480}$, pmol·min$^{-1}$</td>
<td>232±61</td>
<td>314±61</td>
<td>0.01</td>
</tr>
<tr>
<td>$AUC_{120-180}$, pmol·min$^{-1}$</td>
<td>173±51</td>
<td>203±51</td>
<td>0.3</td>
</tr>
<tr>
<td>$AUC_{0-480}$, pmol</td>
<td>136±35</td>
<td>143±35</td>
<td>0.6</td>
</tr>
<tr>
<td>$AUC_{0-480}$, pmol·min$^{-1}$</td>
<td>84±17</td>
<td>67±17</td>
<td>0.07</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SE (range). The $T_{1/2}$ analysis was significant compared with EM ($P = 0.001$) resulting in a 2.1 times faster absorption of paracetamol in OT ($T_{1/2}$ absorption: OT: 6 ± 4 min; EM: 12 ± 7 min) in the absorption phase, i.e., 30 min postprandial.

**Table 4. Paracetamol kinetic measurements in healthy male subjects after consumption of a LM containing 4 g rapeseed oil as an emulsion and after consumption of a fat-free LM and nasogastric administration of 4 g rapeseed oil.**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>EM</th>
<th>OT</th>
<th>$P$ Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paracetamol dose, g</td>
<td>1.5</td>
<td>1.5</td>
<td></td>
</tr>
<tr>
<td>$C_{\text{max}}$, µmol/l</td>
<td>117±9 (59–171)</td>
<td>118±8 (77–169)</td>
<td>1.0</td>
</tr>
<tr>
<td>$T_{\text{max}}$, min</td>
<td>52±8 (20–120)</td>
<td>30±4 (10–60)</td>
<td>0.003</td>
</tr>
<tr>
<td>$T_{1/2}$ elimination, min</td>
<td>119±5 (92–144)</td>
<td>121±9 (73–176)</td>
<td>0.8</td>
</tr>
<tr>
<td>MRT, min</td>
<td>108±3 (94–122)</td>
<td>103±3 (78–119)</td>
<td>0.2</td>
</tr>
<tr>
<td>$AUC_{240}$, µmol·min$^{-1}$</td>
<td>12±2 (5–20)</td>
<td>11±2 (5–15)</td>
<td>0.7</td>
</tr>
<tr>
<td>$T_{1/2}$ absorption, min</td>
<td>12±7</td>
<td>6±4</td>
<td>0.001</td>
</tr>
</tbody>
</table>

$C_{\text{max}}$, $T_{\text{max}}$, half-life ($T_{1/2}$) of elimination and absorption, MRT, and $AUC_{240}$ were calculated by using a non-compartment model (WinNonLin version 5.1). Values are expressed as means ± SE (range). The $T_{1/2}$ of absorption was determined with the software R with the package nlme (R core team, 2007) by use of a population-based model.
observed layered rapeseed oil affected gastrointestinal physiology. We intragastric lipid layering, demonstrated that intragastric meal in combination with nasogastric intubation to mimic of lipid absorption and GE of the aqueous phase of the parameters linked to satiety. The use of markers for determina-

strates that very low lipid loads as an integral part of a meal and GE and potentially in satiety. The present study demonstrates that very low lipid loads as an integral part of a meal indeed have measurable effects on physiological relevant parameters linked to satiety. The use of markers for determination of lipid absorption and GE of the aqueous phase of the meal in combination with nasogastric intubation to mimic intragastric lipid layering, demonstrated that 4 g of intragastric layered rapeseed oil affected gastrointestinal physiology. We observed 1) delayed absorption of lipids, 2) initial faster GE of the aqueous phase of the LM, and 3) increased postprandial CCK levels. However, the perception of satiety, hunger, and fullness was not consistently related to the changes in lipid absorption and CCK throughout the day.

Compared with an equicaloric dose of high-oleic-acid rapeseed oil (labeled with $^{13}$C-C18:1) emulsified in a LM (treatment EM), 4 g of oil when administered via a nasogastric tube on top of a fat-free LM (treatment OT) markedly delayed lipid absorption. The latter was exhibited by a twofold increase in $T_{lag}$ in OT compared with EM. Moreover, also the pulse time, i.e., the difference between $T_{max}$ and $T_{lag}$, was significantly shorter in EM, indicating that the bulk of $^{13}$C-C18:1 reached plasma faster than in the OT group. In addition, the mean time of all $^{13}$C-C18:1 absorbed residing in plasma (MRT) was in line with the shift in $T_{lag}$ of $\sim$40 min. This indicates that intestinal delivery and/or absorption of oleic acid occurred in OT at slower rates compared with EM. The consistently delayed absorption kinetics of oleic acid in OT suggests that the rapeseed oil indeed layered in the stomach and was not mixed with the aqueous phase of the LM by antral grinding, pyloric shearing, or low shear in the small intestine. Furthermore, our test conditions would not have favored spontaneous emulsification. At low gastric pH, the protein of the LM will precipitate and not be available for emulsification processes. All in all this suggests that nasogastrically infused lipids stayed as a layer on top of the LM, although very sensitive radiotracer studies combined with scintigraphy would be needed to confirm this.

In our study we achieved layering of the lipid by nasogastric intubation. The question is whether such a separation of lipid from the aqueous phase of the meal can be achieved by food-based approaches. Recent MRI studies point out that a layering effect indeed occurs in vivo after ingestion of high-fat liquid meals. In these studies it was visualized that the lipid forms a layer on top of the aqueous phase. The lipid layer markedly accelerated the GE rate as in these studies pure oil in water emulsions were used (24, 33). However, from these studies it is unclear whether the lipid layer consists of layered but still intact small emulsified lipid droplets (i.e., “creamed” droplets) or of coalesced emulsion particles creating larger particles or even a single oil layer. It was also reported in a very recent publication (30) that an in vitro validated gastric unstable fat emulsion behaved in vivo only partly as an unstable emulsion. A significant part of the oil fraction stayed emulsified in the oil-water meal (30), suggesting that the creamed oil fraction would at least partly consists of still intact emulsion particles. This is important with respect to the fact that the effect of lipids on inhibition of GE depends on lipid hydrolysis (15). The degree of gastric lipolysis, which is responsible for up to 25% of lipid hydrolysis during meal ingestion (8, 9), is strongly dependent on lipid droplet size and micellization (4). Thus the inhibition of GE by lipids is most likely determined not only by the rate of emptying of lipid from the stomach but also by the rate and degree of gastric hydrolysis of the lipids. In addition, lingual lipase has been suggested to be necessary to induce gastric lipolysis; however, the contribution of lingual lipase to overall lipolysis seems to be negligible in humans (12, 20, 35). Taken together, these facts highlight that the effect of nasogastric infusion of rapeseed oil represents an extreme situation of intragastric layering of lipids, which most likely cannot be achieved in vivo by using gastric unstable emulsions.

Monitoring the intestinal fate of such small amounts of lipids as used in the present study is not possible using the various state-of-the-art MRI technologies. We have therefore chosen to
examine emptying of lipids from the stomach indirectly by following plasma concentrations of $^{13}$C-labeled oleic acid derived from glyceryl-[$^{13}$C]triolein-marked rapeseed oil. The observed increase in $T_{lag}$, $T_{max}$, and MRT in the OT treatment could have been caused by 1) a delayed emptying from the stomach, 2) delayed hydrolysis in the gastric and intestinal lumen, 3) prolonged storage in the enterocyte due to delayed chylomicron formation, or 4) delayed transport by the lymph. We hypothesize that the deferred $^{13}$C-C18:1 appearance in plasma was most likely caused by delayed emptying from the stomach and/or retarded gastric and intestinal hydrolysis. This is supported by the faster GE of the LM in the first 30 min postprandial and by the increased CCK levels 2 h before ingestion of the second meal in treatment OT. CCK and GE are directly influenced by and strongly dependent on the amount of lipid delivered from the stomach to the duodenum. Absorption of paracetamol was used as a marker of GE of the aqueous phase because of its complete and very fast absorption in the duodenum (37). The rate constant of absorption was 2.1-fold higher in OT than in EM, reflecting greater inhibition of GE in EM until 30 min postprandial. This was most likely caused by the higher initial caloric outflow induced by the emulsified oil. However, total GE time was not affected by the treatment, showing that the inhibition of GE was limited to the early postprandial phase. In line with this, although not statistically significant ($P = 0.07$), was the greater AUC value of CCK in EM in the first 45 min after treatment. Concomitantly with the delayed lipid absorption in OT concentrations of CCK were increased 50–90 min postprandial compared with EM. Interestingly, in OT CCK levels were increased after ingestion of the second LM and stayed elevated for the remaining intervention period (AUC 340–480). This suggests, but does not confirm, that the second meal provoked hydrolysis of oil retained in the gastrointestinal tract, leading to increased CCK concentrations via fatty acid sensing in enterendocrine cells.

The small but robust and consistent effect of OT on increased CCK concentrations after the second meal is demonstrated by the individual CCK plasma curves (see Supplementary Fig. S2 for the individual CCK plasma concentrations). Concurrently $^{13}$C-C18:1 plasma concentrations increased again after the second meal at $t = 300$ min (Fig. 3A), although this effect was mainly observed in two of the six subjects. Early triacylglyceride peaks following sequential meals have been suggested to be partly derived from the previous meal by storage in enterocytes (41). In addition, the contribution of lipids ingested at the previous meal to increase plasma triacylglyceride concentrations after the following meal has been demonstrated recently (29). In this study lipid ingested at breakfast was partly retained within the stomach and gut and was mobilized after lunch ingestion (29). Even though it was unclear from these studies whether lipid were stored in the gut lumen or in the enterocytes, our data suggest that in the present study the increase was due to a gastric “washout” via the appearance of the second meal causing subsequent increased CCK concentrations.

Although changes in satiety were not a primary outcome of the study, we tested whether changes in biochemical parameters were related to changes in appetite scores. The study, however, was not powered to pick up small differences in satiety. With the present design we would have been able to detect statistical significant differences of at least 12% between the treatments. Thus the small differences as observed in the present study must be interpreted with care. Notwithstanding the delayed lipid release and concomitantly higher CCK levels, changes in appetite scores were minor and mostly not consistent with changes in CCK. Although CCK is the prototypical satiety hormone, a number of gastrointestinal signals including the peptide hormones PYY and GLP-1 are involved in the regulation of satiety (45). PYY and GLP-1 are released from more distal cells in the small intestine before nutrients have reached the predominant sites of production possibly by a neural reflex (45). Possibly, in treatment OT plasma concentrations of GLP-1 and/or PYY were decreased, leading to lower satiety as observed over the 8-h period compared with EM. However, since these hormones were not assessed and as the mean weighted satiety scores differed only by 3 mm on a 100-mm scale, this remains speculative. We hypothesize that total volume and total energy dominated the satiety perception rather than the intragastric lipid distribution. Perception of satiety is in part modulated by the rate of GE and is at equicaloric loads, dependent mainly on volume and not macronutrients (19). The initial GE rate of the bulk of the meal differed between both treatments, which was reflected by numerical lower satiety scores in OT. The higher CCK levels and the concomitant trend for higher satiety in OT between $t = 150$ and 270 min, however, cannot be attributed to a GE effect because at that time the LM was already emptied (19, 21, 30–33). Overall, the marginal effect observed on appetite scores in combination with the slight increase in CCK suggests that the amount of lipid infused was too low to exhibit a significant and consistent effect. An escalating-dose study should determine which lipid loads are needed to achieve physiological meaningful effects on CCK and satiety.

In conclusion, our study demonstrated modest differences in the effects of low, equicaloric doses of oil administered nasogastrically and as fine emulsions both in combination with a LM. Although delivery of 4 g of oil on top of a LM markedly delayed lipid absorption, plasma CCK concentrations were modestly increased compared with the same amount of oil incorporated as an emulsion into the LM. Intra-gastric-layered oil, but not emulsified oil, led to an initial increase in GE rate of the aqueous phase. This was most likely due to reduced total energy delivery to the duodenal-jejunal region in the early postprandial phase in the OT study group. Feelings of satiety, however, were inversely related to lipid absorption and plasma CCK concentrations. Although CCK levels were modestly increased after OT delivery, overall ratings of satiety were lower compared with the emulsion treatment. Although satiety was decreased when integrated over the whole study period, ratings of satiety and fullness tended to be higher and ratings of hunger to be lower in OT in the 2 h before intake of the second meal, suggesting that at slightly higher lipid loads the time to return to the hunger baseline can be prolonged. Finally, the present study using physiological doses of lipids lends support to other studies that showed that emulsified lipids behave physiologically and biochemically different, dependent on the emulsifier type used. It remains to be determined whether higher doses of lipids than were used in the present study mediate changes both in primary physiological parameters and also in end points, i.e., increased CCK and satiety. The present study demonstrates the potential importance of structuring of dietary lipid formulations for controlling hunger.
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