

# GASTROINTESTINAL METABOLISM OF A VEGETABLE-OIL EMULSION IN HEALTHY SUBJECTS

## INTRODUCTION

The increasing prevalence of overweight and obesity worldwide is well established (1), and the need exists for practical intervention strategies. Although numerous environmental and genetic factors contribute to the increasing prevalence, the development of overweight and obesity is a consequence of energy intake that exceeds energy expenditure. It was estimated that affecting energy balance by only 100 kcal/d, the average weight gain of the adult US population could be prevented (2). One way to prevent excess energy intake is to control appetite; hence, ingredients that affect satiety may play a role in preventing weight gain, especially if these could be incorporated into everyday foods.

Satiety feelings are determined by a coordinated series of neural and humoral signals that originate from the gut in response to mechanical and chemical stimuli from the ingested food (3). Fat in the form of a triacylglyceride emulsion suppresses short-term hunger and food intake more than does an equienergetic infusion of carbohydrates (4, 5). The presence of lipids in the distal small intestine

increases the gastrointestinal transit time, stimulates the release of gastrointestinal hormones, inhibits pancreaticobiliary and gastric acid secretion, and reduces appetite (6, 14).

On the basis of these observations, the ileal brake mechanism has been proposed as a part of a negative feedback loop in appetite regulation. A reduction in the rate of digestion or absorption of dietary fats will increase the intraluminal fat component and lead to an increase in satiety signals in the distal small intestine (7, 8, 13, 15). Short-term studies in both normal and overweight subjects, which measured the effects on food intake with a vegetable-oil emulsion made of palm oil and oat oil in water, showed a suppressive appetitive response (16–18). However, other studies did not show a significant effect (19, 20). The inconsistency of the results may, at least in part, be attributed to heterogeneity in the study design and the methodologic approaches. Alternatively, long-term studies with a positive effect on body-weight control (21) could be regarded as a confirmation of the appetite-suppressing effect. A plausible mechanism has been suggested on the basis of delayed breakdown of

the vegetable-oil emulsion with the presence of unabsorbed nutrients in the ileum that activate the ileal brake (17). An increased gastrointestinal transit time was recently shown with the fat emulsion investigated in this study (22), but additional studies are needed to confirm if digestion or absorption rates are reduced with this vegetable-oil emulsion.

The aim of this experimental clinical study was to investigate the differences in digestion and absorption of this vegetable-oil emulsion compared with those of dispersed milk fat in the gastrointestinal system in healthy subjects by using a well-established multilumen intestinal tube technique with an occluding balloon (23, 24).

## SUBJECTS AND METHODS

### Subjects

Healthy subjects of both sexes were recruited through advertisements in Uppsala University and around the campus. Subjects [aged 20–45 y; body mass index (in kg/m<sup>2</sup>) of 19–29] were eligible for the trial if they were considered healthy before the medical examination and had clinically normal findings in the laboratory measurements. Subjects with a significant

clinical illness or injury within 2 wk before the first administration of the trial, history of cardiac, renal, hepatic or significant gastrointestinal disease, hypersensitive stomach, history of lactose intolerance or other types of food intolerance, symptoms or signs of an ongoing allergy or hypersensitivity, history of drug addiction or alcohol abuse, requirement of concurrent medication during the study, and intake of any prescribed medicine within 2 wk before the study were not eligible to be included. After an initial screening, 16 healthy subjects (4 men and 12 women; age range 23–36 y; mean age: 28.0 y for men and 27.9 y for women; mean body mass index: 23.1 for men and 22.2 for women) participated in the study.

### Ethics

The single pass jejunal-perfusion studies were performed at the Clinical Research Department, University Hospital, Uppsala, Sweden, and were approved by the Ethics Committee (decision no. 2007/324; 19 December 2007) and the Radiation Ethics Committee of the Medical Faculty (Radiation Ethics Committee decision no. 08:03; 19 February 2008) of the Uppsala University.

Signed informed consent was obtained from all participants.

### Study design

The study had a randomized, double-blind, single-product administration and placebo-controlled crossover design and was carried out within 3 wk. The study started on 25 February 2008 and was completed on 23 April 2008. Each subject was studied on 2 separate occasions with a > 5-d interval between study visits.

Within the study, participants were evenly randomly assigned between the 2 study days; one group received the test product first (300 g yogurt that contained 8.5 g fat from a vegetable-oil emulsion), whereas the other group received the control product first (300 g of yogurt that contained 8.5 g dairy fat).

### Clinical method

All subjects were on a standardized diet the day before the experiment because they received all food items to be consumed from the institute. The energy and macronutrient amounts were set according to the Swedish Nutrition Recommendations for men and women, respectively, at age 20–50 y with normal physical activity (men: 2900 kcal; women: 2200 kcal; percentage of energy given at 55%, 30%, or 15% for carbohydrates, fats, and proteins, respectively).

After an overnight fast of 10 h, the perfusion tube (LOC-IGUT; Synectics Medical, Stockholm, Sweden) was introduced through the mouth and positioned in the proximal part of the small intestine during fluoroscopic guidance (Philips BV 21-S; Philips Healthcare, Best, Netherlands). During insertion, a polytetrafluoroethylene-coated guide wire inside the instrument was used to facilitate the passage of the tube into the small intestine (Amplatz extrastiff wire guide; William Cook Europe A/S, Bjaereskov, Denmark). The tube was a multichannel device designed and extensively validated at our laboratory (23–25). The tube could be fitted with 2 occluding balloons for segmental studies of intestinal metabolism in a closed segment of the intestine. In this study only the distal balloon was inflated because we wanted to include all enzymes excreted and transported into the intestine from saliva, the stomach, bile, pancreatic juice, and the intestinal wall and, thereby, mimic the normal intraluminal metabolism of food. With one balloon inflated, the available surface area for absorption was similar between experiments.

This is described in detail elsewhere (25). The tube was placed at approximately the same position in the proximal small intestine in all subjects. After finishing each experiment, the position of the tube was determined. Along with the perfusion tube, another tube was positioned in the stomach for infusion of the dif-

ferent yogurt products as well as for obtaining samples from the stomach for different analyses (Salem sump tube; Sherwood Medical, Tullamore,

Ireland). The detection of the placing of this gastric tube was performed in the same manner, and it ensured that infusion of the 2 different products was carried out in a likewise position in the stomach in all subjects. Once the perfusion tube was in place, a distal balloon was inflated with 26–30 mL air. The distance from the tip of the gastric tube, where the products were infused, to the occluding balloon of the perfusion tube in the proximal jejunum, where the intestinal juice mixed with the different partly digested yogurts was collected, was 35 cm at the beginning of each experiment. All gastric fluid was collected and replaced by 20 mL saline solution before infusion of the yogurt products through the gastric tube. After infusion, samples were drawn at regular time intervals. After 30 min, a gastric sample of 10 mL was taken, whereafter the gastric tube was rinsed with 10 mL saline solution. At the end of the experiment, after 180 min, the total contents of the stomach were retrieved. During 3h, intestinal samples were collected every 30 min in vials that were kept at 37°C. All samples obtained were checked for pH (indicator paper ranged from pH 2.0–9.0 in steps of 0.5 pH units; E Merck AB, Stockholm, Sweden). A minute amount of the samples was taken for light microscopy, accurately weighed, and directly brought to a pH 3 with 0.1g 1 mol/L HCl solution/g sample, immediately frozen with liquid nitrogen, and stored at 280°C until freeze-dried (Alpha 2–4 LD; Martin Christ, Osterode am Harz, Germany and VirTis Wizard 2.0; SP Industries, Gardiner, NY). Thereafter, the dried samples were stored at 280°C.

### Products

In this study, 2 different yogurt products were investigated. The control product contained milk at, and

the test product contained the vegetable-oil emulsion Fabuless (DSM Food Specialties, Delft, Netherlands) (535 and 538 kcal, 28.3 and 28.3 g fat, 32.7 and 33.0 g protein, and 37.7 and 37.7 g carbohydrate, respectively; the content is given per 1000 g product). The laboratory scale production of the test products was described elsewhere (22). Two batches of each yogurt were produced; the shelf life of the products was too short to cover the study period with one batch. The mean (6SD) lipid emulsion particle sizes for the 2 batches each of the control and test yogurts, respectively, were 621 6 429 and 572 6 367 nm with 21.6% and 16.2% .1  $\mu$ m and 589 6 437 and 520 6 340 nm with 13.8% and 11.7% .1  $\mu$ m as determined with an LS 13 320 Laser Diffraction Particle Size Analyzer (Beckman Coulter BV, Woerden, Netherlands).

Before particle-size analysis and for light-microscopy evaluation, the yogurt samples were dispersed into tubes containing 0.2% EDTA in sodium hydroxide solution at pH 12 to dissolve protein (Sigma Aldrich, Zwijndrecht, Netherlands).

### Analytic measurements

Several different analyses were performed such as analyses of lipid composition [monoacylglycerides, diacylglycerides, triacylglycerides, and free fatty acids (FFAs)], fatty acid composition [fatty acid methyl ester and FFA composition], and lipase activity (for gastric juice at the start of the experiment only). Microstructural analyses were done by light microscopy, X-ray diffraction, and differential scanning calorimetry (DSC).

### Lipid-composition analyses by HPLC

For HPLC, the freeze-dried samples were extracted as follows: 20 mg homogenized sample was extracted with 1 mL solvent mixture [heptane chloroform (3:1)], followed by ultrasonic treatment of 15 min (ultrasonic bath model no. USC1700D; VWR,

Amsterdam, Netherlands), and vigorously shaking for 1 h (laboratory shaker, KS501 IKA; Labortechnik, Staufen, Germany). The solid material was removed by filtration with 0.2  $\mu$ m polyvinylidene fluoride membranes (Pall Life Sciences, Ann Arbor, MI), and 10  $\mu$ l was injected into a normal phase HPLC (Agilent 1100 liquid chromatograph; Agilent Technologies, Santa Clara, CA) equipped with a polar (cyano) column with evaporative light-scattering detection (Sedex 75; Polymer Laboratories Ltd, Shropshire, United Kingdom). A gradient of heptane-*t*-butylmethylether was used as a running medium. With the use of the calibration line and the Chromeleon Chromatography Management System program (Dionex Corporation, Sunnyvale, CA), the components were determined. Values were converted into moles by using average molecular masses calculated according to the fatty acid composition of the test products. Lipids were separated with a normal-phase chromatography system by using a gradient of organic solvents (26). Retention times were determined with commercial samples of known composition.

A variation of the method was determined by measuring several samples in triplicate. For low concentrations of lipid material (0–2 mg/mL), the SD on the data was observed to be 0.08 g/mg, for higher concentrations (2–15 mg/mL), the relative SD on log values was determined to be 5.7%. In both cases the deviations were smaller than the total spread within the results and, therefore, were not taken into account for further results.

### Fatty acid analyses

The fatty acid profile of the whole lipid composition was determined by the fatty acid methyl ester method. A sample amount of the crude sample was saponified and esterified to its methyl esters and quantified compared with an internal standard of

pentadecanoic acid methyl ester. Approximately 30 mg of the sample and 20 mg pentadecanoate were weighed accurately in a headspace vial. A magnetic stirrer, 1 mL toluene/butylated hydroxytoluene solution, and 4 mL of 0.5 N NaOH solution were added to each tube. The vials were closed with a crimp cap and gently stirred and heated in a heating module for 5 min at 100°C. After cooling down, 5 mL of boron trifluoride-methanol-complex solution (80 1663; E Merck AB) was added, and the vial was closed again and heated once more for 30 min at 100°C. After cooling down, 4 mL heptane and 5 mL saturated sodium chloride solution was added. After mixing thoroughly, the vial was centrifuged at 3000 rpm for 10 min. Gas-chromatography analyses were performed with a gas chromatography system [Hewlett-Packard 5890 series 2; Hewlett-Packard (now Agilent), Amstelveen, Netherlands] equipped with a flame ionization detector, a fused silica column [CP-Sil-88; Varian (now Agilent), Walnut Creek, CA], and an injection volume of 1  $\mu$ l with nitrogen as the carrier gas. The profile of the FFA part of the lipid composition was determined by a method described previously (27).

### Gastric lipase activity

Gastric lipase activity was determined in gastric samples taken before yogurt infusion as described previously (28). The gastric juice (without pH adjustment) was frozen with liquid nitrogen and stored at 280°C until analysis. The freeze-dried samples were diluted (50 mg + 2.5 mL with demineralized water). The measurements were performed with a pH titrator (pH stat TM854; Radiometer, Brønshøj, Denmark) at 37°C. The first part of the measurements was conducted by using a pH-stat method (1 min at pH 5.4), and the second part of the measurements was conducted by using an endpoint method (quickly from pH 5.4–9.4).

The total dosed volume (parts 1 + 2) of sodium hydroxide was the measure for the activity. A blank measurement of each sample (medium without substrate + sample) was also performed, and this (volume) was subtracted from the total dosed volume.

### Light microscopy

In vivo samples for light-microscopy evaluation were taken after 30 min (gastric samples) and 60 and 180 min (jejunal samples). One drop of the gastrointestinal sample was placed on a slide, covered, and immediately evaluated by light microscopy (Nikon Eclipse E800 with image-analysis program Lucia GF on MV-1500 version 4.6; Nikon Instruments Europe BV, Amstelveen, Netherlands) by using the phase-contrast mode, mostly at a magnification  $\times 40$ . Four images were taken, one from each quadrant of the sample, which usually focused on a part of the sample with more details. Two investigators visually analyzed the images on the basis of the following criteria: droplet size, air bubbles, protein leftovers, rods or lactic acid bacteria, mucus or cells, and a general overview. In jejunal samples where crystals were observed, the amount of crystals was estimated by counting the number of crystals in each image of each quadrant. If in  $\geq 3$  of 4 images,  $\geq 5$  crystals were observed, the sample was characterized as having many crystals. For  $< 3$  images that showed  $\leq 5$  crystals, the sample was characterized as having a few crystals. If no crystals were observed, the sample was characterized as having no crystals.

### X-ray diffraction of crystals

Freeze-dried powders of a subset of samples were subjected to X-ray diffraction. Powder-diffraction measurements were performed with a Bruker D8 Advance equipped with a Vantec detector by using  $\theta$ - $\theta$  geometry (Bruker Austria, Wien, Austria). Experiments were performed with a  $0.3^\circ$  divergence slit by using a scan

speed of  $0.3^\circ/\text{s}$ . A series of patterns was recorded from each sample to monitor possible changes in the materials and improve data quality. Identification of the components was performed with the International Centre for Diffraction Data (www.icdd.com) database.

### DSC

A selected set of freeze-dried samples from the jejunum sampled after 60 min of perfusion time was subjected to DSC with a Mettler Toledo DSC 821 (Mettler, Tiel, Netherlands) in a temperature range from  $-15^\circ\text{C}$  to  $200^\circ\text{C}$  and heated with a rate of  $5^\circ\text{C}/\text{min}$ . Aluminum pans ( $40\text{-}\mu\text{L}$ ) were filled with 10 mg of the freeze-dried sample. An empty pan was used as a reference.

### Raman microscopy

Raman microscopy (29) was performed on freeze-dried material with a Jobin Yvon LabRAM HR800 system (Villeneuve d'Ascq, France; 600 grooves/mm grating and  $\lambda = 514.5\text{-nm}$  laser). An Olympus LM PlanFL objective (X100; Hamburg, Germany) was used in conjugation with a  $100\text{-}\mu\text{m}$  confocal pinhole.

As reference samples, palmitic acid (Sigma Aldrich) was used as well as the sodium and calcium salts, which were made by adding an equimolar amount of the hydroxide to an aqueous dispersion of palmitic acid at  $80^\circ\text{C}$ . Freeze-dried jejunal samples were washed with isopropanol, ethanol, and water and observed by using reflected light microscopy to select objects that were subsequently analyzed by Raman spectroscopy. Four spots of each sample were used to identify the nature of the crystals.

### Statistical data analyses

Data are presented as arithmetic mean values and corresponding SEMs. Statistical analyses were performed by using Student's *t* test for paired and unpaired data regarding the amount of lipids and volume

obtained in each time interval when the subjects were given the vegetable-oil emulsion composition compared with when subjects consumed the control product (GraphPad Prism version 3.0; GraphPad Software, San Diego, CA). Calculations of Pearson's product moment *r* and Spearman rank *r* were used for testing differences in lipase activity. The study of data with multiple measurements within the 2 treatments was performed with analysis of variance for repeated measurements (univariate mixed-effect model approach).

For the mixed-effect analyses, the principal model was restricted to the main effect of the treatment  $\cdot$  time interaction. Statistical analysis of variance was performed with SAS software (version 9.1; SAS Institute, Cary, NC). Differences were considered significant at  $P, 0.05$ .

## RESULTS

In 11 out of 16 subjects, samples were obtained from all time periods and from both gastrointestinal sampling sites. In 5 subjects some gastrointestinal samples were missing because those experiments did not last for 180 min or samples could not be obtained (Table 1). The position of the tip of the perfusion tube had moved slightly further down from the initial position because of normal peristalsis, but the distance did not differ between the 2 treatments (test product compared with control product:  $14 \pm 8$  compared with  $13 \pm 7$  cm, respectively). This consistency together with the use of one occluding balloon ensured that the available intestinal surface area between experiments was the same.

### Total amount of lipids and lipid composition

The absolute amounts of lipids (in mg) detected in the different gastrointestinal samples are shown in Table 1. The lipid composition in the jejunum after consuming test and control yogurt are presented in Figure 1.

TABLE 1 - ABSOLUTE LIPID VALUES (IN MG) OF THE CONTROL-PRODUCT TREATMENT AND TEST-PRODUCT TREATMENT IN GASTRIC AND JEJUNAL SAMPLES<sup>1</sup>

	TG		FFA <sup>2,3</sup>		1,3DG		1,2DG		MG2		Total	
	Control	Test	Control	Test	Control	Test	Control	Test	Control	Test	Control	Test
<b>Gastric samples</b>												
Time												
180 min <sup>4</sup>	176.28 ± 48.65	204.30 ± 60.74	70.86 ± 29.65	156.28 ± 44.16	50.73 ± 23.42	31.91 ± 8.32	43.01 ± 15.39	62.57 ± 23.05	11.13 ± 3.38	19.65 ± 6.34	351.98 ± 104.72	474.70 ± 130.72
<b>Jejunal samples</b>												
Time												
30 min <sup>5</sup>	0.34 ± 0.17	0.03 ± 0.02	20.59 ± 8.00	127.94 ± 64.93	2.93 ± 1.11	10.46 ± 5.17	0.18 ± 0.10	0.76 ± 0.70	2.59 ± 1.49	12.72 ± 7.13	26.62 ± 11.09	151.91 ± 78.02
60 min <sup>6</sup>	0.10 ± 0.08	0.09 ± 0.09	71.29 ± 23.27	120.82 ± 39.29	13.41 ± 3.48	15.87 ± 5.57	0.16 ± 0.12	0.96 ± 0.96	3.01 ± 1.07	7.59 ± 4.02	87.97 ± 27.58	145.32 ± 44.92
90 min <sup>7</sup>	0.10 ± 0.09	0.02 ± 0.02	31.01 ± 8.87	72.29 ± 21.79	7.17 ± 2.06	7.66 ± 2.38	0.13 ± 0.08	0.08 ± 0.08	1.83 ± 0.81	2.69 ± 2.05	40.25 ± 10.99	82.74 ± 23.55
120 min <sup>7</sup>	0.09 ± 0.09	0.04 ± 0.03	22.21 ± 7.91	48.99 ± 21.54	3.62 ± 0.85	3.36 ± 0.92	0.03 ± 0.03	0.03 ± 0.02	0.35 ± 0.18	0.12 ± 0.08	26.29 ± 8.91	52.53 ± 21.99
150 min <sup>8</sup>	0.03 ± 0.03	0.00 ± 0.00	10.38 ± 5.18	4.91 ± 2.08	1.13 ± 0.50	0.47 ± 0.23	0.01 ± 0.01	0.00 ± 0.00	0.24 ± 0.15	0.01 ± 0.01	11.79 ± 5.72	5.39 ± 2.37
180 min <sup>8</sup>	0.06 ± 0.05	0.09 ± 0.08	12.08 ± 6.26	22.06 ± 13.49	1.22 ± 0.57	1.34 ± 0.56	0.03 ± 0.03	0.02 ± 0.02	0.18 ± 0.14	0.08 ± 0.07	13.56 ± 6.82	23.59 ± 14.37

<sup>1</sup> All values are means ± SEMs. TG, triacylglyceride; FFA, free fatty acid; DG, diacylglyceride; MG, monoacylglyceride.

<sup>2</sup> Control-product treatment compared with test-product treatment for gastric samples, P, 0.05 (Student's t-test)

<sup>3</sup> Difference over time between treatments for jejunal samples, P, 0.05 (repeated-measurement ANOVA).

<sup>4,5</sup> Sample numbers are as follows for control-product treatment and test-product treatment, respectively: 416 and 16, 514 and 15, 614 and 16, 715 and 16, 816 and 15

## Stomach

Hydrolysis of the lipids started in the stomach, although 76.2 ± 7.6% of the lipids in the test product and 70.0 ± 13.8% of the control product remained as triacylglycerides after 30 min. There was no significant difference in gastric triacylglyceride concentrations after 30 min.

Triacylglycerides continued to be the major component with both treatments up to 180 min (Table 1). There were significantly more mono-

mg of the test and control product, respectively, was recovered in the stomach after 180 min (P = 0.07).

The amount of the gastric juice that was retrieved after 180 min was significantly higher for the control product (P < 0.05) than for the test product (test product compared with control product: 46.2 ± 12.4 compared with 75.6 ± 17.2 g, respectively).

## Proximal jejunum

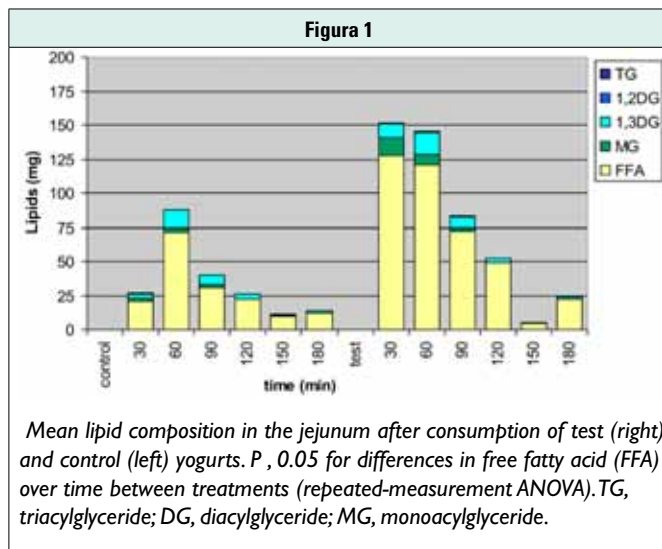
Because the sampling of the intestinal fluid was obtained in the proximal part of the jejunum, the majority of the lipids were almost fully hydrolyzed to FFAs (Table 1). The total amount of lipids collected from the jejunum during the 180-min period was 450 ± 119 compared with 230 ± 50 mg (P < 0.05)

stable 1,3-diacylglyceride form. The concentrations of 1,3-diacylglyceride and 1,2-diacylglyceride did not differ between test and control treatments (Table 1). No significant difference in the total amount of monoacylglycerides was obtained between the 2 groups (P = 0.09). The amount of triacylglycerides was < 0.1 mg in all jejunal samples except in the control treatment after 30 min, where 0.34 mg of triacylglycerides was detected.

The fatty acid profile was determined in the products before ingestion and in the jejunal samples obtained at 60 min. The latter determination was done in 2 ways: the fatty acid profile of the total lipid composition (triacylglycerides, diacylglycerides, monoacylglycerides, and FFAs) was determined, and the fatty acid profile of only the FFA part of the total lipid composition was determined. The fatty acid profiles are listed in Table 2 as relative compositions. After infusion of the test product, an enrichment of the long-chain saturated fatty acids was seen in the jejunum [especially palmitic acid (16:0)] compared with the starting composition. An even further enrichment of the longchain saturated fatty acid was apparent in the FFA fraction of the lipid composition. The control showed a smaller difference in fatty acid composition compared with the starting product.

## Gastric lipase activity

The gastric lipase activity of gastric fluid showed both intra and



acylglycerides and FFAs with the test product than with the control product (P < 0.05 for both monoacylglycerides and FFAs). The amounts of diacylglycerides (both the 1,3-diacylglyceride and 1,2- diacylglyceride) did not differ between the products.

In total, 8.5 g lipids was infused into the stomach in both treatments out of which 475 ± 131 and 352 ± 105

for the test product and milk fat lipids, respectively. There was no significant difference in the intestinal sample volumes obtained after each treatment. Samples from the test-product treatment contained significantly more FFAs than did the control-product treatment (P < 0.05). Diacylglycerides were predominantly in the thermodynamically more

**TABLE 2 - FATTY ACID PROFILE AS RELATIVE COMPOSITION (%) IN THE TEST AND CONTROL PRODUCTS AND IN THE JEJUNAL SAMPLES OF THE CONTROL-PRODUCT AND TEST-PRODUCT TREATMENTS COLLECTED AFTER 60 MIN<sup>1</sup>**

	FA in yogurt <sup>2</sup>	FA in jejunum after 60 min	FFA in jejunum after 60 min
<14			
Control	21.0	5.8 ± 1.7 <sup>3</sup>	4.8 ± 0.7
Test	0.5	0.7 ± 0.1	0.3 ± 0.0
14:0			
Control	12.8	8.6 ± 1.7	13.3 ± 2.9
Test	1.3	1.1 ± 0.1	1.3 ± 0.1
16:0			
Control	32.8	44.0 ± 3.2	40.2 ± 3.4
Test	44.3	57.4 ± 4.9	62.2 ± 4.2
16:1			
Control	2.0	1.7 ± 0.3	0.0 ± 0.0
Test	0.2	0.7 ± 0.1	0.0 ± 0.0
18:0			
Control	9.9	14.3 ± 1.2	19.0 ± 1.5
Test	4.3	5.9 ± 0.5	7.5 ± 0.5
18:1			
Control	18.8	14.5 ± 2.2	16.3 ± 1.4
Test	38.0	18.9 ± 2.7	19.0 ± 1.5
18:2			
Control	1.5	7.5 ± 0.9	6.4 ± 0.7
Test	10.6	12.0 ± 1.0	9.6 ± 0.6
18:3			
Control	0.5	0.5 ± 0.1	0.0 ± 0.0
Test	0.0	0.0 ± 0.0	0.0 ± 0.0
<18			
Control	0.7	3.1 ± 0.2	0.0 ± 0.0
Test	0.7	3.3 ± 0.3	0.0 ± 0.0

<sup>1</sup> FA, fatty acids in the total lipid composition; FFA, fatty acid in the free fatty acid fraction of the total lipid composition.

<sup>2</sup> Measured once.

<sup>3</sup> Mean ± SEM (all such values).

interindividual variability. Subjects were divided into 3 groups on the basis of the level of lipase activity as follows: 1) no activity (below the detection limit; < 300 μmol · min<sup>-1</sup> · g<sup>-1</sup>; 7 persons), 2) low activity (300–1000 μmol · min<sup>-1</sup> · g<sup>-1</sup>; 4 persons), and 3) high activity (1000–5000 μmol · min<sup>-1</sup> · g<sup>-1</sup>; 5 persons). No correlation was seen between the initial gastric lipase activity and the rate of lipolysis patterns in any of the 2 treatments. However, the level of lipase activity had limited effects on the relative concentrations of triacylglycerides when measured in the stomach at 30 min. In the group with the highest lipase activity, 70% of triacylglycerides were detected, and in the 2 groups with a lower activity, 80% of triacylglycerides were detected (data not shown).

## Evaluation of pH

The pH levels in the stomach were 2–4 in the fasted state before yogurt was given and increased to 4.5 at 30 min and dominated by the pH of yogurt. At 180 min, the pH values had returned to the fasting value of 2–3, and there was no difference between the 2 products.

The pH values of the jejunal samples obtained before infusion of the test product ranged between 5.5 and 6.0 and were within the range previously reported with this technique (30). The pH levels of the jejunal samples increased slightly during the experiment; however, there was no statistical difference in the pH values between the 2 products.

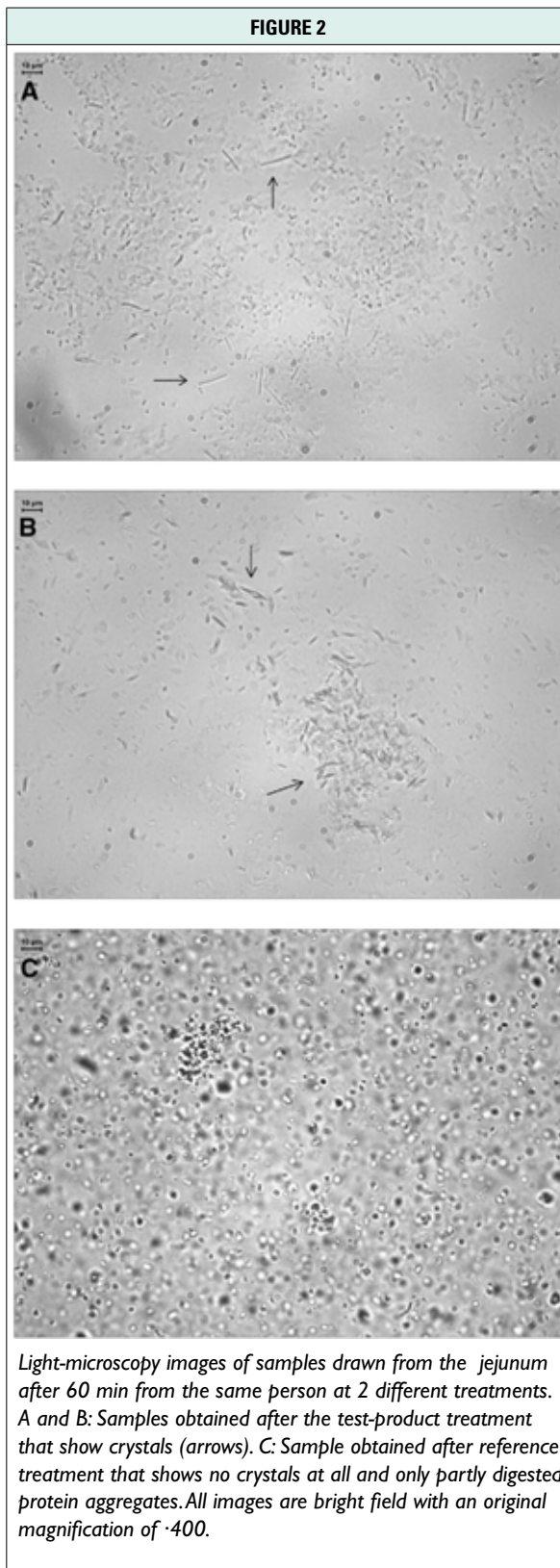
## Microstructural analyses

Microscopic analyses of the yogurt products before ingestion and gastric samples showed no differences. The gastric samples had similar features as the starting material, although the protein aggregates were more broken down.

The majority of the protein aggregates in the jejunal samples had vanished after proteolysis and absorption. No fat droplets were observed in intestinal samples from the test-product group.

Instead, an abundance of crystals

**FIGURE 2**



Light-microscopy images of samples drawn from the jejunum after 60 min from the same person at 2 different treatments. A and B: Samples obtained after the test-product treatment that show crystals (arrows). C: Sample obtained after reference treatment that shows no crystals at all and only partly digested protein aggregates. All images are bright field with an original magnification of 400.

was observed that were recognizable as high-contrast needle-shaped objects of ≥ 20 μm (Figure 2, A and B). In the control-product group, only sporadic

**TABLE 3 - SCORING OF NUMBER OF CRYSTALS IN JEJUNAL SAMPLES COLLECTED AT 60 MIN (J60) AND 180 MIN (J180)<sup>1</sup>**

	J60			J180		
	0	+	++	0	+	++
Control-product treatment	9	7	0	11	5	0
Test-product treatment	2	7	7	6	3	7

<sup>1</sup>0, no crystals; +, few crystals; ++, many crystals

crystals were seen in the intestinal samples (Figure 2C). When the subjects were given the test product, 11 out of 16 subjects had crystals in the intestinal samples and in most cases even many crystals (Table 3). At 180 min, 7 out of 16 intestinal samples contained many crystals (ie, > 5 crystals). In the control product, only a few crystals were seen in some subjects (ie, ≤, 5 crystals) after 60 and 180 min. These crystals were not seen in the starting products or in the gastric samples.

X-ray diffractograms of freeze-dried material were taken of a subset (test and control products) of jejunal samples collected at 60 min. An example of such an X-ray diffractogram obtained from the test-product treatment is shown in Figure 3. The largest signals could be attributed to sodium chloride (2θ = 27.3°, 31.7°, 45.4°, 53.8°, and 56.4°) and the second largest set of signals for potassium chloride (2θ = 28.2°, 40.4°, and 50.1°). However, during the light-microscopy evaluation, the sodium chloride and potassium chloride were still dissolved and, hence, did not appear as crystals.

The other signals mainly represented long-chain saturated fatty acids, such as palmitic acid, characterized by signals at 2θ = 21.5° and 24°, and the smaller peaks at 2θ = 7°, 10°, and 12.5°.

These values match literature values (31) and dif-

fer from literature values for sodium or calcium salts of palmitate (also recorded; data not shown).

Dried jejunal samples were also subjected to the DSC, and samples from the test-product treatment showed a single melting signal with a maximum (melting temperature) at 58°C, which was close to the value of pure palmitic acid of 63°C. Sodium palmitate melts at 270°C. Samples from the control treatment showed a single melting signal at a peak temperature of 52°C, which was too low to be attributed to the melting point of palmitic acid alone.

Objects with crystalline features were analyzed with Raman microscopy. An example of the intestinal sample obtained from the test-pro-

duct treatment that shows both the reflective-light-microscopy image and the Raman spectrum obtained at the indicated spot are presented in Figure 4. This Raman spectrum was compared with recorded reference spectra of palmitic acid and sodium and calcium palmitate and data from the literature (32). From a comparison of the fine details of the spectra, it was concluded that the crystal consisted of palmitic acid and not of palmitate salt.

## DISCUSSION

This study with a validated intestinal perfusion technique generated in-vivo experimental data on digestion and absorption of lipids in the upper gastrointestinal tract in humans after intragastric infusion of yogurt that contained a vegetable-oil emulsion or milk fat. The results showed that the total amount of lipids at the jejunal sampling site was significantly higher for the test product than for the control product. These results suggest a slower digestion or reduced intestinal absorption of the lipids from the test product. This finding was not due to gastric lipase activity or gastric emptying, to different lipolysis kinetics induced by different lipid natures or emulsion microstructures, or to differences in the luminal pH.

In the proximal jejunum the lipids in the 2 test products were hydrolyzed mainly into FFAs, although small amounts of diacylglyceride and monoacylglyceride were still present. To our knowledge, an unexpected and novel finding was the appearance of crystals in the jejunal samples coming from the vegetable-oil emulsion (test product),

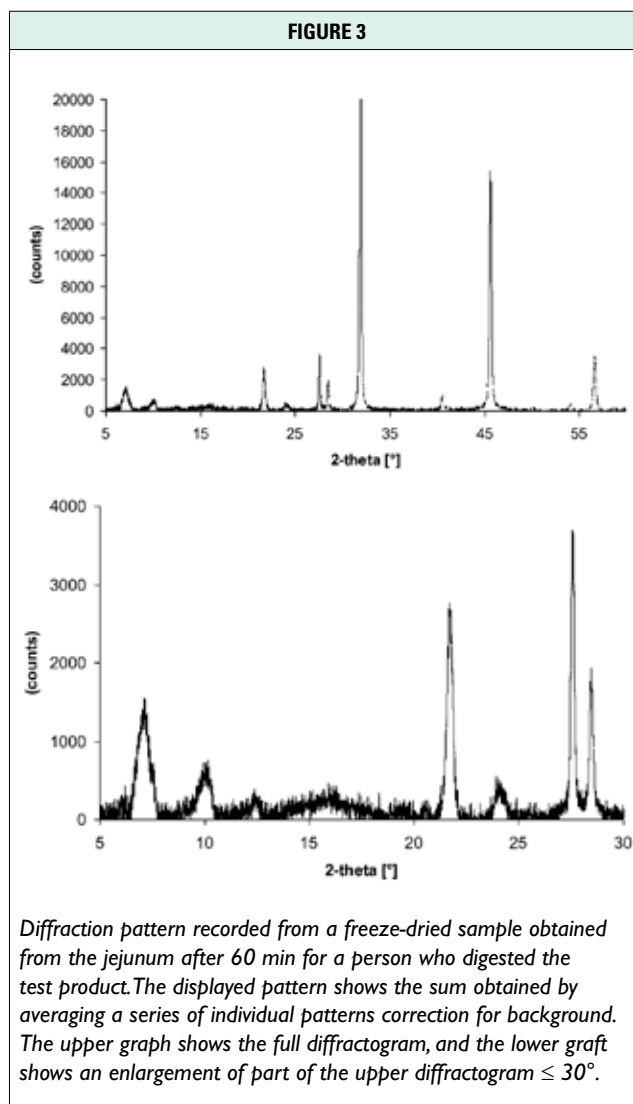
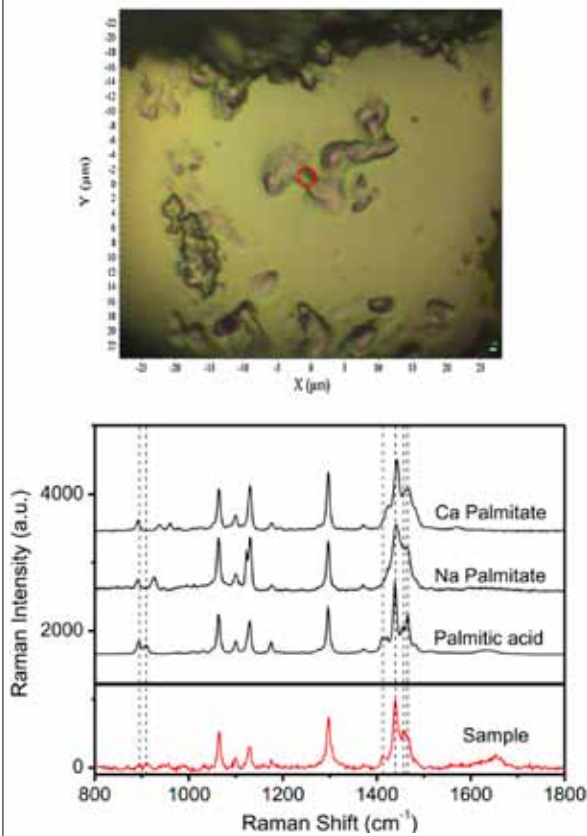


FIGURE 4



Top: Reflective-light-microscopy image from a freeze-dried sample obtained from the jejunum after 60 min for a person that digested the test product. Bottom: Raman spectrum obtained from the marked spot (red circle), corrected for the glass background, and compared with palmitic acid and sodium (Na) and calcium (Ca) salts. Dashed vertical lines indicate the similarity of the experimental sample with palmitic acid. The x and y axes (top panel) show scale (distance in  $\mu\text{m}$ ) relative to the red spot. a.u., arbitrary units.

whereas crystals were only rarely seen in intestinal samples from the control product. Crystals could not be observed in the gastric samples.

Although the amount of fat intake was the same for both product treatments, the higher amount of FFA in the intestinal lumen indicated a slower digestion (lipolysis) or absorption of the lipids with the test-product treatment. Two explanations that contribute to this difference are as follows: 1) the difference in structure of the triacylglycerides of the 2 test products and 2) the occurrence of crystals observed in the jejunal

been confirmed in human adults. Intestinal absorption of palmitic acid is high in human adults regardless of the position on the glycerol moiety (36, 37). Thus, it is unlikely that the difference of the structure of the triacylglycerides in the test and control products influenced the intestinal absorption rate of long-chain saturated fatty acids in this study. A second and more plausible explanation is that the formation and growth of needleshaped palmitic acid crystals in the jejunal samples could have delayed the absorption of fatty acids from the intestine.

samples. First, the amount of long-chain saturated fatty acids, mainly palmitic (16:0) and stearic (18:0) acids, were similar for the 2 test products, but the distribution of these fatty acids on the glycerol moiety differed. Palm oil has predominantly long-chain saturated fatty acids in the outer positions and unsaturated fatty acids in the middle position (33). It has been shown that animals and human infants have better gastrointestinal absorption of palmitic and stearic acids when positioned in the middle of the triacylglycerides compared with those in the outer positions (34, 35). However, that relation between lipid structure and intestinal absorption has not

Lipids, and particularly FFAs, that are rich in long-chain saturated fatty acids with a chain length of  $\geq 16$  carbon atoms are the only class of molecules from the digested food constituents that can form crystals at body temperature (33). All other food constituents present in the intestine after consumption of yogurt-based products, such as peptides and carbohydrates, will not form crystals in the aqueous environment of the intestinal lumen at body temperature. The fatty acid analyses showed a relative enrichment of palmitic acid concentrations in the jejunal samples after the test product treatment compared with the concentrations in the starting products. Palmitic and stearic acids and their sodium or calcium soaps form needle-shaped crystals in an aqueous environment (38). It is possible that the crystals are insoluble soaps of long-chain saturated fatty acids at the jejunal pH, as reported previously (39, 40). However, the FFA was observed in the current study in its protonated form (palmitic acid), not as its salt.

In the acidic stomach, crystallization of hydrolyzed palmitic acid might be initiated, but crystals are too small to be observed by light microscopy and will be disguised in the still present fat globules that contain mostly triacylglycerides. Once emptied into the intestine, these invisible crystals could act as nuclei for the newly hydrolyzed palmitic acid. In the samples that were retrieved from the jejunum, the majority of the lipids were hydrolyzed, fat globules were gone, and palmitic acid crystals became visible.

Although the total amount of FFAs in the jejunal samples from the control treatment contained 60% palmitic and stearic acids, crystal formation hardly occurred. The explanation that underlies the crystal formation could be that the test product contained polar fractions of oat oil rich in galactolipids that emulsified the palm oil (41). The control product contained phospholipids in a



much lower amount (relative to the lipid concentration), which was an amount hardly capable of emulsifying the dairy fat.

We propose that galactolipids have a crucial mediating effect on crystal formation, first as a nucleator and later as a growth facilitator for freshly hydrolyzed saturated long-chain fatty acids by lipase on the droplet surface. Being concentrated close to the droplet surface, the FFA molecules are promoted to form a regular arrangement with the long saturated tails oriented parallel to each other. It is energetically favorable for the FFA molecules to crystallize, even at 37°C. Galactolipids can form lamellar liquid crystalline phases in water (42, 43). Thus, by providing a flat interface, galactolipids stimulate the first ordering of newly hydrolyzed long-chain saturated fatty acids. This mechanism is known as template-assisted nucleation (44). When FFA molecules are released, the crystal can grow out of the droplet environment into the water phase. The surface-active galactolipids can overcome the unfavorable surface energy of this hydrophobic crystal of saturated FFA growing out of the droplet confinement and the poor wetting of the hydrophobic crystal in the aqueous environment. In the control product, only a limited amount of surface-active lipids (as phospholipids) are present to facilitate such a crystal growth. Good emulsifiers can stimulate the formation of needle-shaped lipid crystals in certain lipid compositions that grow from the lipid droplet into the aqueous phase (45).

The occurrence of crystals in the jejunum may alter the conditions for absorption of lipids along the intestine. The molecular solubility of palmitic acid in neutral water is low but not zero [0.72 mg/100 g or 30  $\mu\text{mol/L}$  at 20°C (46)] and is expected to be low in the aqueous phase of the intestinal lumen because the pH is between 6.5 and 7.0. After being

molecularly dissolved in the aqueous phase, dissolution of the hydrophobic palmitic acid into the hydrophobic core of the mixed micelle is favorable. Although the palmitic acid crystals are transported further down the intestine and into the ileum, free palmitic acid is gradually released from the crystals, taken up by the mixed micelles, and transported to the ileal wall. The presence of yet unabsorbed lipids will lead to an activation of the feedback system in the ileum, such as the reduction of gastrointestinal motility and an increase of the release of satiety hormones (12).

In particular, long-chain fatty acids are potent triggers of the ileal brake (6, 7, 13) and induce satiety (6). The results from a previous study (22) with this vegetable-oil emulsion showed a delay in gastrointestinal transit, and the data presented in this study form a hypothesis for a slower digestion or reduced absorption rate as the mechanisms for the observations. A larger part of the intestinal surface area exposed to lipids or the increased exposure of lipids to the ileum could be the explanation behind the suppressive effect on food intake presented in previous studies (16–18).

In the current perfusion study, it was possible to investigate the fate of lipids during the natural digestive route for food, and therefore, this study is different from studies where model emulsions were infused into the ileum (13).

In conclusion, significantly higher amounts of lipids, mainly as FFAs, were observed in the proximal jejunum during 180 min after ingestion of the vegetable-oil emulsion compared with the amounts of lipids observed after ingestion of the control composition.

The FFA appeared as palmitic acid crystals and, hence, caused a reduction of digestion and absorption rates from the small intestine. The crystallization of hydrolyzed

ingested lipids in the gastrointestinal lumen makes it possible that sufficient amounts of lipids may reach the ileum and activate a feedback mechanism, such as an ileal brake. Together, these results form a hypothesis that may explain the increased satiety that has been observed with this vegetable-oil emulsion made of palm oil and oat oil.

We thank all individuals who contributed to this study, in particular, in Uppsala, Lisa Wester, Inger Ohlsson, and Patrik Fridblom for support and, in The Netherlands, Wim Plugge for statistical analysis, Fredoen Valianpour and Robert van den Bos for lipid analysis, Ralf Kleppinger for X-ray diffraction, ChengWei for Raman microscopy, and Wim Saris and Gertjan Smolders for valuable discussions.

The authors' responsibilities were as follows - LK, DJPCK, HF, AV, and AS: conducted the experiments and analyzed data; DJPCK, AV, and AS: provided essential material and databases; DJPCK and AS: performed statistical analysis; HF: wrote the first draft of the manuscript; LK: was the principal investigator and had primary responsibility for the final content of the manuscript; and all authors: participated in the design of the research and made major contributions to the manuscript (especially HL, AS, and AV). DJPCK, AV, and AS are employees of DSM Food Specialties BV. LK, HF, and HL reported no conflicts of interest.

*Lars Knutson, Damiet JPC Koenders, Helle Fridblom, Annika Viberg, Arjen Sein, and Hans Lennernäs - Departments of Surgical Sciences (LK and HF) and Pharmacy (HL), Uppsala University, Uppsala, Sweden, and the DSM Food Specialties BV, Delft, Netherlands (DJPCK, AV, and AS). Supported by DSM Food Specialties BV, the maker of Fabules, after the approval of Uppsala University.*

**Unlimited. DSM**

**DSM Nutritional Products**

[www.dsm.com](http://www.dsm.com)

## References

1. Ayyad C, Andersen T. Long-term efficacy of dietary treatment of obesity: a systematic review of studies published between 1981 and 1999. *Obes Rev* 2000;1:113–9.
2. Hill JO, Wyatt HR, Reed GW, Peters JC. Obesity and the environment: Where do we go from here? *Science* 2003;299:853–5.
3. Woods SC. Gastrointestinal satiety signals I. An overview of gastrointestinal signals that influence food intake. *Am J Physiol Gastrointest Liver Physiol* 2004;286:G7–13.
4. Cook CG, Andrews JM, Jones KL, et al. Effects of small intestinal nutrient infusion on appetite and pyloric motility are modified by age. *Am J Physiol* 1997;273:R755–61.
5. Andrews JM, Doran S, Hebbard GS, Raszias G, Sun WM, Horowitz M. Effect of glucose supplementation on appetite and the pyloric motor response to intraduodenal glucose and lipid. *Am J Physiol* 1998;274:G645–52.
6. Welch I, Saunders K, Read NW. Effect of ileal and intravenous infusions of fat emulsions on feeding and satiety in human volunteers. *Gastroenterology* 1985;89:1293–7.
7. Read NW, McFarlane A, Kinsman RI, et al. Effect of infusion of nutrient solutions into the ileum on gastrointestinal transit and plasma levels of neurotensin and enteroglucagon. *Gastroenterology* 1984;86:274–80.
8. Spiller RC, Trotman IF, Higgins BE, et al. The ileal brake—inhibition of jejunal motility after ileal fat perfusion in man. *Gut* 1984;25: 365–74.
9. Holgate AM, Read NW. Effect of ileal infusion of intralipid on gastrointestinal transit, ileal flow rate, and carbohydrate absorption in humans after ingestion of a liquid meal. *Gastroenterology* 1985;88:1005–11.
10. Keller J, Holst JJ, Layer P. Inhibition of human pancreatic and biliary output but not intestinal motility by physiological intraleal lipid loads. *Am J Physiol Gastrointest Liver Physiol* 2006;290:G704–9.
11. Layer P, Holst JJ, Grandt D, Goebell H. Ileal release of glucagon-like peptide-1 (GLP-1). Association with inhibition of gastric acid secretion in humans. *Dig Dis Sci* 1995;40:1074–82.
12. Maljaars PW, Peters HP, Mela DJ, Masclee AA. Ileal brake: a sensible food target for appetite control. A review. *Physiol Behav* 2008;95:271–81.
13. Maljaars PW, Symersky T, Kee BC, Haddeman E, Peters HP, Masclee AA. Effect of ileal fat perfusion on satiety and hormone release in healthy volunteers. *Int J Obes (Lond)* 2008;32:1633–9.
14. Symersky T, Kee BC, Haddeman E, Peters HP, Masclee AAM. Low dose ileal oil perfusion increases satiety in humans. Spring Meeting, March 18–19, 2004. *Eur J Gastroenterol Hepatol* 2005;17:A9 (abstr).
15. Van Citters GW, Lin HC. The ileal brake: a fifteen-year progress report. *Curr Gastroenterol Rep* 1999;1:404–9.
16. Burns AA, Livingstone MB, Welch RW, et al. Short-term effects of yoghurt containing a novel fat emulsion on energy and macronutrient intakes in non-obese subjects. *Int J Obes Relat Metab Disord* 2000;24:1419–25.
17. Burns AA, Livingstone MB, Welch RW, Dunne A, Reid CA, Rowland IR. The effects of yoghurt containing a novel fat emulsion on energy and macronutrient intakes in non-overweight, overweight and obese subjects. *Int J Obes Relat Metab Disord* 2001;25:1487–96.
18. Burns AA, Livingstone MB, Welch RW, Dunne A, Rowland IR. Dose-response effects of a novel fat emulsion (Olibra) on energy and macronutrient intakes up to 36 h post-consumption. *Eur J Clin Nutr* 2002;56:368–77.
19. Logan CM, McCaffrey TA, Wallace JM, et al. Investigation of the medium-term effects of Olibra fat emulsion on food intake in non-obese subjects. *Eur J Clin Nutr* 2006;60:1081–91.
20. Diepvens K, Steijns J, Zuurendonk P, Westerterp-Plantenga MS. Short-term effects of a novel fat emulsion on appetite and food intake. *Physiol Behav* 2008;95:114–7.
21. Diepvens K, Soenen S, Steijns J, Arnold M, Westerterp-Plantenga M. Long-term effects on consumption of a novel fat emulsion in relation to body-weight management. *Int J Obes (Lond)* 2007;31:942–9.
22. Haenni A, Sundberg B, Yazdanpandah N, Viberg A, Olsson J. Effect of fat emulsion (Fabules) on orocecal transit time in healthy men. *Scand J Gastroenterol* 2009;44:1186–90.
23. Knutson L, Odlind B, Ha'llgren R. A new technique for segmental jejunal perfusion in man. *Am J Gastroenterol* 1989;84:1278–84.
24. Lennerna's H, Ahrenstedt O, Ha'llgren R, Knutson L, Ryde M, Paalzow LK. Regional jejunal perfusion, a new in vivo approach to study oral drug absorption in man. *Pharm Res* 1992;9:1243–51.
25. Nilsson RG, Hansson GI, Lo'fgren LJ, et al. A clinical single-pass perfusion investigation of the dynamic in vivo secretory response to a dietary meal in human proximal small intestine. *Pharm Res* 2006;23:742–51.
26. Persson E, Lo'fgren L, Hansson G, Abrahamsson B, Lennerna's H, Nilsson R. Simultaneous assessment of lipid classes and bile acids in human intestinal fluid by solid-phase extraction and HPLC methods. *J Lipid Res* 2007;48:242–51.
27. de Jong C, Badings HT. Determination of free fatty acids in milk and cheese procedures for extraction, clean up, and capillary gas chromatographic analysis. *J Sep Sci* 1990;13:94–8.
28. Juhel C, Armand M, Pafumi Y, Rosier C, Vandermander J, Lairon D. Green tea extract (AR25) inhibits lipolysis of triglycerides in gastric and duodenal medium in vitro. *J Nutr Biochem* 2000;11:45–51.
29. Mansour HM, Hickey AJ. Raman characterization and chemical imaging of biocolloidal self-assemblies, drug delivery systems, and pulmonary inhalation aerosols: a review. *AAPS PharmSciTech* 2007;8:E99.
30. Lindahl A, Ungell AL, Knutson L, Lennerna's H. Characterization of fluids from the stomach and proximal jejunum in men and women. *Pharm Res* 1997;14:497–502.
31. Lynch ML, Wireko F, Tarek M, Klein M. Intermolecular interactions and the structure of fatty acid-soap crystals. *J Phys Chem B* 2001;105:552–61.
32. Caspers PJ, Lucassen GW, Puppels GJ. Combined in vivo confocal Raman spectroscopy and confocal microscopy of human skin. *Biophys J* 2003;85:572–80.
33. Gunstone FD, Harwood JL, Dijkstra AJ, eds. *The Lipid Handbook*. 3rd ed. Boca Raton, FL: CRC Press, 2007.
34. Tomarelli RM, Meyer BJ, Weaver JR, Bernhart FW. Effect of positional distribution on the absorption of the fatty acids of human milk and infant formulas. *J Nutr* 1968;95:583–90.
35. Kennedy K, Fewtrell MS, Abbott R, et al. Double-blind, randomized trial of a synthetic triacylglycerol in formula-fed term infants: effects on stool biochemistry, stool characteristics, and bone mineralization. *Am J Clin Nutr* 1999;70:920–7.
36. Kubow S. The influence of positional distribution of fatty acids in native, interestrified, and structure-specific lipids on lipoprotein metabolism and atherogenesis. *J Nutr Biochem* 1996;7:530–41.
37. Berry SSE. Triacylglycerol structure and interestrification of palmitic and stearic acid-rich fats: an overview and implications for cardiovascular disease. *Nutr Res Rev* 2009;22:3–17.
38. Vold RD, Ferguson RH. A phase study of the system sodium palmitate – sodium chloride – water at 90%. *J Am Chem Soc* 1938;60:2066.
39. Reid IR. Effects of calcium supplementation on circulating lipids: potential pharmacoeconomic implications. *Drugs Aging* 2004;21:7–17.
40. Vaskonen T. Dietary minerals and modification of cardiovascular risk factors. *J Nutr Biochem* 2003;14:492–506.
41. Hauksson JB, Bergqvist MHJ, Rilfors L. Structure of digalactosyldiacylglycerol from oats. *Chem Phys Lipids* 1995;78:97–102.
42. Carlsson A, So'derlind E, Bergqvist M, Nilsson R, Nylander T. Galactolipids—a new tool in drug formulation. In: Proceedings of the 21st World Congress of the International Society for Fat Research (ISF), The Hague, October 1995. Vol. 2. Bridgewater, United Kingdom: PJ Barnes & Associates, 1996:259–62.
43. Mannock DA, Collins MD, Kreichbaum M, Harper PE, Gruner SM, McElhaney RN. The thermotropic phase behaviour and phase structure of a homologous series of racemic *b*-*d*-galactosyl dialkylglycerols studied by differential scanning calorimetry and X-ray diffraction. *Chem Phys Lipids* 2007;148:26–50.
44. Fujiwara K, Nagahisa S, Yano J, Ueno S, Sato K. Kinetics of heterogeneous nucleation of *n*-alcohol crystals from solution assisted by template thin films of monoacylglycerols and fatty acids. *J Phys Chem B* 2000;104:8116–23.
45. Boode K, Walstra P. Partial coalescence in oil-in-water emulsions I. Nature of the aggregation. *Colloids Surf A Physicochem Eng Asp* 1993; 81:121–37.
46. Lide DR, ed. *CRC handbook of chemistry and physics*. 82nd ed. Boca Raton, FL: CRC Press, 2002.