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Abbreviations: AMF: anhydrous milk fat; AP: atom percent; APE: atom percent excess; AUC: area under curve; iAUC: incremental area under curve; CMRF: chylomicron-rich fraction; CRNH-RA: Human Nutrition Research Center Rhône-Alpes; d_{32}: volume-surface mean diameter; dARF: acetate recovery factor; DSC: differential scanning calorimetry; FA: fatty acid; FAMEs: fatty acid methyl esters; NEFA: non-esterified fatty acid; NW: normal-weight; TAG: triacylglycerol; XRD: X-ray diffraction.

Running title: Fat structuring modifies postprandial metabolism.

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ABSTRACT

Background: Prolonged postprandial hypertriglyceridemia is a potential risk factor for cardiovascular diseases. In the context of obesity, this is associated with a chronic imbalance of lipid partitioning oriented towards storage and not towards β-oxidation.

Objectives: We tested the hypothesis that the physical structure of fat in a meal can modify absorption, chylomicron transport and further metabolic handling of dietary fatty acids.

Design: 9 normal-weight and 9 obese subjects were fed 40g of milkfat (+^{13}C-triacylglycerols), either emulsified or not, in breakfasts of identical composition. We measured the postprandial triglyceride content and size of the chylomicron-rich fraction, plasma kinetics of ^{13}C-fatty acids, exogenous lipid oxidation using breath-test/indirect calorimetry, and fecal excretion.

Results: The emulsified fat resulted in earlier (>1h) and sharper chylomicron and ^{13}C-fatty acids peaks in plasma compared to spread fat in both groups (P<0.0001). After 2h, the emulsified fat increased ApoB48 concentration (9.7 ±0.7 *vs* 7.1 ±0.9 mg/L; P<0.05) in the normal-weight subjects compared to the spread fat. For the obese subjects, emulsified fat resulted in 3-fold larger chylomicrons (218 ±24 nm) compared to the spread fat (P<0.05). The emulsified fat induced higher dietary fatty acid spillover in plasma and sharper $^{13}CO_2$ appearance, provoking increased exogenous lipid oxidation in each group: from 45% to 52% in normal-weight subjects (P<0.05), 40% to 57% in obese (P<0.01).

Conclusions: This study supports a new concept of "slow *vs* fast fat" whereby intestinal absorption can be modulated by structuring of dietary fat to modulate postprandial lipemia and lipid β-oxidation in humans of different BMI.

Key words: intestinal absorption; chylomicron; emulsion; stable isotopes; obesity.

INTRODUCTION

The metabolic importance of intestinal absorption and transport of nutrients in the postprandial period is recognized as important in the context of metabolic diseases such as obesity and type 2 diabetes (1). Regarding lipid metabolism, plasma kinetics, timing of peak of lipemia as well as chylomicron size are recognized as factors determining metabolic complications that are still an open field of research (2-4). For this reason, control of intestinal lipid absorption, the resulting chylomicron transport dynamics and ultimate dietary lipid fate may be an effective tool in the management of metabolic diseases. Recent studies have shown differential effects of oral sensory stimulation with high *vs* low amounts of dietary fat on intestinal lipid absorption (5). The possible effects of fatty acid (FA) profile of an oral fat load on chylomicron size have been suggested (6-10). While both fat load and composition can affect postprandial lipid absorption, few studies have investigated the effects of fat structure on the postprandial metabolism of an identical lipid load. In diabetes, the concept of "slow/low glycemic index carbohydrates and fast/high glycemic index carbohydrates" is well established and has facilitated the development of specific foods and/or cooking methods to control postprandial glycemia (11). We thus raised the question of whether a similar concept may be applicable to dietary fat according to the way it is structured in the meal.

Dietary lipids are incorporated in food products with different physicochemical structures, e.g., in dispersed lipid droplets in oil-in-water emulsions like ice cream or in a continuous lipid phase in butter and margarine. Emulsions are the most widespread fat structures, in processed foods and enteral formulas, and are therefore of interest regarding their role in lipid digestion and absorption (12). Indeed, we have previously demonstrated in rodents the importance of lipid emulsified structure on FA absorption and β-oxidation (13-15). However, the impact of fat structure on the kinetics of lipid absorption and dietary FA handling in humans remains to be elucidated. Such effects also deserve to be elucidated in obese subjects that present altered storage function of dietary FA in the postprandial state (16).

We therefore hypothesized that the physicochemical structure of the fat in a meal could modulate postprandial lipemia and fat partitioning: storage *vs* oxidation, and that the effect would be more pronounced in obese subjects. We investigated the metabolic response to fat-containing meals (40 g) differing only in the structuring of fat, emulsified or not, in healthy young normal weight and obese men. Measurements included chylomicron number and size, FA β-oxidation and FA excretion in feces. The aim of this study was to define the contribution of fat structure and subject BMI on the postprandial lipemia and metabolism of dietary FA.

SUBJECTS AND METHODS

Study design

The study was an open label trial with a cross-over randomized controlled design involving 2 days of metabolic testing separated by at least three weeks (Supplemental Figure 1). It was conducted at the Human Nutrition Research Center Rhône-Alpes (CRNH-RA; Lyon, France) according to the Second Declaration of Helsinki and the French Huriet-Serusclat law. The LIPINFLOX study was approved by the Scientific Ethics Committee of Lyon Sud-Est-II and AFSSAPS and registered at Clinical Trials (#NCT01249378). Volunteers received written and oral information and their medical history was reviewed. In addition they underwent a physical examination and fasting clinical analysis were assessed before enrolment. Informed written consent was obtained from all subjects. Volunteers performed trial in the period from April 2010 to July 2011. During the protocol, all subjects were asked to continue their regular diet and activity except for the week before and the 3-d period following each test day. Subjects were told to avoid foods naturally rich in ^{13}C and were given a list. For 48 h prior to testing subjects were asked to refrain from consuming alcohol and to avoid exercise. In addition, subjects were provided with a standardized dinner

the evening prior to testing. Compliance was checked through diet records, 5 days before and 3 days after each test day.

After an overnight of fast, subjects ingested one of the two test breakfasts. The primary outcome measured was the effect of fat structure on postprandial lipemia. Secondary outcomes measured were the effect of fat structure and BMI on postprandial lipid metabolism. Previous studies on lipemia (17) and lipid oxidation (18) were used for the power analysis: a minimum sample size of 8 subjects per BMI group was calculated to be necessary to detect significant changes in these parameters. The treatments were randomized according to a random allocation sequence performed by a CRNH-RA biostatistician using Stat® v.11; two randomization lists were generated and stratified over BMI. Subjects were anonymized using a number corresponding to randomization sequence order.

Subjects

Twenty-two healthy men were recruited, 11 normal-weight (NW) and 11 obese, and 20 completed the study (see Flow Diagram online). One subject in each group was not included in data analyses due to abnormal postprandial lipid metabolism; therefore 18 healthy subjects divided in two groups, 9 NW and 9 obese with comparable mean age were finally tested for the primary outcome, (**Table 1**). Volunteers were required to be non-smokers, sedentary or having <4h per week of physical activity and non-claustrophobic. We excluded persons under medication interfering with lipid metabolism, with psychological illness, or those with eating/metabolic disorders. In addition, subjects were required to have had stable weight, to be free of diabetes and to have not made blood donation for 3 months prior to the start of the study. Data were collected at CRNH-RA.

Test meals

The test breakfasts were isoenergetic, equal in nutrient composition (**Table 2**) and both consisted of bread (50 g), skimmed milk (160 mL) and anhydrous milk fat (AMF; 40 g), containing 600 mg of tracers, either spread on bread or emulsified in skim milk. Both meals had the same composition as no additional emulsifier was added because milk proteins are sufficient to provide a submicronic milk fat emulsion. Prior to the test day, a mixture of labeled triacylglycerols (TAG) proportionally representing each FA type present in test fat was first incorporated into melted milk fat: 300 mg of [1,1,1-^{13}C3] tripalmitin for long-chain saturated FA, 210 mg of [1,1,1-^{13}C3] triolein for unsaturated FA and 90 mg of [1,1,1-^{13}C3] trioctanoin for short- and medium-chain FA (99 atom% ^{13}C, Eurisotop, Saint-Aubin, France). For the emulsion test, melted labeled milk fat was coarsly pre-mixed in skimmed milk (ProScientific Inc., Oxford, USA) and then further finely emulsified (4x 1 min, Vibra-cell™ Ultrasonic Processor, Sonics, Newtown, USA) (Supplemental Figure 2). Test products were then kept at 4°C overnight.

A second meal was served 5 hours after breakfast, containing pasta (200 g), turkey (100 g), butter (10 g), olive oil (10 g), bread (50 g), stewed fruit (100 g) which provided 713 kcal (2985 kJ) with 29%, 51% and 20% of energy as lipids (22.7 g), carbohydrates (91.5 g) and proteins (35.7 g) respectively. All subjects were given 10 minutes to eat breakfast and 30 minutes for lunch. During the test, participants were allowed to drink 200 mL of water.

Test fat characterization

Emulsion droplet size was measured by Dynamic Light Scattering (Zetasizer Nano S, Malvern, France). Specific surface area of emulsion droplets was calculated using Laser Light Scattering (Mastersizer 2000, Malvern, France). The melting temperature and crystalline state of the fat was characterized by Differential Scanning Calorimetry (DSC) using a Q1000 DSC

(TA Instruments, New Castle, USA) and by powder X-ray Diffraction (XRD) using a D8 Advance diffractometer (Bruker, Germany).

Hunger assessment

Subjective assessment of hunger was measured on a 10-cm visual analogue scale 2 min before breakfast and 2 min before lunch. Specific question to assess hunger was "How hungry do you feel?".

Metabolic explorations

Blood samples were obtained at baseline and at regular intervals after the meal, from an antecubital arm vein through a catheter and collected in vacutainer sterile tubes (with EDTA when necessary). Plasma was separated by centrifugation (1500 *g*, 10 min, 4°C) and stored at -20°C until analysis or at 4°C for separation of the chylomicron-rich fraction (CMRF).

Metabolic tests were divided into postprandial phases including a first period of 5 hours (0 to 300 min) post-breakfast in the morning, a second period of 3 hours post-lunch (300 to 480 min) and the entire exploration day (0 to 480 min).

Indirect calorimetry was performed during metabolic testing using a Deltatrac II™ calorimeter (Sensormedics, Yorba Linda, CA, USA). Respiratory exchanges (VO_2 and VCO_2) were recorded for periods of 30 or 60 minutes during the 8-h test period. Substrate oxidations were calculated using Ferrannini's equations (19). Urine was collected at 0, 300 and 480 min to determine nitrogen excretion for oxidation calculations. For breath test, expired gas samples were obtained at baseline, each 30 min for 8-h and then at 720 and 1440 min to check return to baseline. Subjects had to collect and freeze their stools individually over 72 h after the test day.

Plasma metabolite and hormone measurements

Non esterified fatty acid (NEFA) concentrations were determined by an enzymatic method Wako® (Neuss, Deutschland). ApoB48 was measured by ELISA (Gentaur, France). Insulin concentration was determined by RIA (CISBIO Bi insuline IRMA, France).

Isolation and analysis of chylomicron-rich fractions

To collect the CMRF, containing chylomicrons and their large remnants, 250 µL of plasma was deposited below a layer of 850 µL of distilled/deionized water and centrifuged at 80 000 rpm for 30 min using a Sorvall Kendro ultracentrifuge (Asheville, NC, USA). The floating layer was collected and stored at -80°C. TAG and cholesterol concentrations of CMRF were measured with a lipase glycerokinase and a cholesterol esterase/oxidase method, respectively, on a AU 2700 Beckman Coulter® (O' Cllagan's mils, Ireland) and expressed as differences in concentration over baseline. Hydrodynamic diameter of CMRF was measured by dynamic light scattering at 25°C using a ZetaSizer NanoS (Malvern, UK) using 1.0658 cP and 1.33 as viscosity and refractive index of the aqueous phase, respectively. Gravity-separated fraction containing chylomicrons and their large remnants are reported to size in the range 70-450 nm (20-22), and postprandial increase in cholesterol content of such fraction is about <0.2 mM (23). Our fraction collected by ultracentrifugation contained particles of mean diameter up to 200-300 nm and with postprandial increase in cholesterol of <0.15 mM in NW and <0.25 mM in obese subjects. Our CMRF is thus typical of fractions that are rich in chylomicrons and that also contain large remnants.

^{13}C-fatty acids in plasma lipids, NEFA, CMRF and stools

Sample preparation. Internal standards were added according to the fraction analyzed (heptadecanoic acid or glycerol triheptadecanoate).

Plasma processing. Plasma samples were submitted to direct methylation as described previously (24).

NEFA processing. Total lipids were extracted from plasma aliquots at 120 min after breakfast consumption (700µL) with 3 mL of a mixture of chloroform/methanol (2:1 v/v) according to Folch method (25). NEFA fractions were obtained therefrom by TLC on silica-gel plates with a mobile phase of hexane/diethyl ether/acetic acid (80:20:1 v/v/v). NEFA were derivatized to Fatty Acid Methyl Esters (FAMEs) (24).

CMRF processing. Lipids were extracted from CMRF at 120 min after breakfast according to Folch method (25). TAG fractions were then processed as the NEFA fractions above, to obtain the FAMEs from CMRF.

Stool processing. Fecal collections were weighed, homogenized and a precisely weighed aliquot was collected. Total lipids were extracted according to a modification of the Folch method and derivatized to obtain FAMEs (24).

Sample analysis. The amounts of FA in stools, plasma and NEFA were assessed by GC/MS using a quadrupole mass spectrometer connected to a gas chromatograph (MS 5975 and GC6890, Agilent Technologies, Massy, France). The isotopic enrichment of palmitic and oleic acids was determined using GC/C/IRMS (Isoprime, GV Instruments, Manchester, UK)(24). The ^{13}C enrichments were expressed as atom percent excess (APE). The plasma concentrations of non-esterified labelled palmitic and oleic acids (called [^{13}C-NEFA]) and non-esterified unlabelled palmitic and oleic acids (called [^{12}C-NEFA]) were also obtained from these analyses.

Calculations associated with apparent dietary fatty acid "spillover"

NEFA analysis at 120 min after breakfast was used to calculate ^{13}C enrichment in plasma NEFA as: [^{13}C-NEFA] / ([^{13}C-NEFA] + [^{12}C-NEFA]) (expressed in % enrichment). The proportion of exogenous NEFA in total plasma NEFA, expressed in %, was estimated by the

ratio of the ^{13}C enrichment in plasma NEFA to the ^{13}C enrichment of corresponding FA in the ingested milk fat. The proportion of exogenous fatty acids in plasma that was present in non-esterified form in the sum of pools NEFA+CMRF was calculated as: $[^{13}C\text{-NEFA}]_{plasma}$ / ($[^{13}C\text{-NEFA}]_{plasma} + [^{13}C\text{-FA}_{CMRF}]_{plasma}$), where $[^{13}C\text{-FA}_{CMRF}]_{plasma}$ is the plasma concentration of ^{13}C-FA esterified in CMRF-TAG = $[^{13}C\text{-FA}]_{CMRF}$ / ($[^{13}C\text{-FA}]_{CMRF} + [^{12}C\text{-FA}]_{CMRF}$) x 3 x $[\text{CMRF-TAG}]_{plasma}$.

Calculations of exogenous lipid oxidation from indirect calorimetry & breath tests

Exogenous lipid oxidation was calculated according to Binnert *et al.* (18) from data of indirect calorimetry and breath tests. Here the formula was adapted to our use of 3 labeled triglycerides as follows:

Exogenous lipid oxidation (% of ingested fat) =

$$\frac{\{[\{[AP\ CO_2(t) + AP\ CO_2(t_{-30})]/2\} - AP\ CO_2(t_0)]/100\} \times \dot{V}\ CO_2}{\{(A)+(B)+(C)\} \times 22.4 \times dARF} \times 100$$

With:

$$(A) = \{[\ AP\ ^{13}TG\ C8:0\ ]/100\} \times [(0.09/473.66) \times 27]$$

$$(B) = \{[\ AP\ ^{13}TG\ C16:0\ ]/100\} \times [(0.30/810.30) \times 51]$$

$$(C) = \{[\ AP\ ^{13}TG\ C18:1\ ]/100\} \times [(0.21/888.40) \times 57]$$

Where AP CO_2 (t) is the AP value of the expired CO_2 at time t, AP CO_2 (t_0) is the AP value of the expired CO_2 at time t_0, AP tracers is the calculated AP value of the labeled mixture of TAG (tracers) and $\dot{V}\ CO_2$ is the production rate of expired CO_2 (indirect calorimetry). Mean molecular weights of trioctanoin, tripalmitin and triolein are 473.66 g/mol, 810.30 g/mol and 888.40 g/mol, respectively. Mean number of carbons in trioctanoin, tripalmitin and triolein are 27, 51 and 57, respectively. dARF (Acetate Recovery Factor) is the correction factor for

incomplete recovery of ^{13}C bicarbonate (0.505 for NW; 0.453 for obese (26)) and 22.4 is the molar volume (L) of CO_2.

Kinetic parameters

We calculated the incremental area under curve (iAUC); maximum postprandial concentration, delta and diameter (C_{max}, Δ_{max}, d_{max}); time for appearance of these maximum parameters (t_{max}) and appearance/enlargement-rates between 0 and 60 min.

Statistical analysis

Each subject served as his own control. All data are presented as means ±SEM (n=9 per group) and were analyzed with Statview 5.0 software (Abacus Concept, Berkeley, CA). Postprandial data were compared by analysis of variance (ANOVA) for repeated measures followed by post-hoc test (Fisher PLSD) for statistical effects of (i) time alone (P_{time}) over the first postprandial period (0-300min), (ii) meal alone (P_{meal}) independently of the time in the postprandial period and (iii) interaction of both factors, time and meal ($P_{timexmeal}$). Kinetic parameters were compared by two-way ANOVA followed by Fisher PLSD according to meal and BMI (P_{meal}, P_{BMI}, $P_{mealxBMI}$) and time period before/after lunch ($P_{mealxBMIxtime}$). Multiple comparisons regarding tracer excretion in feces were performed using ANOVA followed by Bonferroni post-hoc test. Comparisons between meals within subject groups were performed using a paired Student's *t*-test and comparisons between subject groups within meals with an unpaired Student's *t*-test. Differences were considered significant at the $P< 0.05$ level.

RESULTS

Properties of emulsion *vs* spread fat

The emulsion droplet size (Table 3; Supplemental Figure 3) indicates the homogenization was effective in producing the emulsions. The emulsion had an approximately ~70000-fold

greater surface area than the spread fat. To control for the possibility that the different metabolic effects could be attributed to the fat melting properties, we measured melting profiles and crystalline structures in all conditions (with or without tracers, emulsified or not, Supplemental Figure 4). According to these analyses, the test fat differed only by their structure (Table 3).

Hunger feeling

At the end of the first postprandial period (0-300 min) just before lunch, normal-weight subjects felt similarly hungry regardless of breakfast type (Supplemental figure 5). In contrast, obese subjects felt hungrier after emulsion than spread fat ($P< 0.05$; Supplemental figure 5). Of note, before breakfasts, all subjects felt equally hungry (not shown).

Postprandial concentration profile and size of chylomicron-rich fraction

Figure 1A-B shows that in both groups, CMRF-TAG rapidly increased (60 min) after ingestion of emulsified fat and peaked at 3-4 h (t_{max} in **Table 4**). The emulsion induced a significantly earlier and sharper increase in CMRF-TAG than the spread fat (Table 4: t_{max} and appearance-rate$_{0\text{-}60min}$; $P< 0.001$). These differences were dramatically marked in the obese subjects, with a significant delay in absorption of the spread fat from 0 to 300 min compared to NW subjects ($P< 0.01$, Table 4). At the end of the test, CMRF-TAG of NW subjects returned to lower values regardless of fat structure. The obese subjects showed different profiles, with CMRF-TAG remaining elevated at the end of the spread fat test: e.g. at 480 min, 0.61 ± 0.15 mmol/L for spread *vs* 0.27 ± 0.06 mmol/L for emulsion above fasting baseline ($P< 0.05$). These differences in profile before and after lunch according to obese state and meal type are supported by different BMI x meal x time interactions for the Δ_{max} and iAUC of CMRF-TAG (Table 4).

Mean CMRF size sharply increased in both groups from the first hour after emulsion (Figure 1 C-D; P_{meal}< 0.05 for enlargment-rate$_{0\text{-}60min}$, Table 4). For NW subjects, CMRF diameters were similar for spread fat and emulsion all along the test. In obese subjects, CMRF diameters became equal for both meals at 300 min. We can note that in the period from 0 to 240 min, CMRF diameter in obese subjects was higher after emulsion *vs* spread fat from 0 to 240 min (P_{meal}< 0.05 and P_{time}< 0.001). Altogether, obese subjects presented larger CMRF than NW subjects (P< 0.01 for d_{max} 0-480 min, Table 4) with persistence of large CMRF after the second meal for spread fat.

Plasma concentration profile of ApoB48

Figure 1E-F shows that plasma ApoB48 changed over time in both groups after both breakfasts (P_{time}< 0.0001) and differently according to the type of breakfast for NW subjects ($P_{timexmeal}$=0.001). At 120 min, NW subjects accumulated more ApoB48 after consumption of emulsion than spread fat (7.08 ±0.86 mg/L for spread fat *vs* 9.73 ±0.69 mg/L for emulsion, P< 0.05) and compared with obese subjects for emulsion (7.47 ±0.78 mg/L, P< 0.05).

Plasma concentration profile of ^{13}C-fatty acid tracers and fecal loss

Figure 2A-B-C-D shows a change in plasma ^{13}C-palmitic and ^{13}C-oleic acids over time in both groups after both breakfasts (P_{time}< 0.0001). ^{13}C-palmitic acid appeared earlier and sharper in plasma when it was in emulsion, differences between breakfasts being greater for obese (P_{meal}= 0.007). Plasma concentrations of ^{13}C-oleic acid were higher during 5h of emulsion digestion, especially for obese subjects (P_{meal}= 0.018 and $P_{timexmeal}$= 0.0002). For both tracers, a second peak was observed at 360 min, after ingestion of the second meal.

During the first 300 min for obese subjects, the iAUC for plasma ^{13}C-FA were significantly higher after consumption of emulsion *vs* spread fat (P< 0.05, Figure 2B-D). iAUC after spread fat were lower for obese *vs* NW subjects (P< 0.05, Figure 2B-D).

Fecal excretion of ^{13}C-palmitic acid was higher than that of ^{13}C-oleic acid (Figure 2E). There was no effect of breakfast type on fecal excretion of ^{13}C-palmitic acid or ^{13}C-oleic acid in the two groups.

Plasma concentration profile of insulin and NEFA and apparent dietary FA spillover

Figure 3A-B-C-D shows a significant change in plasma insulin and NEFA over time after the two breakfasts in both groups (P_{time}< 0.0001). Over the first 300 min, the NEFA profile indicated a meal type x time interaction in both groups, with the decrease in plasma NEFA at 120 min being lower for emulsion than spread (Figure 3C-D). Therefore, we measured ^{13}C enrichment in plasma NEFA at 120 minutes (Figure 3E) to estimate whether this would result from the contribution of exogenous FA, so-called apparent fatty acid "spillover". We observed higher apparent "spillover" during the postprandial phase of emulsion *vs* spread fat, in both groups (*P*< 0.05 for NW and *P*< 0.01 for obese subjects, Figure 3E). The contribution of exogenous FA to total NEFA in NW subjects was 42% for spread *vs* 79% for emulsion, and was lower in obese, 4% for spread *vs* 50% for emulsion (meal effect, *P*< 0.01; BMI effect, *P*< 0.01; no meal x BMI interaction). Moreover, in NW subjects, the proportion of exogenous FA being in non-esterified form in plasma at 120 min was 10.6 % for spread *vs* 18.7 % for emulsion, i.e. 1.8-fold increase, and in obese 10.1 % for spread *vs* 15.0% for emulsion, i.e. 1.5-fold increase (*P*<0.05 for spread *vs* emulsion; no significant effect of BMI nor meal x BMI interaction). In the same time emulsification increased total plasma NEFA by 2.2-fold in NW and by 1.6-fold in obese subjects (Figure 3C-D). Altogether, this means that more than 80 % of the increase of plasma NEFA due to emulsification may be explained by an increased amount of exogenous fatty acids being released non-esterified in plasma (spillover).

Postprandial appearance of label in expired CO_2

The ^{13}C appearance in expired CO_2 represents the final product of FA β-oxidation. A significant change in APE occurred over time in both groups (P_{time}< 0.0001; **Figure 4**A-B). In both groups, APE was higher after consumption of emulsion *vs* spread fat over the first 300 min, indicating improvement of dietary fat β-oxidation using emulsified form. APE returned to baseline after 720 min. An effect of BMI was also observed with higher appearance-rate$_{0\text{-}60min}$ in NW than obese subjects (P< 0.01; Table 4).

Inserts in Figure 4A-B show that AUC of expired $^{13}CO_2$ after 300 min for the emulsion was significantly higher than for spread fat in both groups (P< 0.01 for NW subjects and P< 0.001 for obese subjects). Besides, over 0 to 720 min, obese subjects presented higher AUC of expired $^{13}CO_2$ after consuming emulsion *vs* spread fat (P< 0.05, Figure 4B insert). Altogether, the structuring of fat in the meal significantly affected the kinetic parameters of $^{13}CO_2$ air enrichment regardless of BMI while BMI affected AUC and appearance-rate $_{0\text{-}60min}$ (Table 4).

Exogenous lipid fate

We studied the metabolic handling of exogenous lipids by evaluating the fractions of ingested lipids that have been either oxidized or lost in feces, and so estimated the remaining fraction stored in body pools. For the same quantity and composition of ingested fat, all subjects β-oxidized FA better when fat was emulsified (Figure 4C). In turn, the calculated fraction of ingested lipids oriented towards storage in body pools was lower after emulsion *vs* spread consumption. After accounting for the part of exogenous lipids lost in feces (Figure 4C), the percentage of exogenous lipid oxidation according to the fraction that has been intestinally absorbed was higher for NW and obese subjects after emulsion *vs* spread fat (P< 0.05 and P< 0.01, respectively, Figure 4D). Total lipid oxidation was unchanged with the two

test meals in both groups but with a greater relative contribution of exogenous lipids ingested at breakfast as emulsion *vs* spread fat (Figure 4E).

DISCUSSION

Postprandial triglyceridemia is the first step in the metabolization of dietary lipids. Ingested FA are firstly present in plasma TAG in the form of intestinally secreted chylomicrons, which further lead to large remnants after hydrolysis by lipoprotein lipase (10, 22). The next step concerns trafficking of FA towards β-oxidation or storage that is of utmost importance regarding the metabolic impact of these dietary FA. We therefore investigated whether structuring fat in the meal could modify postprandial lipid metabolism, from the amount and size of chylomicrons to β-oxidation, including fecal loss. To this aim, labeled breakfasts containing either spread or emulsified fat were fed to NW and obese subjects. Test meals were designed to be of equal composition. Thus, factors like FA composition or protein content cannot be involved in the presently observed differences in lipid metabolism, which can be uniquely attributed to the fat physico-chemical structure in the meal. The postprandial chylomicron TAG profile after emulsion consumption differed from that of the spread fat, with the peak being more rapidly achieved, more pronounced and more quickly cleared, especially in obese subjects. This is consistent with reports of enhanced FA absorption when a simple bolus of vegetable oil was emulsified in humans (27) and rodents(14, 15, 28). One explanation is that our emulsion had ~70000-times greater surface area available for lipases than spread fat, which is reported to enhance lipolysis and absorption (29). Enteral emulsions of different droplet sizes, ~1 *vs* ~10 µm (14.5-fold difference in fat surface area), were shown to result in small differences only in postprandial lipemia in humans (17). The dramatic differences observed in the present work are due to the greatest differences in fat structure. Of note, postprandial lipid metabolization was previously found faster using unemulsified than emulsified milk fat in rats (14). Differences with the present results can be explained by (i)

rodent physiology of bile flow that is different from humans (30), and (ii) unemulsified melted milk fat being force-fed intragastrically, prior to the proteinaceous phase. This could have favored lipid emptying in the upper intestine and a rapid rise of plasma TAG in rats. Our study also provides a proof of concept that effects of fat structure in the meal can occur in a real mixed meal, while previous studies used oil or emulsion bolus fed orally or intragastrically (17, 29). Regarding emulsifier type, our fat was emulsified by the proteins naturally present in skim milk. Emulsions stabilized with caseins and monoacylglycerols were recently reported to result in lower postprandial plasma TAG than those formulated with lecithin in non-obese humans (31), which can be explained by lower *in vitro* digestive lipolysis (32). Because obese men were the most affected by emulsification, further work should test the effect of emulsifiers on postprandial lipid metabolism. The importance of sensory exposure to lipids on postprandial metabolism in humans was also recently revealed (5). Therefore, we cannot exclude a contribution of oral fat perception in our results.

TAG-rich lipoproteins remaining elevated all along the postprandial phase are an independent CVD risk factor (3, 4), which can be mechanistically related to the atherogenic potential of small chylomicron remnants (21, 22). In this study, we collected CMRF fractions containing chylomicrons and their large remnants. The latter do not contribute significantly to the formation of small atherogenic remnants because of direct hepatic clearance (33). However, the role of TAG-rich particles is still debated, underlining that this is still an open field needing further studies (1, 2). In this context, our study shows for the first time in NW and obese men that postprandial lipemia profile can be modulated by structuring the fat in a mixed meal. Therefore, the study of atherogenic small particles in the postprandial phase after consumption of differently structured lipids in the meal should now be performed.

Obese men presented a delayed increase of CMRF-TAG after spread fat. Overweight men were also reported to present delayed TAG-rich lipoprotein metabolism after a high-fat load (34). Our observed differences between the two breakfasts in obese men could be explained

by their lower pancreatic secretion and lower levels of gallbladder emptying compared with lean men (35). Therefore, obese subjects can better hydrolyze fat when it is pre-emulsified. Moreover, fine stable emulsions were reported to be emptied faster and to cause greater release of cholecystokinin than those that broke and layer in the stomach (36, 37). We can thus suggest that the fat absorption delay observed with spread fat can be due to layering in the stomach and thus delayed emptying. Of note, immediately after lunch, a peak of ^{13}C-FA appeared in plasma. This so-called "second-meal effect" is known as the contribution of lipids from a meal to lipemia after the next meal (38). For obese men, the marked delay in lipemia appearance after spread fat cumulated with the second meal effect, causing high lipemia until the test ended. In contrast, obese subjects (without fasting hyperlipidemia herein) did not have difficulties in absorbing the emulsion, with a final return of lipemia to baseline.

In NW subjects, higher CMRF-TAG after the emulsion corresponded transiently to an increased number of particles, as shown by the similar CMRF-size with an increased ApoB48 level at 120 min. In obese subjects however, ApoB48 levels remained similar, i.e., the increase in lipemia after emulsion was due to an increased CMRF size. High particle numbers estimated by ApoB48 level are reported to lead to increased chylomicron remnant numbers, hence potentially increased atherosclerotic risk (10, 21). It would now be useful to explore the chronic metabolic impact of fat structure, especially regarding ApoB48-containting particles.

The few reports about metabolic effects of emulsions have solely studied lipemia or plasma FA concentrations as endpoints. For the first time to our knowledge, our study shows that fat emulsification further affects the metabolic handling of exogenous FA, including β-oxidation. Early appearance of $^{13}CO_2$ was due to the rapid β-oxidation of short-chain FA that are directly absorbed in the portal vein and oxidized by the liver (39). Obesity is associated with a defect in the β-oxidation of dietary FA (18, 40-42). Hodson *et al.* recently challenged this idea by showing greater FA β-oxidation in obese men and attributed this to specific FA acid partitioning (43). We highlight that exogenous FA oxidation can be enhanced in obese

men by emulsifying fat. Discrepancies between reports can thus be explained by the present "fast *vs* slow lipid" notion. Indeed, lower β-oxidation in obese *vs* lean subjects was observed using a single oil bolus (18) whereas higher β-oxidation in obese was observed when the tracer was dispersed into an emulsion (43). This aspect had not been taken into account by previous authors. Moreover, emulsification is now advised to enhance the intestinal absorption of essential fatty acids (27). However, our results highlight the risk that such essential FA quickly absorbed can be lost in the β-oxidation process rather than being bioavailable for cell membrane turnover. Therefore, further studies on the structuring of oils rich in essential PUFA should now investigate their final postprandial metabolic fate.

The effect of emulsification on exogenous lipid oxidation cannot be due to differences in intestinal absorption because of similar fecal excretion. Total lipid oxidation during the test day was unchanged by fat structuring, as well as total energy expenditure and diet-induced thermogenesis. However, the source of β-oxidized FA was different: using emulsion, exogenous FA ingested at breakfast were shunted towards β-oxidation pathways. Using spread fat, more endogenous FA and/or exogenous FA ingested at lunch were oxidized so that exogenous FA ingested at breakfast were more oriented towards storage. Therefore, regardless of energy balance, FA metabolism is changed by lipid structure. This is consistent with the greater FA spillover after emulsion. It can be explained by the faster intestinal absorption, resulting in enhanced lipolysis of chylomicrons that generates exogenous NEFA (44). Their early influx can serve as fuel for tissues and explain their higher contribution to total FA oxidation with emulsion. However, high NEFA can also constitute a risk for ectopic fat accumulation (44). Another aspect in obesity research concerns energy balance and satiety regulation (45, 46). Just before lunch, our obese subjects felt hungrier after emulsion than spread fat (visual analog scales, Supplemental figure 5). Further trials could test the impact of fat structuring at breakfast on satiety regulation at lunch and energy balance.

In summary, we demonstrate that the postprandial metabolic handling of dietary FA can be significantly modified by emulsifying the fat in the meal, especially in obese subjects. The clinical perspectives of this first study should thus not be underrated. This study supports the further exploration of a possible dietary concept of "fast *vs* slow lipid" for the nutritional management of metabolic diseases through food formulation. Our results in the postprandial phase raise the questions of whether (i) daily ingestion of "fast *vs* slow fat" would result in different lipid metabolisms, adiposity and/or cardiovascular risk markers in the long term and (ii) the composition and structuring of dietary lipids could be optimized to this aim.

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481 data; SLP: designed research, administrative support; HV: critical revision of the manuscript
482 for important intellectual content; ML: critical revision of the manuscript for important
483 intellectual content; MCM: designed research, study supervision, analyzed data, wrote paper,
484 had primary responsibility for final content. All authors read and approved the final
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REFERENCES

1. Lopez-Miranda J, Williams C, Lairon D. Dietary, physiological, genetic and pathological influences on postprandial lipid metabolism. Br J Nutr 2007;98(3):458-73. doi: 10.1017/S000711450774268X.
2. Nordestgaard BG, Benn M, Schnohr P, Tybjaerg-Hansen A. Nonfasting triglycerides and risk of myocardial infarction, ischemic heart disease, and death in men and women. JAMA 2007;298(3):299-308. doi: 10.1001/jama.298.3.299.
3. Chapman MJ, Ginsberg HN, Amarenco P, Andreotti F, Boren J, Catapano AL, Descamps OS, Fisher E, Kovanen PT, Kuivenhoven JA, et al. Triglyceride-rich lipoproteins and high-density lipoprotein cholesterol in patients at high risk of cardiovascular disease: evidence and guidance for management. Eur Heart J 2011;32(11):1345-61. doi: 10.1093/eurheartj/ehr112.
4. Maggi FM, Raselli S, Grigore L, Redaelli L, Fantappie S, Catapano AL. Lipoprotein remnants and endothelial dysfunction in the postprandial phase. J Clin Endocrinol Metab 2004;89(6):2946-50. doi: 10.1210/jc.2003-031977.
5. Chavez-Jauregui RN, Mattes RD, Parks EJ. Dynamics of fat absorption and effect of sham feeding on postprandial lipema. Gastroenterology 2010;139(5):1538-48. doi: 10.1053/j.gastro.2010.05.002.
6. Sakr SW, Attia N, Haourigui M, Paul JL, Soni T, Vacher D, Girard-Globa A. Fatty acid composition of an oral load affects chylomicron size in human subjects. Br J Nutr 1997;77(1):19-31.
7. Lai HC, Ney DM. Gastric digestion modifies absorption of butterfat into lymph chylomicrons in rats. J Nutr 1998;128(12):2403-10.
8. Renner F, Samuelson A, Rogers M, Glickman RM. Effect of saturated and unsaturated lipid on the composition of mesenteric triglyceride-rich lipoproteins in the rat. J Lipid Res 1986;27(1):72-81.
9. Feldman EB, Russell BS, Hawkins CB, Forte T. Intestinal lymph lipoproteins in rats fed diets enriched in specific fatty acids. J Nutr 1983;113(11):2323-34.
10. Xiao C, Lewis GF. Regulation of chylomicron production in humans. Biochim Biophys Acta 2012;1821(5):736-46. doi: 10.1016/j.bbalip.2011.09.019.
11. Willett W, Manson J, Liu S. Glycemic index, glycemic load, and risk of type 2 diabetes. Am J Clin Nutr 2002;76(1):274S-80S.
12. Hur SJ, Decker A, McClements DJ. Influence of initial emulsifier type on microstructural changes occurring in emulsified lipids during in vitro digestion. Food Chemistry 2009;114:253-62.
13. Michalski MC, Soares AF, Lopez C, Leconte N, Briard V, Geloen A. The supramolecular structure of milk fat influences plasma triacylglycerols and fatty acid profile in the rat. Eur J Nutr 2006;45(4):215-24. doi: 10.1007/s00394-006-0588-9.
14. Michalski MC, Briard V, Desage M, Geloen A. The dispersion state of milk fat influences triglyceride metabolism in the rat--a 13CO2 breath test study. Eur J Nutr 2005;44(7):436-44. doi: 10.1007/s00394-005-0551-1.
15. Laugerette F, Vors C, Geloen A, Chauvin MA, Soulage C, Lambert-Porcheron S, Peretti N, Alligier M, Burcelin R, Laville M, et al. Emulsified lipids increase endotoxemia: possible role in early postprandial low-grade inflammation. J Nutr Biochem 2011.

16. McQuaid SE, Hodson L, Neville MJ, Dennis AL, Cheeseman J, Humphreys SM, Ruge T, Gilbert M, Fielding BA, Frayn KN, et al. Downregulation of adipose tissue fatty acid trafficking in obesity: a driver for ectopic fat deposition? Diabetes 2011;60(1):47-55. doi: 10.2337/db10-0867.
17. Armand M, Pasquier B, Andre M, Borel P, Senft M, Peyrot J, Salducci J, Portugal H, Jaussan V, Lairon D. Digestion and absorption of 2 fat emulsions with different droplet sizes in the human digestive tract. Am J Clin Nutr 1999;70(6):1096-106.
18. Binnert C, Pachiaudi C, Beylot M, Hans D, Vandermander J, Chantre P, Riou JP, Laville M. Influence of human obesity on the metabolic fate of dietary long- and medium-chain triacylglycerols. Am J Clin Nutr 1998;67(4):595-601.
19. Ferrannini E. The theoretical bases of indirect calorimetry: a review. Metabolism 1988;37(3):287-301.
20. Hussain MM, Kedees MH, Singh K, Athar H, Jamali NZ. Signposts in the assembly of chylomicrons. Front Biosci 2001;6:D320-31.
21. Pang J, Chan DC, Barrett PH, Watts GF. Postprandial dyslipidaemia and diabetes: mechanistic and therapeutic aspects. Curr Opin Lipidol 2012;23(4):303-9. doi: 10.1097/MOL.0b013e328354c790.
22. Su JW, Nzekwu MM, Cabezas MC, Redgrave T, Proctor SD. Methods to assess impaired post-prandial metabolism and the impact for early detection of cardiovascular disease risk. Eur J Clin Invest 2009;39(9):741-54. doi: 10.1111/j.1365-2362.2009.02179.x.
23. Dubois C, Beaumier G, Juhel C, Armand M, Portugal H, Pauli AM, Borel P, Latge C, Lairon D. Effects of graded amounts (0-50 g) of dietary fat on postprandial lipemia and lipoproteins in normolipidemic adults. Am J Clin Nutr 1998;67(1):31-8.
24. Gabert L, Vors C, Louche-Pelissier C, Sauvinet V, Lambert-Porcheron S, Drai J, Laville M, Desage M, Michalski MC. 13C tracer recovery in human stools after digestion of a fat-rich meal labelled with [1,1,1-13C3]tripalmitin and [1,1,1-13C3]triolein. Rapid Commun Mass Spectrom 2011;25(19):2697-703. doi: 10.1002/rcm.5067.
25. Folch J, Lees M, Sloane Stanley GH. A simple method for the isolation and purification of total lipides from animal tissues. J Biol Chem 1957;226(1):497-509.
26. Antoun E, Momken I, Bergouignan A, Villars C, Platat C, Schoeller DA, Blanc S, Simon C. The [1-13C]acetate recovery factor to correct tracer-derived dietary fat oxidation is lower in overweight insulin-resistant subjects. The European e-Journal of Clinical Nutrition and Metabolism 2010;5(4):173-9.
27. Garaiova I, Guschina IA, Plummer SF, Tang J, Wang D, Plummer NT. A randomised cross-over trial in healthy adults indicating improved absorption of omega-3 fatty acids by pre-emulsification. Nutr J 2007;6:4. doi: 10.1186/1475-2891-6-4.
28. Couedelo L, Boue-Vaysse C, Fonseca L, Montesinos E, Djoukitch S, Combe N, Cansell M. Lymphatic absorption of alpha-linolenic acid in rats fed flaxseed oil-based emulsion. Br J Nutr 2011;105(7):1026-35. doi: 10.1017/S000711451000454X.
29. Delorme V, Dhouib R, Canaan S, Fotiadu F, Carriere F, Cavalier JF. Effects of surfactants on lipase structure, activity, and inhibition. Pharm Res 2011;28(8):1831-42. doi: 10.1007/s11095-010-0362-9.
30. Martins PN, Neuhaus P. Surgical anatomy of the liver, hepatic vasculature and bile ducts in the rat. Liver Int 2007;27(3):384-92. doi: 10.1111/j.1478-3231.2006.01414.x.

31. Keogh JB, Wooster TJ, Golding M, Day L, Otto B, Clifton PM. Slowly and rapidly digested fat emulsions are equally satiating but their triglycerides are differentially absorbed and metabolized in humans. J Nutr 2011;141(5):809-15. doi: 10.3945/jn.110.131110.

32. Vors C, Capolino P, Guerin C, Meugnier E, Pesenti S, Chauvin MA, Monteil J, Peretti N, Cansell M, Carriere F, et al. Coupling in vitro gastrointestinal lipolysis and Caco-2 cell cultures for testing the absorption of different food emulsions. Food Funct 2012;3(5):537-46. doi: 10.1039/c2fo10248j.

33. Karpe F, Olivecrona T, Hamsten A, Hultin M. Chylomicron/chylomicron remnant turnover in humans: evidence for margination of chylomicrons and poor conversion of larger to smaller chylomicron remnants. J Lipid Res 1997;38(5):949-61.

34. Nabeno-Kaeriyama Y, Fukuchi Y, Hayashi S, Kimura T, Tanaka A, Naito M. Delayed postprandial metabolism of triglyceride-rich lipoproteins in obese young men compared to lean young men. Clin Chim Acta 2010;411(21-22):1694-9. doi: 10.1016/j.cca.2010.07.004.

35. Wisen O, Johansson C. Gastrointestinal function in obesity: motility, secretion, and absorption following a liquid test meal. Metabolism 1992;41(4):390-5.

36. Marciani L, Wickham M, Singh G, Bush D, Pick B, Cox E, Fillery-Travis A, Faulks R, Marsden C, Gowland PA, et al. Enhancement of intragastric acid stability of a fat emulsion meal delays gastric emptying and increases cholecystokinin release and gallbladder contraction. Am J Physiol Gastrointest Liver Physiol 2007;292(6):G1607-13. doi: 10.1152/ajpgi.00452.2006.

37. Marciani L, Faulks R, Wickham MS, Bush D, Pick B, Wright J, Cox EF, Fillery-Travis A, Gowland PA, Spiller RC. Effect of intragastric acid stability of fat emulsions on gastric emptying, plasma lipid profile and postprandial satiety. Br J Nutr 2009;101(6):919-28. doi: 10.1017/S0007114508039986.

38. Silva KD, Wright JW, Williams CM, Lovegrove JA. Meal ingestion provokes entry of lipoproteins containing fat from the previous meal: possible metabolic implications. Eur J Nutr 2005;44(6):377-83. doi: 10.1007/s00394-004-0538-3.

39. Michalski MC. Specific molecular and colloidal structures of milk fat affecting lipolysis, absorption and postprandial lipemia. European Journal of Lipid Science and Technology 2009;111(5):413-31. doi: DOI 10.1002/ejlt.200800254.

40. Schutz Y, Flatt JP, Jequier E. Failure of dietary fat intake to promote fat oxidation: a factor favoring the development of obesity. Am J Clin Nutr 1989;50(2):307-14.

41. Giacco R, Clemente G, Busiello L, Lasorella G, Rivieccio AM, Rivellese AA, Riccardi G. Insulin sensitivity is increased and fat oxidation after a high-fat meal is reduced in normal-weight healthy men with strong familial predisposition to overweight. Int J Obes Relat Metab Disord 2004;28(2):342-8. doi: 10.1038/sj.ijo.0802589.

42. Bessesen DH, Rupp CL, Eckel RH. Dietary fat is shunted away from oxidation, toward storage in obese Zucker rats. Obes Res 1995;3(2):179-89.

43. Hodson L, McQuaid SE, Humphreys SM, Milne R, Fielding BA, Frayn KN, Karpe F. Greater dietary fat oxidation in obese compared with lean men: an adaptive mechanism to prevent liver fat accumulation? Am J Physiol Endocrinol Metab 2010;299(4):E584-92. doi: 10.1152/ajpendo.00272.2010.

44. Fielding B. Tracing the fate of dietary fatty acids: metabolic studies of postprandial lipaemia in human subjects. Proc Nutr Soc 2011;70(3):342-50. doi: 10.1017/S002966511100084X.

45. Halford JC, Harrold JA. Satiety-enhancing products for appetite control: science and regulation of functional foods for weight management. Proc Nutr Soc 2012;71(2):350-62. doi: 10.1017/S0029665112000134.

46. Simpson K, Parker J, Plumer J, Bloom S. CCK, PYY and PP: the control of energy balance. Handb Exp Pharmacol 2012(209):209-30. doi: 10.1007/978-3-642-24716-3_9.

Table 1. Anthropometric and fasting metabolic subject parameters.

	Normal weight (*n*=9)	**Obese (*n*=9)**	***P* value**
Anthropometric parameters			
Age (years)	28.3 ± 1.4	30.2 ± 2.2	*ns*
Body weight (kg)	72.0 ± 2.1	101.2 ± 1.9	< 0.0001
BMI ($kg.m^{-2}$)	22.3 ± 0.5	31.7 ± 0.3	< 0.0001
Waist circumference (cm)	83.3 ± 1.6	105.9 ± 0.8	< 0.0001
Fasting metabolic parameters			
Glucose (mM)	4.94 ± 0.16	5.19 ± 0.15	*ns*
Insulin (mIU/L)	3.75 ± 0.59	7.14 ± 0.95	0.008
HOMA	0.85 ± 0.14	1.69 ± 0.25	0.009
Total cholesterol (mM)	4.85 ± 0.22	4.89 ± 0.24	*ns*
HDL cholesterol (mM)	1.51 ± 0.10	1.09 ± 0.06	0.004
LDL cholesterol (mM)	3.03 ± 0.27	3.11 ± 0.21	*ns*
Triacylglycerols (mM)	0.85 ± 0.06	1.39 ± 0.18	0.017

Data are means ± SEM. Groups are compared using unpaired Student's *t*-test.

Table 2. Nutritional composition of the test breakfasts containing either spread or emulsified fat enriched with ^{13}C-labelled triglycerides.

	Breakfast composition[1]			
	Quantity (g or mL)	**Carbohydrates (g)**	**Proteins (g)**	**Lipids (g)**
Anhydrous Milk Fat[2]	40	-	-	40
Skimmed milk[3]	160	7.5	5.3	0.3
Bread	50	28	4	0.5
[1,1,1-$^{13}C_3$] trioctanoin	0.09	-	-	0.09
[1,1,1-$^{13}C_3$] tripalmitin	0.30	-	-	0.30
[1,1,1-$^{13}C_3$] triolein	0.21	-	-	0.21
Total (g)	250.6	35.5	9.3	41.4
% caloric intake		26	7	67

[1] Identical nutrient composition for both spread and emulsion breakfasts.

[2] Fatty acid profile of TAG includes 68.6% SFA, 28.1% MUFA and 3.3% PUFA.

[3] Natural vanillia-flavoured.

Table 3. Physico-chemical properties of fat used in the formulation of test breakfasts.

	Fat properties[1]			
Structure (type of breakfast)	**Droplet size[2] (µm)**	**d_{32}[3] (µm)**	**Fat surface area in meal[4] (m^2)**	**Melting temperature[5] (°C)**
Spread	-	-	0.006	42
Emulsion	1.04	0.63	410	40

[1] Mixture of milk fat + ^{13}C-TAG tracers.

[2] Diameter of the peak of maximum intensity measured by DLS.

[3] Surface averaged diameter measured by LLS.

[4] For Spread fat: calculated as the surface of an equivalent sphere of 40 g. For Emulsion: calculated from the specific surface area (m^2 per g fat) calculated by the software, further multiplied by fat content in the meal.

[5] Temperature at which the entire fat amount is in liquid form.

Table 4. Kinetic parameters after digestion of test breakfasts in NW *vs* obese subjects.

Parameter	Spread fat 40 g		Emulsified fat 40 g		*P* value			
Δ CMRF TAG	Normal-weight	Obese	Normal-weight	Obese	P_{meal} [1]	P_{BMI} [1]	$P_{mealx\ BMI}$ [1]	$P_{mealx\ BMIxtime}$ [2]
0-480 min:								
Δ_{max} (mmol/L)	0.63 ± 0.13	0.80 ± 0.12	0.75 ± 0.09	0.94 ± 0.25	*ns*	*ns*	*ns*	
iAUC (mmol·min/L)	132.8 ± 29.1	165.6 ± 23.1	180.4 ± 28.2	218.1 ± 53.2	*ns*	*ns*	*ns*	
t_{max} (min)	293 ± 23	367 ± 33	220 ± 42	207 ± 25	< 0.001	*ns*	*ns*	
Appearance-rate$_{0\text{-}60\ min}$ (µmol/L/min)	0.32 ± 0.27	0.14 ± 0.32	2.85 ± 0.72	2.93 ± 0.6	<0.0001	*ns*	*ns*	
0-300 min:								
Δ_{max} (mmol/L)	0.56 ± 0.10	0.53 ± 0.09	0.74 ± 0.09	0.94 ± 0.25	0.05	*ns*	*ns*	
iAUC (mmol·min/L)	81.4 ± 18.2	57.6 ± 10.7	127.7 ± 17.4	159.3 ± 39.6	*ns*	< 0.01	*ns*	
t_{max} (min)	233 ± 16	267 ± 15	167 ± 17	193 ± 17	<0.0001	< 0.1	*ns*	
300-480 min:								
Δ_{max} (mmol/L)	0.54 ± 0.13	0.80 ± 0.12	0.54 ± 0.11	0.56 ± 0.14	*ns*	*ns*	*ns*	< 0.05
iAUC (mmol·min/L)	51.4 ± 12.9	107.9 ± 17.7	52.7 ± 11.2	58.8 ± 14.7	< 0.05	*ns*	*ns*	< 0.01
t_{max} (min)	347 ± 5	390 ± 23	397 ± 19	420 ± 24	< 0.05	< 0.1	*ns*	< 0.1
CMRF Size								
0-480 min:								
d_{max} (nm)	253 ± 34	494 ± 93	262 ± 20	344 ± 58	*ns*	< 0.01	*ns*	
t_{max} (min)	243 ± 25	307 ± 23	207 ± 30	237 ± 33	< 0.1	0.1	*ns*	
Enlargement-rate$_{0\text{-}60min}$ (µmol/L/min)	-0.06 ± 0.07	-0.02 ± 0.11	0.65 ± 0.11	1.26 ± 0.78	< 0.05	*ns*	*ns*	
0-300 min:								
d_{max} (nm)	246 ± 35	296 ± 70	239 ± 12	336 ± 58	*ns*	< 0.1	*ns*	
t_{max} (min)	180 ± 17	200 ± 41	200 ± 26	193 ± 28	*ns*	*ns*	*ns*	
300-480 min:								
d_{max} (nm)	195 ± 10	451 ± 96	207 ± 28	236 ± 46	< 0.1	< 0.05	< 0.05	0.055
t_{max} (min)	337 ± 4	367 ± 17	340 ± 5	360 ± 16	*ns*	< 0.05	*ns*	*ns*
$^{13}CO_2$ enrichment								
0-720 min:								
C_{max} (%)	0.019 ± 0.001	0.013 ± 0.001	0.019 ± 0.001	0.016 ± 0.001	< 0.01	*ns*	*ns*	
AUC (%.min)	6.9 ± 0.5	4.8 ± 0.8	7.7 ± 0.2	6.4 ± 0.5	< 0.01	< 0.001	*ns*	
t_{max} (min)	310 ± 21	347 ± 23	267 ± 26	267 ± 17	< 0.01	*ns*	*ns*	
Appearance-rate$_{0\text{-}60\ min}$ (%/min)	$6.0\ .10^{-5}$ $\pm 1.7\ .10^{-5}$	$2.0\ .10^{-5}$ $\pm\ 0.5\ .10^{-5}$	$13.6\ .10^{-5}$ $\pm\ 1.9\ .10^{-5}$	$9.8\ .10^{-5}$ $\pm\ 0.8\ .10^{-5}$	< 0.0001	< 0.01	*ns*	
0-300 min:								
C_{max} (mmol/L)	0.018 ± 0.001	0.011 ± 0.001	0.019 ± 0.001	0.016 ± 0.001	< 0.05	< 0.0001	< 0.05	
AUC (%.min)	2.9 ± 0.3	1.6 ± 0.4	3.9 ± 0.2	3.2 ± 0.2	< 0.0001	< 0.0001	*ns*	
t_{max} (min)	283 ± 17	293 ± 7	257 ± 23	260 ± 14	< 0.1	*ns*	*ns*	
300-720 min:								
C_{max} (mmol/L)	0.019 ± 0.001	0.013 ± 0.001	0.017 ± 0.001	0.016 ± 0.001	*ns*	0.0001	< 0.05	*ns*
AUC (%.min)	3.9 ± 0.4	3.2 ± 0.5	3.8 ± 0.3	3.1 ± 0.4	*ns*	< 0.1	*ns*	*ns*
t_{max} (min)	333 ± 3	360 ± 18	330 ± 0	330 ± 0	< 0.1	*ns*	*ns*	*ns*

Data are means ± SEM, *n*=9 per group. Parameters calculated over the indicated time period: C_{max} indicates maximum concentration; d_{max} indicates maximum diameter; Δ_{max} indicates maximum concentration delta; iAUC, incremental area under the curve; AUC, area under the curve. *P* values (P_{meal}, P_{BMI} and $P_{mealxBMI}$) obtained by ANOVA followed by post hoc Fisher PLSD.

[1] *P* values of two-way ANOVA for meal and BMI effects and their interactions.

[2] *P* values of two-way ANOVA for repeated measures regarding both time periods (before and after 300 min), for meal x BMI x time period interactions.

Figure Caption

Figure 1. Postprandial profile after consuming spread fat (□, ■) or emulsion (○, ●): CMRF-TAG (mM) in NW (A) and obese subjects (B) and corresponding iAUC; CMRF size (nm) in NW (C) and obese subjects (D); ApoB48 (mg/L) in NW (E) and obese subjects (F). Data are means ±SEM, *n*=9 per group; P_{time}, P_{meal} and $P_{timexmeal}$ for postprandial period from 0 to 300 min (repeated measures-ANOVA followed by post-hoc Fisher PLSD).

(B) ** *P*< 0.01 for time 420 min emulsion *vs* spread fat (paired Student's *t*-test); **P*< 0.05 for emulsion *vs* spread fat at time 480 min and for $iAUC_{0-300}$ (paired Student's *t*-test); § P< 0.05 for obese *vs* NW regarding spread fat $iAUC_{300-480}$ (unpaired Student's *t*-test).

(D) **P*< 0.05 for time 120 min emulsion *vs* spread fat (paired Student's *t*-test)

(C-D) §§ *P*< 0.01 for time 120 min obese *vs* NW subjects (unpaired Student's *t*-test);

(E-F) * *P*< 0.05 for time 120 min emulsion *vs* spread fat (paired Student's *t*-test), § *P*< 0.05 for time 120 min obese *vs* NW subjects (unpaired Student's *t*-test).

Figure 2. Postprandial concentration profile and iAUC of total plasma lipids of ^{13}C-palmitic acid (mM) and ^{13}C-oleic acid (mM) in NW (A & C, respectively) and obese subjects (B & D, respectively) consuming spread fat (□, ■) or emulsion (○, ●). (E) Fecal excretion of ^{13}C-palmitic acid and ^{13}C-oleic acid in NW (white bars) and obese subjects (black bars) consuming spread fat (plain bars) or emulsion (droplet pattern bars).

Data are means ±SEM, *n*=9 per group.

(A-B-C-D) P_{time}, P_{meal} and $P_{timexmeal}$ for postprandial period from 0 to 300 min (repeated measures-ANOVA followed by post-hoc Fisher PLSD).

(A-B) *P< 0.05 for obese iAUC 0-300 min emulsion *vs* spread fat (paired Student's *t*-test), § P< 0.05 for spread fat iAUC 0-300 min obese *vs* NW subjects (unpaired Student's *t*-test), $^{\$}P$< 0.1 for obese iAUC 0-480 min emulsion *vs* spread fat (paired Student's *t*-test); (C-D) *P< 0.05 for obese iAUC 0-300 min emulsion *vs* spread fat (paired Student's *t*-test), § P< 0.05 for spread fat iAUC 0-300 min obese *vs* NW subjects (unpaired Student's *t*-test); (E) no common letter with another bar indicates a statistical difference, P< 0.001 (ANOVA followed by post-hoc Bonferroni).

Figure 3. Postprandial concentration profile of insulin (mUI/L) and NEFA (µM) in NW (A & C, respectively) and obese subjects (B & D, respectively) consuming spread fat (□, ■) or emulsion (○, ●). (E) ^{13}C-enrichment of plasma NEFA at 120 min, estimating so-called apparent fatty acid "spillover" in NW (white bars) and obese subjects (black bars) consuming spread fat (plain bars) or emulsion (droplet pattern bars).

Data are means ±SEM, n=9 per group; P_{time}, P_{meal} and $P_{timexmeal}$ for postprandial period from 0 to 300 min (repeated measures-ANOVA followed by post-hoc Fisher PLSD).

(C) **P< 0.01 for NW subjects at 120 min emulsion *vs* spread fat (paired Student's *t*-test);

(D) *P< 0.05 for obese subjects at 120 min emulsion *vs* spread fat (paired Student's *t*-test);

(E) *P< 0.05 for NW subjects at 120 min emulsion *vs* spread fat (paired Student's *t*-test); **P< 0.01 for obese subjects at 120 min emulsion *vs* spread fat (paired Student's *t*-test); ANOVA analysis revealed meal effect (P<0.01) and BMI effect (P<0.01) but no significant meal x BMI interaction.

Figure 4. Postprandial profile of ^{13}C-appearance in breath in NW subjects (A) and obese subjects (B) consuming spread fat (□, ■) or emulsion (○, ●); (C) Exogenous lipid fate, either oxidized (white), lost in feces (black) or stored (grey, calculated as "total – (lost+oxidized)") in NW and obese subjects over 480 min; (D) Oxidation of intestinally absorbed lipids over 480 min in NW (white bars) and obese subjects (black bars) consuming spread fat (plain bars) or emulsion (droplet pattern bars); (E) Total lipid oxidation (total bar) and fraction of cumulative exogenous lipid oxidation (dashed part) over 480 min after consuming test breakfasts in NW *vs* obese subjects.

Data are means ±SEM, n=9 per group.

(C-D-E) * P< 0.05 for NW subjects and ** P< 0.01 for obese subjects emulsion *vs* spread fat (paired Student's *t*-test). ANOVA analysis revealed no significant meal x BMI interaction (P=0.087).