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**Modulating absorption and postprandial handling of dietary fatty acids  
by structuring fat in the meal: a randomized cross-over clinical trial<sup>1-3</sup>**

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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.

**Clinical trial registry:** Clinical Trials, #NCT01249378

## 1 ABSTRACT

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

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

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

12 **Results:** The emulsified fat resulted in earlier (>1h) and sharper chylomicron and  $^{13}\text{C}$ -fatty  
13 acids peaks in plasma compared to spread fat in both groups ( $P<0.0001$ ). After 2h, the  
14 emulsified fat increased ApoB48 concentration ( $9.7 \pm 0.7$  vs  $7.1 \pm 0.9$  mg/L;  $P<0.05$ ) in the  
15 normal-weight subjects compared to the spread fat. For the obese subjects, emulsified fat  
16 resulted in 3-fold larger chylomicrons ( $218 \pm 24$  nm) compared to the spread fat ( $P<0.05$ ). The  
17 emulsified fat induced higher dietary fatty acid spillover in plasma and sharper  $^{13}\text{CO}_2$   
18 appearance, provoking increased exogenous lipid oxidation in each group: from 45% to 52%  
19 in normal-weight subjects ( $P<0.05$ ), 40% to 57% in obese ( $P<0.01$ ).

20 **Conclusions:** This study supports a new concept of “slow vs fast fat” whereby intestinal  
21 absorption can be modulated by structuring of dietary fat to modulate postprandial lipemia  
22 and lipid  $\beta$ -oxidation in humans of different BMI.

23  
24 **Key words:** intestinal absorption; chylomicron; emulsion; stable isotopes; obesity.

25

## 26 INTRODUCTION

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

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

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

60

## 61 **SUBJECTS AND METHODS**

### 62 **Study design**

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

77 the evening prior to testing. Compliance was checked through diet records, 5 days before and  
78 3 days after each test day.

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

88

## 89 **Subjects**

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

100

## 101 **Test meals**

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

115 A second meal was served 5 hours after breakfast, containing pasta (200 g), turkey (100  
116 g), butter (10 g), olive oil (10 g), bread (50 g), stewed fruit (100 g) which provided 713 kcal  
117 (2985 kJ) with 29%, 51% and 20% of energy as lipids (22.7 g), carbohydrates (91.5 g) and  
118 proteins (35.7 g) respectively. All subjects were given 10 minutes to eat breakfast and 30  
119 minutes for lunch. During the test, participants were allowed to drink 200 mL of water.

120

## 121 **Test fat characterization**

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



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

128

### 129 **Hunger assessment**

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

133

### 134 **Metabolic explorations**

135 Blood samples were obtained at baseline and at regular intervals after the meal, from an  
136 antecubital arm vein through a catheter and collected in vacutainer sterile tubes (with EDTA  
137 when necessary). Plasma was separated by centrifugation (1500 g, 10 min, 4°C) and stored at  
138 -20°C until analysis or at 4°C for separation of the chylomicron-rich fraction (CMRF).

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

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

150

## 151 **Plasma metabolite and hormone measurements**

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

155

## 156 **Isolation and analysis of chylomicron-rich fractions**

157 To collect the CMRF, containing chylomicrons and their large remnants, 250  $\mu$ L of  
158 plasma was deposited below a layer of 850  $\mu$ L of distilled/deionized water and centrifuged at  
159 80 000 rpm for 30 min using a Sorvall Kendro ultracentrifuge (Asheville, NC, USA). The  
160 floating layer was collected and stored at  $-80^{\circ}\text{C}$ . TAG and cholesterol concentrations of  
161 CMRF were measured with a lipase glycerokinase and a cholesterol esterase/oxidase method,  
162 respectively, on a AU 2700 Beckman Coulter® (O' Cllagan's mils, Ireland) and expressed as  
163 differences in concentration over baseline. Hydrodynamic diameter of CMRF was measured  
164 by dynamic light scattering at  $25^{\circ}\text{C}$  using a ZetaSizer NanoS (Malvern, UK) using 1.0658 cP  
165 and 1.33 as viscosity and refractive index of the aqueous phase, respectively. Gravity-  
166 separated fraction containing chylomicrons and their large remnants are reported to size in the  
167 range 70-450 nm (20-22), and postprandial increase in cholesterol content of such fraction is  
168 about  $<0.2$  mM (23). Our fraction collected by ultracentrifugation contained particles of mean  
169 diameter up to 200-300 nm and with postprandial increase in cholesterol of  $<0.15$  mM in NW  
170 and  $<0.25$  mM in obese subjects. Our CMRF is thus typical of fractions that are rich in  
171 chylomicrons and that also contain large remnants.

172

## 173 **$^{13}\text{C}$ -fatty acids in plasma lipids, NEFA, CMRF and stools**

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

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

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

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

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

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

197

#### 198 **Calculations associated with apparent dietary fatty acid “spillover”**

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

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

208

### 209 **Calculations of exogenous lipid oxidation from indirect calorimetry & breath tests**

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

213 Exogenous lipid oxidation (% of ingested fat) =

$$214 \frac{\{[\{[AP \text{ CO}_2(t) + AP \text{ CO}_2(t_{-30})] / 2\} - AP \text{ CO}_2(t_0)] / 100\} \times \dot{V} \text{ CO}_2}{\{(A) + (B) + (C)\} \times 22.4 \times dARF} \times 100$$

215

$$216 \text{ With: } (A) = \left\{ \left[ AP \text{}^{13}\text{TG C8:0} \right] / 100 \right\} \times \left[ (0.09 / 473.66) \times 27 \right]$$

$$217 (B) = \left\{ \left[ AP \text{}^{13}\text{TG C16:0} \right] / 100 \right\} \times \left[ (0.30 / 810.30) \times 51 \right]$$

$$218 (C) = \left\{ \left[ AP \text{}^{13}\text{TG C18:1} \right] / 100 \right\} \times \left[ (0.21 / 888.40) \times 57 \right]$$

219 Where AP  $\text{CO}_2(t)$  is the AP value of the expired  $\text{CO}_2$  at time  $t$ , AP  $\text{CO}_2(t_0)$  is the AP value of  
 220 the expired  $\text{CO}_2$  at time  $t_0$ , AP tracers is the calculated AP value of the labeled mixture of  
 221 TAG (tracers) and  $\dot{V} \text{ CO}_2$  is the production rate of expired  $\text{CO}_2$  (indirect calorimetry). Mean  
 222 molecular weights of trioctanoin, tripalmitin and triolein are 473.66 g/mol, 810.30 g/mol and  
 223 888.40 g/mol, respectively. Mean number of carbons in trioctanoin, tripalmitin and triolein  
 224 are 27, 51 and 57, respectively. dARF (Acetate Recovery Factor) is the correction factor for

225 incomplete recovery of  $^{13}\text{C}$  bicarbonate (0.505 for NW; 0.453 for obese (26)) and 22.4 is the  
226 molar volume (L) of  $\text{CO}_2$ .

227

### 228 **Kinetic parameters**

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

232

### 233 **Statistical analysis**

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

246

## 247 **RESULTS**

### 248 **Properties of emulsion vs spread fat**

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

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

256

### 257 **Hunger feeling**

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

262

### 263 **Postprandial concentration profile and size of chylomicron-rich fraction**

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

276 Mean CMRF size sharply increased in both groups from the first hour after emulsion  
277 (Figure 1 C-D;  $P_{\text{meal}} < 0.05$  for enlargement-rate<sub>0-60min</sub>, Table 4). For NW subjects, CMRF  
278 diameters were similar for spread fat and emulsion all along the test. In obese subjects, CMRF  
279 diameters became equal for both meals at 300 min. We can note that in the period from 0 to  
280 240 min, CMRF diameter in obese subjects was higher after emulsion vs spread fat from 0 to  
281 240 min ( $P_{\text{meal}} < 0.05$  and  $P_{\text{time}} < 0.001$ ). Altogether, obese subjects presented larger CMRF  
282 than NW subjects ( $P < 0.01$  for  $d_{\text{max}}$  0-480 min, Table 4) with persistence of large CMRF after  
283 the second meal for spread fat.

284

### 285 **Plasma concentration profile of ApoB48**

286 Figure 1E-F shows that plasma ApoB48 changed over time in both groups after both  
287 breakfasts ( $P_{\text{time}} < 0.0001$ ) and differently according to the type of breakfast for NW subjects  
288 ( $P_{\text{time} \times \text{meal}} = 0.001$ ). At 120 min, NW subjects accumulated more ApoB48 after consumption of  
289 emulsion than spread fat ( $7.08 \pm 0.86$  mg/L for spread fat vs  $9.73 \pm 0.69$  mg/L for emulsion,  $P <$   
290  $0.05$ ) and compared with obese subjects for emulsion ( $7.47 \pm 0.78$  mg/L,  $P < 0.05$ ).

291

### 292 **Plasma concentration profile of <sup>13</sup>C-fatty acid tracers and fecal loss**

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

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

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

305

### 306 **Plasma concentration profile of insulin and NEFA and apparent dietary FA spillover**

307 **Figure 3A-B-C-D** shows a significant change in plasma insulin and NEFA over time after  
308 the two breakfasts in both groups ( $P_{\text{time}} < 0.0001$ ). Over the first 300 min, the NEFA profile  
309 indicated a meal type x time interaction in both groups, with the decrease in plasma NEFA at  
310 120 min being lower for emulsion than spread (Figure 3C-D). Therefore, we measured  $^{13}\text{C}$   
311 enrichment in plasma NEFA at 120 minutes (Figure 3E) to estimate whether this would result  
312 from the contribution of exogenous FA, so-called apparent fatty acid “spillover”. We  
313 observed higher apparent “spillover” during the postprandial phase of emulsion vs spread fat,  
314 in both groups ( $P < 0.05$  for NW and  $P < 0.01$  for obese subjects, Figure 3E). The contribution  
315 of exogenous FA to total NEFA in NW subjects was 42% for spread vs 79% for emulsion,  
316 and was lower in obese, 4% for spread vs 50% for emulsion (meal effect,  $P < 0.01$ ; BMI  
317 effect,  $P < 0.01$ ; no meal x BMI interaction). Moreover, in NW subjects, the proportion of  
318 exogenous FA being in non-esterified form in plasma at 120 min was 10.6 % for spread vs  
319 18.7 % for emulsion, i.e. 1.8-fold increase, and in obese 10.1 % for spread vs 15.0% for  
320 emulsion, i.e. 1.5-fold increase ( $P < 0.05$  for spread vs emulsion; no significant effect of BMI  
321 nor meal x BMI interaction). In the same time emulsification increased total plasma NEFA by  
322 2.2-fold in NW and by 1.6-fold in obese subjects (Figure 3C-D). Altogether, this means that  
323 more than 80 % of the increase of plasma NEFA due to emulsification may be explained by  
324 an increased amount of exogenous fatty acids being released non-esterified in plasma  
325 (spillover).

326



### 327 **Postprandial appearance of label in expired CO<sub>2</sub>**

328 The <sup>13</sup>C appearance in expired CO<sub>2</sub> represents the final product of FA β-oxidation. A  
329 significant change in APE occurred over time in both groups ( $P_{\text{time}} < 0.0001$ ; **Figure 4A-B**). In  
330 both groups, APE was higher after consumption of emulsion *vs* spread fat over the first 300  
331 min, indicating improvement of dietary fat β-oxidation using emulsified form. APE returned  
332 to baseline after 720 min. An effect of BMI was also observed with higher appearance-  
333 rate<sub>0-60min</sub> in NW than obese subjects ( $P < 0.01$ ; Table 4).

334 Inserts in Figure 4A-B show that AUC of expired <sup>13</sup>CO<sub>2</sub> after 300 min for the emulsion  
335 was significantly higher than for spread fat in both groups ( $P < 0.01$  for NW subjects and  $P <$   
336  $0.001$  for obese subjects). Besides, over 0 to 720 min, obese subjects presented higher AUC  
337 of expired <sup>13</sup>CO<sub>2</sub> after consuming emulsion *vs* spread fat ( $P < 0.05$ , Figure 4B insert).  
338 Altogether, the structuring of fat in the meal significantly affected the kinetic parameters of  
339 <sup>13</sup>CO<sub>2</sub> air enrichment regardless of BMI while BMI affected AUC and appearance-rate<sub>0-60min</sub>  
340 (Table 4).

341

### 342 **Exogenous lipid fate**

343 We studied the metabolic handling of exogenous lipids by evaluating the fractions of  
344 ingested lipids that have been either oxidized or lost in feces, and so estimated the remaining  
345 fraction stored in body pools. For the same quantity and composition of ingested fat, all  
346 subjects β-oxidized FA better when fat was emulsified (Figure 4C). In turn, the calculated  
347 fraction of ingested lipids oriented towards storage in body pools was lower after emulsion *vs*  
348 spread consumption. After accounting for the part of exogenous lipids lost in feces (Figure  
349 4C), the percentage of exogenous lipid oxidation according to the fraction that has been  
350 intestinally absorbed was higher for NW and obese subjects after emulsion *vs* spread fat ( $P <$   
351  $0.05$  and  $P < 0.01$ , respectively, Figure 4D). Total lipid oxidation was unchanged with the two

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

354

## 355 **DISCUSSION**

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

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

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

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

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

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

422 The few reports about metabolic effects of emulsions have solely studied lipemia or  
423 plasma FA concentrations as endpoints. For the first time to our knowledge, our study shows  
424 that fat emulsification further affects the metabolic handling of exogenous FA, including  $\beta$ -  
425 oxidation. Early appearance of  $^{13}\text{CO}_2$  was due to the rapid  $\beta$ -oxidation of short-chain FA that  
426 are directly absorbed in the portal vein and oxidized by the liver (39). Obesity is associated  
427 with a defect in the  $\beta$ -oxidation of dietary FA (18, 40-42). Hodson *et al.* recently challenged  
428 this idea by showing greater FA  $\beta$ -oxidation in obese men and attributed this to specific FA  
429 acid partitioning (43). We highlight that exogenous FA oxidation can be enhanced in obese

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

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

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

463

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474

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478 and material support, analyzed data; CLP: conducted research, technical support; CD:  
479 acquisition of data, technical support; DL: critical revision of the manuscript for important  
480 intellectual content; MD: conducted research, analyzed data; SD: technical support, analyzed

481 data; SLP: designed research, administrative support; HV: critical revision of the manuscript  
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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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**Table 1.** Anthropometric and fasting metabolic subject parameters.

	<b>Normal weight</b> <b>(n=9)</b>	<b>Obese</b> <b>(n=9)</b>	<b>P value</b>
<b>Anthropometric parameters</b>			
Age (years)	28.3 ± 1.4	30.2 ± 2.2	<i>ns</i>
Body weight (kg)	72.0 ± 2.1	101.2 ± 1.9	< 0.0001
BMI (kg.m <sup>-2</sup> )	22.3 ± 0.5	31.7 ± 0.3	< 0.0001
Waist circumference (cm)	83.3 ± 1.6	105.9 ± 0.8	< 0.0001
<b>Fasting metabolic parameters</b>			
Glucose (mM)	4.94 ± 0.16	5.19 ± 0.15	<i>ns</i>
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	<i>ns</i>
HDL cholesterol (mM)	1.51 ± 0.10	1.09 ± 0.06	0.004
LDL cholesterol (mM)	3.03 ± 0.27	3.11 ± 0.21	<i>ns</i>
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  $^{13}\text{C}$ -labelled triglycerides.

<b>Breakfast composition<sup>1</sup></b>				
	<b>Quantity (g or mL)</b>	<b>Carbohydrates (g)</b>	<b>Proteins (g)</b>	<b>Lipids (g)</b>
Anhydrous Milk Fat <sup>2</sup>	40	-	-	40
Skimmed milk <sup>3</sup>	160	7.5	5.3	0.3
Bread	50	28	4	0.5
[1,1,1- $^{13}\text{C}_3$ ] trioctanoin	0.09	-	-	0.09
[1,1,1- $^{13}\text{C}_3$ ] tripalmitin	0.30	-	-	0.30
[1,1,1- $^{13}\text{C}_3$ ] triolein	0.21	-	-	0.21
Total (g)	250.6	35.5	9.3	41.4
<b>% caloric intake</b>		26	7	67

<sup>1</sup> Identical nutrient composition for both spread and emulsion breakfasts.

<sup>2</sup> Fatty acid profile of TAG includes 68.6% SFA, 28.1% MUFA and 3.3% PUFA.

<sup>3</sup> Natural vanillia-flavoured.

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

Structure (type of breakfast)	Fat properties <sup>1</sup>			
	Droplet size <sup>2</sup> ( $\mu\text{m}$ )	$d_{32}$ <sup>3</sup> ( $\mu\text{m}$ )	Fat surface area in meal <sup>4</sup> ( $\text{m}^2$ )	Melting temperature <sup>5</sup> ( $^{\circ}\text{C}$ )
Spread	-	-	0.006	42
Emulsion	1.04	0.63	410	40

<sup>1</sup> Mixture of milk fat + <sup>13</sup>C-TAG tracers.

<sup>2</sup> Diameter of the peak of maximum intensity measured by DLS.

<sup>3</sup> Surface averaged diameter measured by LLS.

<sup>4</sup> For Spread fat: calculated as the surface of an equivalent sphere of 40 g. For Emulsion: calculated from the specific surface area ( $\text{m}^2$  per g fat) calculated by the software, further multiplied by fat content in the meal.

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

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;  $\Delta_{max}$  indicates maximum concentration delta; iAUC, incremental area under the curve; AUC, area under the curve.  $P$  values ( $P_{meal}$ ,  $P_{BMI}$  and  $P_{meal \times BMI}$ ) obtained by ANOVA followed by post hoc Fisher PLSD.

<sup>1</sup>  $P$  values of two-way ANOVA for meal and BMI effects and their interactions.

<sup>2</sup>  $P$  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  $\pm$ SEM,  $n=9$  per group;  $P_{\text{time}}$ ,  $P_{\text{meal}}$  and  $P_{\text{time}\times\text{meal}}$  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  $\text{iAUC}_{0-300}$  (paired Student's  $t$ -test); §  $P < 0.05$  for obese vs NW regarding spread fat  $\text{iAUC}_{300-480}$  (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  $^{13}\text{C}$ -palmitic acid (mM) and  $^{13}\text{C}$ -oleic acid (mM) in NW (A & C, respectively) and obese subjects (B & D, respectively) consuming spread fat (□, ■) or emulsion (○, ●). (E) Fecal excretion of  $^{13}\text{C}$ -palmitic acid and  $^{13}\text{C}$ -oleic acid in NW (white bars) and obese subjects (black bars) consuming spread fat (plain bars) or emulsion (droplet pattern bars).

Data are means  $\pm$ SEM,  $n=9$  per group.



(A-B-C-D)  $P_{\text{time}}$ ,  $P_{\text{meal}}$  and  $P_{\text{time} \times \text{meal}}$  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 ( $\mu\text{M}$ ) in NW (A & C, respectively) and obese subjects (B & D, respectively) consuming spread fat ( $\square$ ,  $\blacksquare$ ) or emulsion ( $\circ$ ,  $\bullet$ ). (E)  $^{13}\text{C}$ -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  $\pm$ SEM,  $n=9$  per group;  $P_{\text{time}}$ ,  $P_{\text{meal}}$  and  $P_{\text{time} \times \text{meal}}$  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  $^{13}\text{C}$ -appearance in breath in NW subjects (A) and obese subjects (B) consuming spread fat ( $\square$ ,  $\blacksquare$ ) or emulsion ( $\circ$ ,  $\bullet$ ); (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  $\pm$ 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 ( $P=0.087$ ).