Changes in plasma triacylglycerol concentrations after sequential lunch and dinner in healthy subjects

F Maillot¹, MA Garrigue², M Pinaut³, M Objois¹, V Théret¹, F Lamisse¹, C Hoinard¹, JM Antoine³, D Lairon⁴, C Couet¹

**Summary**

**Objectives:** The present study examines the kinetic of plasma triacylglycerol (TAG) after sequential ingestion of lunch and dinner as well as the contribution of dietary fat ingested to lunch to subsequent post-dinner TAG composition.

**Method:** Six healthy subjects were included. After standardized breakfast (7:30AM), 2 mixed meals with fat loads composed of 44 g olive oil (rich in oleic acid) at lunch (12AM) and 44 g sunflower oil (rich in linoleic acid) at dinner (7PM) were ingested. [1-13C] palmitate was added in lunch only. Plasma TAG and chylomicron-TAG (CM-TAG) levels were measured sequentially after meals. [1-13C] palmitate enrichment and concentrations of oleic acid and linoleic acid were measured in all lipid fractions.

**Result:** Post-dinner plasma TAG peak was delayed as compared to lunch (3 hours vs 1 hour, p = 0.002) whereas the magnitude of the postprandial peaks was not significantly different between lunch and dinner (2.4 ± 0.3 vs 2.0 ± 0.4 mmol/L, p = 0.85). [1-13C] palmitate enrichment was maximal 5 hours after lunch in all lipid fractions and decreased slowly thereafter. After dinner ingestion, the rate of decline of [1-13C] palmitate enrichment plateaued during the first 60 minutes. Oleic acid increased slightly and immediately after dinner and remained the predominant fatty acid in all lipid fractions during the first hour after dinner. A delayed peak of plasma and CM-TAG was observed after dinner as compared to lunch without difference in the magnitude of the peaks.

**Conclusion:** The contribution of dietary fat ingested at lunch to post-dinner lipemia is confirmed despite the relatively long lasting interval between the 2 meals (7 h) and the absence of any early peak of plasma TAG after dinner.

**Key-words:** Postprandial lipemia · Dietary fat · [1-13C] palmitate · Triacylglycerol · Chylomicron.

**Résumé**

**Objectif :** Cette étude examine la cinétique des triglycérides plasmatiques (TAG) après ingestion successive d’un déjeuner et d’un dîner, ainsi que la contribution des graisses ingérées au déjeuner à la lipémie postprandiale du dîner.

**Méthode :** Six sujets sains furent inclus. Après un petit déjeuner standardisé (7 h 30), 2 repas mixtes ont été ingérés, avec des charges en graisses de 44 g d’huile d’olive (riche en oléate) au déjeuner (12 h 00) et 44 g d’huile de tournesol (riche en linoléate) au dîner (19 h 00). Du [1-13C] palmitate était ajouté uniquement au déjeuner. Les concentrations de TAG et triglycérides des chylomicrons (CM-TAG) étaient régulièrement mesurées après les repas. L’enrichissement en [1-13C] palmitate et les concentrations en oléate et linoléate ont été mesurés dans toutes les fractions lipïdiques.

**Résultats :** Le pic de TAG après dîner était retardé par rapport à celui du déjeuner (3 h vs 1 h, p = 0.002) alors que l’amplitude des pics n’était pas différente entre le déjeuner et le dîner (2,4 ± 0,3 vs 2,0 ± 0,4 mmol/L, p = 0,85). L’enrichissement en [1-13C] palmitate était maximal 5 h après le déjeuner dans toutes les fractions lipidiqques et diminuait progressivement. Après le dîner, l’enrichissement en [1-13C] restait en plateau pendant 60 minutes. L’oléate augmentait discrètement et immédiatement après le dîner et restait l’acide gras prédominant dans toutes les fractions lipidiques pendant une heure. Le pic de CM-TAG était retardé après le dîner par rapport au déjeuner, sans différence dans l’amplitude des pics.

**Conclusion :** La contribution des graisses ingérées au déjeuner à la lipémie postprandiale du dîner est confirmée, malgré l’intervalle de temps relativement long entre les 2 repas (7 h) et l’absence de pic précoce de TAG après le dîner.

**Mots-clés :** Lipémie postprandiale · Graisses alimentaires · [1-13C] palmitate · Triglycérides · Chylomicron.
Since the initial work of Zilmer mit in 1979 [1], many studies showed evidence of an association between postprandial lipoprotein metabolism abnormalities and coronary atherosclerosis [2, 3, 4, 5]. Vessels walls are exposed to triglyceride-rich lipoproteins (TRL) during the postprandial state, especially intestine-derived TRL, namely chylomicrons (CM) and chyomicron remnants. Assessment of lipid profile as a cardiovascular risk factor is usually determined on blood samples taken in the fasted state, but most of the day [14-18/24 h] is spent in the postprandial state. Thus, the postprandial state, rather than the fasting state should be recognized as the usual metabolic state [6].

There has been a resurgence of interest in nutritional influences on postprandial lipemia [7, 8]. Numerous studies have focused on the effect of oral carbohydrate or fat load [9, 10, 11, 12] and fatty acid composition of the meal on postprandial lipemia [13, 14, 15] and both monophasic [16] and biphasic [17, 18] patterns of postprandial lipoproteins response were reported. The origin of the plasma triacylglycerol (TAG) peaks in response to a high-fat meal was further investigated. When the test meal was preceded by a pre-test meal, a biphasic pattern of plasma TAG response to fat ingestion was observed [14, 18]. Further investigations by Fielding et al. showed that fat from a breakfast contributed to TRL-triglyceride response to a lunch eaten 5h later [19], in the form of early postprandial plasma TAG peak. The presence of an early TAG peak after lunch was confirmed whatever the oil ingested during breakfast (safflower oil, a mixture of fish and safflower oil or olive oil) [20].

On the other hand, the time lag between lunch and dinner is usually longer than between breakfast and lunch, and the influence of the fat content of lunch on the plasma TAG response seen after dinner is not known. The present study was designed to describe the lipemic response after dinner. For this purpose, the appearance of labeled ingested at lunch to subsequent post-dinner lipemia and to look for a possible contribution of dietary fat was designed to describe the lipemic response after dinner [7h interval]. For this purpose, the appearance of labeled 

Subjects and methods

Protocol

Six healthy male volunteers aged 19-25 y (median 23 y) with body mass indexes (BMI, in kg/m²) of 18.3-23.7 (median 21.8) were studied. Their good health was assessed from a detailed medical history, a thorough physical examination, an electrocardiogram, a routine biochemical screening of blood samples (fasting plasma glucose 4.62 ± 0.13 mmol/L, plasma TAG 1.10 ± 0.16 mmol/L, total cholesterol 4.46 ± 0.25 mmol/L, m ± SEM) and an oral glucose tolerance test (75 g). All subjects were non or occasional light smokers (< 4 cigarettes/day). Usual dietary habits were estimated from a 7-day dietary recall. They were asked to keep their usual diet before the test and to avoid smoking and intensive physical activity for 3 days before the test. All subjects signed an informed consent and were paid for their participation in the study. Human Investigations Ethics Committee of our institution approved the experimental protocol, according to the current French regulations. After a 12 h overnight fast, subjects were admitted in our Metabolic Unit at 7AM and a standardized breakfast was given at 7:30AM (Tab I). Then, subjects continued with their normal activities and returned to the Metabolic Unit at 11AM. They were placed at rest in a bed. An indwelling intravenous catheter cannula was inserted in a forearm vein and blood samples were taken at 30 min, 15 min and 1 min before lunch (baseline level). A lunch meal, containing 35 g protein, 48 g fat including 44 g of olive oil, 127 g carbohydrate and 250 ml water was given at 12AM (Tab I). One and a half g of [1-13C] palmitate (99 mol % excess, EURISOTOP, Saint-Aubin, France) was added to the lunch meal. Blood samples were sequentially taken every hour between 12AM and 7PM. The test meal (dinner) was given at 7PM. This dinner meal had exactly the same composition than lunch meal but contained 44 g

<table>
<thead>
<tr>
<th>Meal and foods</th>
<th>carbohydrate</th>
<th>Fat</th>
<th>Protein</th>
<th>Energy</th>
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<tr>
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<td>0</td>
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<td>100</td>
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<tr>
<td>Olive oil, 44 g</td>
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<td>0</td>
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<tr>
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<td>2</td>
<td>184</td>
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<tr>
<td>Pasta uncooked, 150 g</td>
<td>110</td>
<td>0</td>
<td>18</td>
<td>2140</td>
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<tr>
<td>Tomato sauce, 400 g</td>
<td>12</td>
<td>0</td>
<td>4</td>
<td>268</td>
</tr>
<tr>
<td>Yoghurt 0% fat, 125 g</td>
<td>5</td>
<td>0</td>
<td>5</td>
<td>167</td>
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<tr>
<td>Total</td>
<td>127</td>
<td>48</td>
<td>35</td>
<td>4514</td>
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<tr>
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<tr>
<td>Egg white raw, 60 g</td>
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<td>6</td>
<td>100</td>
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<tr>
<td>Sunflower oil, 44 g</td>
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<td>Total</td>
<td>127</td>
<td>48</td>
<td>35</td>
<td>4514</td>
</tr>
</tbody>
</table>

1Determined from food composition tables [35]. [1-13C] palmitate was mixed with pasta, tomato sauce, oil and egg and ingested at lunch (12AM).
of sunflower oil instead of olive oil (Tab I). No \[^{1-13}C\] palmitate was ingested at dinner. After dinner, blood samples were taken at 30 min, then every 20 min for 2 hours and every hour during the last 5 hours. Meals were consumed within 15 min each. All subjects stayed at rest in a bed during the study and were not allowed to sleep.

**Analytical methods**

Blood was collected in dry tubes and plasma was separated rapidly by centrifugation at 800 \( g \) for 10 min at 4°C and frozen at -80°C until time of assay, except for a 2 ml portion of each sample used immediately to prepare CM-rich fraction. CM-rich fractions (Svedberg flotation index \( > 1 \ 000 \)) were separated using a 0.9% NaCl solution and centrifugation at 25 000 \( g \) for 65 min at 10°C [10]. CMs were aspirated and frozen at -80°C until lipid extraction. TAG concentrations were measured in plasma and CM-rich fraction by an enzymatic method (GPO-PAP, Boehringer Mannheim, Germany). Plasma immunoreactive insulin concentrations were measured by radio-immunoassay (Bi-insuline RIA Pasteur, ERIA diagnostics Pasteur, France). Plasma NEFA concentrations were determined by enzymatic colorimetric method with the use of a commercial kit (NEFA C, WAKO chemicals, Neuss, Germany). Plasma glucose concentrations were measured by the glucose oxidase technique using a Beckman Glucose Analyser II (Beckman Instruments, Gagny, France). Total lipids were extracted from 2 ml portions of plasma and 1 ml portions of CM-rich fractions according to Bligh and Dyer [21] into chloroform - methanol 2:1. Trinonadecanoic acid (19:0 TAG, 300 nmol, SIGMA, France) and nonadecanoic acid (19:0 NEFA, 300 nmol, SIGMA, France) were added before extraction as internal standards. All evaporation steps were performed under nitrogen. TAG and NEFA were separated by thin-layer chromatography on silica gel plates (type linear-K5, Whatman, Maidstone, UK) using hexane - diethyl ether - acetic acid (70:30:1 vol/vol/vol) as mobile phase. Lipid spots were scraped and extracted; NEFA were treated with 14% boron trifluoride in methanol (2 ml) and TAG were treated with hexane-methanol and 14% boron trifluoride in methanol at 100°C for 15 min for fatty acid methyl ester preparation.

**Fatty acid methyl esters of plasma TAG, CM-TAG, plasma NEFA, and ingested oils were analysed by capillary gas chromatography using an on-column injector under conditions already described [22]. Fatty acid composition of olive oil and sunflower oil is summarized in Table II. Isotopic enrichment analysis was performed by a mass spectrometer (5971 series, Hewlett-Packard, Les Ulis, France) interfaced with a gas chromatograph (5890 series II, Hewlett-Packard, Les Ulis, France) equipped with a capillary column (DB Wax 0.32 \( 3 \) 60 m, J and W Scientific, USA), using the electron impact ionization mode. Isotopomers at m/z 270 and 271 were measured (selected ion monitoring). The \(^{13}C\) abundance in 16:0 was measured in plasma TAG, CM-TAG and plasma NEFA as a function of the ratio 16:0 tracer molecules to 16:0 tracer plus natural molecules (mol % excess), using calibration curves.

**Statistics**

Descriptive statistics for continuous variables are reported as means \( \pm \) SEM. The mean of the three samples before lunch was used to determine the baseline value. Plasma TAG and CM-TAG peaks were assessed with Graphpad Prism software (Graphpad software inc., USA). Analysis of variance (ANOVA) with repeated measures and grouping factor (time) was performed to assess the effect of mealtime (lunch and dinner) on plasma and CM-TAG concentrations. The level of significance was set at \( p < 0.05 \). Statistica\(^{\text{\textregistered}}\) software version 6 (Statsoft, Paris, France) was used for this analysis.

**Results**

**Plasma and CM triacylglycerol**

The changes in mean plasma TAG concentrations with time are presented in figure 1a. Total plasma TAG concentration rose from 1.0 \( \pm \) 0.1 mmol/L to 2.4 \( \pm \) 0.3 mmol/L 60 min after lunch, and then progressively decreased to basal level 7 hours after lunch (1.1 \( \pm \) 0.2 mmol/L). After dinner, TAG concentration slowly increased and peaked at 180 min after dinner (2.0 \( \pm \) 0.4 mmol/L). Graphpad prism software identified a first peak of plasma TAG 60 min after a lunch, and a second one 180 min after dinner with a significant effect of time on postprandial TAG levels after both lunch and dinner (ANOVA with repeated measures; \( p = 0.002 \)). The magnitude of the plasma TAG peaks was not statistically significant (2.4 \( \pm \) 0.3 vs 2.0 \( \pm \) 0.4 mmol/L; lunch vs dinner respectively; \( p = 0.85 \)). The time changes in mean CM-TAG concentrations closely mirrored the changes in total plasma TAG (Fig. 1b) including two CM-TAG peaks, one at 60 min after a lunch, and the second at 180 min after dinner with no difference in the magnitude of the CM-TAG peak between lunch and dinner (0.7 \( \pm \) 0.3 vs 0.6 \( \pm \) 0.3 mmol/L; lunch vs dinner respectively; \( p = 0.81 \)).

<table>
<thead>
<tr>
<th>Palmitic acid</th>
<th>Oleic acid</th>
<th>Linoleic acid</th>
</tr>
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<tbody>
<tr>
<td>g/100g</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Olive oil</td>
<td>11.5</td>
<td>73.7</td>
</tr>
<tr>
<td>Sunflower oil</td>
<td>6.2</td>
<td>24</td>
</tr>
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</table>

\(^{1}\)Determined by capillary gas chromatography. Olive oil was ingested at lunch (12AM) and sunflower oil at dinner (7PM).
Changes in [1-13C] palmitate enrichment in plasma TAG, CM-TAG and NEFA

[1-13C] palmitate enrichment in plasma TAG, CM-TAG and plasma NEFA is shown in figure 2. A maximum enrichment was observed 300 min after lunch, whatever the lipid fraction considered. Maximal [1-13C] palmitate enrichment was higher in CM-TAG (mean MPE = 15.07 ± 0.9%) than in plasma TAG and NEFA (mean MPE = 10.54 ± 0.06% and 10.46 ± 0.46% respectively). A slight decrease was observed thereafter until dinner time (420 min). [1-13C] palmitate enrichment plateaued for the first 60 minutes after dinner and then decreased again with time, depicting a regular parabolic curve in all lipid fractions.

Specific fatty acids measurements

The time changes of palmitic (16:0), oleic (18:1n-9) and linoleic (18:2n-6) acid concentrations in total plasma TAG, CM-TAG and plasma NEFA are depicted in figure 3a, b, c respectively. Oleic acid was the major fatty acid found in all plasma lipid fractions during the postprandial lunch period and remained the predominant fatty acid during the first 90 min following the ingestion of dinner. There was no change in linoleic acid concentration during the first hour following dinner, whatever the plasma lipid fraction considered. Then, linoleic acid concentration progressively increased and peaked at 260 min after dinner in all lipid fractions. From that time up to the end of the test period,
linoleic acid concentration remained above the oleic acid concentration in all lipid fractions analysed.

**NEFA, plasma insulin and glucose**

The mean plasma NEFA, glucose, and insulin concentrations are depicted in figure 4a, 4b, 4c respectively. Mean plasma NEFA concentration decreased from 464 ± 47 μmol/L before lunch to 277 ± 67 μmol/L at 180 min after lunch and reached a plateau until 420 min (277 ± 18 μmol/L). Mean plasma NEFA concentration slightly increased to 306 ± 21 μmol/L 30 min after dinner, and then followed a U-shaped curve up to 210 min after dinner. NEFA concentrations remained stable afterward and reached the concentration 290 ± 13 μmol/L at the end of the test period (Fig. 4a). Mean plasma glucose concentration increased from 4.9 ± 0.1 mmol/L at baseline to 6.4 ± 0.2 mmol/L 60 min after lunch, stayed in plateau for two hours and progressively decreased to baseline value just before dinner (5.0 ± 0.4 mmol/L). The time changes in mean plasma glucose concentration showed a very different pattern after dinner (Fig. 4b), with two peaks, one 30 min after dinner and the second 160 min after dinner. Mean plasma glucose concentration did not return to baseline concentration at the end of the test (5.5 ± 0.4 mmol/L). Mean plasma insulin concentrations mirrored plasma glucose concentrations (Fig. 4c).

**Discussion**

In the present study, similar meals given at lunch and dinner induce different plasma TAG and CM-TAG changes over time. Maximal concentrations occurred 3 hours after dinner with no evidence for an early peak of TAG after dinner in any of the 6 subjects, although large interindividual variations were observed. In contrast, maximal plasma TAG and CM-TAG concentrations were observed 1 hour after lunch. Neither fat load [9, 10] nor pre-meal plasma TAG levels [15, 23] were different between lunch and dinner in our study. There are two factors that could contribute to the slow and delayed increase in the plasma TAG and CM-TAG concentrations after dinner as compared to lunch. First, a delay in the lipemic response has been already reported after dinner compared to lunch [24] and may be related to circadian variation in gastric emptying of meals [25]. The higher plasma glucose and insulin concentrations seen at the end of the post-dinner period as compared to the pre-dinner levels is consistent with a delayed gastric emptying after dinner. Secondly, oils of different fatty acid composition were ingested at lunch (olive oil) and dinner (sunflower oil) in our study, and there is some evidence for different metabolic handling in the gut of oils with different fatty-acid composition [20] as well as for a dietary fatty acid-dependent metabolism of postprandial lipoproteins [26, 27]. However and with respect to the postprandial lipoprotein metabolism, the difference is marginal [8] if any [28, 29] unless n-3 fatty acids were concerned which is not the case in the present study. On the other hand, when ingested during the pre-test meal as in our study, olive oil has been shown to produce more chylomicrons after test meal than safflower oil, palm oil and a mixture of fish and safflower oil (50/50) [20]. Increased postprandial levels in TAG responses has been reported in heavy smokers (>20 cigarettes/day; smoking years: 24.8 ± 2.0), [30]. In the present study, only 3 subjects were light and occasional smokers, and were not allowed to smoke 3 days before the test.

There is some evidence for a re-entry of specific fatty acids provided at lunch into post-dinner TAG. As specific fatty acid composition of plasma TAG and CM-TAG is concerned, we found a slight and transient rebound in their oleic acid content 30 minutes after dinner while the linoleic acid content increased with some delay thereafter.
Although sunflower oil contains both oleic and linoleic acids, the short time which separates the ingestion of the dinner and the increase in oleate content of TAG, as well as the time discrepancy between the increase in the oleate and linoleate content of TAG, support a re-entry of oleate coming from the fat load ingested at lunch. Such re-entry is also
consistent with the interruption in the time decay in the [1-\(^{13}\)C] palmitate enrichment in TAG and CM-TAG lipid fractions during the first hour after dinner. However, the metabolic shifts induced by the sharp increase in insulin concentrations seen soon after dinner might also be involved in the [1-\(^{13}\)C] palmitate enrichment plateau that we observed after dinner. Thus, in our study, the fat ingested at lunch might have been to some extend retained within the jejunal tissue and released into plasma TAG following dinner ingestion [31, 32].

Figure 4
Plasma non esterified fatty acids (NEFA, panel a), glucose (panel b) and insulin (panel c) concentrations obtained after lunch and dinner. Lunch was taken at time 0 and dinner at time 420. Mean ± SEM.
The contribution of fat ingested at lunch to post-dinner-TAG composition as stigmatized by our results seems less intense compared to the evidences provided by other studies [19, 20, 31, 33, 34]. This might be because we did not found any early TAG peak after dinner. The reasons for the absence of an early peak of TAG in our study are not known. It is noteworthy to stress that the time lag between lunch and dinner was 7 h 00 in our study but only 04 h 30 in other studies [19, 20, 32] in which an early peak of plasma TAG and CM-TAG was also observed 50-60 min after a high-fat lunch [19] or a low-fat lunch [20, 32]. Peel et al. also reported plasma TAG peak one hour after dinner in healthy males but, according to their study protocol, subjects were allowed to take snacks (2.24 MJ, 23 g of fat) between lunch and dinner [34]. Thus, whether the occurrence of an early postprandial TAG peak depends on the time-interval which separates two consecutive meals requires further investigations.

In the present study, the plasma TAG peak induced by ingestion of similar fat loads is delayed after dinner as compared to lunch in young healthy males. Despite the lack of early plasma TAG peak after dinner, fat originated from previous lunch are found in the postdinner TAG.

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References


