Ratio of oleic to palmitic acid is a dietary determinant of thrombogenic and fibrinolytic factors during the postprandial state in men¹–⁴

Yolanda M Pacheco, Beatriz Bermúdez, Sergio López, Rocío Abia, José Villar, and Francisco JG Muriana

ABSTRACT

Background: The nature of dietary fats affects the postprandial activation of the hemostatic system.

Objective: We investigated whether the ratio of oleic to palmitic acid [and that of monounsaturated to saturated fatty acids (MUFA:SFA)] in the diet affects postprandial concentrations of triacylglycerols, tissue factor (TF), fibrinogen, tissue-type plasminogen activator (t-PA), and plasminogen activator inhibitor 1 (PAI-1).

Design: We studied the effects of diets enriched in olive oil (ROO), high-palmitic sunflower oil (HPSO), butter, or a mixture of vegetable and fish oils (VEFO) on circulating concentrations of the aforementioned factors in 14 healthy men. The fats had ratios of oleic to palmitic acid (MUFA:SFA) of 6.83 (5.43), 2.36 (2.42), 0.82 (0.48), and 13.81 (7.08).

Results: The largest and longest-lasting postprandial changes in plasma triacylglycerol concentrations were found with the butter-based diet (all P < 0.05). No correlation was observed between the net incremental area under the curve (netAUC) for triacylglycerol and the ratio of oleic to palmitic acid (or MUFA:SFA) in the dietary fats. The netAUCs for TF and PAI-1, however, were inversely related to the ratio of oleic to palmitic acid (and MUFA:SFA) in ROO, HPSO, butter, and VEFO. Similar results were found for the fibrinogen netAUC when VEFO was omitted from the analysis. The netAUC for t-PA was inversely correlated with postprandial concentrations of triacylglycerol.

Conclusions: Postprandial concentrations of TF, fibrinogen, and PAI-1 are associated with the ratio of oleic to palmitic acid (MUFA:SFA) in dietary fats. The postprandial t-PA response is related to postprandial concentrations of triacylglycerol.

KEY WORDS Oleic acid, palmitic acid, postprandial responses, diet, monounsaturated fatty acids, saturated fatty acids, fatty acid ratio

INTRODUCTION

Among the main complications of atherosclerosis are the impaired thrombogenesis and fibrinolysis that cause most myocardial events (1). The extrinsic clotting cascade is thought to play a crucial role in the shift in hemostatic balance, and it is triggered by tissue factor (TF) bound to or shed from blood cells and the disrupted endothelium (2). TF is a small, integral transmembrane glycoprotein that acts as a cofactor in the proteolytic activity of factor VII/VIIa toward factor IX and factor X. Increases in concentrations of TF are reflected by increases in plasma fibrinogen concentrations (3). In contrast, plasminogen and its activators, including tissue-type plasminogen activator (t-PA; 4), mediate the proteolytic degradation of fibrin. Indeed, the endothelial release of t-PA is considered a primary endogenous defense mechanism against thrombosis. Plasminogen activator inhibitor 1 (PAI-1), a member of the serine protease inhibitor (serpin) superfamily, is the main physiologic inhibitor of t-PA in the fibrinolytic system (5). Thus, endogenous t-PA is rapidly neutralized by PAI-1, which binds to the active site of t-PA and forms a stable 1:1 stoichiometric (t-PA:PAI-1) complex (6).

Most persons who consume a Western-style diet are postprandial most of the time. Postprandial triacylglycerol-rich lipoproteins may affect endothelial function and hemostasis in a prothrombotic or a profibrinolytic manner (7, 8). The magnitude of the postprandial lipidic response is determined by several factors, such as the amount and composition of the fat ingested, fasting triacylglycerol concentrations, age, lifestyle, and habitual dietary fat composition (9). It is generally agreed that consumption of butter fat [which contains short-chain and medium-chain saturated fatty acids (SFAs)] but not fats containing long-chain SFAs produces higher postprandial lipemic responses than does the consumption of monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids (10, 11). This suggests that the nature of dietary lipids may have an important regulatory role in the activation of the hemostatic system during the postprandial period (12). It is generally recommended that the dietary intake of fats rich in SFAs (mainly palmitic acid, 16:0) be replaced with those rich in MUFAs (mainly oleic acid, 18:1 n−9), which highlights the importance of investigating the effects of high-oleic fats on...
postprandial events. Thus, we set out to study the effects of natural fats containing different relative amounts of oleic and palmitic acid on postprandial concentrations of TF, fibrinogen, t-PA, and PAI-1 in healthy subjects. We hypothesized that a meal rich in oleic acid (typical of a MUFA-rich Mediterranean diet) and with high ratio of oleic to palmitic acids might be more favorable for the postprandial hemostatic variables than meals with less oleic acid (MUFA) and more palmitic acid (SFA).

SUBJECTS AND METHODS

Subjects

This study was conducted according to good clinical practice guidelines. All protocols were approved before the start of the study by the Human Clinical Commission and the Ethics Committee, and informed consent was obtained from each subject. The study conformed with the principles set out in the Helsinki Declaration.

The study was carried out with 14 healthy, male nonsmokers with no evidence of established coronary artery disease who were recruited by advertising (Table 1). Subjects were excluded if they displayed biochemical evidence of renal impairment, hypothyroidism, or liver dysfunction.

Study design

This was a randomized crossover study and it was carried out with the investigators blinded to the treatments. The subjects were maintained for a lead-in baseline period of 1 wk on a National Cholesterol Education Program (NCEP) Step I diet (control diet). Thereafter, the subjects were instructed to follow the same diet or they were switched to an NCEP diet supplemented with refined olive oil (ROO), high-palmitic sunflower oil (HPSO; 13,14), butter, or a mixture of vegetable and fish oils (VEFO) for an additional 1 wk (Table 2). The evening before the postprandial study, the subjects ate a meal relatively high in carbohydrates (60% of energy) made up of mainly plain pasta. Fasting blood samples were taken 12 h after the evening meal (baseline values).

Immediately after the fasting blood samples were taken, the subjects were administered a fat-rich meal consisting of the corresponding dietary fats (ROO, HPSO, butter, or VEFO, 50 g/m² body surface area) along with a portion of plain pasta (50 g), one slice of brown bread (28 g), and one skim-milk yogurt. The average total energy provided by the meals was 3700 kJ (885 kcal) with a macronutrient profile of 72% fat, 22% carbohydrate, and 6% protein. The fatty acid composition of the meals is shown in Table 3. The subjects were asked to consume the meal in <15 min, after which time blood was collected from a cubital vein catheterized with a small-bore extension set and a Smartsite needleless valve port (Alaris, San Diego, CA). A blood sample was drawn every hour for a total of 8 h into precooled tubes containing sodium citrate (final concentration, 0.129 mmol/L). The plasma was separated immediately by centrifugation (2000 × g, 4 °C, 20 min), and the aliquots were transferred into sterile, 1-mL cryovials and stored at −70 °C until analyzed further. For a blank test, the subjects who followed the NCEP diet during the second week consumed a test meal containing no fat, to determine the diurnal fluctuations in the thrombogenic and fibrinolytic variables (15, 16). The test meals were well tolerated by the subjects and had no unpleasant side effects. The subjects rested and read during the postprandial period. Only water was available on request.

Each subject followed 5 dietary cycles lasting 2 wk each. Each cycle included 1 wk of the NCEP diet in between the corresponding test fat meal as a washout and adaptation period to eliminate any carry-over effects on the next diet being tested. The diets were prepared by the subjects themselves under the direction of a registered dietitian and consisted of whole foods according to

**TABLE 2**

<table>
<thead>
<tr>
<th>Nutrient characteristics†</th>
<th>NCEP Step I diet</th>
<th>ROO diet</th>
<th>HPSO diet</th>
<th>Butter diet</th>
<th>VEFO diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (kJ/d)</td>
<td>9623</td>
<td>9623</td>
<td>9623</td>
<td>9623</td>
<td>9623</td>
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<tr>
<td>Energy (kcal/d)</td>
<td>2300</td>
<td>2300</td>
<td>2300</td>
<td>2300</td>
<td>2300</td>
</tr>
<tr>
<td>Fat (% of energy)</td>
<td>28.8</td>
<td>37.9</td>
<td>37.9</td>
<td>37.9</td>
<td>37.9</td>
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<td>Saturated</td>
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<td>9.8</td>
<td>14.3</td>
<td>22.2</td>
<td>9.5</td>
</tr>
<tr>
<td>Monounsaturated</td>
<td>13.9</td>
<td>22.9</td>
<td>18.1</td>
<td>10.7</td>
<td>21.2</td>
</tr>
<tr>
<td>Polyunsaturated</td>
<td>5.3</td>
<td>5.2</td>
<td>5.5</td>
<td>5.0</td>
<td>7.2</td>
</tr>
<tr>
<td>Protein (% of energy)</td>
<td>13.7</td>
<td>13.1</td>
<td>13.1</td>
<td>13.1</td>
<td>13.1</td>
</tr>
<tr>
<td>Carbohydrate (% of energy)</td>
<td>57.5</td>
<td>49</td>
<td>49</td>
<td>49</td>
<td>49</td>
</tr>
<tr>
<td>Cholesterol (mg/4184 kJ)</td>
<td>80</td>
<td>80</td>
<td>80</td>
<td>80</td>
<td>80</td>
</tr>
<tr>
<td>Fiber (g/4184 kJ)</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>

† Carbohydrate intake was calculated by subtracting the values for fat and protein intakes from the value for total energy intake. NCEP, National Cholesterol Education Program; ROO, olive oil; HPSO, high-palmitic sunflower oil (a gift from Manuel Mancha) obtained from field-grown mutant sunflower seeds CAS-12 (Advanta Semillas, Balcarce, Argentina); VEFO, mixture of vegetable and fish oils used in functional dairy products (Puleva Omega 3; Puleva Food SL, Granada, Spain). 4184 kJ = 1000 kcal.
TABLE 3
Fatty acid composition of the test meals

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>ROO</th>
<th>HPSO</th>
<th>Butter</th>
<th>VEFO</th>
</tr>
</thead>
<tbody>
<tr>
<td>% by wt of total fatty acids</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10:0</td>
<td>—</td>
<td>—</td>
<td>2.0</td>
<td>0.0</td>
</tr>
<tr>
<td>12:0</td>
<td>—</td>
<td>—</td>
<td>2.5</td>
<td>0.0</td>
</tr>
<tr>
<td>14:0</td>
<td>—</td>
<td>—</td>
<td>9.9</td>
<td>0.0</td>
</tr>
<tr>
<td>16:0 (palmitic acid)</td>
<td>11.7</td>
<td>24.9</td>
<td>31.8</td>
<td>5.4</td>
</tr>
<tr>
<td>16:1n=7</td>
<td>1.0</td>
<td>6.8</td>
<td>2.4</td>
<td>0.6</td>
</tr>
<tr>
<td>18:0</td>
<td>2.8</td>
<td>2.0</td>
<td>13.3</td>
<td>4.2</td>
</tr>
<tr>
<td>18:1n–9 (oleic acid)</td>
<td>79.8</td>
<td>58.7</td>
<td>26.2</td>
<td>74.4</td>
</tr>
<tr>
<td>18:2n–6</td>
<td>3.6</td>
<td>6.9</td>
<td>3.0</td>
<td>10.3</td>
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<tr>
<td>20:5n–3</td>
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<td>—</td>
<td>1.0</td>
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<td>22:6n–3</td>
<td>—</td>
<td>—</td>
<td>2.3</td>
<td></td>
</tr>
<tr>
<td>Others</td>
<td>1.1</td>
<td>0.6</td>
<td>8.9</td>
<td>1.8</td>
</tr>
<tr>
<td>SFA</td>
<td>14.9</td>
<td>27.2</td>
<td>65.3</td>
<td>10.7</td>
</tr>
<tr>
<td>MUFA</td>
<td>81.0</td>
<td>65.8</td>
<td>31.3</td>
<td>75.4</td>
</tr>
<tr>
<td>PUFA</td>
<td>4.0</td>
<td>7.0</td>
<td>3.4</td>
<td>13.9</td>
</tr>
<tr>
<td>Oleic/palmitic acid</td>
<td>6.83</td>
<td>2.36</td>
<td>0.82</td>
<td>13.81</td>
</tr>
<tr>
<td>MUFA:SFA</td>
<td>5.43</td>
<td>4.22</td>
<td>0.48</td>
<td>7.08</td>
</tr>
</tbody>
</table>

1 ROO, olive oil; HPSO, high-palmitic sunflower oil; VEFO, mixture of vegetable and fish oils; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

calculated menus and standardized recipes. The planned carbohydrate, protein, and fat distribution was similar for all high-fat diets. The daily consumption of ROO, HPSO, butter, and VEFO per 9623 kJ (2300 kcal) was 52 g. Overall dietary compliance was estimated as >94% on the basis of weekly supervision of the subjects at home by an investigator and on the basis of the personal diaries of the dietary records kept by the subjects. For subjects to maintain a constant body weight during the study, energy intake was periodically adjusted. None of the subjects used tobacco, consumed special diets, or took vitamins, antioxidants, or medication. They were asked to maintain their usual exercise pattern and to abstain from alcohol.

Analysis of dietary fats

Fatty acid composition was determined according to the method described in EC/796/2002 (17) with a gas chromatography system (HP-5890; Hewlett-Packard, Palo Alto, CA) with a flame ionization detector and an SP-2380 (Supelco, Bellefonte, PA) fused silica capillary column (50 m × 0.25 mm internal diameter) coated with cyanopropylpolysiloxane (0.25 μm film thickness). The oven temperature program was isothermal at 165 °C for 10 min before rising to 200 °C at 1.5 °C/min. The injector and detector temperatures were 220 °C and 250 °C, respectively. Hydrogen was used as the carrier gas at a column head pressure of 130 kPa. Sample injections were performed in the split mode.

Laboratory measurements

Triacylglycerols and other plasma lipids were quantified by using commercially available reagents on a Hitachi Modular Analytics D-2400 analyzer (Roche Diagnostics, Basel, Switzerland). Hemostatically active fibrinogen was measured according to the method of Clauss by using fibrinogen reagent (Stago Diagnostica, Asnieres-sur-Seine, France). An STA Unicalibrator was used as a reference standard and Precicot I/II were used as quality controls for accuracy and reproducibility (Stago Diagnostica). TF was assayed by enzyme-linked immunosorbent assay (matched-pair test for human TF; Kordia, Leiden, Netherlands). As reference standards, several dilutions of a recombinant human tissue factor (not lipidated) were used (American Diagnostica, Stamford, CT).

t-PA and PAI-1 were measured with commercial kits (Imulyse t-PA and Imulyse PAI-1, Biopool; Kordia). In addition to active and latent PAI-1, the PAI-1 kit measured the t-PA:PAI-1 and urokinase-PA:PAI-1 complexes, albeit less efficiently. The assay included the use of quenching and normal antibodies to exclude false positives (immunologic specificity and accuracy control, or ISAC). The t-PA kit enabled single-chain and two-chain t-PA antigen to be quantified and used the same double-antibody method (ISAC).

Statistical analysis

Individual data from each subject were plotted and evaluated qualitatively. Statistical analyses were carried out to compare the effects of each fat on the fasting and postprandial values and to analyze the values from each fat at different time intervals. The net incremental area under the curve (netAUC), including the entire incremental area below the curve and the area below the fasting concentration, was analyzed by a one-factor repeated-measures analysis of variance (ANOVA). A Bonferroni correction was used for the post hoc detection of significant pairwise differences. The netAUC was calculated by the trapezoidal method by using Microsoft EXCEL 2000 v.9 (Microsoft Corp, Redmond, WA). Univariate correlation analysis between variables was performed with Pearson’s product-moment correlations. The data were analyzed by using STATVIEW v.5 for WINDOWS (SAS Institute, Cary, NC). The designated level of significance was P < 0.05.

RESULTS

Postprandial triacylglycerol concentrations induced by the different fat-enriched diets are shown in Figure 1. As expected, no plasma triacylglycerol was apparent when the diet was not supplemented with fat. Conversely, there was a marked increase in plasma triacylglycerol when supplementary fat was consumed, which peaked at different times postprandially depending on the fat tested (P < 0.05). Overall, the HPSO supplement was associated with a lower plasma triacylglycerol response (netAUC was 3.75 ± 0.51 mmol · h/L; P < 0.05) than were ROO (4.45 ± 0.67 mmol · h/L) and VEFO (4.42 ± 0.45 mmol · h/L). The largest and longest-lasting changes in plasma triacylglycerol concentrations were found with the butter supplement, which produced the highest postprandial response (6.02 ± 0.82 mmol · h/L). No correlation was observed between postprandial triacylglycerol responses (netAUC) and the ratio of oleic to palmitic acid (MUFA:SFA) with ROO, HPSO, butter, and VEFO (Table 4).

Postprandial thrombogenic factors

The postprandial study showed that TF concentrations peaked 2 h after the ingestion of all the fat-enriched meals (Figure 2). There was then a sudden clearance of plasma TF to baseline values, except when butter was used for fat enrichment. Indeed,
the butter supplement induced the highest TF postprandial response (netAUC = 33.03 ± 4.52 g · h/L; *P < 0.05; Figure 3). Moreover, the netAUC for TF differed significantly (*P < 0.05) after the ingestion of the different fat supplements analyzed in the following descending order: HPSO (23.57 ± 5.11 g · h/L), ROO (11.09 ± 1.65 g · h/L), and VEFO (−13.75 ± 2.47 g · h/L). No correlation was observed between the triacylglycerol and TF postprandial responses (netAUC) after the ingestion of any of the fat supplements. Nevertheless, a significant inverse correlation was observed between the netAUC for TF and the ratio of oleic to palmitic acid (MUFA:SFA) with ROO, HPSO, butter, and VEFO (*P < 0.05; Table 4).

Postprandial fibrinogen concentrations and netAUC values did not differ significantly after ingestion of ROO (netAUC was 1.47 ± 0.24 g · h/L) and HPSO (1.05 ± 0.28 g · h/L), but were higher than the values found with the other fat supplements (Figures 2 and 3; *P < 0.05). When butter or no fat was added to the meal, there was a similar slight decrease in plasma fibrinogen with respect to the baseline value (−0.47 ± 0.07 g · h/L for butter, −0.38 ± 0.08 g · h/L in the absence of the fat supplement).

However, VEFO enrichment produced an even greater decrease (−2.29 ± 0.35 g · h/L; *P < 0.05). No correlation was observed between the postprandial triacylglycerol and fibrinogen responses (netAUC) after the ingestion of any test fat, but there was a significant positive correlation between the netAUC for fibrinogen and the ratio of oleic to palmitic acid (MUFA:SFA) after ROO, HPSO, and butter enrichment (Table 4; *P < 0.05).

### Postprandial fibrinolytic factors

Normal diurnal variations in t-PA and PAI-1 were observed in each subject (Figure 2). However, when the meal was enriched with butter or when no fat was supplemented, there was a comparable drop in the plasma t-PA response from the baseline value (the netAUC was −1.65 ± 0.21 g · h/L for butter and −1.24 ± 0.26 g · h/L in the absence of the fat supplement; Figure 3). HPSO enrichment induced a significantly smaller change in the netAUC value for t-PA (−9.13 ± 1.24 g · h/L; *P < 0.05), whereas ROO and VEFO enrichment had a similar effect (−12.39 ± 1.63 g · h/L for the ROO meal and −11.79 ± 1.37 g · h/L for the VEFO meal). No correlation was observed...
FIGURE 2. Mean (± SD) postprandial concentrations of tissue factor (TF), fibrinogen, tissue-type plasminogen activator (t-PA), and plasminogen activator inhibitor 1 (PAI-1) after the ingestion of a meal containing no fat (○), olive oil (ROO; △), high-palmitic sunflower oil (HPSO; ■), butter (□), or a mixture of vegetable and fish oils (VEFO; ■). n = 14. There was a significant effect of the type of fat, a significant time effect, and a significant meal × time interaction, *P* < 0.05 (repeated-measures ANOVA with Bonferroni correction).

FIGURE 3. Mean (± SD) tissue factor (TF), fibrinogen, tissue-type plasminogen activator (t-PA), and plasminogen activator inhibitor 1 (PAI-1) postprandial responses [net incremental area under the curve (netAUC)] after the ingestion of a meal containing no fat, olive oil (ROO), high-palmitic sunflower oil (HPSO), butter, or a mixture of vegetable and fish oils (VEFO). n = 14. Bars with different letters are significantly different, *P* < 0.05 (repeated-measures ANOVA with Bonferroni correction).
between the t-PA postprandial responses (netAUC) and the ratio of oleic to palmitic acid (MUFA:SFA) after any fat supplement, but there was a significant inverse correlation between the netAUC for t-PA and triacylglycerol after the ingestion of ROO, HPSO, butter, and VEFO (Table 4; \( P < 0.05 \)).

Diurnal postprandial PAI-1 concentrations were significantly higher after the ingestion of butter than after the other fat supplements (Figure 2; \( P < 0.05 \)). Moreover, butter and HPSO enrichment significantly reduced the changes in the netAUC value for PAI-1 (\(-55.17 \pm 8.35 \, \mu g \cdot h/L \) for the butter meal and \(-64.95 \pm 9.16 \, \mu g \cdot h/L \) for HPSO; \( P < 0.05 \); Figure 3). When ROO or VEFO was added to the meal, there was a comparable decrease in plasma PAI-1 with respect to the baseline value (\(-76.13 \pm 8.83 \, \mu g \cdot h/L \) for ROO and \(-75.42 \pm 7.46 \, \mu g \cdot h/L \) for VEFO). No correlation was observed between the triacylglycerol and PAI-1 postprandial responses (netAUC) after the ingestion of any test fat. However, there was a significant inverse correlation between the netAUC for PAI-1 and the ratio of oleic to palmitic acid (MUFA:SFA) after ROO, HPSO, butter, and VEFO enrichment (Table 4; \( P < 0.05 \)).

**DISCUSSION**

It is well accepted that among other factors, dietary fatty acids influence the development and progression of atherosclerosis (18). We hypothesized that the postprandial metabolism of dietary fats might represent a procoagulant challenge that disturbs thrombogenesis and fibrinolysis in healthy subjects. Moreover, we proposed that this phenomenon could at least partially depend on the ratio of oleic to palmitic acid (MUFA:SFA) in the fats. Hence, we compared the dietary effects of ROO, HPSO, and butter, having oleic to palmitic acid (MUFA:SFA) ratios of 6.83 (5.43), 2.36 (2.42), and 0.82 (0.48), respectively. In addition, we analyzed VEFO, which had an oleic to palmitic acid ratio of 13.81 (MUFA:SFA = 7.08; containing 1.0% EPA and 2.3% DHA).

Postprandial triacylglycerol responses were similar for dietary enrichment with ROO and VEFO. This agrees with a recent study in which no difference was found in postprandial concentrations of triacylglycerol after MUFA enrichment in the presence or absence of n-3 long-chain polyunsaturated fatty acids (LCPUFAs; 19). Our results also showed that the postprandial triacylglycerol response was significantly higher after the butter supplement, whereas it was lowest after HPSO enrichment. Indeed, butter as a fat source was previously shown to cause an increase in postprandial triacylglycerol concentrations when compared with ROO (11). In contrast, no differences were observed in other studies involving diets enriched in high-palmitic or high-oleic fats (20), nor were differences identified between butter fats and ROO in terms of lower postprandial triacylglycerol concentrations (21). These discrepancies are probably due to differences in the amount and nature of the fats administered, the composition of the test meals, the standardization of the diet before the postprandial study, and the subjects involved. In accordance with a recent study with interesterified fats in healthy young males, we did not observe any association between the postprandial triacylglycerol response and the oleic to palmitic acid ratio (MUFA:SFA) in dietary fats (22).

The present study focused on the influence of the oleic to palmitic acid ratio (MUFA:SFA) in dietary fats on the postprandial responses of TF, fibrinogen, t-PA, and PAI-1. Despite observations indicating that thrombotic complications might be mediated by high circulating concentrations of TF (23) and might be accelerated during postprandial lipemia (24), few data are available regarding the postprandial effects of dietary fats on TF. In contrast, much more information exists regarding the effects of dietary fat composition on postprandial concentrations of activated factor VII (FVIIa; 12). Activation of FVII by TF represents a critical event in thrombogenesis. Indeed, the transient rise in FVIIa after a fat-rich meal is generally detectable 2–3 h postprandially and persists for \( \geq 8 \) h, thus displaying dose-response characteristics similar to those reported here for TF. The increase in FVIIa is correlated with fasting triacylglycerol concentrations, but not with postprandial triacylglycerol concentrations (25). Interestingly, the main dietary determinants of postprandial changes in FVIIa are oleic and palmitic acid (26), which agrees with the correlation found between the netAUC for TF and the ratio of oleic to palmitic acid (MUFA:SFA) in the ROO-, HPSO-, butter-, and VEFO-enriched meals. Nevertheless, the link between TF and FVIIa and their equivalence as procoagulant postprandial markers given a controlled dietary background should be studied further.

Elevated fibrinogen appears to be a risk factor for arterial thrombosis (27), which is relevant in the postprandial period because fibrinogen can bind to newly created triacylglycerol-rich lipoproteins (28). The coating of fibrinogen with triacylglycerol-rich lipoproteins involves hydrophobic patches on the surface of the lipoproteins. It is therefore likely that the high postprandial fibrinogen response observed after the intake of the ROO supplement was a consequence of an increase in surface lipoproteins due to the small number and larger size of nascent postprandial triacylglycerol-rich lipoproteins from ROO (29, 30). In our study, the netAUCs for fibrinogen after the ROO, HPSO, and butter supplements were significantly correlated with the ratio of oleic to palmitic acid (MUFA:SFA). The lower netAUC for fibrinogen after VEFO enrichment and the absence of such a correlation when the VEFO meal was included in the analysis suggest that ingestion of a low amount of n-3 LCPUFAs could reverse this effect on postprandial fibrinogen concentrations. Thus, our data stress importance of n-3 LCPUFAs as a determinant of the direction and magnitude of the postprandial response of fibrinogen to dietary fats. Similar observations were made regarding postprandial vascular reactivity to high-MUFA meals (19), with the presence of n-3 LCPUFAs reverting the pattern of fibrinogen while exerting no effect on postprandial triacylglycerol concentrations. The present study confirms that n-3 LCPUFAs may partially reduce the thrombotic potential of fat-rich meals (31, 32).

In prospective clinical (33) and in multicenter (34) studies of angina pectoris and postinfarction patients, the t-PA antigen can serve to predict subsequent acute coronary syndromes. Our data showed a marked decline in postprandial t-PA and PAI-1 concentrations from 0 to 8 h after all meals, which agrees with the strong diurnal (characteristic U-shaped) variations in net fibrinolytic activity (15, 16). We found that butter enrichment induced the lowest postprandial t-PA response, as is commonly observed for SFA-rich diets (35). This suggests that butter enrichment does
not decrease postprandial clot lysis mediated by t-PA when compared with ROO, HPSO, and VEFO. We also observed a significant correlation between the netAUC for t-PA and the netAUC for triacylglycerol, but not with the ratio of oleic to palmitic acid (MUFA:SFA) after ROO, HPSO, butter, and VEFO enrichment. Notably, fasting t-PA antigen is linked to the metabolic syndrome, and it is positively related to fasting triacylglycerol and insulin, as well as to the waist-to-hip ratio in familial combined hyperlipidemia (36). However, our data show that the postprandial triacylglycerol response and not dietary fatty acid composition influences postprandial concentrations of t-PA antigen in healthy subjects.

Circulating PAI-1 is derived from a variety of sources, including the vascular endothelium, adipose tissue, and liver, and it is involved in the onset of obesity, diabetes, and cardiovascular disease (37). Notable ROO, HPSO, and butter enrichment induced a peak in PAI-1 2 h postprandially. This novel finding of postprandial PAI-1 peaks had remained undetected in other studies, possibly because of the failure to take blood samples under fasting conditions and hourly over 8 h. In addition, the netAUC for PAI-1 was significantly correlated with the ratio of oleic to palmitic acid (MUFA:SFA) in the ROO-, HPSO-, butter-, and VEFO-enriched meals. Butter enrichment produced the lowest ratio of oleic to palmitic acid (MUFA:SFA) and induced the highest PAI-1 postprandial response. A similar temporary increase in postprandial concentrations of PAI-1 antigen and impaired fibrinolytic activity after a butter-enriched meal was reported previously (38). Moreover, improved fibrinolytic activity has been associated with a decline in postprandial PAI-1 after high-MUFA (oleic acid) meals (39, 40). Together, these observations could have important clinical consequences, because the morning peak in PAI-1 antigen corresponds with the circadian peak in the incidence of acute myocardial infarction (41).

One limitation of the current study was that we evaluated the effects of meals containing large amounts of specific dietary fats that did not correspond in fatty acid content to the background diets to which the subjects had become accustomed. To date, the effect of varying the fatty acid composition in individuals who habitually consume comparable background diets is uncertain. In conclusion, our novel data are consistent with the view that the ratio of oleic to palmitic acid (MUFA:SFA) in dietary fatty acids on the postprandial hemostatic system.

REFERENCES
11. Bysted A, Hølmer G, Lund P, Sandström B, Tholstrup T. Effect of 6 dietary fatty acids on postprandial lipemia and triacylglycerol response and not dietary fatty acid composition influences postprandial concentrations of t-PA antigen in healthy subjects. We thank Eduardo López-Huertas and Julio Boza (Puleva Biotech SA) for helpful comments on this manuscript.

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