Postprandial variations in fibrinolytic activity in middle-aged men are modulated by plasminogen activator inhibitor I 4G-675/5G genotype but not by the fat content of a meal\textsuperscript{1–3}

Thomas AB Sanders, Tamara de Grassi, Jayshree Acharya, George J Miller, and Steve E Humphries

ABSTRACT
Background: Decreased fibrinolytic activity is associated with an increased risk of ischemic heart disease and elevated plasma triacylglycerol concentrations.
Objective: The goal was to determine whether plasminogen activator inhibitor I (PAI-1) and fibrinolytic activities are influenced by 1) dietary fat intake from a test meal and 2) the PAI-1 4G allele.
Design: A parallel randomized controlled trial was used to compare the effect on fibrinolytic activity, measured as dilute clot lysis time, of high-oleate or high-palmitate test meals (both containing 50 g fat; both \( n = 18 \)) with that of a low-fat test meal (15 g fat; \( n = 15 \)) in men aged \( > 52 \) y. In a second study, postprandial changes in PAI-1 activity were measured in 32 men in response to a high-oleate meal containing 50 g fat. The results from both studies were analyzed according to PAI-1 4G-675/5G genotype.
Results: Fasting dilute clot lysis time was positively associated with body mass index \(( r = 0.326, P = 0.02 \) and was shortened postprandially \(( P < 0.00001 \) independent of the fat content of the meal. Fasting PAI-1 activity was higher in those carrying the 4G allele and was correlated with fasting plasma triacylglycerol concentrations \(( r = 0.48, P = 0.008 \) and factor VII coagulant activity \(( r = 0.46, P = 0.012 \) after adjustments for age, body mass index, and genotype. Plasma PAI-1 activity decreased significantly after a meal but was not associated with postprandial changes in plasma triacylglycerols after a high-fat meal. The postprandial increase in plasma triacylglycerols was higher in subjects carrying the 4G allele.

KEY WORDS Plasminogen activator inhibitor I, PAI-1, monounsaturated fatty acids, saturated fatty acids, PAI 4G-675/5G, fibrinolysis

INTRODUCTION
The Northwick Park Heart Study (NPHS) identified decreased fibrinolytic activity as measured by dilute clot lysis time (DCLT) as an independent predictor of both fatal and nonfatal ischemic heart disease (1). Subsequent studies showed that fibrinolytic activity measured by this method is strongly negatively correlated with plasminogen activator inhibitor I (PAI-1) activity (2). Elevated PAI-1 activity has been shown to be associated with elevated fasting plasma triacylglycerol concentrations, obesity, and the insulin resistance syndrome (3), and the reduction in plasma triacylglycerol concentrations after weight loss and a fat-modified diet results in increased fibrinolytic activity (4). However, it is uncertain whether the lower fibrinolytic activity is due to the elevated plasma triacylglycerols per se or whether it is secondary to insulin resistance.

The presence of a 4G allele at a common insertion-deletion polymorphism in the promoter of the PAI-1 gene has been associated with elevated plasma PAI-1 concentration and activity (5) and also with inducible expression of PAI-1 in response to interleukin I (6). Carriers of the 4G allele may be at increased risk of ischemic heart disease (7, 8), but this may be dependent on an interaction with other environmental factors, which may explain why results in Western populations have been equivocal (5, 9). In one study, an increase in PAI-1 activity was reported 8 h after a test meal very high in butter fat in subjects carrying the 4G allele but not in those with the 5G/5G genotype (10). The aims of the present study were to compare, in middle-aged men, the acute effects of meals high in palmitate or oleate with those of a low-fat meal on fibrinolytic activity measured by the DCLT method and to determine PAI-1 activity during fasting and in response to a high-oleate meal according to PAI-1 4G-675/5G genotype.

SUBJECTS AND METHODS
The protocol was reviewed and approved by the Human Experimentation Committees of King’s College London and the Hertfordshire Health Authority, and all participants gave their written informed consent before the study commenced. The study subjects were men aged \( > 52 \) y who had been recruited into the second NPHS (NPHS-II). Exclusion criteria for NPHS-II were

\begin{itemize}
  \item From the Nutrition Food and Health Research Centre, King’s College London (TABS and TiG); the Medical Research Council Cardiovascular Research Group, Wolfson Institute, Royal London & St Bartholomew’s Hospital Medical School, London (GJM); and the Centre for the Genetics of Cardiovascular Disease, British Heart Foundation Laboratories, Royal Free and University College London Medical School, London (JA and SEH).
  \item Supported by the Ministry of Agriculture, Fisheries and Food Dietary: Lipids Research Programme (project AN0219); the British Heart Foundation (grant RG2000/015); and the Medical Research Council.
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  \item Received March 7, 2003.
  \item Accepted for publication September 3, 2003.
\end{itemize}
were a clinical history of myocardial infarction, unstable angina, stroke, cardiac surgery, malignancy, or other life-threatening malignancy; use of aspirin or anticoagulant therapy; any known condition that would make blood sample handling hazardous (eg, HIV, hepatitis); and an inability to give informed consent or attend annual reexamination visits. The subjects in NPHS-II were examined annually.

In the first study, 54 men selected on the basis of having nonfasting triacylglycerol concentrations > 2 mmol/L were recruited to compare the effects of test meals high in oleate or palmitate with those of a low-fat diet on fibrinolytic activity measured by using the DCLT method. In the second study, the subjects were selected on the basis of their factor VII R353Q genotype (11); measurements of PAI-1 activity were made in response to a test meal high in oleate, and comparisons were made post hoc with PAI-1 genotype. Subjects were invited by letter to take part in the test meal studies and received no remuneration for their cooperation in the study. Background medication, use, blood pressure, nonfasting plasma triacylglycerol and cholesterol concentrations, and body mass index (BMI; in kg/m²) were obtained annually.

The subjects were requested to avoid foods high in fat on the day preceding the test meal study and to avoid strenuous exercise. They were given a list of foods to avoid and were asked to fast from 2200 overnight. The following morning, a fasting venous blood sample for determination of DCLT and plasma triacylglycerol concentrations was taken in the clinic of the general practice. The subjects were then provided with a test meal that they consumed within 20 min. Further venous blood samples were obtained 3 and 6 h postprandially for the same measurements. After the 3-h postprandial venous sample had been taken, the subjects were advised to avoid any strenuous activity throughout the study period but were allowed to leave the clinic to return home or to work between blood samplings.

To compare the effects of palmitate- and oleate-rich meals with those of a low-fat meal, we randomly allocated the subjects into 3 groups of 18 each. The high-fat test meals contained 50 g fat provided either as high-oleic acid sunflower oil or as palm oil and the low-fat meal contained 15 g fat (high-oleic acid sunflower oil). The test meals were previously described (11). The high-oleate test meal provided 40 g 18:1n–9, 5 g 18:2n–6, 2 g 18:0, and 2 g 16:0; the high-palmitate test meal provided 20 g 18:1n–9, 5 g 18:2n–6, 2 g 18:0, and 22 g 16:0; and the low-fat test meal provided 11.9 g 18:1n–9; 1.4 g 18:2n–6, 0.6 g 18:0, and 0.6 g 16:0.

Venous blood samples were collected by using the evacuated tube technique with minimal compression necessary to display the vein. The first 4.5 mL blood was drawn into a tube containing EDTA. Plasma was separated by centrifugation at 1500 × g for 15 min at 4 °C and was stored at 4 °C for determination of triacylglycerols within 48 h by enzymatic assay (GPO-PAP; Roche Diagnostics, Lewes, East Sussex, United Kingdom). For determination of fibrinolytic activity, blood was collected into 0.5 mL solution containing 38 g trisodium citrate/L at room temperature and centrifuged at 1000 × g for 15 min at 20 °C and plasma was separated. The sample was transferred to the main laboratory for measurement of DCLT on the same day (12).

RESULTS

In the first study, measurements of DCLT were successfully made in 51 men, and PAI-1 genotype was available for 49 men. Owing to equipment failure, DCLT measurement data were available for only 15 subjects allocated to the low-fat meal. In the second study, PAI-1 activity was determined in 32 men in response to a test meal high in oleate (this included 3 men in whom DCLT was measured in the first study), and PAI-1 genotype was available for all these men. Details of the subjects in study 1 and study 2 on whom measurements were made are shown in Table 1.

The results for DCLT and plasma triacylglycerol concentrations are shown in Table 2. As expected, plasma triacylglycerol concentrations were significantly higher after the test meals high in fat than after the low-fat meal. DCLT decreased similarly from 2200 overnight. The following morning, a fasting venous blood sample for determination of DCLT and plasma triacylglycerols within 48 h by enzymatic assay (GPO-PAP; Roche Diagnostics, Lewes, East Sussex, United Kingdom). For determination of fibrinolytic activity, blood was collected into 0.5 mL solution containing 38 g trisodium citrate/L at room temperature and centrifuged at 1000 × g for 15 min at 20 °C and plasma was separated. The sample was transferred to the main laboratory for measurement of DCLT on the same day (12).
DIETARY FAT AND PAI-1

this was not statistically significant. Only 2 subjects in the groups given the high-fat meals were homozygous for the 5G allele. The increase in plasma triacylglycerols and the changes in PAI-1 activity after a 50-g high-oleate test meal according to whether subjects were carriers of the 4G allele are shown in Table 3. PAI-1 activity in subjects according to PAI-1 genotype is shown in Figure 1. Fasting plasma triacylglycerol concentrations were strongly positively correlated with fasting PAI-1 activity (r = 0.48, P = 0.005), and this association remained after adjustment for age, genotype, and BMI (r = 0.48, P = 0.008). Fasting PAI-1 activity was also strongly correlated with FVII:c activity after adjustment for age, BMI, and PAI-1 genotype (r = 0.46, P = 0.012). Plasma triacylglycerol concentrations increased to a lesser extent in subjects homozygous for the 5G allele than in carriers of the 4G allele. Repeated-measures analysis of variance showed the significant effect of meal on PAI-1 activity (P = 0.001), with the values at 3 and 6 h being significantly lower than the fasting values (P = 0.012 and P = 0.002, respectively). Further analysis of variance including PAI-1 genotype as a factor and age and BMI as covariates showed a significant effect of PAI-1 genotype on PAI-1 activity (P = 0.01), but the time effect was no longer significant. There was no significant genotype × time interaction.

DISCUSSION

The primary aim of the present study was to ascertain whether meals high in palmitate or oleate result in impaired fibrinolytic activity compared with a low-fat meal. We chose to use fibrinolytic activity as measured by the DCLLT method because this was

TABLE 2

<table>
<thead>
<tr>
<th>Plasma triacylglycerol (mmol/L)</th>
<th>Low-fat test meal (n = 15)</th>
<th>High-oleate test meal (n = 18)</th>
<th>High-palmitate test meal (n = 18)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting</td>
<td>1.74 ± 0.57</td>
<td>1.95 ± 0.59</td>
<td>2.13 ± 0.59</td>
</tr>
<tr>
<td>3 h</td>
<td>1.92 ± 0.83</td>
<td>3.53 ± 0.86&lt;sup&gt;4,6&lt;/sup&gt;</td>
<td>3.51 ± 0.86&lt;sup&gt;4,6&lt;/sup&gt;</td>
</tr>
<tr>
<td>6 h</td>
<td>1.99 ± 0.77</td>
<td>2.44 ± 0.80</td>
<td>2.42 ± 0.80</td>
</tr>
<tr>
<td>Percentage change at 3 h (%)</td>
<td>13.9 ± 35.9</td>
<td>88.6 ± 37.0&lt;sup&gt;4&lt;/sup&gt;</td>
<td>66.9 ± 37.1&lt;sup&gt;4&lt;/sup&gt;</td>
</tr>
<tr>
<td>Percentage change at 6 h (%)</td>
<td>20.5 ± 27.5</td>
<td>24.1 ± 28.4</td>
<td>15.7 ± 28.4</td>
</tr>
</tbody>
</table>

Dilute clot lysis time (h)<sup>5</sup>

| Fasting                         | 11.3 ± 6.3               | 12.9 ± 7.0                    | 11.8 ± 7.1                    |
| 3 h                              | 8.1 ± 6.2                | 7.9 ± 4.1                     | 7.8 ± 4.3                     |
| 6 h                              | 7.3 ± 4.7                | 8.6 ± 4.6                     | 7.9 ± 3.8                     |
| Percentage change at 3 h (%)     | -28.1 ± 33.6             | -38.0 ± 37.5                  | -34.7 ± 57.3                  |
| Percentage change at 6 h (%)     | -35.6 ± 34.6             | -32.6 ± 41.1                  | -33.2 ± 40.9                  |

<sup>1</sup> All values are ± SD.
<sup>2</sup> Adjusted for age and BMI; significant effect of age and BMI; significant mean × time interaction by repeated-measures ANOVA (P < 0.00001).
<sup>3</sup> Significantly different from the fasting value in the same column (Dunn’s test).
<sup>4</sup> Significantly different from the corresponding value for the 5G/5G group, P < 0.01 (Bonferroni’s multiple-comparison test).
<sup>5</sup> Significant effect of time, P < 0.00001; no significant interaction with meal.

FIGURE 1. Influence of plasminogen activator inhibitor 1 (PAI-1) genotype on mean (± SEM) PAI-1 activity after a high-oleate test meal. n = 9 4G/4G, 15 4G/5G, and 8 5G/5G subjects. Repeated-measures ANOVA with age and BMI as covariates showed significant effects of genotype (P = 0.03) and time (P = 0.001) but no significant genotype × time interaction.

TABLE 3

Plasma triacylglycerol concentrations and plasminogen activator inhibitor 1 (PAI-1) activity according to 4G-675/5G genotype after the high-oleate test meal<sup>1</sup>

<table>
<thead>
<tr>
<th>4G/4G + 4G/5G (n = 24)</th>
<th>5G/5G (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma triacylglycerol (mmol/L)&lt;sup&gt;2&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Fasting</td>
<td>1.55 ± 0.64</td>
</tr>
<tr>
<td>3 h</td>
<td>2.62 ± 0.98&lt;sup&gt;4,6&lt;/sup&gt;</td>
</tr>
<tr>
<td>6 h</td>
<td>1.92 ± 0.63&lt;sup&gt;4,6&lt;/sup&gt;</td>
</tr>
<tr>
<td>Percentage change at 3 h (%)</td>
<td>69.0 ± 48.7</td>
</tr>
<tr>
<td>Percentage change at 6 h (%)</td>
<td>23.8 ± 23.7</td>
</tr>
<tr>
<td>PAI-1 activity (U/mL)&lt;sup&gt;6&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Fasting</td>
<td>12.9 ± 6.6</td>
</tr>
<tr>
<td>3 h</td>
<td>8.4 ± 3.5</td>
</tr>
<tr>
<td>6 h</td>
<td>8.2 ± 3.3</td>
</tr>
<tr>
<td>Percentage change at 3 h (%)</td>
<td>-34.9 ± 37.5</td>
</tr>
<tr>
<td>Percentage change at 6 h (%)</td>
<td>-36.4 ± 77.2</td>
</tr>
</tbody>
</table>

<sup>1</sup> All values are ± SD. Values were adjusted for age and BMI.
<sup>2</sup> Repeated-measures ANOVA showed a significant time × genotype interaction, P = 0.03.
<sup>4</sup> Significantly different from the corresponding value for the 5G/5G group, P < 0.01 (Bonferroni’s multiple-comparison test).
<sup>6</sup> Significant effect of allele, P = 0.01; no significant interaction with time.
predictive of future risk of ischemic heart disease in the NPHS cohort. The values for fasting DCLT in the present study are in the range of those reported by Meade et al (1). DCLT is strongly affected by PAI-1 activity and tissue-type plasminogen activator activity, which are known to vary diurnally and with physical activity. Care was taken to ensure that the subjects avoided strenuous activity before and during the study, although they undertook activities that were representative of everyday life. Fibrinolytic activity increased from fasting values after all test meals and did not appear to be affected by postprandial lipemia. These observations are consistent with reports of circadian variations in fibrinolytic activity (14, 15). This increase in fibrinolytic activity is consistent with the decline in PAI-1 activity after the oleate meal and conforms with previous reports by our group in middle-aged subjects (16, 17). These findings suggest that postprandial lipemia induced by oils high in oleate or palmitate does not impair fibrinolytic activity. Kozima et al (18) reported that PAI-1 antigen concentrations were elevated 8 h after the consumption of 100 g butter. However, there was no control treatment for comparison. Oakley et al (13), using a crossover design, compared meals containing 95 g fat provided either by high-oleate sunflower (as in the present study) or a mixture of oleate and medium-chain triacylglycerols or butter fat with a low-fat test meal and found that PAI-1 declined from fasting to the same extent with all 4 treatments.

In contrast with the findings of the present study, Byrne et al (10) reported an increase in PAI-1 activity after a high-fat meal. In that study, the subjects had an in-dwelling catheter and thus were relatively immobile. Because physical activity acutely decreases PAI-1 activity (19), immobility might have accounted for the increase in PAI-1 activity reported in that study. However, the amount of fat used in the test meal was much higher (130 g) than in the present study, and the type of fat also differed (butter fat). In that study, PAI-1 activity differed after the test meal between subjects carrying the 4G allele and subjects homozygous for the 5G allele by an increase of ~4 units/mL. This difference is similar to that in PAI-1 activity between genotypes in the present study.

Our results are consistent with recent reports that there is significant diurnal variation in carriers of the 4G allele compared with subjects homozygous for the 5G allele (20, 21). The reasons for the diurnal variations in PAI-1 activity are uncertain.

A novel observation that requires confirmation was the finding of a lower plasma triacylglycerol concentration in the subjects homozygous for the 5G allele. We did not analyze the data for the comparison in the first study because the subjects were selected on the basis of their nonfasting serum triacylglycerol concentrations.

In common with previous reports, we observed that PAI-1 activity was positively associated with fasting plasma triacylglycerol concentrations (3). However, because the elevation in plasma triacylglycerols after a high-fat meal was accompanied by a decrease in PAI-1 activity, this implies that the association between fasting plasma triacylglycerols and PAI-1 activity is not causal and may be related to the underlying cause of elevated fasting plasma triacylglycerols, most likely insulin resistance. Although we did not measure plasma insulin or glucose in these subjects, we found that FVIIIC, another index of the insulin resistance syndrome, was also positively correlated with PAI-1 activity. These findings are consistent with the observation that weight loss associated with decreased energy intake (4) and regular physical activity (22) results in improvement in fibrinolytic activity.

These data confirm the findings of many previous reports that PAI-1 activity in 5G/5G subjects is 40–50% lower than that in subjects who carry one or more 4G alleles (5, 6, 20, 21). However, the diurnal variations appeared to be greater in subjects with one or more 4G allele. This could be an important source of confounding in studies assessing diet-genotype interactions with regard to PAI-1. Meta-analysis of published studies show that the PAI-1 promoter 4G variant is associated with a 30% higher risk of myocardial infarction than that in 5G/5G subjects (23), with little evidence of heterogeneity of effect in subjects from different countries who might be expected to have different dietary habits. In summary, the data in the present study show that postprandial variations in fibrinolytic activity are modulated by the PAI-1 4G/5G genotype but not by the fat content of a meal.

We are grateful to David Howarth for the measurements of PAI-1 activity. TABS and GJM were the principal investigators and contributed to the design, interpretation, and writing of the manuscript. TdG recruited the subjects, designed the test meals, and undertook the dilute clot lysis activity measurements. JA undertook the PAI-1 genotyping analyses, and SEH contributed to the writing of the manuscript. None of the authors had any conflicts of interests.

REFERENCES


