A low-glycemic-index diet reduces plasma plasminogen activator inhibitor-1 activity, but not tissue inhibitor of protease-1 or plasminogen activator inhibitor-1 protein, in overweight women1–4

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ABSTRACT

Background: The development of obesity has been suggested to involve plasminogen activator inhibitor-1 (PAI-1) and tissue inhibitor of protease-1 (TIMP-1). Plasma PAI-1 is elevated in obesity. A low-glycemic-index (LGI) diet may have a beneficial effect on obesity through a decrease in plasma PAI-1, but whether it affects plasma TIMP-1 in healthy humans has not been studied.

Objective: We investigated whether a 10-wk intake of an LGI or a high-glycemic-index (HGI), high-carbohydrate, low-fat, ad libitum diet is associated with decreases in plasma PAI-1 and TIMP-1 concentrations in overweight women.

Design: Forty-four overweight women [body mass index (BMI; in kg/m²): 27.5 ± 0.23] were randomly assigned to consume an HGI or an LGI diet for 10 wk. A subgroup of 29 women was assigned to participate in an additional 4-h meal test on the last day of the 10-wk intervention.

Results: PAI-1 activity decreased after 10 wk of the LGI diet and was significantly different between groups. Changes in PAI-1 antigen followed the same trend, but no significant difference was observed between groups. No difference in plasma TIMP-1 concentrations was observed between groups. PAI-1 and TIMP-1 concentrations after the 4-h meal test were not significantly different between groups.

Conclusion: An LGI diet reduces fasting plasma PAI-1 activity and therefore may be useful for diminishing the adverse cardiovascular effects of obesity. This trial was registered at clinicaltrials.gov as NCT00324090. Am J Clin Nutr 2008;87:97–105.

KEY WORDS Plasminogen activator inhibitor-1, PAI-1, tissue inhibitor of protease-1, TIMP-1, glycemic index, obesity, women

INTRODUCTION

The glycemic index (GI) is a physiologic measure based on the incremental area under the response curve for blood glucose after ingestion of a given weight of carbohydrate compared with the incremental area under the response curve after ingestion of an equivalent amount of a reference food (glucose or white bread). Foods of low glycemic index (LGI) result in lower, but more sustained, increases in blood glucose and insulin (1). In recent years the clinical relevance of the GI has been vigorously debated. Several studies have described improvements in endpoints related to obesity, diabetes, and cardiovascular diseases after LGI diets (2), although the results are inconsistent.

The development of obesity is characterized by an excessive accumulation and modification of adipose tissue, involving adipogenesis, angiogenesis, and extracellular matrix (ECM) degradation. Two major proteolytic systems, the plasminogen activators and the matrix metalloproteinases (MMPs), have been implicated in several pathophysiological processes involving extensive vascular and ECM remodeling (3–5). Plasminogen activator inhibitor-1 (PAI-1) has been shown to regulate angiogenesis and apoptosis (6, 7), and, as such, it could play an important role as a regulator of adipogenesis. In support of this it has been shown that PAI-1 deficiency in mice protects against fat accumulation induced by a high-fat diet (8, 9), and a synthetic inactivator of PAI-1 has been shown to prevent the development of diet-induced obesity in mice (10). Several studies have shown that PAI-1 is increased in adipose tissue and plasma in obese humans and that fasting plasma PAI-1 concentrations decrease after weight loss (11–13), which suggests a role for this proteinase inhibitor in the development and maintenance of obesity and therefore also the metabolic syndrome.

In contrast, very little is known about the potential involvement of MMPs and their tissue inhibitors (TIMPs) in the development of obesity and the metabolic syndrome. TIMP-1, which inhibits a large array of MMPs, is an interesting candidate in the regulation of fat accumulation. Studies in mice have shown that a deficiency of TIMP-1 impairs nutritionally induced obesity, whereas an overexpression of TIMP-1 has no effect on adipogenesis or adipose tissue development (14, 15). TIMP-1 mRNA
expression is stimulated by glucose (16), and experiments have suggested that PAI-1 and TIMP-1 interact (16). Because TIMP-1 and PAI-1 share many biological functions, they may also interact in the development of obesity and the metabolic syndrome.

A decrease in fasting plasma PAI-1 concentrations after consumption of an LGI diet has been shown in 2 randomized controlled trials involving diabetic patients (17, 18). A 12-mo study in obese young adults showed decreased fasting plasma PAI-1 concentration with a low glycemic load (GL) diet compared with a low-fat diet (19). The effects of the GI of a meal on postprandial plasma concentrations of PAI-1 and TIMP-1 are still unknown.

In the present study we compared the effect of an LGI diet with that of an HGI diet on fasting PAI-1 and TIMP-1 concentrations in plasma. The macronutrient composition, dietary fiber content, and energy density were the same in both diets. In addition, we investigated the effects of an LGI diet compared with those of an HGI diet on plasma concentrations of PAI-1 and TIMP-1 4 h postprandially.

SUBJECTS AND METHODS

Experimental design

The study was a parallel, randomized trial. Two matched groups of nondiabetic obese women participated in a 10-wk intervention in which a major part of the carbohydrate-rich foods in their habitual diet was replaced with either LGI or HGI test foods. Height, weight, blood pressure, heart rate, sagittal height, waist and hip circumferences, and body composition (by dual-energy X-ray absorptiometric scanning) were measured, and fasting blood samples were collected at weeks 0 and 10. At week 0, 5, and 10, all subjects completed 7-d weighed dietary records. On the last day of the intervention, a subgroup from each diet group participated in a 4-h meal test, in which baseline and postprandial energy expenditure, substrate oxidation, appetite ratings, ad libitum energy intake, and blood variables were measured after consumption of a breakfast meal with either an LGI or an HGI carbohydrates. Only measurements relevant to the present study will be described in further detail.

Subjects

The inclusion criteria for the study were as follows: female, age 20–40 y, body mass index (BMI; in kg/m²) of 25–30, body weight fluctuations ≤ 5 kg over the past 2 mo, free of any physiologic or psychological illnesses that could influence the study results, no regular use of medicine other than birth control pills, blood pressure normal to mildly hypertensive (≤159/99 mm Hg), no food allergies, no special diets (eg, vegetarian) or special food dislikes, intake of ≤14 alcoholic drinks/wk (1 drink = 10 g alcohol), nonsmoker (defined as ≤1 cigarette/d), not an elite athlete and not planning to change physical activity during the study, not pregnant and with no planned pregnancy within the study period, not lactating, premenopausal with regular menstrual cycle, and no blood donation within the past 3 mo before entering the study. A subgroup that was representative of the entire group participated in the meal test. Of 55 enrolled subjects, 7 dropped out and 3 were excluded, as described in detail previously (20). Data from one subject (in the LGI group, also participating in the subgroup) was excluded from the analysis because the blood samples showed pathological concentrations of insulin (110 pmol/L) and triacylglycerols (2.9 mmol/L). Characteristics of the 44 subjects completing the study at weeks 0 and 10 are presented in Table 1.

All subjects gave written consent after having received verbal and written information about the study. The clinical study was carried out at the Department of Human Nutrition and was approved by the Municipal Ethical Committee of Copenhagen and Frederiksberg. The study was conducted in accordance with the Helsinki-II declaration (KF 01-249/01).

Diets

10-wk Intervention

The 10-wk intervention and test foods were described in detail previously (20). In brief, the study involved a highly controlled diet. Diets were ad libitum, high-carbohydrate (55–60% of energy as carbohydrate), low-fat (≤30% of energy as fat) diets that were rich in either LGI or HGI foods. The 44 subjects received a certain amount of carbohydrate-rich test foods from the Department of Human Nutrition every week and were instructed to eat a minimal amount of test foods according to a 7-d rotation plan. Instructions were given to note the exact amounts eaten every day by using a digital scale and a food diary. The minimum requirements for intake of the carbohydrate-rich test foods covered 49% of subjects estimated daily energy requirements (based on the assumption that 55% of energy was from carbohydrates, and we wanted to cover ≈75% of carbohydrate intake). Briefly, the test foods consisted of wheat and rye breads in both groups (LGI: whole intact grain; HGI: whole-meal flour, rice (LGI: long-grain; HGI: round-grain), and pasta (LGI) or mashed potato

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>Subject characteristics in the main group before and after a 10-wk high-glycemic-index (HGI) or low-glycemic-index (LGI) diet</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HGI diet (n = 22)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>80.2 ± 1.4</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.6 ± 0.3</td>
</tr>
<tr>
<td>Waist-to-hip ratio</td>
<td>0.80 ± 0.01</td>
</tr>
<tr>
<td>Waist (cm)</td>
<td>86.9 ± 7.3</td>
</tr>
<tr>
<td>Sagittal height (cm)</td>
<td>21.5 ± 0.4</td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg)</td>
<td>76.2 ± 1.8</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>123.7 ± 2.3</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>59.0 ± 1.8</td>
</tr>
</tbody>
</table>

1 All values are ± SD. No differences between groups were observed at 0 wk.

2 ANCOVA with baseline values as covariates.
Total fat 810
Protein 810
Carbohydrates 362
Energy density (kJ/100 g with drinking water) 672
Weight (g/d) 232
Dietary fiber (g/d) 29.3
Water (g/d) 401

**Test meal at the end of the intervention in the subgroup**

The breakfast provided in the meal test consisted of rye bread with a GI of 82 for the LGI group and a GI of 107 for the HGI group served with butter, artificially sweetened strawberry marmalade, cheese, low-fat and low-sugar yogurt with berries, and water. Energy requirements were calculated from body weight, height, age, and physical activity according to the questionnaire of Baekke et al (22) by using the FAO/WHO/UNU formulas (23). The energy content of the meal was designed to provide 20% of the subject’s daily energy requirement adjusted to the nearest 0.5 MJ. The distribution of energy in the test meals was as follows: 57% of energy as carbohydrates, 14% of energy as protein, and 29% of energy as fat; the fiber content was 3.9 and 4.2 g/100 g for the LGI and HGI diets, respectively. The energy density was 659 kJ/100 g for the LGI and HGI groups, respectively. The carbohydrate content of the rye bread constituted 79% of weight of both of the LGI and HGI meals. All nutrient calculations for the meals were done with the computer database of foods from The National Food Agency of Denmark (Dankost 2000) (24).

**Measurements**

**10-wk Intervention**

All measurements were performed in the morning after subjects had fasted for 10 h. Height, body weight, sagittal height, waist and hip circumferences, blood pressure, heart rate, and body-composition measurements were all described in detail previously (20). Fasting blood samples for the measurement of PAI-1, TIMP-1, glucose, insulin, nonesterified fatty acids (NEFAs), triacylglycerols, and total and HDL cholesterol were obtained at weeks 0 and 10. Fasting plasma concentrations of blood lipids, glucose, and insulin before and after the 10-wk intervention period were presented previously (20), but with the inclusion of data from one subject who was excluded from the present study, as described above.

**Test meal at the end of the intervention in the subgroup**

On the last day of the 10-wk study period, the subjects participating in the meal test study arrived in the morning after having fasted for 10 h. A Venflon catheter (reference 391467; Becton Dickinson & Co, Helsingborg, Sweden) was inserted in an antecubital arm vein, and the subjects rested for 15 min before all of the measurements listed above were performed. A breakfast meal was then served, and the subjects were instructed to finish the entire meal within 15 min. Blood samples were collected in the fasting state and 30, 60, 90, 120, 180, and 240 min postprandially. The samples were analyzed for glucose, insulin, NEFAs, and triacylglycerol. PAI-1 and TIMP-1 concentrations were measured in fasting and 240-min samples. Water was provided on request; a maximum of 200 mL was provided during the entire test period.

**Laboratory analyses**

Blood was sampled without stasis through an indwelling catheter. Samples were centrifuged for 15 min at 2800 \( \times \) g and 4 °C within 60 min of sampling, and the supernatant fluid was stored at \(-20\) °C until analyzed. Blood for analysis of PAI-1 and TIMP-1 was collected in iced Biopool Stabilyte tubes (Dandig Pipetter Aps, Copenhagen, Denmark), and the middle third of the supernatant plasma was quick frozen to \(-50\) °C and stored at \(-80\) °C. Blood for glucose analysis was collected in iced tubes containing EDTA prepared with sodium fluoride. Blood for determination of NEFAs was collected in iced tubes containing EDTA. Blood for analysis of triacylglycerol, insulin, and total and HDL cholesterol was collected in plain tubes.

**PAI-1**

PAI-1 antigen concentrations were measured in plasma with an IMUBIND PAI-1 enzyme-linked immunosorbent assay (ELISA) from American Diagnostica Inc (Stamford, CT), which detects latent and active PAI-1 and PAI-1 complexed with tissue-type plasminogen activator and urokinase-type plasminogen activator. Although the plasma was cleansed of platelets, some will have been present and these could be responsible for an elevated concentration of latent PAI-1 in some plasma samples. To avoid the measurement of inactive PAI-1, a Spectrolyse PAI-1 activity chromogenic assay (which does not detect the latent PAI-1) was used.
performed on all plasma samples (American Diagnostica Inc). For both assays, the samples were not diluted, and duplicates of a control plasma pool were included on every assay plate as internal control. All measurements were well within the detection limit.

**TIMP-1**

An established TIMP-1 ELISA (25) was used for measurements of TIMP-1 concentrations in plasma samples. Serial dilutions of duplicates of recombinant TIMP-1 and duplicates of a control plasma pool were included on every assay plate. Plasma samples were diluted 1:100 in sample dilution buffer. All measurements were well within the detection limit. The intraassay CV was previously shown to be 5.3%, and the interassay CV 6.2% (25).

**Glucose, insulin, and lipid concentrations**

Glucose, NEFA, triacylglycerol, and total and HDL-cholesterol concentrations were measured with the use of an enzymatic colorimetric method on a Cobas Mira Plus spectrophotometer (Roche Diagnostic Systems, F Hoffmann-La Roche, Basel, Switzerland). Plasma glucose concentrations were measured with a gluco-quant Glucose/HK kit (GLU Roche/Hitachi 1447513; Roche Diagnostics GmbH, Mannheim, Germany) (26). Plasma NEFA concentrations were measured with a Wako 994-75409 NEFA-C test kit (ACS-ACOD method; Wako Chemicals GmbH, Neuss, Germany). Serum triacylglycerol concentrations were measured with a Triacylglycerol GPOPAP kit (TAG Roche/Hitachi 2016648; Roche Diagnostics GmbH, Mannheim, Germany) (27). Serum total cholesterol concentrations were measured with a CHOL Cholesterol CHOD-PAP kit (Roche/Hitachi 2016630; Roche Diagnostics GmbH). Serum HDL-cholesterol concentrations were measured with the HDL-Cholesterol plus 1930672 kit (Roche Diagnostics GmbH) (28). Serum LDL-cholesterol concentrations were calculated by using the equation of Friedewald et al (29) as described earlier (20). Serum insulin concentrations were determined by using an ELISA technique (AutoDELFIA Insulin kit B080-101; Wallac Oy, Turku, Finland) on an assay system (AutoDELFIA 1235–514; Wallac Oy) (30).

**Statistical analysis**

Descriptive data are reported as means ± SDs, and the results are reported as means ± SEMs. Results were considered significant when the P value was <0.05. Differences between groups in subject characteristics and fasting blood values were analyzed with the use of Student’s unpaired t test. Differences between groups in changes in fasting blood samples from week 0 to week 10 were analyzed by using analysis of covariance (ANCOVA) with baseline values as covariates. In the case of significant differences between groups, a subsequent within-group analysis of differences from week 0 to week 10 was performed. After the exclusion of one subject from the LGI subgroup, body weights between groups tended to be different at week 0, and this became significant by week 10. However, there was no difference between groups in body weight changes from week 0 to week 10. Because of the difference between groups in body weight at week 10, the postprandial changes were analyzed with body weight as a covariate in addition to analyses without body weight as a covariate. Postprandial response curves were evaluated by comparing peak and nadir values by ANCOVA with fasting values and body weight as covariates and time to peak and nadir values by using an unpaired t test. For postprandial response curves, a 2-factor repeated-measures analysis of variance (ANOVA) with group, time, and the group × time interaction as the main factors to test whether entire curves were parallel; a 2-factor ANCOVA was performed with baseline as covariate to test whether postprandial curves were parallel. Analysis with body weight as a covariate was also performed to test the effects independently of body weight changes. When the group × time interactions were not significant, the model was reduced. Residual plots of data were examined to consider homogeneity of variance, Shapiro-Wilk’s test was performed for normal distribution of data, and logarithmic transformation was used when required. Student’s t test analyses were performed by using GraphPad PRISM version 4.02 (GraphPad Software, Inc, San Diego, CA). All other statistical analyses were performed by using SAS version 8.02 (SAS Institute, Cary, NC).

**RESULTS**

Body weight and BMI decreased in both groups after 10 wks intervention with no significant differences between groups, as described previously (20). No significant differences between the LGI and HGI groups were observed in week 0 to week 10 changes in waist-to-hip-ratio, waist circumference, sagittal height, blood pressure, or heart rate (Table 1).

There was a significant difference between groups in fasting plasma glucose, with a significant increase from week 0 to 10 in the LGI group (Table 3). A significant difference in week 0 to week 10 changes in LDL cholesterol between groups was observed (Table 3). A tendency to a larger decrease in total cholesterol after 10 wk of the LGI diet relative to the HGI diet was observed (Table 3). Differences from week 0 to week 10 in any other of the fasting plasma variables were not significantly different between groups (Table 3). In the meal test subgroup at week 10, there were no significant differences between groups in fasting glucose (LGI: 4.82 ± 0.08 mmol/L; HGI: 4.75 ± 0.09 mmol/L; P = 0.57) or insulin (LGI: 26.9 ± 2.6 pmol/L; HGI: 28.9 ± 2.7 pmol/L; P = 0.63) concentrations, but the immediate postprandial glucose and insulin responses were higher in the HGI group than in the LGI group, and visa versa, in the late postprandial period for the glucose response (Figure 1). No differences between groups were observed in the week 10 fasting concentrations of triacylglycerol (LGI: 0.97 ± 0.08 mmol/L; HGI: 1.07 ± 0.06 mmol/L; P = 0.43) or NEFA (LGI: 540.5 ± 33.3 μmol/L; HGI: 583.3 ± 28.9 μmol/L; P = 0.38). Postprandial profiles for triacylglycerol and NEFAs were also not different between groups (Figure 1).

The change in fasting plasma PAI-1 activity from 0 to 10 wk varied between HGI and LGI groups (P = 0.007) (Table 4). PAI-1 activity decreased (P = 0.002) after 10 wk in the LGI group, with no significant changes observed in the HGI group. During the 10-wk intervention, fasting PAI-1 antigen or TIMP-1 changes did not differ significantly between groups (Table 4).

In the subgroup participating in the postprandial study, no differences between groups in fasting PAI-1 or TIMP-1 plasma concentrations were observed either at week 0 or at week 10 (data not shown). A decrease in PAI-1 activity from fasting to 4 h was
observed in the HGI group (Table 5); this observation was independent of body weight. Fasting to 4-h changes in PAI-1 antigen or TIMP-1 concentrations did not differ between the LGI and HGI groups in response to the meal test (Table 5).

**DISCUSSION**

The present study showed that an LGI diet decreased the activity of fasting plasma PAI-1 more so than did an HGI diet. However, even though PAI-1 antigen concentrations followed the same pattern as did PAI-1 activity, neither the LGI nor the HGI diet induced significant changes in the plasma PAI-1 antigen. No changes in plasma TIMP-1 concentrations were observed with either the LGI or HGI diet. The present study was the first to show that a 10-k LGI diet is sufficient to decrease fasting PAI-1 concentrations in overweight but otherwise healthy subjects.

**PAI-1 concentration**

We observed a 15% decrease in fasting PAI-1 activity after only 10 wk of an LGI diet. In an earlier study in healthy obese subjects, a 39% and 58% decrease in plasma PAI-1 antigen concentration was shown after a low-GL diet of 6 and 12 mo, respectively (19). The subjects in that study weighed more (93.3 ± 3.5 kg) and achieved a greater weight loss (−8.4% compared with −2.6%) than did the subjects in the present study. Thus, our results suggest that even less overweight subjects can lower their plasma PAI-1 concentrations after only 10 wk of an LGI diet, and the study by Ebbeling et al (19) suggests that this lowering can be sustained for 12 mo. A greater decrease in PAI-1 activity (38% and 50%) compared with the present study has been reported in 2 other studies (17, 18). However, the subjects in these studies had a significantly higher basal concentration of PAI-1, most probably because of diabetes (31).

The elevated concentration in plasma PAI-1 activity in obese subjects may be a result of a increased amount of hormones, metabolites, and cytokines that are themselves regulated by obesity (eg, tumor necrosis factor-α, insulin, glucose, blood lipids, angiotensin II, and NEFAs) (32–34). Therefore, some of these factors could be responsible for the effect of GI on PAI-1 activity observed in the present study. Because LDL cholesterol showed a tendency to be lower only with the LGI diet, and differed between the LGI and HGI groups, LDL is a possible candidate. No differences between groups in any of the other measured blood lipids were observed in the present study, which suggests no important involvement of any of these factors. The fiber content of the diet has also been shown to be associated with PAI-1 concentrations (35), but, because fiber was controlled for in the present study, this does not explain the observed differences in fasting PAI-1 concentrations. Another factor that has been correlated with PAI-1 concentrations is body weight (11, 36), and adipose tissue has been shown to express and secrete PAI-1 (12). However, there was no significant difference in weight loss between the HGI and LGI groups. This finding is corroborated by a study that included 2 groups, a low-GL diet group and a conventional low-fat-diet group, with no differences between groups in weight loss after a 6-mo intervention. This study found a decrease of ≈58% in the PAI-1 concentration in the LGI group compared with an increase of ≈30% in the conventional low-fat-diet group (19), which further indicates that body weight is not the only factor responsible for a decrease in PAI-1. Because both glucose and insulin are able to increase PAI-1 expression and release (32, 33), these compounds could be responsible for the reduction in PAI-1. The insulin response was lower in the LGI group, and this could be a factor in the reduction in PAI-1 activity.

The observed changes in plasma PAI-1 activity in the present study were not confirmed statistically when PAI-1 antigen was measured in the same samples. This might have been due to the detection of latent, bound, and active PAI-1 when PAI-1 antigen was measured, and the presence of latent and bound PAI-1 might mask any possible changes in PAI-1 activity. However, in the present study, PAI-1 antigen concentrations followed the same trend as observed for PAI-1 activity, which confirmed a decrease in fasting plasma PAI-1 concentrations after an LGI diet.

**Postprandial PAI-1 concentrations**

In the present study, 4-h postprandial concentrations of PAI-1 were found not to differ significantly between groups after either an HGI or an LGI meal. However, a decrease in plasma PAI-1 activity and antigen 4-h after an HGI meal was observed. The 23% decrease in PAI-1 activity is in agreement with the findings of others, who observed decreases in PAI-1 of between 3% and

**TABLE 3**

Results from fasting blood samples in the main group before and after 10 wk of a high-glycemic-index (HGI) or low-glycemic-index (LGI) diet

|                      | HGI diet (n = 22) | LGI diet (n = 22) | P value for difference from week 0 to week 10 between GI groups
<table>
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<tr>
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<tbody>
<tr>
<td></td>
<td>Week 0</td>
<td>Week 10</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Week 0</td>
<td>Week 10</td>
<td></td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>4.79 ± 0.22</td>
<td>4.70 ± 0.18</td>
<td>4.58 ± 0.22</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/L)</td>
<td>1.63 ± 0.07</td>
<td>1.52 ± 0.07</td>
<td>1.53 ± 0.07</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/L)</td>
<td>2.63 ± 0.18</td>
<td>2.68 ± 0.15</td>
<td>2.50 ± 0.18</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>4.75 ± 0.1</td>
<td>4.72 ± 0.07</td>
<td>4.70 ± 0.06</td>
</tr>
<tr>
<td>Insulin (pmol/L)</td>
<td>39.3 ± 3.5</td>
<td>34.1 ± 2.6</td>
<td>36.1 ± 3.3</td>
</tr>
<tr>
<td>Triacylglycerol (mmol/L)</td>
<td>1.18 ± 0.10</td>
<td>1.11 ± 0.06</td>
<td>1.20 ± 0.13</td>
</tr>
<tr>
<td>NEFAs (μmol/L)</td>
<td>479 ± 38</td>
<td>569 ± 26</td>
<td>444 ± 30</td>
</tr>
</tbody>
</table>

1 All values are x ± SEM. No differences between groups were observed at 0 wk.
2 ANCOVA with baseline values as covariates.
3 Significantly different from week 0, *P < 0.05 (paired t test).*
up to 36% in 3-, 4-, and 6-h postprandial blood samples not dependent on the time points (37–39). After observing a higher postprandial insulin response along with a tendency to a higher glucose peak in the HGI than in the LGI group, we expected an elevated concentration of PAI-1 at 4 h. We observed no increased PAI-1 concentrations and speculate that some other factors re-

FIGURE 1. Mean (±SEM) plasma concentrations of glucose, insulin, triacylglycerol, and nonesterified fatty acids (NEFAs) in 29 overweight women after consumption for 10 wk of a high-glycemic-index (HGI; n = 15) or a low-glycemic-index (LGI; n = 14) diet. Data reflect changes from fasting concentrations. A repeated-measures 2-factor ANOVA was performed to test whether entire curves were parallel, and a two-factor ANCOVA with baseline as covariate was performed to test whether postprandial curves were parallel. Analysis with body weight as covariate was also performed to test the effects independently of body weight changes. Peak and nadir values were analyzed by ANCOVA with fasting values as covariates (PROC GLM; SAS Institute Inc, Cary, NC). Time to peak and nadir values was analyzed with the use of a Student’s unpaired t test. Glucose: entire curve including baseline, Pgroup×time = 0.03; with baseline value as covariate, Pgroup×time = 0.08; and with both baseline value and body weight as covariates, Pgroup×time = 0.08; peak, P = 0.07; time to peak, P = 0.37. Insulin: entire curve including baseline, Pgroup×time = 0.01; with baseline value as covariate, Pgroup×time = 0.05; and with both baseline value and body weight as covariates, Pgroup×time = 0.004; peak, P = 0.07; time to peak, P = 0.51. Triacylglycerol: entire curve including baseline, Pgroup = 0.43; with baseline value as covariate, Pgroup = 0.73; and with both baseline value and body weight as covariates, Pgroup = 0.83; peak, P = 0.73; time to peak, P = 0.08. NEFAs: entire curve including baseline, Pgroup = 0.43; with baseline value as covariate, Pgroup = 0.64; and with both baseline value and body weight as covariates, Pgroup = 0.41; nadir, P = 0.53; time to nadir, P = 0.08.

up to 36% in 3-, 4-, and 6-h postprandial blood samples not dependent on the time points (37–39). After observing a higher postprandial insulin response along with a tendency to a higher glucose peak in the HGI than in the LGI group, we expected an elevated concentration of PAI-1 at 4 h. We observed no increased PAI-1 concentrations and speculate that some other factors re-
leased in response to a meal might be able to produce a protecting decrease in plasma PAI-1 concentration. We did observe a decrease in NEFAs in both groups, which could explain at least a part of the decrease in fasting to 4 h postprandial plasma PAI-1 concentrations. The fasting to 4 h postprandial decrease in plasma PAI-1 concentrations in the HGI group might have been partly a result of the postprandial (0 to 2 h) decrease in triacylglycerol in this group (40). Because humans are mostly in the postprandial state, the avoidance of elevated postprandial plasma PAI-1 concentrations might protect against a reduced concentration of fibrinolysis, which could lead to thrombosis and other cardiovascular diseases. Why the postprandial effect of an HGI meal on plasma PAI-1 activity is not sustained and measurable in fasting blood samples cannot be answered by the present study, but these findings raise some interesting questions about how GI affects PAI-1 concentrations over time.

PAI-1 concentrations have mostly been found to decrease after a meal (37, 39), as in the present study, but increases have also been reported (41, 42). The difference in postprandial PAI-1 responses has been explained by differences in PAI-1 genotypes, fat types and content, and physical activity levels and by the fact that PAI-1 has a circadian rhythm (PAI-1 concentration decreases during the day) (38, 43, 44).

### TIMP-1 concentration

We found no difference between the effects of the HGI or LGI diet on the fasting plasma TIMP-1 concentration. No changes in plasma TIMP-1 concentration were observed in response to weight loss. Information on the clinical correlates of plasma TIMP-1 concentrations in humans is limited. In hypertensive patients, both elevated (45) and decreased (46) plasma TIMP-1 concentrations have been reported, and plasma TIMP-1 is elevated in diabetic, hypercholesterolemic, and some cancer patients (25, 47, 48). However, as far as we know, no studies have investigated the role of diet or weight loss on TIMP-1 concentrations in humans. In the present study, we only measured TIMP-1 in plasma so it could be speculated that the expression of TIMP-1 is only affected in tissue and not in blood. Additional work is needed to evaluate the importance of TIMP-1 in obesity.

A positive correlation between TIMP-1 concentration and BMI, cholesterol, and other serum lipids has been observed (48, 49), but the results are inconsistent (50). In the present study the postprandial changes in glucose, insulin, and NEFAs were not associated with any changes in 4-h postprandial TIMP-1 values.

### Conclusion

Although both PAI-1 and TIMP-1 have been suggested to be involved in adipose tissue development (51, 52), and PAI-1 to be decreased during weight loss (52), we showed that plasma TIMP-1 concentrations are not affected by weight loss or GI in slightly overweight healthy women, whereas fasting plasma PAI-1 activity is decreased after 10 wk of an LGI diet with no decrease after the HGI diet. Thus, an LGI diet in overweight adults could be beneficial in regulating fasting concentrations of the cardiovascular disease risk factor PAI-1.

We thank the technical staff at the Department of Human Nutrition for help with data collection and analysis, Christina Cuthbertson for proofreading, and all the subjects for their endurance throughout the study.

The authors' responsibilities were as follows—AR: responsible for the first study protocol; BS, AR, and IK-M: responsible for conducting the trial and for data collection; LJ and NB: responsible for the protease inhibitor analyses; AA: medical counselor for the project; LJ and BS: drafted the first

### Table 4

<table>
<thead>
<tr>
<th></th>
<th>HGI diet (n = 22)</th>
<th>LGI diet (n = 22)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Week 0</td>
<td>Week 10</td>
</tr>
<tr>
<td>PAI-1 activity (U/mL)</td>
<td>10.3 ± 0.8</td>
<td>11.1 ± 0.8</td>
</tr>
<tr>
<td>PAI-1 antigen (ng/mL)</td>
<td>19.8 ± 3.0</td>
<td>20.1 ± 3.1</td>
</tr>
<tr>
<td>TIMP-1 (ng/mL)</td>
<td>47.6 ± 1.1</td>
<td>47.5 ± 1.5</td>
</tr>
</tbody>
</table>

1 All values are ± SEM. No significant differences between groups were observed at week 0.
2 Differences between the LGI and HGI diet groups in changes from week 0 to week 10 tested by 2-factor ANCOVA.
3 Significantly different from week 0 (paired t test).

### Table 5

<table>
<thead>
<tr>
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<th>Fasting</th>
<th>4 h</th>
<th>Δ Fasting − 4 h</th>
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<tr>
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<tr>
<td>PAI-1 activity (U/mL)</td>
<td>12.1 ± 0.8</td>
<td>9.3 ± 0.4</td>
<td>−2.8 ± 0.7</td>
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<tr>
<td>PAI-1 antigen (ng/mL)</td>
<td>19.9 ± 3.2</td>
<td>11.8 ± 2.4</td>
<td>−8.1 ± 1.6</td>
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<tr>
<td>TIMP-1 (ng/mL)</td>
<td>49.4 ± 1.4</td>
<td>46.9 ± 1.5</td>
<td>−2.5 ± 1.2</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Fasting</th>
<th>4 h</th>
<th>Δ Fasting − 4 h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LGI diet (n = 14)</td>
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<tr>
<td>PAI-1 activity (U/mL)</td>
<td>10.8 ± 0.8</td>
<td>10.7 ± 0.7</td>
<td>−0.1 ± 0.6</td>
</tr>
<tr>
<td>PAI-1 antigen (ng/mL)</td>
<td>14.4 ± 1.9</td>
<td>12.4 ± 2.6</td>
<td>−2.0 ± 2.3</td>
</tr>
<tr>
<td>TIMP-1 (ng/mL)</td>
<td>50.4 ± 1.8</td>
<td>49.7 ± 1.9</td>
<td>−0.7 ± 1.0</td>
</tr>
</tbody>
</table>

1 All values are ± SEM. No significant differences between groups were observed at fasting.
2 Differences between the LGI and HGI diet groups from fasting to 4-h changes tested by 2-factor ANOVA (time × treatment interaction).
3 Significantly different from fasting, P < 0.01 (paired t test).


43. Andreotti F, Davies GJ, Hackett DR, et al. Major circadian fluctuations in fibrinolytic activity and the possibility to measure persistent of myo-


