From the Departments of Pathology, Nutritional Research and Clinical Chemistry, University of Umeå, Umeå, Sweden.

**FOOD INTAKE, FIBRINOLYSIS AND RISK FACTORS FOR CARDIOVASCULAR DISEASE**

Studies with special focus on plasminogen activator inhibitor type 1 (PAI-1)

*Birgitta Sundell-Rånby*

Umeå University
Umeå 1993
Er föda skall vara ert läkemedel och ert läkemedel skall vara er föda.

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Birgitta Sundell-Rånby

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ABSTRACT

Food intake, fibrinolysis and risk factors for cardiovascular disease. Studies with special focus on plasminogen activator inhibitor type 1 (PAI-1).

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Elevated plasminogen activator inhibitor (PAI-1) activity levels, hyperlipemia, hypertension, impaired glucose tolerance and obesity, in particular central obesity, are all related to increased risk for the development of cardiovascular disease. Some risk factors are known to be and shown to be influenced by dietary habits. One aim of this study was to determine the distribution of PAI-1 activity and its linkage to serum lipids, body build, glucose and insulin (including glucose tolerance) among healthy men and women. Another aim was to elucidate the effects of different diet programmes on the relationship between PAI-1 activity, serum lipid, glucose and insulin levels.

Two cross-sectional studies, involving 260 individuals, the Norsjö study 1986, the mean PAI-1 activity among 30-60 year-old men was 7.9 U/mL and among women 7.8 U/mL. Both men and women with a body mass index over 27 kg/m² had higher PAI-1 activity, tPA antigen, fasting insulin and insulin responses following an oral glucose tolerance test than persons with body mass index <27. They also had lower HDL-cholesterol. Women with a high waist/hip circumference ratio had a higher mean PAI-1 activity, tPA antigen, triglyceride, blood pressure and insulin response to an oral glucose tolerance test than women with low or normal waist/hip ratio. Men with high waist/hip ratio had higher tPA antigen, glucose and insulin responses to an oral glucose tolerance test than men with low or normal waist/hip ratio.

In two dietary studies different low-energy diets (a juice fast or a weight reduction program) were followed. PAI-1 activity was decreased in both cases. In a third dietary study, transition from a high-fat/low-carbohydrate diet to a low-fat/high-carbohydrate diet decreased PAI-1 activity provided that it did not also cause a substantial increase in triglycerides or glucose. In a fourth dietary study the regular diet was supplemented with oat-husk. PAI-1 activity was reduced; a small increase in glucose but not in triglyceride levels was observed.

On the basis of these results it is concluded that PAI-1 activity levels are associated with constitutional factors such as body mass index and waist/hip ratio. PAI-1 is elevated in obesity. Nutritional factors are also of importance for the PAI-1 activity levels. PAI-1 activity levels can be reduced by dietary regiments such as low-energy diets or high-fiber diets.

Key-words: Body build, diet, fiber, fibrinolysis, glucose, insulin, lipids, obesity, plasminogen activator inhibitor.

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**ABBREVIATIONS**

ADP = adenosine diphosphate
BMI = body mass index
CHO = carbohydrate
GAG = glycosamineglycanes
HDL = high density lipoprotein
LDL = low density lipoprotein
Lp(a) = lipoprotein(a)
PAI-1 = plasminogen activator inhibitor type 1
PUFA = polyunsaturated fatty acids
tPA = tissue plasminogen activator
TXA$_2$ = thromboxane A$_2$
TXA$_3$ = thromboxane A$_3$
uPA = urokinase
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Key-words: Body build, diet, fiber, fibrinolysis, glucose, insulin, lipids, obesity, plasminogen activator inhibitor.
LIST OF ORIGINAL PAPERS

This thesis is based on the following papers, which will be referred to in the text by their Roman numerals.


VI Sundell IB, Rånby M. Oat fiber supplement decreases PAI-1 activity. Haemostasis 1993; 23: 45-50.
INTRODUCTION

Diet and eating habits

In the first Swedish pathology textbook, *Genera Morborum*, published in 1759, Carl von Linné presented a general hypothesis according to which a proper diet, including good eating habits, prevents disease. Linné believed that the human body consists of marrow (brain) and cortex (solids and liquids) of which the solid parts of the cortex are constantly damaged through wear and tear and in constant need of replacement materials. Insufficient diet and bad eating habits deprive the body of necessary replacement materials and hamper the repair processes. Linné thus considered diet and eating habits to be of importance for the maintenance of health. Today, some 200 years later, we no longer describe the body in terms of marrow and cortex, but we generally accept that insufficient diet and bad eating habits deprive the body of fundamental prerequisites for health.

Jean-Jacque Rosseau also recognized the importance of diet. In 1750, he described a proper diet as consisting of fruits, vegetables, herbs and small servings of meat prepared without addition of salt. This is an early description of what we now call a low-salt, low-fat and high-fiber diet.

During the period 1920 to 1978, the average carbohydrate intake in the Swedish diet decreased from 60 to 48 energy% and fat intake increased from 27 to 40 energy% (Hambraeus 1979). The great change in fat intake is partly due to the large increase in butter and margarine consumption -- from 4 to 22 kg/person/year. The consumption of cereals (wheat and rye)
has been cut in half -- from 110 down to 54 kg/person/year; the sugar consumption has increased from 9 to 42 kg, and the pork consumption has increased from 10 to 34 kg. Other basic food items such as potatoes and other meats are consumed in approximately the same amounts.

**Cardiovascular disease**

In ancient Egypt the difference between the blood vessels of old and young humans was observed during mummification procedures (Sinzinger & Widhalm 1981). For a very long time only the morphological changes were discussed and the atherosclerosis was regarded as a degenerative process. The terms “arteriosclerosis” and “atherosclerosis” were first used by Lobstein in 1833 and later by Marchand in 1904. The definition was based solely on morphological observations (McMillan GC 1985). In 1856, Virchow presented a theory according to which the causes of cardiovascular disease are to be found in disturbances in the functions of the plasma proteins, disturbances in the functions of the vessel wall, and/or disturbances in the functions of the blood cells. Virchow’s view continues to be a foundation for our understanding of cardio-vascular disease.

**Plasma proteins**

Already Aristoteles (384-322 B.C.) assumed that blood coagulation was caused by fibrous material in the blood. However, as reviewed by Blombäck 1966, it was not until the 17th century that fibrin, an important plasma protein component of the clot, was described. During the 17th century it was generally believed that coagulation was caused by
aggregation of the red blood cells. Although John Hunter and Hewson believed that blood is a living tissue they reported 1746 on experiments in which plasma samples were separated to obtain "coagulable lymph". We known now that Hunter and Hewson made observations on fibrinogen. Purification and characterization of fibrinogen from human plasmas were later performed by Denis de Commercy (1859) and by Olof Hammarsten (1875). Alexander Schmidt discovered that the substance that causes fibrinogen to clot is an enzyme (thrombin); it converts fibrinogen to fibrin. In 1861 Schmidt presented a first theory of the clotting process in 1861. This theory was modified and extended by Arthus & Pages in 1890 (Ca$^{2+}$ necessary for coagulation), by Hammarsten 1896 (Ca$^{2+}$ only needed for thrombin synthesis) and by Morawitz in 1905 (thrombin is formed from prothrombin in the presence of calcium and/or thromboplasmin) (Blombäck 1966).

The term fibrinolysis was first used by Dastre in 1893 to describe dissolution of fibrin in a coagulated clot. The general features of the fibrinolysis process had been described by Hunter earlier. Fibrinolysis was reported to occur in the blood of humans who had suffered sudden death (Morawitz 1906) and in patients with liver insufficiency (Goodpasture 1914). In a report presented in 1933 fibrin clot lysis was reported to occur when streptococci cultures were added to clotted plasma (Tillett & Garner 1933). This report proved to be important, since it initiated intense research leading to the discovery of the enzyme plasmin. Plasmin resided in blood plasma in the form of an inactive pro-enzyme, plasminogen (Kaplan 1944, Christensen 1945, Christensen & Macleod 1945).
This plasminogen was activated by specific activators of which some were found in plasma (intrinsic plasminogen activators, Astrup & Permin 1947), whereas others were firmly associated to tissue (extrinsic or tissue plasminogen activators) in the vascular endothelium (Todd in 1959). However, details of the components in the fibrinolytic system and their physiological action have only recently been elucidated.

The fibrinolytic process is an important protective effect directed against thrombus formation. It is generally believed that the activity of the fibrinolytic system influences the growth, size and dissolution of the thrombus. The extrinsic fibrinolytic system is described below (Fig 1). As shown in Figure 1 the primary enzyme involved in fibrinolysis, the breakdown of fibrin, is plasmin. The tissue plasminogen activator (tPA) plays a major role in the fibrinolytic system since it converts the inactive plasminogen into the active enzyme plasmin. However, in the absence of fibrin tPA is a poor activator of plasminogen (Rånby & Wallén 1985).

The plasminogen activator inhibitor (PAI-1) is a rapid inhibitor of tPA (Chmielewska et al 1983) and is secreted not only from the endothelium but also from platelets and hepatocytes. The reaction between tPA and PAI-1 is typical of serine proteases and their inhibitors (Lindahl et al 1990). In human plasma the tPA concentration measured as tPA antigen is low, 5 - 10 ng/mL (Rånby et al 1986). Most of the circulating tPA is inactive and bound to PAI-1 (Bergsdorf et al 1983, Kruithof 1987, Sprenger & Kluft 1987), the active fraction was originally estimated to
about 5%. This has proved to be an underestimation due to improper handling of blood samples. When proper sampling procedures were introduced (paper IV) it was revealed that the tPA activity in plasma is about 0.5 IU/mL or about 1 ng/mL of active enzyme. Thus, typically about 15% of the circulating tPA is active. In individuals with high PAI-1 levels the active fraction can be 1% or less (paper IV).

![Diagram of the fibrinolytic system]

**Figure 1.** The fibrinolytic system.

In a series of clinical reports published over the last eight years (Hamsten et al 1985, Nilsson et al 1985, Paramo et al 1985a, Paramo et al 1985b, Wiman et al 1985, Keber & Keber 1992), elevated PAI-1 levels are shown to be associated with thrombotic disorders such as deep venous thrombosis and myocardial infarction. PAI-1 activity determination are routinely performed at a several leading coagulation and cardiology centers.
**Vessel wall**

The arterial wall is prone to the disease of atherosclerosis, and atheromatous plaques lead to the complication of thrombosis. When an atheromatous plaque has become large it can rupture or become ulcerated. Rupture or ulceration are necessary for initiation of thrombus formation (Davies et al 1979). There are two major reasons for plaque rupture: hemorrhage into the plaque (Crawford 1977) and the forces of the contractile movements within the vessel wall (Richardson et al 1989).

Components of the blood respond to rupture or ulceration by activation of the platelets and the coagulation system so that a thrombus forms on the surface of the plaque, causing an incomplete or a complete vessel occlusion. In cases of partial occlusion the thrombus subsequently becomes incorporated into the plaque (Constantinides 1966, Crawford 1977) which increases in size. Atheromatous plaques can be divided into two stages: white fibrous plaques and yellow fatty plaques which may accumulate cholesterol-rich material (Lindop 1985). The latter type is instable and liable to rupture (Richardson et al 1989).

Endothelial cell surfaces are covered with complex carbohydrates such as glycosaminoglycans (often referred to as GAGs). These GAGs act as receptors for lipoproteins, mediating their passage into the cell interior. Interactions of GAGs in the arterial wall and circulating low-density lipoproteins (LDL) are important in the pathogenesis of atherosclerosis (Tracy et al 1965, Bihari-Varga & Gero 1966); complexes of the two are often found in atherosclerotic lesions (Sirivasan et al 1980, Camejo et al 1985). Following an endothelial cell injury the smooth muscle cells of the
vessel wall are exposed to the blood stream. Cell culture studies show that smooth muscle cells and macrophages synthesize chondroitin sulphate proteoglycans whereas the endothelial cells synthesize heparin sulphate proteoglycans (Wight & Hascall 1983, Christner 1988, Vijayagopal et al 1988). In atherosclerotic lesions the concentration of chondroitin sulphate is higher than in normal aorta sections, and this component is likely to form insoluble complexes with LDL (Alves & Mourao 1988). Accumulation of LDL after an endothelial cell injury may promote chondroitin sulfate excretion by proliferating smooth muscle cells. LDL bound to cell receptors is then available for GAG-LDL complex formation in the arterial wall (Horn-Brahimi et al 1988). Macrophages are one of the most important cells in the atherosclerotic plaque. Foam cell formation is enhanced by LDL uptake in the macrophages. After oxidative or enzymatic degradation, which inhibits the interaction with lipoprotein receptors on the arterial wall, LDL is taken up by receptors on the macrophages (Morton et al 1986, Steinberg et al 1989, Kodama et al 1990).

Lipoprotein(a) binds more easily to GAGs than to LDL and may be able to enhance the binding of cholesterol by macrophages (Bihara-Varga et al 1988). Lp(a) is a lipid-protein particle with a spherical structure (diameter 236-255 Å) resembling that of low-density lipoprotein (LDL, diameter 200-225 Å). The density of Lp(a), 1.050 -1.080 g/mL, is greater than that of LDL, 1.021-1.063 g/mL, but not as great as that of high-density lipoprotein (HDL), 1.063-1.221 g/mL. Lp(a) resembles LDL in that it carries one copy of apoprotein B100 embedded in its phospholipid
surface membrane. In contrast to LDL, Lp(a) also carries one copy of an additional specific protein, often referred to as apo(a), (Gaubatz et al 1983). The apo(a) protein gives Lp(a) properties that distinguish it from LDL. Purified Lp(a) is more prone to aggregate in vitro and weakly inhibits fibrin-stimulated tissue plasminogen activator (tPA) mediated plasminogen activation, i.e. it inhibits fibrinolysis (Brändström et al 1988). In cell cultures, Lp(a) interacts more strongly than LDL with membrane structures of skin fibroblasts (Fless et al 1985) and binds to plasminogen receptors on umbilical vein endothelial cells, inhibiting plasminogen binding (Hajjar et al 1989). Levels of Lp(a) greater than 300 mg/L are associated with an increased prevalence of atherosclerosis manifested as an increased risk for cardiovascular disease (Dahlén et al 1986, Utermann 1989, Scanu & Scandiani 1991, Scanu & Fless 1991) and claudicatio intermittens (Dahlén et al 1979). Lp(a) determinations are now routinely performed at several leading lipid and cardiology centers.

**Blood cells**

As an early physiological process to arrest bleeding, platelets accumulate at sites of vascular injury (Weiss 1975); von Willebrand factor plays a key role by forming bridges between platelets and the damaged vessel wall (Ruggeri & Zimmerman 1987). The bridging function of von Willebrand factor is thought to be due to interactions with subendothelial factors such as collagen or heparin-like molecules and surface glycoproteins on the platelets. Exposure of subendothelial structures results in thrombogenic responses, and in pathological situations, platelet
accumulation at sites of atherosclerotic lesions represents the actual event which leads to thrombotic occlusion of the vessel (Fuster et al 1985, Davies et al 1986). It is well known that this process of platelet deposition can be divided into two steps: the initial platelet adhesion (the formation of a platelet layer that covers the lesion), and the platelet aggregation (accumulation of platelets adhering to each other which eventually results in a thrombus). This is illustrated in Figure 2 (page 17).

Platelet-vessel wall interaction plays an essential role in acute coronary syndromes such as unstable angina, myocardial infarction and vascular occlusion (Wilerson et al 1989, Colier 1990, Fuster et al 1990, Maseri 1990). Normally, platelets remain inactivated even under the high shear stress conditions present in the arterial circulation. However, under pathophysiological conditions, platelets can be activated to release a variety of substances such as thromboxane A$_2$ (TXA$_2$) and serotonin, which evoke potent vasoconstriction and further activation of the platelets (De Clerck & Jansen 1990). In vessels with intact endothelium the contraction is inhibited by the simultaneous thrombin-induced release of nitric oxide and prostacyclin (Yang et al 1991), but in injured vessels thrombin promotes further TXA$_2$ release (Yang et al 1991). During platelet activation, thrombin is formed after activation of the coagulation cascade. The production of thrombin converts soluble fibrinogen to a fibrin network (Fig 2).

As previously described, von Willebrand factor mediates adhesion of platelets to the sub-endothelium at the early stages of platelet plug
formation. Once platelets have adhered, they undergo the release reaction, liberating substances such as serotonin, histamine and platelet derived growth factor (Packham et al 1968). In addition, release of ADP and TXA$_2$ results in secondary platelet aggregation causing a platelet plug to grow at the site of damage. The risk factors of smoking, hypercholesterolemia and hypertension may act via the platelets or endothelium or both. Hypercholesterolemic animals have increased platelet deposition at areas where the endothelium has been lost (Ross & Harker 1976). The fatty acid composition of the phospholipids in the platelets appears to be related to platelet activity. Platelets from individuals living in areas with high intake of saturated fats and high incidence of vascular mortality are more susceptible to thrombin than platelets from individuals living in areas with a low clinical incidence (Renaud & Gautheron 1975). Studies of Greenland Eskimos, who have a very low incidence of heart disease, show reduced platelet reactivity and prolonged bleeding times (Dyerberg & Bang 1979). The Eskimos consume a marine diet rich in polyunsaturated fatty acids of the n-3 family (PUFA's), and their platelets release TXA$_3$ upon stimulation instead of the normally released pro-aggregatory TXA$_2$ (Moncada & Vane 1978). There are presently no data available from large scale prospective studies supporting these experimental results.

**Diet and cardiovascular disease**

The relationship between eating habits and health has been well established. Many population studies show a correlation between serum cholesterol levels and incidence of coronary heart disease (Keys 1975).
Reports from the Surgeon General of the United States 1988, the National Cholesterol Education Program 1988 and the National Research Council 1989 show that the risk of developing coronary heart disease can be decreased through dietary intervention. However, in cross-sectional studies, the association between the individual's diet and the cholesterol level has been weak (Gordon 1970, Nichols et al 1976, Gordon et al 1982). This might be due to the fact that the fat intake is above the level where further effects on the cholesterol level can be noted (Connor & Connor 1985). In the Framingham Study 1958-60, fat intake was 37-39 energy% (Dawber et al 1962) and in the Harvard Study 38 energy% (Willett et al 1985, Willett et al 1987). After 5 years of intervention in the Family Heart Study (Connor et al 1992), the number of subjects eating a current American diet (37 energy% fat) decreased from 256 to 139. The subjects that had reduced their fat intake to 25 energy% or less had lower cholesterol and LDL-cholesterol than the subjects still consuming an American diet. In population studies a decrease in fat consumption from 37 to 30 energy% has no effect on the cholesterol level, suggesting that in a diet where 30 energy% is from fat there is no relation between diet and serum cholesterol level. Since diet is the main environmental determinant of serum lipid levels dietary modification is the first approach to be tried in the treatment of hyperlipemia (European Atherosclerosis Society 1988). In cases of atherosclerosis a low-fat diet can reduce atheromas as demonstrated by angiography (Duffield et al 1983, Arntzenius et al 1985, Watts et al 1992). Lipid-rich plaques, also the most likely to precipitate acute coronary thrombosis, have been reported to decrease in size during lipid-lowering trials (Ambrose et al 1988, Hackett et al 1989). Fifteen
subjects with high risk for coronary heart disease were selected for a diet intervention program (Andersen et al 1988). Half of them responded to the dietary treatment by reducing their triglyceride levels by at least 20%. Among the good diet responders the fibrinolytic potential increased.

A reduction of elevated triglyceride levels has also been reported following fish oil and n-3 polyunsaturated fatty acid (PUFA) supplementation (Mehta et al 1987, Beil et al 1991). Lipoprotein(a) (Herrmann et al 1989, Gavish et al 1990, Beil et al 1991) and PAI-1 (Mehta et al 1987) were also reduced during n-3 PUFA supplementation, and the reductions seemed greater the higher the baseline levels were. A major goal for therapies is to prevent occlusive thrombus formation, or to prevent a non-occlusive thrombus from becoming occlusive.
Aims of this study

The aims of this study were:

• to study PAI-1 activity and tPA antigen levels in a population to learn how these fibrinolytic factors are distributed related to age and sex (paper I)

• to study PAI-1 activity and tPA antigen in subjects of different age and sex in relation to serum lipids and obesity (paper II)

• to study the effect of a weight change on PAI-1 activity and serum lipids (paper III)

• to study the effect of a fasting period on PAI-1 and tPA activity levels (paper IV)

• to study the possibility of changing PAI-1 activity through dietary changes (paper V)

• to study if PAI-1 activity, serum lipids and fasting insulin levels are affected by a fiber supplement (paper VI).
Table 1 presents the clinical and biochemical characteristics of the subjects included in the different studies.

**TABLE 1. Characteristics of the subjects in studies I-VI**

<table>
<thead>
<tr>
<th>Study Group</th>
<th>Diet</th>
<th>N</th>
<th>Sex M/F</th>
<th>Age years</th>
<th>BMI kg/m²</th>
<th>PAI-1 act U/mL</th>
<th>tPA ag ng/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>I - II Cross section</td>
<td>-</td>
<td>35</td>
<td>M</td>
<td>30</td>
<td>25.1±2.4</td>
<td>8.2±5.6</td>
<td>6.4±2.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>31</td>
<td>M</td>
<td>40</td>
<td>24.6±3.2</td>
<td>7.3±4.6</td>
<td>7.3±3.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>27</td>
<td>M</td>
<td>50</td>
<td>26.3±3.4</td>
<td>8.4±5.3</td>
<td>9.0±3.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>32</td>
<td>M</td>
<td>60</td>
<td>25.4±2.7</td>
<td>7.7±2.7</td>
<td>9.2±3.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>27</td>
<td>F</td>
<td>30</td>
<td>22.5±2.3</td>
<td>6.1±4.3</td>
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<td></td>
<td></td>
<td>37</td>
<td>F</td>
<td>40</td>
<td>25.4±5.9</td>
<td>6.9±4.0</td>
<td>6.1±3.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>35</td>
<td>F</td>
<td>50</td>
<td>26.3±4.3</td>
<td>7.5±3.9</td>
<td>7.5±3.9</td>
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<tr>
<td></td>
<td></td>
<td>32</td>
<td>F</td>
<td>60</td>
<td>26.1±4.7</td>
<td>10.5±6.6</td>
<td>9.4±4.1</td>
</tr>
<tr>
<td>III Slightly over-weight low-fiber</td>
<td></td>
<td>10</td>
<td>2F 54.2±11.2</td>
<td>26.8±3.4</td>
<td>21.5±18.9</td>
<td>9.5±3.4</td>
<td></td>
</tr>
<tr>
<td>IV Healthy Juice</td>
<td>10</td>
<td>F</td>
<td>44.0±10.3</td>
<td>24.6±3.1</td>
<td>11.4±11.6</td>
<td>7.3±2.9</td>
<td></td>
</tr>
<tr>
<td>V Healthy High- or low-Cho</td>
<td></td>
<td>24</td>
<td>F 23.9±3.5</td>
<td>21.7±2.5</td>
<td>6.0±3.5</td>
<td>3.1±1.1</td>
<td></td>
</tr>
<tr>
<td>VI Healthy +10g oat husk/day</td>
<td></td>
<td>11</td>
<td>9F 31.7±6.4</td>
<td>23.9±2.2</td>
<td>5.8±3.2</td>
<td>6.1±3.0</td>
<td></td>
</tr>
</tbody>
</table>

Data are presented as mean±SD. M=males and F=females. Body mass index (BMI) were calculated using the formula body weight in kg/height in m².

The subjects in studies IV-VI had a mean BMI of 21.7 to 24.6 kg/m², which is in good agreement with the mean BMI of the Norsjö population of...
similar age. Norsjö is a North Swedish inland community with 5500 inhabitants located approx. 600 km northeast of Stockholm and about 200 km northeast of Umeå. It has been selected for the implementation of a health intervention program (1985-1994), which includes a “high-risk strategy” screening for individuals at high risk of cardiovascular disease (Brännström et al 1988). Annually all men and women 30, 40, 50 and 60 years of age during that particular year are invited to participate in the screening.

The subjects in study III had a mean BMI of 26.8 kg/m², slightly over the upper 95% confidence limit in the Norsjö population (26.0 kg/m²) and very close to the upper reference value for BMI of 19-27 kg/m² (Metropolitan Life Insurance Company 1983). The subjects in studies IV-VI also had PAI-1 activity levels in good agreement with the values for individuals of the same age in studies I-II.

**MATERIAL AND METHODS**

**Planning of the diets**

Details of the nutritive content of the diets in studies III-VI are presented in Table 2 (page 25).

**The high-fiber and low-fiber diets (study III)**

Ten subjects consumed 2 different energy-controlled diets (1 week each) during 2 consecutive weeks with a cross-over design. A dietician prepared
the meals and meal boxes were distributed to the subjects. All leftovers were collected and the weight recorded to ensure full compliance. The diets were isocaloric and contained an equivalent energy percent protein, fat and carbohydrate. Only the amount of dietary fiber differed: the high-fiber diet contained 35 g dietary fiber and the low-fiber diet 9 g dietary fiber: the fiber source was brewer’s spent grain (barley). For details see Table 2, page 25.

**The juice fast (study IU)**

Detailed menus describing the drinks, including the volume, were given each subject before the 24-hour fasting period. In addition, the subjects were instructed to drink 2-3L water. All the fruit and vegetable juices were delivered to the subjects to ensure full compliance.

**The high-carbohydrate and low-carbohydrate diets (study IU)**

The study had a cross-over design: 15 subjects started on a high-carbohydrate (high-CHO) diet (60 hours) and 9 started on a low-carbohydrate diet (36 hours). Detailed menus describing the meals and how these were to be prepared, including the weight of each food item, were given to each subject at the start of each period. The high-CHO diet was based on high-starch/high-fiber foods (pasta, rice, bread and potatoes); the low-CHO diet was based on high-fat/high-protein (butter, cream, mayonaise, prawns, meat and fish). The subjects, students at the Department of Home Economics, Umeå University, were highly motivated and could easily follow the menus prescribed.
Normal diet supplemented with oat-husk tablets (study VI)

The subjects were instructed to consume their normal diet throughout the study. In addition they were instructed to consume oat-fiber tablets containing mainly husk. Each tablet contained 0.45 g fiber, 99% of which was insoluble as analyzed according to Asp et al 1983.

### TABLE 2. Mean and standard deviation of the energy and nutrient contents in the diets in studies III-VI calculated for one day.

<table>
<thead>
<tr>
<th></th>
<th>Energy (MJ)</th>
<th>Protein energy%</th>
<th>Fat energy%</th>
<th>Carbohydrate energy%</th>
<th>Dietary fiber, g</th>
</tr>
</thead>
<tbody>
<tr>
<td>High-fiber</td>
<td>12.3±2.1</td>
<td>17±2</td>
<td>28±3</td>
<td>59±4</td>
<td>35±0</td>
</tr>
<tr>
<td>Low-fiber</td>
<td>12.4±2.2</td>
<td>16±2</td>
<td>27±2</td>
<td>58±3</td>
<td>9±1</td>
</tr>
<tr>
<td>(study III)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High-CHO</td>
<td>7.1</td>
<td>12</td>
<td>18</td>
<td>70</td>
<td>17</td>
</tr>
<tr>
<td>Low-CHO</td>
<td>8.5</td>
<td>22</td>
<td>66</td>
<td>12</td>
<td>6</td>
</tr>
<tr>
<td>(study V)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Juice fast</td>
<td>0.7</td>
<td>1</td>
<td>0</td>
<td>99</td>
<td>0</td>
</tr>
<tr>
<td>(study IV)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>8.8±2.0</td>
<td>15±3</td>
<td>30±7</td>
<td>55±7</td>
<td>20±8</td>
</tr>
<tr>
<td>+5g oat husk</td>
<td>7.4±2.1</td>
<td>16±2</td>
<td>29±12</td>
<td>55±13</td>
<td>28±16</td>
</tr>
<tr>
<td>+10g oat husk</td>
<td>7.7±2.1</td>
<td>15±3</td>
<td>29±8</td>
<td>55±10</td>
<td>25±9</td>
</tr>
<tr>
<td>(study VI)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The nutritive content of the diets was calculated with the aid of the Swedish Food Composition tables (SLV 1986). Data are presented as mean±SD.

### Anthropometric and blood pressure determinations

Anthropometric and blood pressure determinations are easy to perform and therefore well suited for large-scale clinical studies and the clinical routine.
Body height, body weight and body mass index

Body height and body weight were measured with the subjects lightly dressed and without shoes. Height was measured to the nearest cm and weight to the nearest 0.1 kg. In studies I-II, the measurements were performed between 08.00 and 16.00 following a 4-hour fasting period. In studies III-VI, the measurements were performed in the morning following an overnight fast.

Body mass index (BMI) is probably the most widely recognized obesity index. It is calculated as body weight divided by height$^2$ and given in kg/m$^2$ (Keys et al 1972, Thomas et al 1976). Ideal values for men and women have been suggested to be in the range of 19 - 27 kg/m$^2$ (Metropolitan Life Insurance Company 1983); this corresponds to the 25th - 75th percentile values recorded in the 1971-74 National Health and Nutrition Survey (Cronk & Roche 1982). Several authors have defined obesity as a BMI of ≥30 (Bray 1978, Thomas et al 1976), a definition also adopted by the World Health Organization (WHO) 1987. The average BMI is about the same for both sexes during the adolescent and young adult years (Cronk & Roche 1982, Rolland-Cachera et al 1982) despite the difference in body fat content. The definition has been questioned since there is no correlation between BMI and fatness in adolescent males (Haschke 1983). However, both in adult men and women the relationship is significant (Revicki & Israel 1986, WHO 1987). In the 1986 Norsjö study the subjects were adults of 30-60 years (Studies I-II).
**Waist and hip circumference measurements (studies I-III)**

Waist and hip circumferences were measured after a 4-hour fast. The subjects were measured in standing position using a soft measuring tape. Waist circumference was measured in the midaxillary line, midway between the lowest rib margin and the iliac crest (WHO 1987). Hip circumference was measured over the widest part of the gluteal region. The waist/hip circumference ratio was calculated (Krotkiewski et al 1983). “High” waist/hip ratio among men is considered to be ≥1.00 and among women ≥0.80. It has been concluded that waist/hip ratio and other measures of obesity are good predictors of poor health (Larsson et al 1984, Lapidus et al 1984).

**Skinfold thickness (studies I-III)**

The method of estimating body fat by skinfold thickness is based on two assumptions: the thickness of the subcutaneous fat mantle reflects the total amount of fat in the body, and the sites chosen for the measurements (singly or together) represent the average thickness of the entire mantle. As reviewed by Lohman 1981, skinfold thickness measurements are a simple, rapid, easily interpreted method to obtain reasonably accurate predictions of body density. However, both biological and technical errors might contribute to the uncertainty of calculation of body density and fatness from skinfold measurements. Lohman has estimated the error to be approx 3.3%. In studies I-II, skinfold thickness was measured on two of the most commonly used sites: the abdomen at the periumbilical region and the upper arm over the triceps midway between elbow and shoulder using a Harpenden caliper (HE Morse Co, Holland, Michigan, USA).
Blood pressure measurements (study 1)

Blood pressure was measured on the right arm with a blood pressure cuff (12 cm) connected to a mercury column, performed twice after a 5-min rest in the sitting position, and finally after a 5-min bed rest. The latter value was recorded for this study.

Accuracy and precision of plasminogen activator inhibitor-1 (PAI-1) activity determination

The existence in plasma of specific inhibitors of plasminogen activators was suspected for more than 20 years, but their discovery eluded investigators for decades. This was due to a combination of two circumstances: sensitive and specific assay methods for plasminogen activator (PA) in plasma were not developed, and the specific PA-inhibitors occurred in surprisingly low concentrations. The availability of highly purified plasminogen activators, urinary plasminogen activator (uPA or urokinase) and tissue plasminogen activator (tPA) and antibodies against these proteins represented a considerable advance. However, even with these tools, straightforward attempts to detect specific PA inhibitors failed. The first clear evidence for the existence of a rapid inhibitor to tPA in plasma came from researchers engaged in the problem of specific and accurate determination of tPA activity (Chmielewska et al 1983, Kruithof et al 1984, ). Once clearly identified, the rapid plasma inhibitor of tPA, named PAI-1, was shown to be closely related to an inhibitor isolated from bovine endothelial cell cultures (Loskutoff & Edgington 1976) and identical to an
inhibitor from human umbilical vein endothelial cell cultures (Dosne et al 1978). However, the poor methodology in use in fibrinolytic research continued to cause some investigators to raise doubts concerning the feasibility of PAI-1 activity determinations (Samama et al 1985) and prompted others to make ad hoc postulations on the existence of additional PA-inhibitors.

The reaction between tPA and PAI-1 is typical of serine proteases and their inhibitors (Lindahl et al 1990). An initial, tight, inactive 1:1 complex is formed through a reversible reaction between tPA and PAI-1. The reaction is characterized by a second-order reaction constant of about \(10^7 \text{M}^{-1}\text{s}^{-1}\) in the forward direction (Chmielewska et al 1983, Kruithof et al 1984, Lindahl et al 1990). The initial reversible complex is rearranged by an incomplete proteolytic reaction to form a more stable complex (Lindahl et al 1990). Although the reaction between tPA and PAI-1 is very fast, the low levels of tPA and PAI-1 in plasma still necessitate many minutes for completion. The PAI-1 activity level is defined by the amount of tPA that quenches the fast kinetics described above. This definition that intimately links the PAI-1 activity unit to the tPA activity unit, has attained general acceptance (Loskutoff & Edgington 1976, Dosne et al 1978, Chmielewska et al 1983, Emeis et al 1983, Kruithof et al 1984, Gaffney & Curtis 1985, Hamsten et al 1985, Nilsson et al 1985, Paramo et al 1985a, Paramo et al 1985b, Samama et al 1985, Wiman et al 1985, Eriksson et al 1988, Lindahl et al 1990, Rånby et al 1990) and focuses interest on tPA activity standards.
The first internationally recognized standard preparation for tPA activity was made available in 1984 through the courtesy of Dr Patrik Gaffney of the National Institute for Biological Standards and Control, South Mimms, London (Gaffney & Curtis 1985). This international tPA standard, coded 83/517, and its successor, the second international standard, coded 86/670, provided a foundation for quantitative determination of tPA activity and PAI-1 activity. Highly purified human one-chain tPA preparations typically show a specific activity of 600 000 IU per mg of the 64-kD glycosylated tPA moiety. One IU/mL of tPA is equivalent to 26 pM and, with the definitions given above, 1 U/mL of PAI-1 is also equivalent to 26 pM. This, in turn, is equivalent to 1.66 ng/mL of active 64-kD tPA and 1.35 ng/mL of active glycosylated 52-kD PAI-1. It also follows that the specific activity of fully active 52-kD PAI-1 is 0.740 U/ng and fully active 42.5-kD PAI-1 0.900 U/ng.

RESULTS AND DISCUSSION

PAI-1 activity, tPA antigen and serum lipids; relation to sex and age (studies I-III)

As shown in study I, blood plasma PAI-1 levels in Swedish people are skewed, with a mean of approx. 8 U/mL for both males and females. Individuals with elevated levels of PAI-1 are typically defined as those who have more than 0.4 nM or more than 20 IU/mL of PAI-1 in their plasma (Rånby et al 1990). In study I we found no age-dependent PAI-1 activity increase in males but the levels increased after 50 years of age in females (Table 1, page 22 & Study I, Fig 1). In a healthy population PAI-1
activities among 20-49-year-old men were higher than among women of
the same age (Rånby et al 1990). PAI-1 activity levels in men and women
50-59 years of age did not differ. In some studies PAI-1 activity has been
found to increase with age, at least in women (Aillaud et al 1986,
Hashimoto et al 1987), and in some no age-related increases have been
observed (Stegnar & Pentek 1992). Consistently increased levels of PAI-1
activity (Scarabin et al 1990a) and factor VII coagulant activity (Meade et
al 1983, Balleisen et al 1985, Scarabin et al 1990b) have been reported in
postmenopausal as compared with premenopausal women. These
observations may contribute to the increased risk for coronary heart
disease in the postmenopausal state (Gordon et al 1978, Colditz et al
1987).

The mean plasma tPA antigen levels in a Swedish population were
7.9 ng/mL among men and 7.1 ng/mL among women (Study I, Table 1). In
both males and females tPA antigen levels were correlated with age;
males between 30 and 50 years of age tended to have higher tPA levels
than females (Table 1 & Study I, Fig 1). In a cross-section sample of men
and women 30-60 years of age (Study II) tPA antigen levels were found to
correlate positively with total cholesterol, triglycerides and inversely
with HDL-cholesterol. In multiple regression analysis, however, only
triglycerides were found to contribute significantly to the variance of tPA
antigen levels when BMI and abdominal skinfold thickness were entered
into the model (Study II). In a previous population study using multiple
regression analyses with tPA antigen as dependent variable there were
significant associations with age, sex, BMI, total cholesterol, insulin and
HDL-cholesterol observed (Stegnar & Pentek 1992). An age-dependent increase in tPA antigen levels up to 10 ng/mL has been reported (Rånby et al 1986). In a population study of 100 men and 91 women aged 19-80 years old, tPA antigen was found to increase with age from 3.8 ng/mL among 19-30-year-olds to 9.3 ng/mL among 71-80-year-olds (p<0.01) (Stegnar & Pentek 1992); women had approx. 25% lower tPA antigen levels than men. Fibrinolytic response measured as the absolute amount of tPA antigen after venous occlusion increased with increasing age; it was also higher in men than in women (Stegnar & Pentek 1993). Most early studies showed that fibrinolytic activity was unrelated to age (Sawyer et al 1960, Moser & Hajjar 1966, Rosing et al 1973), but contrary findings have also been reported (Meade et al 1975). As reviewed by Ogston, fibrinolytic activity was generally believed to be similar in men and women (Ogston 1983).

Lipoprotein(a) levels among the 30-60-year-old men and women living in Norsjö are presented in Table 3 (page 33). The distribution was highly skewed with a mean of between 109 - 142 mg/L (study I and unpublished data from samples obtained in 1987). Lp(a) was largely unrelated to glucose and insulin responses to an oral glucose tolerance test and to anthropometric variables (Table 5, Studies I-II). Only age and serum cholesterol were related to Lp(a) concentration. In study V Lp(a) was also unrelated to the other measured variables and was not affected by the dietary change. Scanu (1992) points out that there are marked inter-individual differences in Lp(a) concentrations (up to 1000 fold) but the level is relatively stable in the same individual. Cardiovascular disease is...
increased in patients in whom the plasma levels of the whole Lp(a) lipoprotein molecule are above 250 to 300 mg/L.

**TABLE 3.** Plasma levels of lipoprotein (a) [Lp(a)] among 500 subjects aged 30, 40, 50 or 60 years.

<table>
<thead>
<tr>
<th>Plasma Lp(a) conc, mg/L</th>
<th>AGE, years</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>30</td>
</tr>
<tr>
<td>mean±SD</td>
<td>108.8±118.0</td>
</tr>
<tr>
<td>n</td>
<td>123</td>
</tr>
<tr>
<td>range</td>
<td>9.0-683.0</td>
</tr>
<tr>
<td>skewness</td>
<td>1.95</td>
</tr>
<tr>
<td>kurtosis</td>
<td>4.57</td>
</tr>
</tbody>
</table>

Total serum cholesterol and triglyceride levels were higher in individuals 60 years of age as compared with the 30- and 40-year-olds (Figs. 3 & 4 page 34 & Study II). In both men and women cholesterol and triglyceride levels are significantly correlated to age. Age has previously been shown to be strongly positively correlated to serum cholesterol, triglyceride and inversely correlated to HDL-cholesterol levels (Hopkins et al 1989). On the average, females had lower triglyceride and higher HDL-cholesterol levels than males.

**PAI-1 activity, tPA antigen, serum lipids and body build:**

**Interrelationships (studies I-II)**

In studies I-II PAI-1 activity among women was correlated to BMI, waist/hip ratio, abdomen and triceps skinfold thicknesses, total cholesterol, triglycerides, tPA antigen and inversely correlated to HDL-cholesterol levels. In men PAI-1 activity was correlated to BMI, waist/hip
**Figure 3.** Serum triglyceride concentration in 260 subjects at 30-60 years of age. The male/female ratio was 26/36 among 30-year-olds, 31/32 among 40-year-olds, 27/35 among 50-year-olds and 34/33 among 60-year-olds. ■ denotes men and □ denotes women. *=p<0.05 and **=p<0.01 when compared with the 60-year-olds.

**Figure 4.** Serum cholesterol □ and HDL-cholesterol △ concentration in 260 men and women at 30-60 years of age. Solid symbols=males and open symbols=females. For further explanation see legend to figure 3.
ratio, triglycerides, tPA antigen and inversly correlated to HDL-cholesterol levels. In all individuals (study II) PAI-1 activity was positively related to BMI, waist/hip ratio, abdomen and triceps skinfold thicknesses. BMI has previously been shown to be strongly positively correlated to serum cholesterol, triglyceride and inversely correlated with HDL-cholesterol levels (Hopkins et al 1989).

Several studies show that fibrinolytic activity is lower in obese than in non-obese subjects (Goldrick 1961, Fearnley et al 1963, Ogston & Mc Andrew 1964; Bennet et al 1966, Meade et al 1979, Craveri et al 1990). Obesity and elevated PAI-1 activity levels are also associated (Vague et al 1986, Juhan-Vague et al 1987b, Craveri et al 1990). The fibrinolytic response, measured as the absolute amount of tPA antigen after venous occlusion, was higher in obese than in slim subjects (Stegnar & Pentek 1993).

The mean BMI±SD among the men was 25.3±2.9 and among the women 25.2±4.8 kg/m$^2$ (Study I). The 50-60 year old men and women in Norsjö have a mean BMI in good agreement with earlier reported population data. In Norway mean BMI is 25.3 kg/m$^2$ among men and 26.7 among women (Waal 1984). In Gothenburg, Sweden the corresponding values are 25.0 among men (Larsson et al 1984) and 25.2 kg/m$^2$ among women (Noppa et al 1979).

Among women with a BMI>27 PAI-1 activity, tPA antigen, triglycerides, fasting insulin, systolic and diastolic blood pressures and insulin
TABLE 4. Fasting plasma PAI-1 activity, tPA antigen, serum lipids, glucose, insulin levels, blood pressures, glucose and insulin responses to an OGTT among men and women with high or low BMI.

<table>
<thead>
<tr>
<th></th>
<th>MEN</th>
<th>WOMEN</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BMI&lt;27 n=88</td>
<td>BMI&gt;27 n=32</td>
</tr>
<tr>
<td>PAI-1 act, U/mL</td>
<td>7.10±3.99</td>
<td>9.96±5.75*</td>
</tr>
<tr>
<td>tPA ag, ng/mL</td>
<td>7.07±2.86</td>
<td>10.40±3.45**</td>
</tr>
<tr>
<td>Cholesterol, mmol/L</td>
<td>6.52±1.47</td>
<td>6.74±1.10</td>
</tr>
<tr>
<td>HDL-chole, mmol/L</td>
<td>1.43±0.38</td>
<td>1.24±0.32*</td>
</tr>
<tr>
<td>Triglyceride, mmol/L</td>
<td>1.45±0.92</td>
<td>1.88±0.82</td>
</tr>
<tr>
<td>Lp(a), mg/L</td>
<td>139.5±151.5</td>
<td>78.2±94.2*</td>
</tr>
<tr>
<td>Syst BP, mm Hg</td>
<td>125±19</td>
<td>126±13</td>
</tr>
<tr>
<td>Diast BP, mm Hg</td>
<td>79±11</td>
<td>82±9</td>
</tr>
<tr>
<td>Glucose, mmol/L</td>
<td>4.5±0.7</td>
<td>5.4±2.9</td>
</tr>
<tr>
<td>Insulin, µU/mL</td>
<td>6.61±1.63</td>
<td>11.31±12.29*</td>
</tr>
<tr>
<td>Waist/hip ratio</td>
<td>0.918±0.053</td>
<td>0.969±0.046**</td>
</tr>
</tbody>
</table>

Responses to an OGTT

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose, mmol·h/L</td>
<td>11.3±2.8</td>
<td>12.2±3.0</td>
</tr>
<tr>
<td>Insulin, µU·h/mL</td>
<td>50.7±26.3</td>
<td>86.9±46.5**</td>
</tr>
</tbody>
</table>

Results by unpaired t-test. * p<0.05 and ** p<0.01 as compared with the individuals of the same sex but with BMI<27 kg/m². Data presented as mean±SD.
responses to an OGTT were elevated (Table 4, page 36). They also had lower HDL-cholesterol levels.

Among men with a BMI $\geq 27$ PAI-1 activity, tPA antigen, triglycerides, fasting insulin, Lp(a) and the insulin response to an OGTT were greater than in men with BMI $< 27$ (Table 4, page 36).

Larsson et al (1984) found average waist/hip ratio to be $0.927 \pm 0.054$ (SD) among 792 men in Gothenburg. In study I, Norsjö 1986, waist/hip ratios (mean $\pm$ SD) were $0.931 \pm 0.056$ in men and $0.815 \pm 0.066$ in women. The distribution of waist/hip ratio is Gaussian.

In women with a waist/hip ratio $\geq 0.80$ insulin responses to an OGTT were larger, systolic and diastolic blood pressures, triglyceride, PAI-1 activity, tPA antigen levels were higher and HDL-cholesterol lower than in women with a waist/hip ratio $< 0.80$. Blood pressures have previously been reported to vary with waist/hip ratio among women (Lapidus et al 1984), and obese women with central obesity are known to have higher fasting insulin, glucose, triglyceride and PAI-1 activity levels than obese women with low waist/hip ratios (Landin et al 1990).

In men with a waist/hip ratio $\geq 1.00$ both glucose and insulin responses to an OGTT were greater, and tPA antigen levels higher than in men with a waist/hip ratio $< 1.00$ (Table 5, page 38).

Obese men and women with a waist/hip ratio over the average had higher
TABLE 5. Fasting plasma PAI-1 activity, tPA antigen, serum lipid, glucose, insulin levels, glucose and insulin responses to an OGTT, and blood pressures in men and women with high or low waist-to-hip girth ratio.

<table>
<thead>
<tr>
<th></th>
<th>MEN</th>
<th>WOMEN</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>w/h&lt;1.00 n=110</td>
<td>w/h&gt;1.00 n=12</td>
</tr>
<tr>
<td>PAI-1 act, U/mL</td>
<td>7.59±4.32</td>
<td>10.25±6.96</td>
</tr>
<tr>
<td></td>
<td>tPA ag, ng/mL</td>
<td>7.76±3.36</td>
</tr>
<tr>
<td>Cholesterol, mmol/L</td>
<td>6.54±1.39</td>
<td>7.00±1.33</td>
</tr>
<tr>
<td>HDL-chole, mmol/L</td>
<td>1.40±0.38</td>
<td>1.24±0.33</td>
</tr>
<tr>
<td>Triglyceride, mmol/L</td>
<td>1.53±0.91</td>
<td>1.93±0.88</td>
</tr>
<tr>
<td>Lp(a), mg/L</td>
<td>124.2±138.5</td>
<td>116.4±159.6</td>
</tr>
<tr>
<td>Syst BP, mm Hg</td>
<td>125±18</td>
<td>129±14</td>
</tr>
<tr>
<td>Diast BP, mm Hg</td>
<td>79±11</td>
<td>85±12</td>
</tr>
<tr>
<td>Glucose, mmol/L</td>
<td>4.6±1.3</td>
<td>5.7±3.7</td>
</tr>
<tr>
<td>Insulin, µU/mL</td>
<td>7.76±7.07</td>
<td>9.07±3.32</td>
</tr>
<tr>
<td></td>
<td>Responses to an OGTT</td>
<td></td>
</tr>
<tr>
<td>Glucose, mmol·h/L</td>
<td>11.3±2.8</td>
<td>13.6±3.5*</td>
</tr>
<tr>
<td>Insulin, µU·h/mL</td>
<td>55.2±29.5</td>
<td>108.6±58.9*</td>
</tr>
</tbody>
</table>

Results by unpaired t-test. (* p<0.05 and ** p<0.01 as compared with individuals of the same sex but with a lower waist/hip (w/h) girth ratio. Data presented as mean±SD.)
fasting insulin and glucose and greater responses to an oral glucose tolerance test (Krotkiewski et al 1983). Both among men (Larsson et al 1984) and women (Lapidus et al 1984), high waist/hip ratio is related to increased morbidity and mortality from heart attack or stroke. Waist/hip ratio is associated with high PAI-1 activity levels (Landin et al 1990, Vague et al 1989, De Pergola et al 1992). In lean women, however, waist/hip ratio is not related to impaired fibrinolysis to the same extent as in obesity (Landin et al 1990, De Pergola et al 1992). Metabolic status may influence the relationship between PAI-1 and obesity. In normal-weight women depleted of glycogen stores, waist/hip ratio was related to PAI-1 activity (Sundell et al 1988). When the same women had repleted glycogen stores, PAI-1 and waist/hip ratio were unrelated. It has been shown that insulin resistance is related to increased PAI-1 activity levels, but PAI-1 and fasting insulin levels are also correlated in normal populations (Vague et al 1986, Juhan-Vague et al 1987b, Sundell et al 1988).

**Fibrinolytic factors, serum lipids, BMI and eating habits**

A frequent intake of fruit, vegetables and root vegetables was associated with low PAI-1 activity levels (Nilsson et al 1990). Frequent fruit intake is also related to low triglyceride levels, BMI, age, cholesterol and tPA antigen. Fruits, vegetables and root vegetables are high-fiber foods and effects of dietary fibers on PAI-1 activity were also observed in study VI. A decrease in triglyceride concentration associated with eating more fruit and vegetables has been observed in a population study (Hopkins et al 1989).
Data obtained in population surveys generally evaluate current food intake, biochemical data reflect recent nutritional status, and clinical and anthropometric examinations evaluate more long-term nutritional history. In spite of this, relations between dietary and biochemical data and clinical findings have been shown (Kerr et al 1982, Wenger et al 1979).

**Diet-induced changes in metabolic status, PAI-1 activity and serum lipids (studies III & V)**

The changes in body weight following energy-controlled diets (study III) were correlated with changes in PAI-1 activity ($r=0.68$, $p<0.01$) and with changes in triglycerides ($r=0.55$, $p<0.05$). The changes in PAI-1, triglycerides, total cholesterol and FFA were not correlated. In study V, 24 healthy young subjects volunteered to maintain a high-fat/low-carbohydrate (CHO) diet followed by a low-fat/high-CHO diet or vice versa in a cross-over design. Diet-induced changes in PAI-1 activity and changes in triglycerides ($r=0.53$, $p<0.05$) and glucose ($r=0.52$, $p<0.05$) were correlated. Following the high-fat/low-CHO diet PAI-1 activity was $7.1\pm3.9$ U/mL as compared with $6.0\pm3.5$ U/mL ($p=0.058$) following the low-fat/high-CHO diet. This tendency towards higher PAI-1 activity at the end of the high-fat/low-CHO diet might be explained by the extremely high fat content, 66.6 energy% (Sundell et al 1988). Postprandial fibrinolytic activity is lower following a high-fat meal than following a low-fat meal (Greig 1956).

An increase in vascular plasminogen activator level and a reduction of inhibitors of plasminogen activator have been reported following a
2-week supplementation with 5 g omega-3 polyunsaturated fatty acids (Barcelli et al 1985). In the diet-anti-smoking trial of the Oslo Study the incidences of myocardial infarction and sudden death were lower in the intervention group as compared with the controls (Hjermann et al 1981). The diet intervention was aimed at lowering the fat intake and increasing dietary fiber intake. On further examination of a subgroup of 42 subjects (Andersen et al 1983) and in subjects with elevated triglyceride levels (Andersen et al 1988), clot lysis time and serum triglyceride levels were found to be correlated. Reduction of elevated triglyceride levels resulted in a fibrinolytic activity increase (Andersen et al 1983, Andersen et al 1988).

Effects of low-energy intake (studies III-IV)

During a 24-hour fast the tPA activity increased and PAI-1 activity decreased. The subjects' tPA or PAI-1 antigen levels were not affected. Following a fasting period, PAI-1 activity falls at the same time as euglobulin fibrinolytic activity increases (Juhan-Vague et al 1987b). In study IV PAI-1 activity was decreased in 9 of the 10 subjects during the fast, whereas in one subject PAI-1 activity was unchanged. That it is possible to reduce PAI-1 activity without affecting PAI-1 antigen was also observed in Study VI. However, since PAI-1 antigen reflects the total mass of PAI-1 present, after a longer diet regimen, there would most likely be a correlation between PAI-1 activity and antigen levels.

During a 2-week experiment slightly obese subjects consumed two different energy-controlled diets, one high in fiber and one low in fiber.
The subjects' mean body weight dropped from 78.6±13.2 to 77.6±12.5 kg 
(p<0.05). No significant drop in PAI-1 activity over the 2 study weeks 
(21.5±18.9 to 12.3±8.1 U/mL) was observed. One subject was found to have 
pathological liver function tests. That subject had, following 3 weeks' of 
extensive alcohol abuse, a PAI-1 activity level of 71.3 U/mL which 
decreased rapidly to 22.1 U/mL after the first week and to 14.1 U/mL 
after the second week of the diet experiment. After that subject was 
excluded, the means were still not significantly different (16.0±7.5 vs 
11.7±4.9 U/mL). The subjects' mean PAI-1 activity level was slightly over 
the mean among 50-year-old men and women in study I: 8.4±5.3 vs 7.5±3.9 
U/mL, but their BMI was in good agreement with the BMI of the population 
(Table 1, page 22). The body weight changes correlated positively with 
both PAI-1 activity changes (r=0.68, p<0.01, n=18) and with tPA antigen 
changes (r=0.60, p<0.05, n=18).

Subjects with inflammatory bowel disease (Mb Crohn or ulcerative 
colitis) have the same basal PAI-1 activity and tPA antigen levels as 
healthy subjects (Gris et al 1990). However, when subjects with 
inflammatory bowel disease were compared with controls following a 
venous occlusion test, the patients with bowel disease had a less marked 
tPA antigen increase and PAI-1 activity decrease. Venous thrombosis is a 
common complication in inflammatory bowel disease (Koenigs et al 1987, 
Sisbegee & Rottenberg 1978), especially in association with exacerbation 
of the disease (Talbot et al 1986).

In this case it is interesting that a body weight change is correlated with
the change in PAI-1 activity, although the PAI-1 change did not reach statistical significance \((p=0.09)\). It is not possible to rule out the influence of nutritional status on PAI-1 activity levels through energy-controlled diet in subjects with inflammatory bowel disease.

**Effects of a high-carbohydrate and high-fiber diet (studies V-VI)**

A second-degree polynomial response surface model suggested that transition from a high-fat/low-carbohydrate to a low-fat/high-carbohydrate diet is associated with reduced levels of PAI-1 provided that glucose and triglycerides are not elevated more than 1.2 and 0.5 mmol/L, respectively (study V). In study VI 11 healthy subjects were instructed to consume oat-husk tablets as a supplement to their ordinary diet. During a 5-week adaptation period they consumed 5 g oat husk/day. PAI-1 activity was unaffected following the 5-g/day supplement but the triglyceride and insulin levels were even higher than at entry. Dietary carbohydrates may increase triglyceride levels during a short period (Gonen et al 1981, Ullman et al 1991). Immediately after the 5-week adaptation the subjects began consuming 10-g/day for a further 2-week experimental period. Following the 10-g/day oat-husk supplement, PAI-1 activity was reduced by 50%, and serum lipids, insulin and glucose levels were back at baseline. Six weeks after discontinuation of the oat-husk supplement PAI-1 activity went back to baseline levels. High consumers of high-fiber foods such as fruits, root vegetables and vegetables have lower PAI-1 activities (Nilsson et al 1990).

Some dietary fibers have previously been reported to reduce serum
cholesterol and LDL-cholesterol levels (Kirby et al 1981, Anderson & Chen 1983, Anderson et al 1984, Anderson 1987, Anderson & Gustafson 1988, Cerda et al 1988, Superko et al 1988, Demark-Wahnefried et al 1990), and high-fiber foods are considered to be favorable in preventing coronary atherosclerosis. Epidemiological data suggest that in populations eating high-fiber diets, coronary artery disease is less common (Liu et al 1979, Kromhout et al 1982, Kushi et al 1985, Kris-Etherton et al 1988, Burr et al 1989). In study IV the subjects' serum cholesterol or LDL-cholesterol levels were not affected by the fiber supplement. Insoluble fibers such as the fibers used in this study have previously been reported to have a modest effect on serum cholesterol levels but they significantly increase the stool weight (Eastwood et al 1983). Insoluble fibers are known to increase fecal bulk 4 fold (Madar & Odez 1990) and decrease transit time (Staniforth et al 1991). The increase in fecal weight has been suggested to stimulate colonic motility by producing volatile fatty acids in the colon (Eastwood 1987).

Triglyceride level was also unaffected by the supplement. A negative correlation between fibrinolytic activity and hyperlipemia has been proposed repeatedly (Grieg 1956, Ogston & Fullerton 1962, Howell 1964, Skrzydlewski & Niewiarowski 1967, Grace & Goldrick 1968, Andersen 1976). In many previous studies a low fibrinolytic activity among hyperlipemic patients has been shown to increase when the subjects reduced their lipid levels; body weight was also reduced (Elkeles et al 1980, Simpson et al 1983, Andersen et al 1988). In study III the subjects' PAI-1 activity and body weight were reduced but serum triglyceride

**Perspectives**

Diet intervention can improve fibrinolysis. However, the exact mechanism of this is still largely unknown. Some general dietary guidelines can be given from the present knowledge. In Western populations total fat and cholesterol in the daily diet should be reduced and the consumption of carbohydrates, mainly dietary fibers, should be increased. In general, high-fiber diets are recommended for the prevention of cardiovascular disease, cancer and for metabolic control of diabetes. It is well known that disorders such as insulin resistance in obesity and type II diabetes are associated with elevated PAI-1 activity levels. Irrespective of the pathways involved it is clear that there are links between risk factors for atherosclerosis and fibrinolytic activity. Treatment of patients should aim at normalization of the whole metabolic risk factor profile in which dietary treatment is one important component.
CONCLUSIONS

- PAI-1 activity in the Norsjö population was skewed with a mean of 7.9 U/mL among men and 7.8 U/mL among women. PAI-1 activity were higher in 60 year old women compared with 60 year old men.

- tPA antigen level in the Norsjö population was 7.9 ng/mL among men and 7.1 ng/mL among women.

- Both in men and women with BMI ≥27 kg/m², PAI-1 activity, tPA antigen, fasting insulin levels and insulin responses to an OGTT were higher than in men and women with a BMI<27 kg/m².

- Among women with a waist/hip ratio ≥0.80, PAI-1 activity, tPA antigen, triglyceride, insulin response to an OGTT, systolic and diastolic blood pressures were higher than among women with a waist/hip ratio <0.80. HDL-cholesterol was lower in the high waist/hip ratio group.

- Among men with a waist/hip ratio ≥1.00, tPA antigen, glucose and insulin responses to an OGTT were higher than among men with a waist/hip ratio <1.00.

- Both following a 24-hour fast and body weight loss the PAI-1 activity levels were reduced.

- Transition from a low-fiber diet to a high-fiber diet lowered PAI-1
activity provided that no or modest increases in glucose and triglyceride levels were induced.

- Following a 10-g/day oat-husk supplement PAI-1 activity levels were decreased. The serum lipid levels were unaffected.

- Lp(a) was found to be largely unrelated to endocrine-metabolic and anthropometric variables.
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