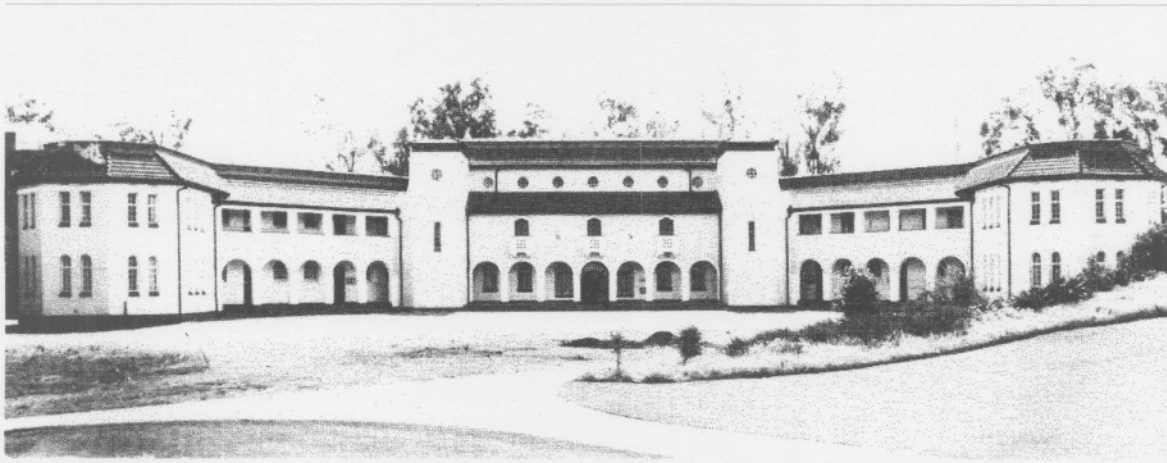# COMPARISON OF THE ASSOCIATION OF PAI-1$_{act}$ WITH THE METABOLIC SYNDROME MARKERS IN CAUCASIAN AND BLACK SOUTH AFRICAN WOMEN

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Dissertation submitted for the degree Magister Scientiae in Nutrition at the North-West University, Potchefstroom Campus

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ACKNOWLEDGEMENTS

AFRIKAANSE TITEL

Vergelyking van die assosiasie van plasminogeen-aktiveerder-inhibeerder-1 met merkers van die metaboliese sindroom tussen swart en blanke vrouens.

OPSOMMING

Motivering:

Die nadelige gevolge van obesiteit en insulienweerstand in blanke en swart Amerikaanse populasies was die fokus van verskeie onlangse publikasies, en die assosiasie van PAI-1-aktiwiteit met merkers van die metaboliese sindroom is reeds bevestig. Data van swart Afrika populasies is egter nog onvoldoende.

Doel:

Om ondersoek in te stel na moontlike verskille in die assosiasie van PAI-1-aktiwiteit met merkers van die metaboliese sindroom tussen swart en blanke vrouens.

Metodes:

Ons het van twee dwarsdeursnit studies (die POWIRS I en II studies) gebruik gemaak. Hierdie studies het respektiewelik 95 Swart en 114 Blanke vrouens uit die Potchefstroom distrik van die Noordwes provinsie, Suid Afrika, ingesluit.

Resultate:

Gemiddelde PAI-1-aktiwiteit van die swart vrouens was betekenisvol laer as dié van die blanke vrouens ($p < 0.001$). Merkers van die metaboliese sindroom het 60% van die variasie in PAI-1-aktiwiteit in die blanke groep, maar slegs 2.8% van die variasie in PAI-1-aktiwiteit in die swart groep verklaar. Middelomtrek was die sterkste onafhanklike voorspeller van PAI-1-aktiwiteit in die blanke (34%) sowel as in die swart (11%) groep.

Gevolgtrekking:

Hierdie studie het duidelike verskille in PAI-1 aktiwiteit tussen swart en blanke vrouens getoon. Daar was ook verskille in die assosiasies van PAI-1 aktiwiteit met merkers van die metaboliese sindroom tussen die 2 groepe. Moontlike genetiese verskille tussen die twee groepe, veral die rol van die 4G/5G genotipe, mag 'n belangrike invloed op PAI-1 aktiwiteit hê. Die rol van PAI-1 aktiwiteit in die metaboliese sindroom is waarskynlik verskillend tussen Swart en Blanke vrouens.

Sleutelwoorde:

PAI-1, metaboliese sindroom, obesiteit, insulien weerstand

ABSTRACT

Motivation

The detrimental effects of obesity and insulin resistance in Caucasians and African-Americans have been the focus of many recent publications, and the association between PAI-1$_{act}$ and markers of the metabolic syndrome is well established but data on African subjects are still lacking.

Objectives

To investigate possible differences between the association of PAI-1$_{act}$ with markers of the metabolic syndrome in Caucasian and African women.

Methods

We used crossectional data from the POWIRS I and II studies, involving 95 African and 114 Caucasian women respectively in the Potchefstroom district of the North West Province, South Africa.

Results

Mean plasma PAI-1$_{act}$ was significantly higher in the Caucasian than in the African subjects ($p < 0.001$). Markers for the metabolic syndrome explained 60% of the variance of PAI-1$_{act}$ in the Caucasian group, but only 2.8% of the variance of PAI-1$_{act}$ in the African group. Waist circumference emerged as the strongest independent predictor of PAI-1$_{act}$ in the Caucasian (34%) as well as the African subjects (11%).

Conclusion

This study showed clear differences in PAI-1$_{act}$ between African and Caucasian subjects, along with differences in the association of PAI-1$_{act}$ with markers of the metabolic syndrome. Apparent genetic differences between the two groups (especially the role of the 4G/5G genotype) may have an important influence on PAI-1$_{act}$. The role of PAI-1$_{act}$ in the metabolic syndrome may differ between Caucasians and Africans.

Keywords

PAI-1, metabolic syndrome, obesity, insulin resistance

LIST OF ABBREVIATIONS

ANOVA - Analysis of variance

ATP III - National Cholesterol Education Program's Adult Treatment Panel III

BMI – Body Mass Index

CI - Confidence intervals

CRP - C-reactive protein

CVD - Cardiovascular disease

GPAQ - Global Physical Activity Questionnaire

HDL-C - High Density Lipoprotein Cholesterol

HOMA - Homeostasis model assessment

hs-CRP - High sensitivity C-reactive protein

kDa – Kilodalton

LDL-C - Low Density Lipoprotein Cholesterol

MAPK - Mitogen-activated protein kinase

Na – Sodium

NO - Nitric oxide

$PAI-1_{act}$ - Plasminogen activator inhibitor-1 activity

PHLA - post-heparin lipolytic activity

PI3K - Phosphoinositide- 3 kinase

POWIRS – Profiles of Obese Women with Insulin Resistance Syndrome

PP - Post-prandial

RAAS - Renin-angiotensin-aldosterone system

SNS - sympathetic nervous system

TBG - Thyroxine-binding globulin

TC - Total Cholesterol

TF - Tissue factor

TG – Triacylglycerol

TGF-β - Transforming growth factor-beta

TNF-α – Tumour necrosis factor alpha

tPA - Tissue-type plasminogen activator

tPA_{ag} - tissue plasminogen activator antigen

TZDs – Thiazoladinediones

uPA - Urokinase-type plasminogen activator

VLDL-C – Very Low Density Lipoprotein Cholesterol

vWF - von Willebrand factor

WHO - World Health Organisation

CONTENTS

CHAPTER 1

PREFACE

CHAPTER 1: PREFACE

1. Aims and objectives

The aims and objectives of this dissertation were:

Main aim

To investigate possible differences between the association of PAI-1$_{act}$ with markers of the metabolic syndrome in a group of African and Caucasian women

Objectives

- To determine whether there are differences in PAI-1$_{act}$ between African and Caucasian women in our study population.
- To determine whether there are differences in PAI-1$_{act}$ between the groups of African and Caucasian women when subdivided on the basis of:
  - o BMI
  - o Android and gynoid obesity
  - o Subjects with established metabolic syndrome and those without any markers
- To determine whether there are differences in the association of PAI-1$_{act}$ with markers of the metabolic syndrome between African and Caucasian women in our study population and when subdivided on the basis of:
  - o BMI
  - o Android and gynoid obesity
  - o Subjects with established metabolic syndrome and those without any markers

2. Structure of this dissertation

This dissertation is presented in article format. The experimental work consisted of two epidemiological studies: Creating profiles of African and Caucasian women with insulin resistance syndrome.

Following this preface chapter, Chapter 2 provides background information necessary for the interpretation of the data in the article. An overview of the metabolic syndrome is given, after which plasminogen activator inhibitor 1 (PAI-1) is described in detail. The role of PAI-1 in insulin resistance and obesity is discussed. The influences of cytokines and lipids on PAI-1 are also discussed along with its role in CVD. The relevant references of Chapter 2 are provided at the end of the chapter in the mandatory style stipulated by the North-West University.

Chapter 3 consists of a manuscript discussing the differences in the association of PAI-1 with markers of the metabolic syndrome between two groups of African and Caucasian women. This article has been submitted to the Journal of Thrombosis and Haemostasis for publication. The relevant references of Chapter 3 are provided at the end of the chapter in the technical style stipulated by the journal.

3. Authors' contributions

The studies reported in this dissertation were planned and executed by a team of researchers. The role of each of the researchers is given in the table hereunder. Also included in this section is a statement from the co-authors confirming their individual roles in each study and giving their permission that the article may form part of this dissertation.

Name	Role in the study
Mr. A. Greyling (Hons. B.Sc. Nutrition)	Responsible for laboratory analysis of samples from POWIRS II, literature searches, statistical analysis and writing up of data. First author of the paper.
Dr. M. Pieters PhD. (Dietician, Nutritionist)	Supervisor of MSc dissertation. Involved in statistical analysis and writing of paper.
Prof. W. Oosthuizen PhD. (Nutritionist)	Assistant Supervisor. Critically revised paper
Dr. A.E. Schutte PhD. (Physiologist)	In charge of the planning and execution of the POWIRS I and II studies

I declare that I have approved the above-mentioned article, that my role in the study, as indicated above, is representative of my actual contribution and that I hereby give my consent that it may be published as part of the M.Sc. dissertation of Mr A. Greyling.

Dr. M. Pieters   Prof. W. Oosthuizen   Dr. A.E. Schutte

CHAPTER 2

LITERATURE REVIEW

1. Introduction

The detrimental effects of obesity and insulin resistance in Caucasians and African-Americans have been the focus of many recent publications, but data of Africans are still lacking. The Profiles of Obese Women with Insulin Resistance Syndrome (POWIRS) I and II studies aimed at assessing the health determinants of two groups of urban, African and Caucasian women by comparing the lifestyle and risk factors associated with the metabolic syndrome of lean, overweight and obese subjects. These two studies included 95 African and 114 Caucasian women respectively. Each group was further sub-divided into three groups (lean [Body mass index (BMI): 18.5-24.9 kg/m^2], overweight [BMI: 25-29.9 kg/m^2], and obese [BMI $\geq$ 30 kg/m^2]). Measurements included various questionnaires (including demographic, psychological, and quantitative food frequency questionnaires), anthropometric measurements, cardiovascular measurements with a Finometer device (recording parameters such as blood pressure and arterial compliance), and an oral glucose tolerance test. Different biochemical analyses included lipids (total cholesterol (TC), low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C) and triacylglycerol (TG)), haemostatic factors (plasminogen activator inhibitor-1 activity (PAI-1$_{act}$) and fibrinogen), leptin, C-reactive protein (CRP) and liver enzymes. Data from the POWIRS studies were utilised for the purpose of this dissertation.

The insulin resistance syndrome, also known as the metabolic syndrome, is a clustering of metabolic abnormalities amongst which are central obesity, hyperinsulinaemia and glucose intolerance, dyslipidaemia characterised by high TG and low HDL-C concentrations and hypertension, all of which are associated with an increased risk for the development of cardiovascular disease (CVD) (1;2).

A number of haemostatic abnormalities have recently been associated with the metabolic syndrome, amongst which elevated concentrations of plasminogen activator inhibitor-1 (PAI-1) and tissue plasminogen activator antigen (tPA$_{ag}$) share the strongest associations and have been studied in the most detail (3). Consistent associations have also been found with fibrinogen concentrations, vitamin K dependant coagulation factors (factors- VII, IX and X), CRP and von Willebrand factor (vWF) (1;3). It is now well established that impaired fibrinolysis due to elevated PAI-1$_{act}$ is an important feature of the metabolic syndrome (4).

Haemostasis describes the systems that prevent loss of blood from an organism by clotting at sites of injury. Normal haemostasis maintains blood in a fluid state within the vessel walls, but retains the ability to prevent excessive blood loss when injured. There are four primary

elements that contribute to haemostasis. These are: the vessel wall (or vascular endothelium), the platelets and the coagulation and fibrinolytic pathways (5).

The normal vascular endothelium maintains blood fluidity by inhibiting blood coagulation and platelet aggregation while promoting fibrinolysis. The endothelium also provides a protective barrier that separates the blood cells and plasma factors from the highly reactive and thrombogenic elements in the deeper layers of the vessel wall. These thrombogenic elements include adhesive proteins, such as collagen and vWF (both of which promote platelet adhesion) and tissue factor (TF) that triggers blood coagulation. When a vessel is severed, it constricts to divert blood from the site of injury and the shed blood comes into contact with the exposed subendothelial matrix, which stimulates the formation of the haemostatic plug by promoting activation of platelets and blood coagulation (6;7).

Platelets play a fundamental role in haemostasis. When a blood vessel injury occurs, platelets exhibit a sequence of events. These events include 1) adhesion of platelets to the injury site, 2) spreading of adherent platelets over the exposed subendothelial surface, 3) secretion of platelet granule constituents, 4) platelet aggregation and 5) platelet coagulant activity (8).

PAI-1 and fibrinogen are major role-players in the haemostatic process – each making up an important part of the fibrinolytic and coagulation pathways respectively (9).

Cell surfaces and fibrin provide sites for local activation of the haemostatic system. Coagulation is primarily initiated by cell surface expression of tissue factor, which acts as a focus for plasma coagulation factors ending in the formation of thrombin, which converts fibrinogen to fibrin to produce an insoluble network. Fibrinolysis depends on bringing plasminogen, which is inactive in plasma, together with its activators, tissue-type plasminogen activator (tPA) and urokinase-type plasminogen activator (uPA) on fibrin or cells, where plasmin is generated and degrades fibrin (5).

PAI-1 is the major negative regulator of both tPA and uPA and thus inhibits the fibrinolytic process. The essential balance in plasma is between the proteolytic activities of tPA and uPA and their inhibitor, PAI-1. In general PAI-1 is present in 4 - 5 fold excess over the activators, favouring the stabilization of fibrin (10).

Elevated concentrations of PAI-1 are considered a potential risk factor for coronary heart disease due to its regulating role in fibrinolysis, and it is now well established that hypofibrinolysis due to high plasma PAI-1 concentrations is a core feature of the metabolic syndrome (11).

2. The metabolic syndrome

When the notion of syndrome X was first conceived, it suggested that insulin resistant, non-diabetic individuals would be glucose intolerant, hypertensive and present a dyslipidaemia with low HDL-C and elevated TG concentrations (2).

Recently there has been some confusion and controversy regarding the definition of the metabolic syndrome, since both the National Cholesterol Education Program's Adult Treatment Panel III (ATP III) and the World Health Organisation (WHO) have their own views regarding this condition (12).

The ATP III identified the metabolic syndrome as a multiplex risk factor for CVD that is deserving of more clinical attention. It defines the metabolic syndrome as a state in which at least three of the five characteristics listed in Table 1 are present in an individual. The primary clinical outcome of metabolic syndrome was identified as CVD. Abdominal obesity, recognized by increased waist circumference, is the first criterion listed. Its inclusion reflects the priority given to abdominal obesity as a contributor to metabolic syndrome. Also listed are raised TG, reduced HDL-C, elevated blood pressure and raised plasma glucose. Explicit demonstration of insulin resistance is, however, not required for diagnosis (13).

A WHO consultation group outlined a provisional classification of diabetes that included a working definition of the metabolic syndrome (see Table 2) (14). CVD is recognized as the primary outcome of the metabolic syndrome. However, insulin resistance is viewed as a required component for diagnosis. A higher blood pressure than in the ATP III criteria is required. BMI (or increased waist:hip ratio) is used instead of waist circumference and microalbuminuria is listed as an additional criterion. The requirement of objective evidence of insulin resistance should give more power to predict diabetes than does ATP III, but like ATP III, the presence of type 2 diabetes does not exclude a diagnosis of metabolic syndrome (13;14).

Table 1: ATP III clinical identification of the metabolic syndrome (13)

Criteria:	Cut-off values:
Abdominal obesity (as determined by waist circumference):	
Men	> 102 cm
Women	> 88 cm
TG	≥ 1.7 mmol/L
HDL-C: Men	< 1.0 mmol/L
Women	< 1.3 mmol/L
Blood pressure	≥ 130/ ≥ 85 mm Hg
Fasting glucose	≥ 6.0 mmol/L

TG, triacylglycerol, HDL-C, high density lipoprotein cholesterol.

Table 2: WHO Clinical Criteria for Metabolic Syndrome (14)

Insulin resistance, defined by one of the following:
• Type 2 diabetes.   • Impaired fasting glucose.   • Impaired glucose tolerance.   • Or, for those with normal fasting glucose levels (< 6.0 mmol/L), glucose uptake below the lowest quartile for background population under investigation under hyperinsulinemic, euglycemic conditions.
Plus any two of the following:
• Use of antihypertensive medication and/or high blood pressure (≥ 140/90 mm Hg).   • Plasma TG ≥ 1.7mmol/L and/or HDL-C < 0.9mmol/L for men, < 1.0mmol/L for women.   • Central obesity: BMI > 30 kg/m^2 and/or waist-to-hip ratio > 0.90 for men and waist-to-hip ratio > 0.85 for women.   • Urinary albumin excretion rate ≥ 20µg/min or albumin:creatinine ratio ≥ 30 mg/g.

TG (triacylglycerol), HDL-C (high density lipoprotein cholesterol), BMI (body mass index).

The common ground between these two sets of criteria is, however, that they were both developed to identify a group of risk factors that would have a higher probability of predicting subsequent development of CVD (12).

In the previous decade since the notion of Syndrome X was first introduced, the number of metabolic abnormalities linked to insulin resistance and hyperinsulinaemia has grown to a

considerable degree. Amongst these are abnormalities of glucose and uric acid metabolism, dyslipidaemia as well as haemodynamic and haemostatic abnormalities, as summarised in Figure 1. These abnormalities tend to cluster in the same individual and represent major risk factors for developing CVD (15).

Genetic influences   Environmental influences

Insulin resistance
+
Hyperinsulinaemia

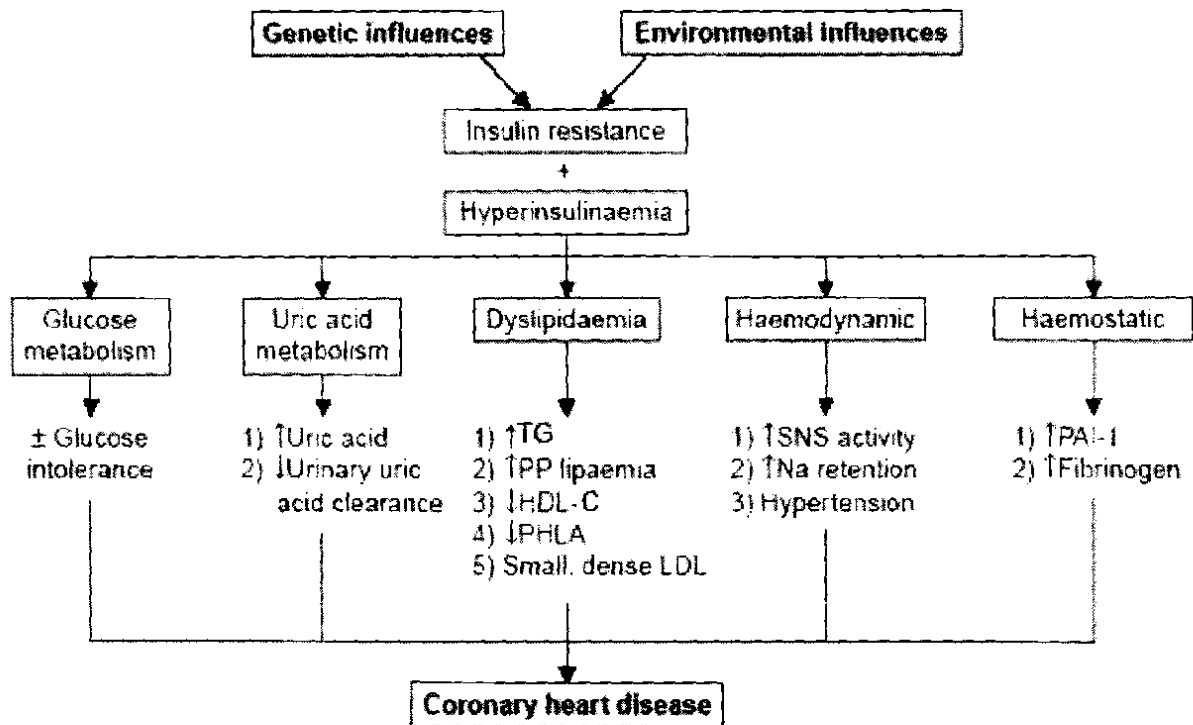| Glucose metabolism	Uric acid metabolism	Dyslipidaemia	Haemodynamic	Haemostatic
± Glucose intolerance	1) ↑Uric acid 2) ↓Urinary uric acid clearance	1) ↑TG 2) ↑PP lipaemia 3) ↓HDL-C 4) ↓PHLA 5) Small, dense LDL	1) ↑SNS activity 2) ↑Na retention 3) Hypertension	1) ↑PAI-1 2) ↑Fibrinogen

Coronary heart disease

Figure 1. Proposed role of insulin resistance and compensatory hyperinsulinaemia in CHD. HDL-C (high density lipoprotein cholesterol), LDL (low density lipoprotein), PAI-1 (plasminogen activator inhibitor-1), PHLA (post-heparin lipolytic activity), PP (post-prandial), Na (sodium), SNS (sympathetic nervous system), TG (triacylglycerol) (adapted from (15)).

Although there are many abnormalities associated with the metabolic syndrome, this review will focus only on PAI-1. Potential mechanisms of increased PAI-1 synthesis in the metabolic syndrome as well as the potential role of PAI-1 in obesity and the metabolic syndrome will be investigated. The role of increased PAI-1 concentrations in CVD risk along with the differences in the association of different CVD risk factors with PAI-1 concentrations in different populations (possibly due to genetic and environmental variables) will also be discussed.

3. Plasminogen activator inhibitor-1

PAI-1 belongs to the serine protease inhibitors (serpin) super-family. Serpins represent about 10% of the total protein in plasma. Among the serpins, two groups can be distinguished, i.e. the inhibitory serpins (e.g. PAI-1) and the non-inhibitory serpins (e.g. thyroxine-binding globulin (TBG)). The inhibitory serpins inhibit the serine protease by the formation of a covalent complex by mimicking the interaction of the substrate with its target protease. PAI-1 is a single-chain glycoprotein consisting of 379 or 381 amino acids (N-terminal heterogeneity) and a 23 amino acid signal peptide indicating that it is a secreted protein. It has a molecular weight of approximately 45 kDa (16).

During fibrinolysis, tPA converts the inactive protein plasminogen into plasmin. Plasmin, in turn, plays a critical role in fibrinolysis by degrading fibrin. PAI-1 is the primary inhibitor of tPA and thus limits the production of plasmin and serves to keep fibrinolysis in check. Uncontrolled plasmin production can result in excessive degradation of fibrin and fibrinogen, leading to an increased risk of bleeding (17).

Plasma PAI-1 is derived from several sources, including the vascular endothelium, adipose tissue, hepatocytes and vascular smooth muscle cells, and its production and secretion can be stimulated by a number of activators such as thrombin, endotoxin and cytokines (18). Platelets are also known to store large quantities of PAI-1 that are secreted following platelet aggregation (19;20).

In healthy individuals, PAI-1 concentrations exceed tPA by a greater than 4:1 ratio on a molar basis, and mean PAI-1$_{ag}$ antigen levels vary between 15 and 30 ng/ml in blood plasma. Functionally there are two forms of PAI-1, namely an active and a latent form and only the active form binds to tPA and uPA to inhibit their activities.

PAI-1 is released from cells as an active form, with a circulating half-life of approximately 5 minutes, thus only a fraction of the secreted active PAI-1 has the opportunity to react with t-PA

and form inert covalent complexes (10). The active form of PAI-1 is not stable, however, and spontaneously transforms into an inactive or latent conformation that has a half-life of ~90 min in vitro. Vitronectin, an abundant plasma protein, stabilises PAI-1 in the active form and prolongs its half-life to 120 min in vitro and appears to prolong the circulating half-life of PAI-1 in vivo as well (21).

Increased PAI-1 concentrations have been shown to be associated with a number of atherosclerotic risk factors (17;22). Insulin and proinsulin correlate with PAI-1 concentrations, and patients with the metabolic syndrome or diabetes tend to have increased PAI-1 concentrations (23). Weight loss and treatment aimed at lowering TG and/or cholesterol levels have also been shown to lower PAI-1 concentrations (17). PAI-1 has been shown to act as a prothrombic factor in both arterial and venous thromboembolic disorders (17;22), and increased concentrations of PAI-1 have been associated with an increased incidence of CVD (24).

Table 3 provides a summary of factors that have been shown to influence PAI-1 concentrations.

Table 3: Factors influencing PAI-1

Variable	Effect	References
Blood lipids	↑ VLDL-C and TG concentrations associated with ↑ PAI-1 concentrations	(17;25;26)
Renin-angiotensin-aldosterone system (RAAS)	Significantly contributes to the upregulation of PAI-1 concentration via a receptor-mediated mechanism	(17)
Dietary factors:		
Vitamin E	1study, decrease, 1study, no effect	(27;28)
Tocorienols	No effect	(29)
Vitamin C/E combination	Decrease in PAI-1$_{ag}$	(30)
L-arginine	No effect	(31;32)
Garlic	No effect on PAI-1$_{act}$	(33;34)
Alcohol	1 study, no effect on PAI-1$_{act}$ 2 others – increased PAI-1$_{act}$, 1 study increased PAI-1$_{ag}$.	(35-38)
Red wine	Increased	(36)
Black tea	No effect on PAI-1$_{act}$ or PAI-1$_{ag}$	(39;40)
Guar gum	Decrease in PAI-1$_{act}$	(41)

Dried beans	Decrease in PAI-1$_{act}$	(42)
Plant sterols/stanols	No effect on PAI-1$_{act}$	(43)
Niacin	No effect on PAI-1$_{act}$	(44)
Vitamin A	No effect on PAI-1$_{act}$ or PAI-1$_{ag}$	(45;46)
Smoking	Apparently not a major determinant, but does seem to ↑ PAI-1 concentrations	(47-50)
Physical activity	Dose-dependant ↓ in PAI-1 concentrations	(51-53)
Insulin resistance	PAI-1 concentrations elevated throughout the spectrum of insulin resistance	(23)
Obesity	Obesity, especially central adiposity, associated with increased concentrations of PAI-1	(54)
Genetic influences	PAI-1 concentrations generally higher in 4G/4G homozygotes compared to 5G/5G homozygotes or heterozygotes, but still some conflicting results	(49;55-56)

Of these factors listed, those associated with the metabolic syndrome, as well as genetic influences will be discussed in more detail, as they are directly related to the topic of this dissertation.

3.1. Genetic variations of PAI-1

Certain polymorphisms in the PAI-1 gene are associated with increased concentrations thereof. The most extensively studied of these polymorphisms, is the 4G/5G polymorphism in the promoter region of the gene (18).

This is a common single guanosine insertion/deletion polymorphism (4G or 5G), situated at -675 bp in the PAI-1 gene promoter. The two alleles have approximately equal frequencies in the general population, but this polymorphism is most significantly associated with plasma PAI-1 concentration of all of those studied to date (18). The nature of this polymorphism can be described as response oriented. This implies that the potential of the allele to determine PAI-1 concentrations is exaggerated in the presence of the relevant environmental or disease factors, but less so in healthy subjects (49).

Subjects homozygous for the 4G allele present higher plasma PAI-1 concentrations than the 5G/5G- or heterozygous genotype, whether they be healthy or suffering from CVD or type 2 diabetes (57-60). The reason for this might be that, even though both alleles bind a transcriptional activator, the 5G allele also binds a repressor protein to an overlapping binding site, which decreases binding of the activator due to interference caused by steric hindrance (54).

There are, however, still some conflicting results regarding the 4G/5G polymorphisms association with PAI-1 concentrations.

In the HIFMEC study, the 4G allele was associated with significantly higher PAI-1 concentrations in survivors of a first myocardial infarction, but not in their healthy, age-matched controls, and taken independently, did not alter the risk for myocardial infarction (61).

In a study by Sartori *et. al.* (62) the 4G/5G polymorphism was a determinant of PAI-1 antigen concentrations in obese subjects, with the highest concentrations in 4G/4G allele carriers. These associations, were however, not seen in the lean controls.

Conversely, in a study of obese children, no influence of the 4G/5G polymorphism on PAI-1 concentrations could be found (56).

There also seems to be some ethnic differences regarding the occurrence of the 4G/5G polymorphism. In the Insulin Resistance Atherosclerosis Study, during which genotyping

of 1564 subjects was performed, the genotype distribution was significantly different across the three ethnic groups (Caucasian, Hispanic and African-American) studied. The allele frequencies for 4G and 5G respectively, were 52% and 48% in Caucasians, 38% and 62% in Hispanics and 28% and 72% in African Americans. Corresponding differences in circulating PAI-1 concentrations were consistently seen amongst all three ethnic groups and were unaffected by metabolic covariates, including insulin resistance. However, although circulating PAI-1 concentrations corresponded with the present ethnic differences in the 4G/5G polymorphism, the genotype explained very little of the variation in PAI-1 concentrations (55).

It seems that there is a definite association between the 4G/5G polymorphism and PAI-1 expression. This association, however, seems to be influenced by several factors that still need to be investigated in order to give us a better understanding of its nature (55). Even though this polymorphism may, in some cases, not be strongly associated with PAI-1 concentrations (56), it has been suggested that it may be associated with PAI-1 responses after triggering (for example after vessel injury) and, therefore, is worthwhile studying. An association between the 4G/5G polymorphism and CVD would contribute to evidence for a causal role of PAI-1 in CVD (49).

4. PAI-1 and the metabolic syndrome

The mechanisms involved in increased PAI-1 production in the metabolic syndrome are not completely understood and PAI-1's origin is also not quite clear. Obviously, induction of PAI-1 overproduction in the metabolic syndrome is a complex process and it is possible that several different inducers at several different sites of synthesis are involved (24).

4.1. PAI-1 and insulin resistance

Insulin resistance is widely recognized to promote the development of vascular inflammation and thrombosis even before the onset of type 2 diabetes. In the Insulin Resistance Atherosclerosis Study, markers of inflammation and PAI-1 levels were higher in insulin-resistant subjects who later developed diabetes than in subjects who did not (63).

Positive associations between fasting insulin concentrations and PAI-1 have been found in subjects with normal and impaired glucose tolerance, as well as in type 2 diabetics

(23;64-66). It was also found that PAI-1 was an independent risk factor for the development of type 2 diabetes in a prospective study of 1047 nondiabetic subjects (63).

Plasma PAI-1 concentrations are elevated throughout the spectrum of insulin resistance, from the metabolic syndrome (normal or impaired glucose tolerance) to prediabetes (period of impaired glucose tolerance) to diabetes (23). Hyperinsulinaemia accompanies insulin resistance through the stages of this spectrum until late in the course of diabetes. Insulin can stimulate PAI-1 release from fat and other tissues (67). In fact, changes in insulin throughout the physiologic range can influence plasma PAI-1 concentrations (20). For example, consumption of high-calorie, high-carbohydrate meals that stimulate insulin release is associated with increased plasma PAI-1 concentrations, whereas a fasting state or administration of metformin or insulin sensitizers are associated with decreased circulating insulin and PAI-1 concentrations (68).

Induction of a diabetic milieu (hyperinsulinaemia combined with hyperglycaemia and hypertriglyceridaemia) for 6 hours, increased concentrations of PAI-1 in normal human subjects (69). Chronic or acute infusions of insulin alone, however, resulted in variable effects on human PAI-1 concentrations (70-72).

An imbalance between the 2 major pathways mediating insulin action (the phosphoinositide 3 kinase (PI3K) and mitogen-activated protein kinase (MAPK) pathways) presents itself in subjects with insulin resistance (73). The MAPK pathway mediates cell growth, migration and PAI-1 expression, whereas the PI3K pathway mediates insulin action to promote cellular glucose uptake and endothelial nitric oxide production. It has been suggested that the PI3K response to insulin is blunted in the adipose tissue of obese subjects without diabetes compared with lean individuals, but that the MAPK pathway responds similarly in both groups (20).

Elevated PAI-1 concentrations are associated with insulin resistance, irrespective of obesity (74), and numerous studies indicate that the increase in CVD risk cannot be solely ascribed to the role of PAI-1 in either obesity or insulin resistance (19).

4.2. PAI-1 and Obesity

Obesity, especially central adiposity, is associated with increased concentrations of PAI-1 (54) and several studies have shown that BMI correlates positively with PAI-1 concentrations in a variety of different types of subjects (75-80).

The PAI-1 promoter is remarkable for its responsiveness to a variety of metabolic and hormonal factors that are associated with obesity, namely tumour necrosis factor alpha (TNF-α), very low density lipoprotein cholesterol (VLDL-C), TG, aldosterone, agiotensin II, glucose and insulin, and decreased nitric oxide (NO) concentrations amongst others. Illustrated in Figure 2 are a number of transcriptional response sites that have been identified in the upstream regulatory region of the PAI-1 promoter. In light of the number of response elements to hormonal and metabolic factors that are linked to obesity, it is not surprising that PAI-1 plasma concentrations are associated with it as well (19).
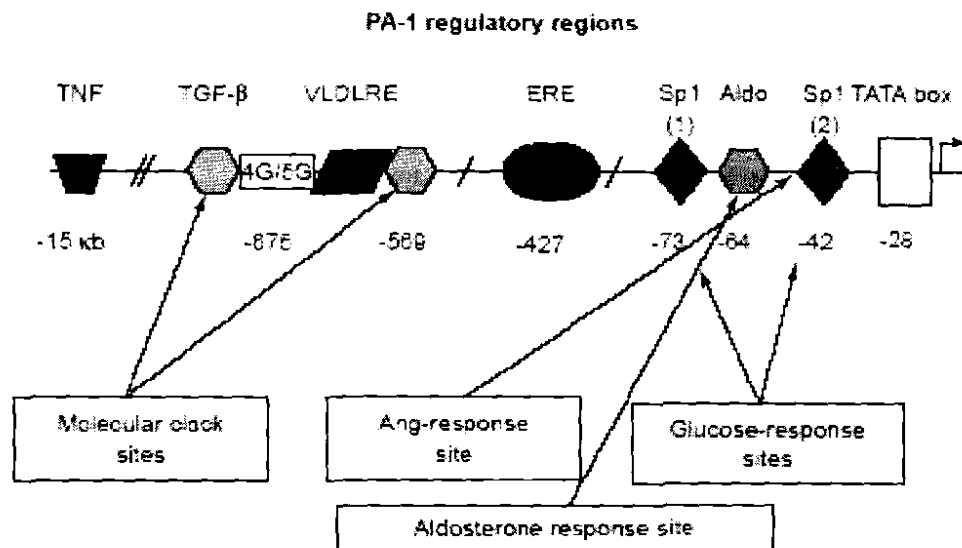

Figure 2. Schematic representation of enhancer elements in the upstream regulatory region of the human PAI-1 promoter. Ang (angiotensin), TGF-b (transforming growth factor-beta) (19).

It has been suggested that adipose tissue itself may contribute to the elevated expression of PAI-1 in obesity (81-83). Several in vitro studies have shown significantly higher PAI-1 production in human visceral adipose tissue than in subcutaneous adipose tissue (84-86).

These regional differences in PAI-1 production may be explained by a recent study that suggested that stromal cells, and not the adipocytes themselves, are the most important source of PAI-1 within adipose tissue (86), and since visceral fat contains more stromal cells than subcutaneous adipose tissue, it would make sense that production of PAI-1 in visceral adipose tissue is higher.

A study of morbidly obese patients did not, however, find a difference in PAI-1 expression between subcutaneous and visceral adipose deposits and the authors concluded that in such extreme conditions, the entire fat mass contributes to plasma PAI-1 concentrations, rather than primarily the visceral adipose tissue (82).

Obesity and insulin resistance are increasingly recognised as states of vascular inflammation and thrombosis, even before the onset of type 2 diabetes. In addition to PAI-1, CRP concentrations are generally elevated in obesity and insulin resistance, reflecting subclinical chronic inflammation. Obesity is well associated with this chronic low-grade proinflammatory state as evidenced by leukocytosis, elevated acute phase proteins, increased plasma levels of markers of endothelial cell dysfunction and activation, along with increased levels of the proinflammatory cytokines transforming growth factor beta (TGF-β) and TNF-α as well as interleukin- 1 and 6 (19). A variety of observations implicate specific hormones and/or cytokines in the increased expression of PAI-1 by adipose tissue in obesity (87). The next section will describe in more detail the role of two prominent cytokines involved in PAI-1 expression.

4.2.1. PAI-1 and cytokines

Adipose tissue synthesizes TNF-α and expression of this cytokine is chronically elevated in adipose tissue from obese individuals (88). TNF-α is known to stimulate PAI-1 biosynthesis by a variety of cultured cells and by many murine tissues in vivo (89), and administration of TNF-α to lean mice significantly increased PAI-1 mRNA expression in the adipocytes, adventitial cells and vascular smooth muscle cells in the adipose tissues (90). This pattern is similar to the pattern of PAI-1 mRNA expression observed in the adipose tissues of obese mice.

Recent studies have shown that human adipose tissue explants also respond to exogenous TNF-α with increased PAI-1 mRNA and protein expression and that the addition of pentoxifylline (an inhibitor of TNF-α mRNA synthesis) decreased PAI-1 mRNA and protein expression. Taken together, these observations support the hypothesis that the chronic elevation in TNF-α that occurs locally in the adipose tissues in human and rodent obesity may act via an autocrine manner to stimulate PAI-1 biosynthesis by the adipocyte and other cells in the adipose tissue. This cytokine may thus contribute to the elevated plasma PAI-1 levels observed in the metabolic syndrome (87).

TGF-β stimulates PAI-1 biosynthesis by a large variety of cultured cells and infusion thereof into rabbits (91) and mice (89;92) dramatically increased plasma PAI-1 activity and induced PAI-1 mRNA in numerous tissues. Adipose tissue seemed to be the most TGF-β–responsive tissue in terms of PAI-1 in mice

(89;92). TGF-β also stimulated PAI-1 gene expression by cultured mouse (92;93) and human adipocytes (85). Interestingly, the level of TGF-β mRNA was significantly higher in the adipose tissue of both *ob/ob* and *db/db* mice when compared with their lean counterparts, (92) and this increase was due to increased expression of TGF-β mRNA by mature adipocytes and stroma/vascular cells. The increase in TGF-β gene expression in adipose tissue in obesity may have broad implications in the pathophysiology of obesity and its related complications.

In summary, it is evident that obesity, especially central obesity, is an important determinant of PAI-1 concentrations. However, the mechanisms that lead to this elevation of PAI-1 concentrations in obesity are of a complex nature. In light of recent studies, adipose tissue is emerging as a secretory organ and PAI-1 is among its products. Whether this tissue contributes directly to circulating PAI-1 is, however still not clear. Cytokines and growth factors are synthesized by adipose tissue and are known to up regulate PAI-1 synthesis. Visceral fat seems to be an important determinant of PAI-1 concentrations, but questions still remain on the depots that are most important for PAI-1 synthesis and on the subtleties of its regulation.

4.3. PAI-1 and blood lipids

PAI-1 has been positively associated with cholesterol, LDL-C, VLDL and TG and negatively with HDL-C concentrations (4;25). In Vitro, VLDL resulted in increased PAI-1 concentrations in endothelial and hepatic cells (94;95). LDL and oxidized LDL have also been found to stimulate increased PAI production by endothelial cells (96-98), and LDL particle size has been found to be inversely related to PAI-1 concentrations (99). In another study, small, dense LDL particle concentration correlated with PAI-1 activity (100).

Associations between TG concentrations and PAI-1 activity and concentrations have been found in a number of studies (101-104). Dietary induced changes in TG concentrations have also been associated with changes in PAI-1 concentrations (105). PAI-1 gene expression increased in HepG2 cells exposed to free fatty acids or TG. Deletion analyses demonstrated that FFA and TG induce PAI-1 expression through distinct regions of the promoter (106). Reductions of plasma TG by lipid lowering drugs such as gemfibrozil and niacin have decreased PAI-1 plasma concentrations and PAI-1 mRNA expression. Treatment of hypercholesterolaemia with statins may not only

reduce plasma PAI-1 indirectly by reducing cholesterol and TG, but also through a direct action of statins on human vascular smooth muscle cells and endothelial cells (17).

4.4. PAI-1 and CVD

There is substantial experimental and epidemiological evidence that PAI-1 might contribute to the development of ischaemic CVD (107-111). In a study including approximately 3000 patients with angina, plasma PAI-1 activity and antigen levels were 30% higher in patients with coronary events compared with the event-free controls (25).

Transgenic mice that over-express a stable form of human PAI-1 develop spontaneous coronary thrombosis and subendocardial myocardial infarction without the presence of hypertension or hyperlipidaemia (112). These animals have no t-PA activity in their plasma and also exhibit significant reductions in plasma levels of activated protein C. The spontaneous coronary thrombosis seen in these mice appears to be as a result of this, since plasminogen activator function and protein C activity are the two critical pathways implicated in the defence against clotting in the coronary circulation (113).

In the ECAT study (a prospective multicenter study of 3043 patients with angina pectoris followed for 2 years), higher baseline concentrations of PAI-1 were shown to predict myocardial events. The associations of PAI-1 with risk of events disappeared after adjustment for parameters reflecting insulin resistance, but were not affected by other adjustments. This suggests that the prognostic role of PAI-1 in predicting coronary events is related principally to insulin resistance (25).

Type 2 diabetes has been associated with an increased PAI-1 expression in the arterial wall (114;115). This increased PAI-1 concentration in the vessel wall, as well as the increased PAI-1 level in plasma could participate in increased cardiovascular risk and unfavourable plaque evolution in insulin resistance syndrome and diabetes (4). Several groups have reported excess PAI-1 in atherosclerotic plaques in humans, a finding that is exaggerated in patients with type 2 diabetes (115).

Animal models have been used by a number of groups to test prospectively whether elevated PAI-1 expression promotes thrombosis and atherosclerotic lesion development (116-119). While it is clear that over-expression of PAI-1 favours the development of thrombosis, the exact role of PAI-1 in vascular remodelling remains controversial (118;120). It seems that PAI-1 may limit cell migration in one early remodelling process, but enhance fibrin accumulation at later time points, promoting cell proliferation

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(116;117;119;120). In mice lacking PAI-1, larger plaques at all sites of the vasculature were observed, but only at advanced stages of atherosclerosis (121). In tissues in which PAI-1 is over-produced, local plasminogen activation is impaired, which in turn has profound effects on vascular housekeeping and remodelling capacity and it has been shown that PAI-1 deficiency effectively prevents the development of arteriosclerosis and hypertension in mice treated with the nitric oxide synthase inhibitor L-NAME for periods of 8–16 weeks (122).

Local impairment of the plasmin/plasminogen activator system appears to play an important role in the progression of atherosclerotic cardiovascular disease in general. It has furthermore been hypothesized that increased vascular PAI-1 production and accumulation plays a major role in the arterial remodelling that contributes to the development of hypertension in obesity and the metabolic syndrome (19).

In summary, circulating PAI-1 levels are elevated in patients with cardiovascular disease and may affect the progression of this disease by directly stimulating the remodelling of the vessel wall and decreasing the capacity to degrade fibrin, thus both promoting atherosclerotic plaque formation and enhancing the chance for a damaging thrombus to develop on plaque rupture. Studies suggest that increases in tissue PAI-1 expression contribute to thrombus formation and vascular injury.

4.5. Therapeutic considerations

As PAI-1 appears to be a common thread in the pathology of obesity, diabetes and cardiovascular disease it can be identified as an attractive target for direct inhibition. Treatment options ameliorating both metabolic changes associated with insulin resistance syndrome and decreasing PAI-1 levels might decrease prothrombotic and proinflammatory states (19).

Administration of thiazoladinediones (TZDs), which are PPAR-γ agonists/ligands, is generally associated with decreased circulating PAI-1 levels in humans with type 2 diabetes. In a double blind, randomized study comparing effects of glibenclamide 10 mg twice daily alone or in combination with rosiglitazone, the combination was associated with a 22% and 34% decrease in both plasma PAI-1 concentration and activity respectively, compared with placebo (123).

Both direct and indirect mechanisms are likely to mediate the effect of TZDs on plasma PAI-1 levels. Administration of TZDs to insulin-resistant subjects is associated with decreased plasma insulin levels, which generally correlate with PAI-1 levels. The TZDs also decrease non-esterified free fatty acids, which are reported to stimulate PAI-1 production (20).

5. Conclusion

The role of PAI-1 in obesity, the metabolic syndrome and CVD has been extensively studied. There is convincing evidence that PAI-1 is associated with CVD and type 2 diabetes, reflecting the overall prothrombotic and proinflammatory milieu in the metabolic syndrome.

It is difficult to ascertain exactly where PAI-1 fits into this "cause or consequence relationship" with the several pathophysiological conditions with which it has been associated. Are PAI-1 concentrations elevated because of a certain condition, or does said condition occur as a result of elevated PAI-1 concentrations? Does one condition appear simply as a result of another already present, with PAI-1 concentrations being elevated as a result of the inflammatory response? Until its precise role in the metabolic syndrome is discovered, would it suffice to say that PAI-1 links the triad of pathophysiological situations: obesity, diabetes and cardiovascular events that make up the metabolic syndrome? This would be consistent with the hypothesis that one metabolite or one hormone alone is probably not sufficient to affect PAI-1 concentrations (19).

There is still much speculation regarding the causality of PAI-1 in the metabolic syndrome, but there is extensive experimental and clinical evidence demonstrating that PAI-1 is at the centre of obesity, insulin resistance and CVD. This makes PAI-1 an attractive target for further study to determine if it is a viable target for therapeutic interventions aiming to reduce the risk of CVD associated with obesity and the metabolic syndrome.

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CHAPTER 3

DIFFERENCE IN THE ASSOCIATION OF PAI-1 ACTIVITY WITH THE OCCURRENCE OF MARKERS FOR THE METABOLIC SYNDROME IN AFRICAN AND CAUCASIAN WOMEN.

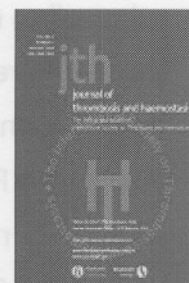A. Greyling, M. Pieters, W. Oosthuizen, A.E. Schutte

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Differences in the association of PAI-1 activity with the occurrence of markers for the metabolic syndrome in African and Caucasian women.

RUNNING HEAD: PAI-1 and the metabolic syndrome.

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Summary

Background: The detrimental effects of obesity and insulin resistance in Caucasians and African-Americans have been the focus of many recent publications and the association between PAI-1$_{act}$ and markers of the metabolic syndrome is well established, but data on African subjects are still lacking. *Objectives:* To investigate possible differences between the association of PAI-1$_{act}$ and markers of the metabolic syndrome in Caucasian and African women. *Patients and methods:* Cross-sectional data were collected from 95 African and 114 Caucasian women respectively in the Potchefstroom district of the North West Province, South Africa. *Results:* Mean plasma PAI-1$_{act}$ was significantly higher in the Caucasian than in the African subjects ($p < 0.001$). Markers for the metabolic syndrome explained 60% of the variance of PAI-1$_{act}$ in the Caucasian group, but only 2.8% of the variance of PAI-1$_{act}$ in the African group. Waist circumference emerged as the strongest independent predictor of PAI-1$_{act}$ in the Caucasian as well as the African subjects. *Conclusion:* This study showed clear differences in PAI-1$_{act}$ between African and Caucasian subjects, along with differences in the association of PAI-1$_{act}$ with markers of the metabolic syndrome. Apparent genetic differences between the two groups (especially the role of the 4G/5G genotype) may have an important influence on PAI-1$_{act}$. The role of PAI-1$_{act}$ in the metabolic syndrome may differ between Caucasians and Africans.

Keywords: PAI-1, metabolic syndrome, obesity, insulin resistance

Introduction

The insulin resistance syndrome, also known as the metabolic syndrome, is a clustering of metabolic abnormalities amongst which are central obesity, hyperinsulinemia, glucose intolerance, and dyslipidemia characterised by high triacylglycerol (TG) and low high density lipoprotein cholesterol (HDL-C) concentrations and hypertension, all of which are associated with an increased risk for the development of cardiovascular disease (CVD) [1;2].

A number of haemostatic abnormalities have recently been associated with the metabolic syndrome, amongst which elevated concentrations of plasminogen activator inhibitor-1 (PAI-1) and tissue plasminogen activator antigen (tPA_{ag}) share the strongest associations and have been studied in the most detail [3]. Consistent associations have also been found with fibrinogen concentrations, vitamin K dependant coagulation factors (factors- VII, IX and X), C-reactive protein (CRP) and von Willebrand factor (vWF) [3;2]. It is now well established that impaired fibrinolysis due to elevated PAI-1 activity (PAI-1_{act}) is an important feature of the metabolic syndrome [4].

The detrimental effects of obesity and insulin resistance in Caucasians and African-Americans have been the focus of many recent publications [2;3;5-8], and the association between PAI-1_{act} and markers of the metabolic syndrome in Caucasians is well established [2;4], but data of Africans are still lacking. However, data from a few studies on African American and African subjects, suggest that these populations have lower circulating PAI-1 concentrations than Caucasians [9-11]. This study, therefore, aims to investigate whether this apparent race difference in PAI-1_{act} may result in differences between the association of PAI-1_{act} and markers of the metabolic syndrome in the two groups (Caucasian and African) that were studied. Based on the importance

of weight for increasing the risk of developing metabolic syndrome, the groups were stratified into three body-mass-index-based subdivisions. They were also statistically divided for android and gynoid obesity as well as for diagnosed metabolic syndrome and the absence of any markers thereof.

Materials and methods

Study design and subject selection

The study was cross-sectional by design and involved 95 African and 114 Caucasian urban women, who were recruited from the Potchefstroom district of the North West Province, South Africa. The inclusion criteria were apparently healthy women aged between 20 and 50 years. Only HIV negative subjects were recruited (according to their status as determined three months before the study), but the negative status of all subjects could not be guaranteed. Subjects were recruited based on their BMI. Three groups of subjects were selected based on guidelines of the Report of a World Health Organization Consultation on Obesity [12]: i) normal range (lean) with BMI: 18.5-24.9 kg/m^2; ii) overweight (pre-obese) with BMI: 25-29.9 kg/; and iii) obese with BMI ≥ 30 kg/m^2. Pregnant and lactating women, diabetics and those with oral temperatures above 37°C were excluded. More details regarding the methods of this study are described elsewhere [13].

Ethical considerations

The study was approved by the Ethics Committee of the North-West University (Potchefstroom Campus). All subjects were fully informed about the objectives and procedures of the study prior to their inclusion and assistance was available to provide information in their home language. All subjects signed informed consent.

Questionnaires

Physical activity levels were assessed using the vallidated Global Physical Activity Questionnaire (GPAQ) [14]. Alcohol intakes were measured with a quantitative food frequency questionnaire [15]. Nutrient intakes were analysed with a programme based on the South African Food Composition Tables [16]. The method of Willet *et al.* [17] was used to adjust for under-reported energy intake.

Anthropometric measurements

Height (stature), weight and body circumferences of subjects in their underwear were measured using calibrated instruments (Precision Health Scale, A & D Company, Japan; Invicta Stadiometer, IP 1465, UK; Holtain metal tape). Measurements were taken using standard methods [18]. The researchers' measurements were standardized and taken in triplicate.

Blood, serum and plasma samples

A qualified nursing sister, using a 21-gauge scalp infusion set, collected venous blood samples after a 10-hour overnight fast. Samples were drawn with minimum stasis between 06:00 and 10:00 to. For the lipid, insulin and uric acid analyses, 20 ml blood was drawn and left to clot. For the determination of coagulation factors, 10 ml citrated blood was drawn. For the determination of fasting glucose concentrations, 5 ml blood was collected in tubes containing sodium fluoride. The clotted, citrated and fluoride blood samples were centrifuged at 3500 rpm for 10 min at 10 °C, within 1 hour of collection, to yield serum and plasma respectively. Serum and plasma were divided into aliquots and stored at -82°C until analysis.

Experimental methods

Serum lipids and uric acid were measured using a Vitros DT60 II Chemistry Analyser (Ortho-Clinical Diagnostics, Rochester, NY, USA). Serum LDL-C was calculated using the Friedewald *et al.* [19] formula. Plasma for the determination of coagulation factors was thawed at 37°C. Plasma fibrinogen was measured by a modified method of Clauss (20)], using the ACL-200 automated coagulation analyser and reagents from Instrumentation Laboratories (IL) Milan, Italy. PAI-1$_{act}$ was measured using an indirect enzymatic method (Spectrolyse pL, Biopool, Umeå, Sweden, Cat. No. 101201; inter-plate CV = 8.1%). High sensitivity C-reactive protein (hs-CRP) was analysed with a high sensitivity C - reactive protein Kit from Immage® Immunochemistry Systems (Beckman Coulter, Inc, Fullerton, USA, Cat. No. 474630). Analysis of insulin concentrations was performed by enzyme immunoassay (BioSource EUROPE S.A. Belgium). Plasma glucose was measured with a hexokinase method [21]. Insulin resistance was calculated using the homeostasis model assessment (HOMA) as: (fasting insulin x fasting venous glucose) /22.5 [22]. Blood pressure was determined by means of a 7-minute continuous measurement of cardiovascular parameters using a Finometer™ device (FMS, Finapres Medical Systems, Amsterdam, Netherlands).

Statistical analysis

Statistical analysis was conducted using Statistica® 7.0 (Statsoft, Inc., Tulsa, USA). Variables were tested for normality with the Shapiro-Wilks test. Normally distributed data are expressed as mean [95% confidence intervals (CI)] and data not normally distributed as median [25, 75 percentiles]. Differences between groups were tested for significance using the *t*-test for independent samples for parametric data and the Mann-Whitney U test for non-parametric data. Differences between the three weight classes were determined using analysis of variance (ANOVA). Significance was set at $p \leq 0.05$.

Correlations between PAI-1$_{act}$ and variables related to the metabolic syndrome in the two groups were obtained with Spearman and Pearson correlations. The total groups were also subdivided on the basis of weight class (BMI <25.0; 25.0-29.9 and ≥ 30.0), fat distribution (BMI>25 with waist circumference cut off of 88cm) and prevalence of the metabolic syndrome (based on the National Cholesterol Education Program's Adult Treatment Panel III (ATPIII) criteria[23]) for determining correlations. There was very little difference between the Pearson and Spearman correlations and since some of the subdivided groups were very small, only Spearman correlations are reported. Multiple regression analysis, performed on variables associated with the metabolic syndrome, was used to determine possible predictors for PAI-1$_{act}$ in the two groups. Standard multiple regression analysis was used to determine the independent contribution of possible predictors and forward stepwise regression analysis was used to determine the best overall predictors of PAI-1$_{act}$. There were no differences in the results from the raw data, or those adjusted for alcohol consumption, smoking, age and physical activity, thus only the results from the raw data are reported.

Results

Baseline characteristics

The general characteristics of the subjects are presented in Table 1. The Caucasian group had higher waist and hip circumferences ($p < 0.01$) than the African group. Despite this, the Caucasian group was more physically active than the African group. LDL-C ($p < 0.01$), TC and TG ($p < 0.001$) concentrations were significantly lower in the African group. The Caucasian group had higher PAI-1 and uric acid concentrations ($p < 0.001$), there were also more smokers in this group and daily intake of alcohol was higher. The African group, however, showed higher diastolic blood pressure and fibrinogen concentrations.

Table 1: Subject characteristics

Variables	African (n = 95) Mean (95% CI)	Caucasian (n = 114) Mean (95% CI)
Age (yr)	30.5 (28.9 , 32.2)	31.4 (29.6 , 33.1)
BMI (kg/m²)	27.6 (26.3 , 28.9)	28.9 (27.6 , 30.3)
Waist Circumference (cm)	80.7 (78.1 , 83.4) *	85.8 (83.1 , 88.6) *
Hip Circumference (cm)	105.8 (103.3 , 108.2) *	111.0 (108.3 , 113.7) *
Waist-hip ratio (cm)	0.76 (0.75 , 0.78)	0.77 (0.76 , 0.78)
Systolic BP (mmHg)	127.9 (124.7 , 131.2)	125.4 (123.2 , 127.6)
Diastolic BP (mmHg)	77.0 (74.9 , 79.1) §	72.5 (70.8 , 74.1) §
TC (mmol/L)	4.18 (4.00 , 4.36) §	4.95 (4.76 , 5.14) §
HDL-C (mmol/L)	1.27 (1.20 , 1.34)	1.21 (1.15 , 1.27)
LDL-C (mmol/L)	2.78 (2.61 , 2.95) *	3.16 (2.98 , 3.33) *
TG(mmol/L)	0.67 (0.60 , 0.74) §	1.28 (1.15 , 1.41) §
Glucose (mmol/L)	4.98 (4.89 , 5.08)	5.03 (4.95 , 5.10)
Insulin (µIU/ml)	13.3 (12.1 , 14.5)	13.2 (12.3 , 14.12)
HOMA score	2.98 (2.68 , 3.28)	2.99 (2.74 , 3.23)
hs-CRP (mg/L)	4.31 (2.83 , 5.80)	3.21 (2.51 , 3.92)
Uric acid (µmol/L)	292.9 (276.1 , 309.7) §	331.7 (319.1 , 344.36) §
PAI-1act (U/ml)	5.71 (4.80 , 6.62) §	12.9 (11.3 , 14.49) §
Fibrinogen (g/L)	3.87 (3.64 , 4.09) §	3.04 (2.94 , 3.15) §
Alcohol intake (g/day)	2.58 (1.60 , 3.56) §	6.53 (4.74 , 8.32) §
Smokers (n)	7	14
Physical Activity (MJ/Wk)	20.6 (17.8 , 23.3) §	34.1 (29.6 , 38.6) §
Metabolic syndrome (n)**	9	17

Note: Means with the same symbol differed significantly between groups: * p<0.01; § p<0.001

Abbreviations: 95% CI, 95% confidence interval, BP, blood pressure, TC, total cholesterol, HDL-C, high density lipoprotein cholesterol, LDL-C, low density lipoprotein cholesterol, TG, triacylglycerol, HOMA, homeostasis model assessment, MS, metabolic syndrome, BMI, body mass index, hs-CRP, high sensitivity C reactive protein, PAI-1$_{act}$, plasminogen activator inhibitor-1 activity

** As identified using the ATP III criteria [23].

Effect of race on mean plasma PAI-1$_{act}$

Mean PAI-1$_{act}$ in the Caucasian group was significantly (p<0.001) higher than in the African group (Fig. 1).
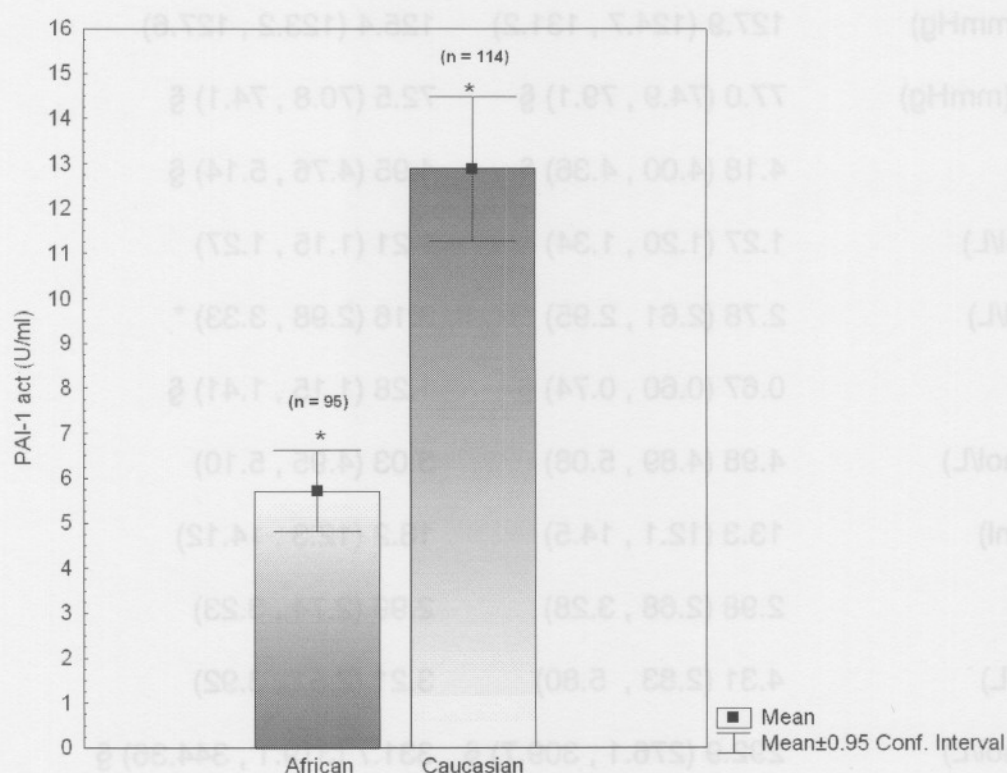

Fig. 1: Mean plasma PAI-1$_{act}$ stratified by race

Means with the same symbol differed significantly between groups

* p < 0.001

Effect of BMI on median plasma PAI-1ₐₜ

Median plasma PAI-1$_{act}$ differed significantly only between lean and obese African subjects (p = 0.014). These values (between 4.7 U/ml and 6.7 U/ml) were, however, still within normal physiological ranges (2.6 - 13.2 U/ml) [24]. In the Caucasian subjects a definitive increasing trend could be seen through the three BMI categories. There were significant differences between the lean and obese (p = 0.001) and also between the overweight and obese (p = 0.003) weight classes. These differences were more pronounced than in the African group and median PAI-1$_{act}$ was on average higher (Fig. 2).
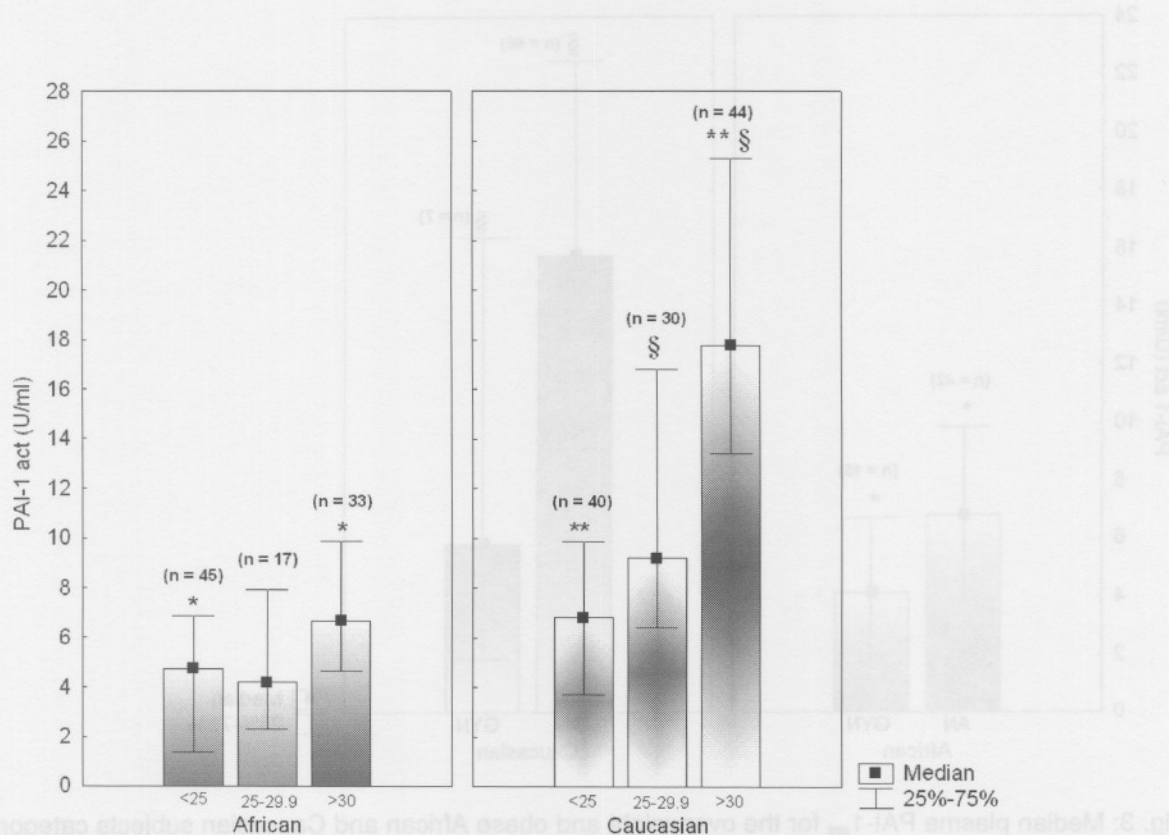

Fig. 2: Median plasma PAI-1$_{act}$ for the African and Caucasian groups, stratified for BMI categories

Medians with the same symbol differed significantly between groups

* p = 0.014, ** p = 0.001, § p = 0.003

Effect of fat distribution on median plasma PAI-1$_{act}$

In the android obese African group, median PAI-1$_{act}$ was significantly higher than in the group with a gynoid fat distribution (p = 0.048), although both median values still fell within the normal range (6.7 U/ml *vs* 4.1 U/ml). In the Caucasian group the difference in PAI-1$_{act}$ between android and gynoid fat distribution (p = 0.009) was more pronounced than the difference in the African group, with the median value (15.8 U/ml) of the android obese group being higher than normal and the median value of the gynoid obese group (5.8 U/ml) comparable with that of the android obese African group (Fig. 3).
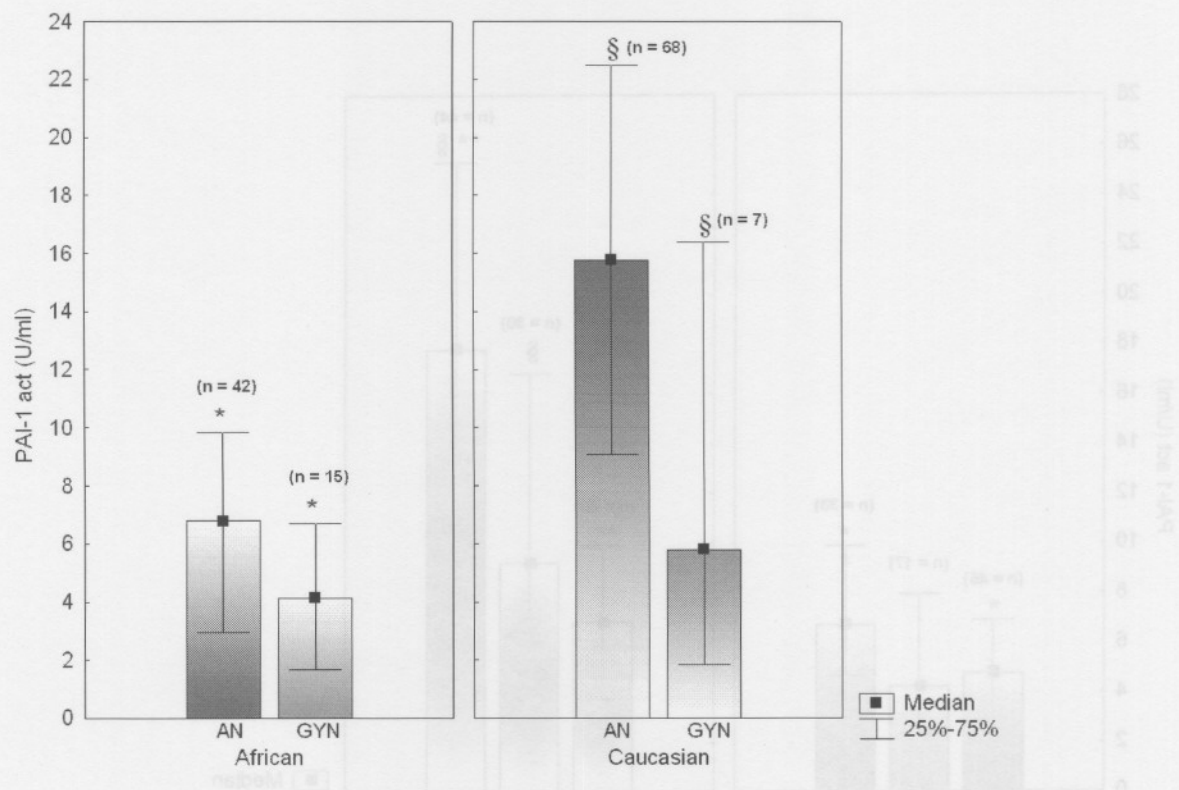

Fig. 3: Median plasma PAI-1$_{act}$ for the overweight and obese African and Caucasian subjects categorized by android or gynoid fat distribution

AN, android fat distribution, GYN, gynoid fat distribution

Medians with the same symbol differed significantly between groups

* p = 0.048, § p = 0.009

Effect of prevalence of the metabolic syndrome on mean plasma PAI-1$_{act}$

The same pattern can be seen here as with the android and gynoid fat distribution, with median PAI-1$_{act}$ being higher in subjects with the metabolic syndrome than those without any markers at all (Fig. 4). Again, the difference in the Caucasian group (p < 0.001) was much more pronounced than in the African group (p = 0.032), with the corresponding PAI-1$_{act}$ values being much higher in the Caucasians (22.5 U/ml *vs* 9.1 U/ml) than in the Africans (6.7 U/ml *vs* 4.1 U/ml).
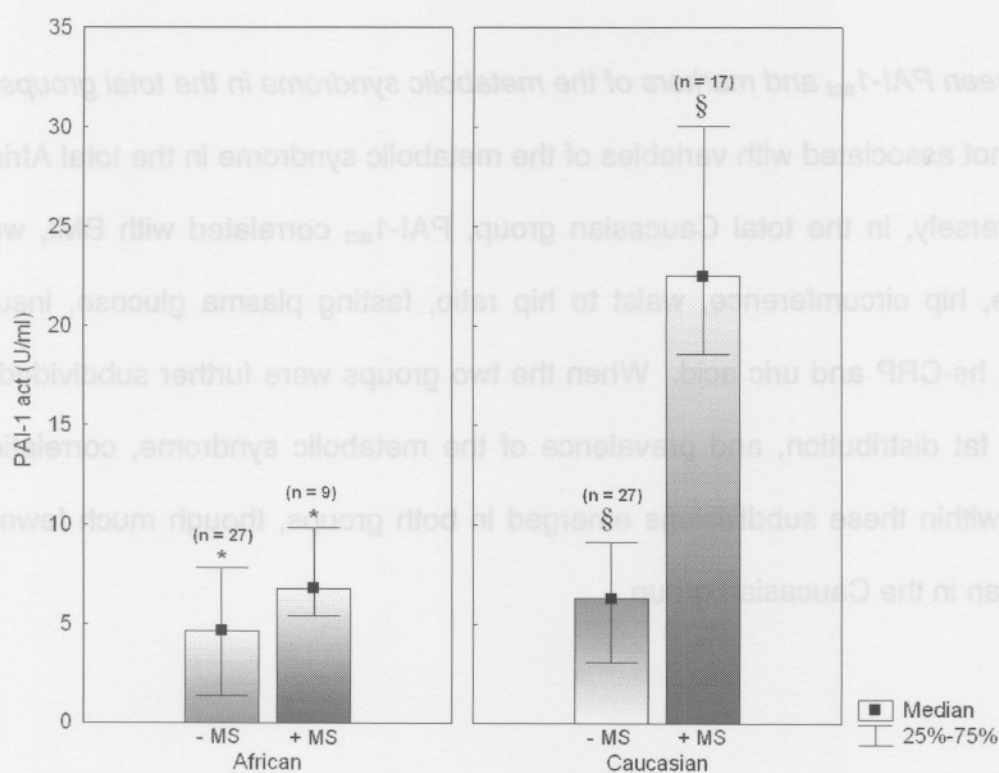

Fig. 4: Median plasma PAI-1$_{act}$ for the African and Caucasian groups, stratified by incidence of the metabolic syndrome

+MS, subjects with metabolic syndrome, -MS, subjects without any metabolic syndrome markers

Medians with the same symbol differed significantly between groups

* p = 0.032, § p < 0.001

Spearman rank order correlations were used to indicate the differences in the association of PAI-1$_{act}$ with markers of the metabolic syndrome in the total groups as well as in the subdivisions: BMI classes, gynoid and android obesity and prevalence of the metabolic syndrome. Only subjects with identified metabolic syndrome and those without any markers for the metabolic syndrome were compared in the subdivision for metabolic syndrome. Subjects with 1 to 2 markers (Africans n = 59, Caucasians n = 70) were excluded. This enabled us to compare the extremes whilst excluding the middle group. Also, only significant correlations were reported (Table 2).

Relation between PAI-1$_{act}$ and markers of the metabolic syndrome in the total groups:
PAI-1$_{act}$ was not associated with variables of the metabolic syndrome in the total African group. Conversely, in the total Caucasian group, PAI-1$_{act}$ correlated with BMI, waist circumference, hip circumference, waist to hip ratio, fasting plasma glucose, insulin, HOMA score, hs-CRP and uric acid. When the two groups were further subdivided by weight class, fat distribution, and prevalence of the metabolic syndrome, correlations with PAI-1$_{act}$ within these subdivisions emerged in both groups, though much fewer in the African than in the Caucasian group.
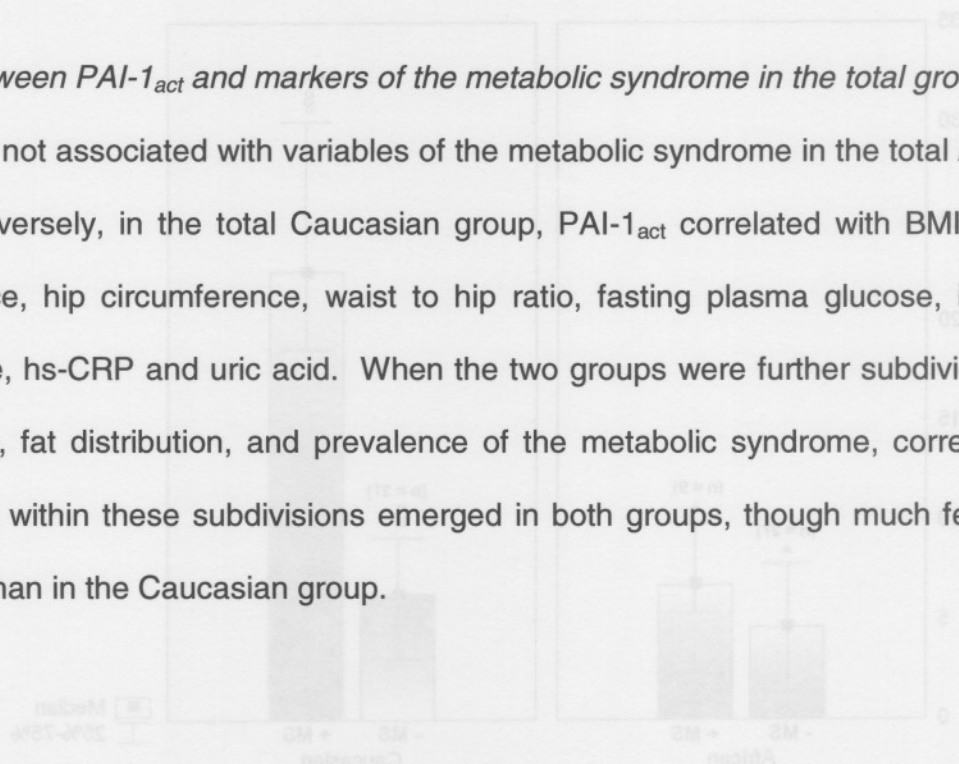
Table 2: Spearman rank order correlations (r) of PAI-1$_{act}$ with metabolic syndrome markers for the total groups as well as different subdivisions

	Total group	+ MS	- MS	Android obesity	Gynoid obesity	BMI < 25	BMI 25 – 29.9	BMI > 30
African								
n	95	9	27	42	15	45	17	33
Alcohol intake (g/day)						0.46		
Smoking (n)						0.35		
Glucose (mmol/L)		0.78	0.38					
Insulin (µIU/ml)			0.40				0.55	
HOMA-score			0.47				0.50	
Uric acid (µmol/L)						-0.37		
Caucasian								
n	114	17	27	68	7	40	30	44
Age (yr)				0.47		-0.32	0.60	
BMI (kg/m²)	0.60						0.60	0.39

Table 2: Spearman rank order correlations (r) of PAI-1$_{act}$ with metabolic syndrome markers for the total groups as well as different subdivisions (cont.)

	Total group	+ MS	- MS	Android obesity	Gynoid obesity	BMI < 25	BMI 25 – 29.9	BMI > 30
	Caucasian							
Waist Circumference (cm)	0.65			0.58			0.60	0.53
Hip Circumference (cm)	0.58			0.41				0.33
Waist-hip ratio	0.52						0.51	0.34
TG (mmol/L)		0.58						0.34
Glucose (mmol/L)	0.37							
Insulin (µIU/ml)	0.58	0.57		0.57			0.63	0.48
HOMA-score	0.60	0.62		0.58			0.66	0.49
hs-CRP (mg/L)	0.35							
Uric acid (µmol/L)	0.39			0.42				0.38

NOTE. Correlations are significant at $p < 0.05$

Abbreviations: MS, metabolic syndrome, BMI, body mass index, HOMA, homeostasis model assessment, hs-CRP, high sensitivity C reactive protein, TG, triacylglycerol, +MS, subjects with metabolic syndrome, -MS, subjects without any metabolic syndrome markers

Correlations with mean plasma PAI-1$_{act}$ in subgroups of subjects with the metabolic syndrome or without any markers for the metabolic syndrome:

PAI-1$_{act}$ correlated with more markers of the metabolic syndrome in the subgroup of African subjects classified without the syndrome than those with the metabolic syndrome. These correlations were, however, relatively weak (fasting plasma glucose, r = 0.38, serum insulin, r = 0.40, and HOMA score, r = 0.42). Only fasting plasma glucose concentrations correlated strongly (r = 0.78) with PAI-1$_{act}$ in African subjects with the metabolic syndrome. In the Caucasian subjects, however, there was a clear distinction between subjects with the metabolic syndrome and those without any markers for it. Strong correlations emerged between PAI-1$_{act}$ and serum TG (r = 0.58), serum insulin (r = 0.57) and HOMA score (r = 0.62) in the subjects with the metabolic syndrome, whilst no correlations emerged in the subjects without any markers for the syndrome.

Correlations with mean plasma PAI-1$_{act}$ in the android and gynoid obese subgroups:

When dividing all of the subjects with a BMI ≥ 25 into subgroups on the basis of android or gynoid fat distribution (as determined by waist circumference), PAI-1$_{act}$ had no correlation with any of the markers of the metabolic syndrome in either the android or gynoid obese African subgroups. However, in the Caucasian subjects, there was a definite distinction between these subgroups. PAI-1$_{act}$ correlated significantly with BMI, waist circumference, hip circumference, serum insulin, HOMA score and uric acid in the android obese subgroup, but shared no association with markers of the metabolic syndrome in the gynoid obese subgroup.

Correlations with mean plasma PAI-1$_{act}$ across the 3 different BMI categories:

There was no clear trend in the associations of PAI-1$_{act}$ with markers of the metabolic syndrome across the different weight classes in the African subjects. In the lean African subjects (BMI < 25), PAI-1$_{act}$ correlated with alcohol intake (r = 0.46) and smoking (r = 0.35), and negatively with uric acid (r = -0.37). These correlations were, however, not very strong. Serum insulin (r = 0.55) and HOMA score (r = 0.50) correlated more strongly with PAI-1$_{act}$ in the overweight African group, but no correlations emerged in the obese group. A clear trend in the association of PAI-1 with markers of the metabolic syndrome emerged in the Caucasian subjects. In the lean group, age correlated relatively weakly (r = -0.32) with PAI-1$_{act}$. In the overweight Caucasian subjects, PAI-1$_{act}$ correlated with BMI, waist circumference, waist to hip ratio, serum insulin and HOMA score. In the obese group, PAI-1$_{act}$ also correlated with BMI, waist circumference, waist to hip ratio, serum insulin and HOMA score, as well as hip circumference, TG and uric acid.

Predictors of PAI-1$_{act}$

Metabolic syndrome variables explained only 2.8% of the variance in PAI-1$_{act}$ in the African group. In the Caucasian group, the metabolic syndrome variables explained a much larger percentage of PAI-1$_{act}$ (60%). Waist circumference (34%) followed by uric acid (15%) had the largest independent contributions. These two variables respectively made up 47% and 3% of the total prediction, followed by HOMA score (8%) and alcohol intake (2%).

Discussion

In the Africans, PAI-1$_{act}$ for all subgroups were within suggested normal ranges, whereas in the Caucasians, the groups typically associated with the metabolic syndrome (obese, android obesity and identified metabolic syndrome cases) had PAI-1$_{act}$ values that were all elevated above the suggested normal ranges. Although relatively higher PAI-1$_{act}$ was also seen in these subgroups in the Africans, it was on average much lower than was seen in the Caucasians and still within suggested normal ranges. It would seem that PAI-1$_{act}$ is more tightly controlled in the African group, in spite of the occurrence of metabolic syndrome markers. Fibrinogen concentrations, however, were higher in the African than in the Caucasian subjects. This finding is in agreement with several previously conducted studies [10;37].

Definite differences in the association of PAI-1$_{act}$ with the markers of the metabolic syndrome could be seen between the African and Caucasian subjects. PAI-1$_{act}$ did not correlate with any markers of the metabolic syndrome in the total African group. Only 2.8% of the variance in PAI-1$_{act}$ was explained by these variables. In the total Caucasian group, however, uric acid and weight parameters, along with markers of insulin resistance correlated strongly with PAI-1$_{act}$. A clear trend of increasing PAI-1$_{act}$ with increased prevalence of markers of the metabolic syndrome could be seen in the Caucasian group. These markers explained 60% of the total variance in PAI-1$_{act}$ in the Caucasian subjects. As in the African subjects, waist circumference had the largest independent contribution, emphasizing the apparent importance of visceral fat distribution in the production of PAI-1 as found by several other studies [25-28].

Obesity, especially central adiposity, has been associated with increased concentrations of PAI-1 [28] and several studies have shown that BMI correlated

positively with PAI-1 concentrations in a variety of different types of subjects [29]. It has been suggested that adipose tissue itself may contribute to the elevated expression of PAI-1 in obesity [30;31]. Several *in vitro* studies have shown significantly higher PAI-1 production in human visceral adipose tissue than in subcutaneous adipose tissue [25;26;32]. These regional differences in PAI-1 production might be explained by a recent study that suggested that stromal cells, and not the adipocytes themselves, are the most important source of PAI-1 within adipose tissue [26], and since visceral fat contains more stromal cells than subcutaneous adipose tissue, it could explain the increased production of PAI-1 in visceral adipose tissue.

The results in the Caucasian group are consistent with those of several studies that found a positive association between PAI-1 concentrations and markers of the metabolic syndrome [28;33-36]. The results in the African subjects are in contrast to those of the previously mentioned studies, since markers of insulin resistance were weakly correlated with PAI-1$_{act}$ and predicted very little of its variability.

Race seems to have a definite influence on PAI-1$_{act}$, as previous studies conducted on African Americans [27;38;39] have found similar differences to those found in the Caucasian and African subjects. It has been speculated that genetic factors and potentially modifiable environmental factors could affect circulating PAI concentrations [27]. Certain polymorphisms in the PAI-1 gene are associated with increased concentrations thereof. The most extensively studied of these polymorphisms is the 4G/5G polymorphism in the promoter region of the gene [40]. Subjects homozygous for the 4G allele present higher plasma PAI-1 concentrations than the 5G/5G or heterozygous genotype, whether they be healthy or suffering from CVD or type 2 diabetes [41-44]. The reason for this might be that, even though both alleles bind a

transcriptional activator, the 5G allele also binds a repressor protein to an overlapping binding site, which decreases binding of the activator due to interference caused by steric hindrance [28].

In a previous study, it was found that African American subjects had a higher frequency of the 5G/5G genotype, along with lower circulating PAI-1 concentrations compared to Caucasian subjects [27]. The apparent role of the 4G/5G genotype in the ethnic differences seen in PAI-1 concentrations seems to be important and may explain the differences in PAI-1$_{act}$ between the African and Caucasian subjects in this study. Limited data are, however, available on genetic and environmental variables in African subjects and more studies need to be conducted in this regard. Concurrently, a large epidemiological study is underway, involving this group of researchers and several others, in which the prevalence of the 4G/5G genotype will be investigated in a large number of urban and rural African subjects. A study comparing urban Caucasian males with rural African males also found significantly lower PAI-1$_{act}$ in the Africans. The authors concluded that differences in environmental factors between the two groups, such as diet were an important determinant of PAI-1$_{act}$ [38].

Elevated PAI-1 concentrations are now well established as a core feature of the metabolic syndrome [40;45]. A question that now arises is whether this assumption can be made for ethnic groups other than Caucasians? Most notably, could this assumption be made for Africans, since the association of PAI-1$_{act}$ with markers of the metabolic syndrome is much less apparent than in Caucasians and since median PAI-1$_{act}$ in this study remained relatively low in spite of the presence of the metabolic syndrome and android obesity?

Acknowledgements

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