

**The association of uric acid and plasminogen activator inhibitor-1 (PAI-1) with cardiovascular function in South African women:
The POWIRS-study.**

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DECLARATION BY AUTHORS

The contribution of each of the researchers involved in this study is given in the following table:

<u>Name</u>	<u>Role in the study</u>
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The following is a statement from the co-authors confirming their individual role in the study and giving their permission that the article may form part of this dissertation.

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

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AFRIKAANSE TITEL: Die assosiasie van uriensuur en plasminogeen aktiveerder inhibeerder-1 (PAI-1) met kardiovaskulêre funksie in Suid-Afrikaanse vroue: Die POWIRS-studie

OPSOMMING

Motivering: Hipertensie is 'n gesondheidsrisiko wat vinnig aan die toeneem is, en wat lei tot verhoogde insidensie van kardiovaskulêre disfunksie en mortaliteit. Die voorkoms van hipertensie is egter hoër in sekere etniese populasies. Verskeie Suid Afrikaanse studies het bevind dat die Afrika populasie meer vatbaar is vir die ontwikkeling van hipertensie in vergelyking met die Kaukasiër populasie. Kardiovaskulêre disfunksie gaan gereeld gepaard met verhoogde vlakke van uriensuur (US) en plasminogeen aktiveerder inhibeerder-1 (PAI-1) en beide is faktore wat geassosieer word met die metaboliese sindroom. 'n Gebrek aan data omtrent die assosiasies van US en PAI-1 met kardiovaskulêre disfunksie in 'n Suid-Afrikaanse konteks, dien as motivering vir die uitvoer van hierdie studie.

Doelstelling: Om die assosiasie van US en PAI-1 met kardiovaskulêre disfunksie in Afrika- en Kaukasiër-vroue te bepaal.

Metodologie: Die manuskrip wat in Hoofstuk 2 vervat is, het gebruik gemaak van die data wat versamel is tydens die POWIRS ("Profiles of Obese Women with the Insulin Resistance Syndrome") studie. 'n Groep van 102 Afrika vroue en 115 Kaukasiër vroue woonagtig in die Noordwes Provinsie van Suid-Afrika, is volgens hulle liggaamsmassa-indeks gewarf. Die groepe is verdeel in skraal, oorgewig en obees volgens hulle liggaamsmassa-indeks. Antropometriese en kardiovaskulêre metings is geneem en bepalinge is gedoen van bloedlipiedprofile, US, PAI-1, vastende insulien en glukose vlakke, HOMA-IR

("homeostasis model assessment-insulin resistance") en leptienvlakke. Die proefpersone se totale proteïen inname is bepaal deur middel van 'n dieet vraelys. Vergelykings tussen die groepe is getref deur gebruik te maak van 'n onafhanklike t-toets asook 'n veelvuldige analise van kovariansie (MANCOVA) terwyl daar vir sekere veranderlikes gekorrigeer is. Elke etniese groep is verdeel in US en PAI-1 tertiele, waarvan die 1^{ste} en 3^{de} tertiele met mekaar vergelyk is. Korrelasie koëffisiënte was bepaal om enige assosiasies met US en PAI-1 met kardiovaskulêre veranderlikes asook veranderlikes van die metaboliese sindroom, aan te dui. Voorwaartse stapsgewyse meervoudige regressie analise is ook uitgevoer deur van US en PAI-1 as die afhanklike veranderlikes gebruik te maak.

Die studie is goedgekeur deur die Etiekkomitee van die Noordwes-Universiteit en al die proefpersone het skriftelik ingeligte toestemming gegee. Die leser word verwys na die *eksperimentele prosedure* afdeling in Hoofstuk 2 vir 'n meer breedvoerige bespreking van die proefpersone, studie-ontwerp en analitiese prosedure wat gevolg is.

Resultate en gevolgtrekking: Resultate van die POWIRS-studie toon aan dat ten spyte van die Afrika-vroue se hoër bloeddrukvlakke, hulle betekenisvolle laer vlakke van US en PAI-1 het in vergelyking met die Kaukasiër-vroue. Alhoewel die Kaukasiër-vroue betekenisvolle hoër vlakke van US en PAI-1 het, toon hulle geen teken van kardiovaskulêre disfunksie nie. Die nadelige effekte van die verhoogde vlakke van US en PAI-1 kan dalk egter in die toekoms meer merkbaar word met 'n toename in ouderdom. Vanuit hierdie studie word dit dus afgelei dat US en PAI-1 nie geassosieer word met die verhoogde bloeddruk wat by jong Afrika-vroue waargeneem word nie.

SLEUTERWOORDE: uriensuur, plasminogeen aktiveerder inhibeerder-1, kardiovaskulêre disfunksie, Afrika-vroue, Kaukasiër-vroue.

SUMMARY

Motivation: Hypertension is a fast growing health risk, leading to increased incidences of cardiovascular dysfunction and mortality. However, the prevalence of hypertension is higher in some ethnic populations than others. Several South African studies have found that the African population is more susceptible to the development of hypertension, compared to the Caucasian population. Cardiovascular dysfunction is often accompanied by elevated levels of uric acid (UA) and plasminogen activator inhibitor-1 (PAI-1) and both are factors associated with the metabolic syndrome. A lack of data regarding the association of UA and PAI-1 with cardiovascular dysfunction in a South African context, serves as a motivation for conducting this study.

Objective: To determine the association of UA and PAI-1 with cardiovascular dysfunction in African and Caucasian women from South Africa.

Methodology: The manuscript presented in Chapter 2 made use of the data obtained in the POWIRS (Profiles of Obese Women with the Insulin Resistance Syndrome) study. A group of 102 African women and 115 Caucasian women, living in the North West Province of South Africa, were recruited according to their body mass indexes. The groups were divided into lean, overweight and obese according to their body mass index. Anthropometric and cardiovascular measurements were taken and determinations were done of their blood lipid profiles, UA, PAI-1, fasting insulin and glucose levels, HOMA-IR (homeostasis model assessment-insulin resistance) and leptin levels. The subject's total dietary protein intake was determined by means of a dietary questionnaire. Comparisons between the groups were done using an independent t-test as well as a multiple analysis of covariance (MANCOVA) whilst adjusting for certain variables. Each ethnic group was divided into UA

and PAI-1 tertiles, for comparison between the 1st and 3rd tertiles. Correlation coefficients were determined to show any associations between UA and PAI-1 with cardiovascular variables as well as variables associated with the metabolic syndrome. Forward stepwise multiple regression analyses were performed using UA and PAI-1 respectively as dependent variables.

The study was approved by the Ethics committee of the North-West University and all the subjects gave informed consent in writing. The reader is referred to the *experimental procedure* section in Chapter 2 for a more detailed description of the subjects, study design and analytical procedures used in this dissertation.

Results and conclusion: Results from the POWIRS-study showed that despite the African women's higher blood pressure, they had significantly lower levels of UA and PAI-1 compared to the Caucasian women. Although the Caucasian women had significantly higher circulating levels of UA and PAI-1, they showed no sign of cardiovascular dysfunction. The detrimental effects might, however, become more noticeable with an increase in age. From this study it is concluded that UA and PAI-1 is not associated with the increased blood pressure in young African women.

KEYWORDS: uric acid, plasminogen activator inhibitor-1, cardiovascular dysfunction, African women, Caucasian women.

PREFACE

For the structure of this study it was decided to use the article format. This chapter (Chapter 1) serves as an introduction and provides a motivation, background and a synopsis of the knowledge that is needed for the interpretation of the data. At the beginning of Chapter 2 is a summary of the instructions for authors of the journal aimed for publication. The format of the article (and the rest of the dissertation) complies with the *Journal of Hypertension*. Chapter 3 is a summary of the study results which includes recommendations for future research. The appropriate references are provided at the end of each chapter.

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LIST OF ABBREVIATIONS

ANCOVA:	Analysis of covariance
Ang II:	Angiotensin II
AT1:	Angiotensin receptor type 1
BMI:	Body mass index
CO:	Cardiac output
C _w :	Windkessel compliance
DBP:	Diastolic blood pressure
HDL:	High-density lipoproteins
HIV:	Human immunodeficiency virus
HOMA-IR:	Homeostasis model assessment-insulin resistance
HR:	Heart rate
hsCRP:	High sensitive C-reactive protein
IDF:	International Diabetes Federation
MANCOVA:	Multiple analysis of covariance
MAP:	Mean arterial pressure
MAP:	Mitogen activated protein
MCP-1:	Monocyte chemoattractant protein-1
mRNA:	Messenger ribonucleic acid

NO:	Nitric oxide
PAI-1:	Plasminogen activator inhibitor-1
PDGF:	Platelet derived growth factor
POWIRS:	Profiles of Obese Women with the Insulin Resistance Syndrome
RAS:	Renin-angiotensin system
ROS:	Reactive oxygen species
SBP:	Systolic blood pressure
TG:	Triglycerides
tPA:	Tissue plasminogen activator
uPA:	urokinase plasminogen activator
TPR:	Total peripheral resistance
UA:	Uric acid
VSMC:	Vascular smooth muscle cells
WC:	Waist circumference
WHO:	World Health Organization

CHAPTER 1

INTRODUCTION

GENERAL INTRODUCTION

During recent years, rates of cardiovascular events have escalated rapidly in many parts of the world to epidemic proportions, causing nearly 17 million deaths per year [1]. According to the first South African National Demographic and Adult Health Survey conducted in 1998 [2], 23.7% of African women have hypertension. Several epidemiological studies revealed that the African population has a higher prevalence of hypertension, compared to the Caucasian population [3-5].

Hypertension *per se* is a serious health risk, but it is often associated with other non-communicable diseases such as type 2 diabetes/ insulin resistance [6-8] and obesity [5] [9-11] to form the well known metabolic syndrome [12].

In some African cultures, increased body weight is a sign of wealth and health, and esthetically looked upon [13], and this phenomenon might explain the high prevalence of obesity in black South African women. According to the THUSA (Transition and Health during Urbanisation of South Africans) study conducted in the North West province of South Africa, data revealed that 53.8% of black women are above the normal body mass index of 25kg/m², 25.2% of these women were overweight while 28.6% were obese [14]. Both hypertension and obesity are often associated with elevated levels of uric acid (UA) [15] [16] and plasminogen activator inhibitor-1 (PAI-1) [17] [18].

UA is an end product of purine metabolism with serum concentrations ranging between 120-420 µmol/L [19]. For years researchers have debated over the possible causal association of UA in cardiovascular diseases and vascular dysfunction. Some researchers believe that

elevated levels of UA act only as a prognostic marker, reflecting existing cardiovascular and metabolic conditions such as hypertension, insulin resistance and type 2 diabetes, while others consider it a causative factor, leading to cardiovascular diseases such as hypertension [16] and atherosclerosis [20].

It is speculated that the adipose tissue, especially visceral adipocytes, play an important role in the production of UA as well as a decrease in the excretion of UA [21]. Therefore an increase in body fat will lead to an increase in circulating UA levels.

Adipose tissue also acts as an endocrine organ, secreting a variety of biologically active compounds known as adipokines [22]. One such an adipokine is PAI-1 [22]. PAI-1 is involved in the fibrinolytic system, working antagonistically with plasminogen activator to maintain the homeostasis of the blood coagulation process [23]. An abnormal level of PAI-1 will disrupt the homeostasis, resulting in increased risk of thrombus formation, myocardial infarction and associated vascular diseases [24]. Because of the high frequency of hypertension especially in the black South African population, compared to Caucasians, it can therefore be speculated that this population will have higher levels of circulating UA and PAI-1, which might be associated with the development of cardiovascular dysfunction. Several studies have been conducted on these topics but many of them have been done in other countries [25-29]. The lack of adequate data concerning UA and PAI-1 related cardiovascular dysfunction in a South African population group serves as the motivation for conducting this study.

AIM

The aim of this study was to investigate the possible association of uric acid and plasminogen activator inhibitor-1 in cardiovascular dysfunction in African and Caucasian women from South Africa.

HYPOTHESIS

1. Due to the higher prevalence of hypertension in the African population [3] [4] [5], African women will have higher levels of serum uric acid and plasminogen activator inhibitor-1 compared to Caucasian women, as well as within each level of obesity.
2. There are associations between serum uric acid, plasminogen activator inhibitor-1 and cardiovascular dysfunction and components of the metabolic syndrome in African and Caucasian women.

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LITERATURE STUDY

The positive association between elevated uric acid (UA) levels and cardiovascular diseases has been recognized as far back as the early 1970's [1]. Several studies conducted during the years reveal that elevated levels of UA can be considered a cardiovascular risk factor [2] [3]. Despite the results of previous studies revealing the importance of elevated UA levels in cardiovascular diseases, there are still controversial opinions. Some researchers believe that UA should be considered a risk marker, rather than a risk factor, because of its association with other risk factors such as obesity, hyperinsulinemia, hypertension and other metabolic disorders.

Uric acid

UA (Figure 1) is the final oxidation product of purine catabolism [4] [5] formed from the breakdown of adenosine and guanine [6]. UA is mainly produced in the liver and then secreted into the bloodstream [7]. Mutations on the uricase gene render it nonfunctional in humans [8] and as a result humans are unable to degrade UA further to allantoin [9]. Under normal physiological circumstances, the renal handling of UA involves 4 pathways – 1) filtration; 2) reabsorption; 3) secretion, and 4) postsecretory reabsorption [10] [11]. The kidneys excrete approximately 70% of the daily production of UA and the rest undergoes intestinal elimination [10] [12].

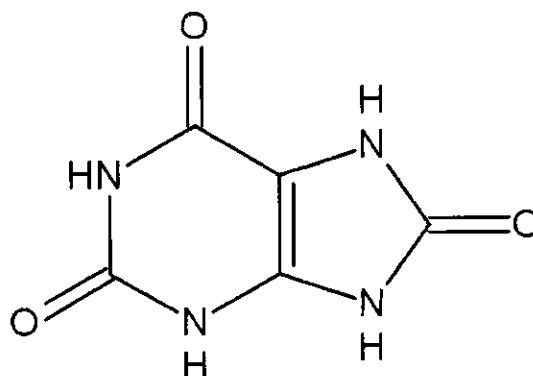


Fig. 1. Chemical structure of uric acid. Uric acid is an organic compound of carbon, nitrogen, oxygen and hydrogen formed during the degradation of purines in the human body [13] .

Uric acid and cardiovascular function

The endothelium plays a critical role in sustaining vascular homeostasis [14]. Besides its role in homeostasis of vasodilation and vasoconstriction, fibrinolysis and thrombogenesis, the endothelium also has a vasoprotective role, control of smooth muscle cell growth and migration, as well as suppression of inflammatory responses [15] [16]. The endothelium plays a role in the control of blood flow and responds to it by releasing vasoactive substances [15]. However, when the endothelial wall is damaged or injured, it loses its protective characteristics and converts to one that is vasoconstrictive [17].

The hallmark of endothelial dysfunction is impaired nitric oxide release [18]. Nitric oxide (NO) is a potent vasodilator [19], opposing the effects of endothelium-derived constrictors such as

Angiotensin II (Ang II) and Endothelin-1 [14]. A reduction in the bioavailability of NO is an important step in the development of endothelial dysfunction and atherosclerosis [18]. It is speculated that UA *per se* is responsible for a decrease in NO bioavailability [20] [21], however, the exact mechanism is not fully understood.

Besides its possible direct effect on NO bioavailability, UA may also exert other detrimental effects on the vascular system. Cells, especially vascular smooth muscle cells (VSMC), do not express receptors for UA, but rather have organic anion transporters that allow UA uptake [22]. Once inside the VSMC, UA activates a series of pathways, which include cyclooxygenase-2, up regulation of platelet-derived growth factor [23] and local thromboxane formation [4]. These pathways have the ability to induce cell proliferation and inflammation [4], contributing to the development of cardiovascular diseases.

Uric acid and hypertension

Hypertension (Table 1) has been reported to be one of the most common causes of cardiovascular events [24]. In the early 1900's there were continuous reports of the association between uric acid and hypertension [25]. The prevalence of hyperuricemia is almost 25% in hypertensive subjects and 75% in malignant hypertensive subjects [26]. Despite the controversy surrounding UA as a causative factor or a representative marker, it was not until recently that possible mechanisms linking UA and hypertension were explained.

One possible mechanism is that UA causes renal vasoconstriction induced by a decrease in the bioavailability of NO (endothelial dysfunction) and stimulation of the renin-angiotensin system (RAS) [27]. The RAS is one of the major physiological regulators of blood pressure.

In the vascular system the binding of Ang II on AT1 receptors causes vascular constriction, as well as expression of plasminogen activator inhibitor type 1 (PAI-1) [28]. According to Kang *et al.* [29], UA induces the upregulation of Angiotensin type 1 receptors (AT1). In the smooth muscle cells, the responsiveness of Ang II will depend on the expression of the AT1 receptors and an upregulation of these receptors will lead to an increased vascular reactivity [30].

Table 1. Guidelines for blood pressure classification.

Classification	Systolic (mmHg)		Diastolic (mmHg)
Normal	<120	and	<80
Prehypertension	120-139	and/or	80-89
Stage 1 hypertension	140-159	and/or	90-99
Stage 2 hypertension	≥160	and/or	≥100

Guidelines Committee – European Society of Hypertension [31]

Uric acid and the metabolic syndrome

It is difficult to identify the specific role of UA because of its association with certain cardiovascular risk factors [32]. It is known that elevated UA levels (>327 $\mu\text{mol/L}$) are

associated with many components of the metabolic syndrome [33] such as obesity [34], hypertension [35], insulin resistance [36] and dyslipidemia [37]. These risk factors cluster together to form the well-known metabolic syndrome. The metabolic syndrome is a complex disorder as well as an emerging clinical challenge. It is considered a "multiplex" cardiovascular risk factor, in that each component of the cluster of abnormalities is a risk factor in its own right [38].

According to the International Diabetes Federation (IDF) [39], the metabolic syndrome can be defined as: central obesity (waist circumference ≥ 94 cm for men and ≥ 80 cm for women) plus any two of the following:

- 1) Raised triglycerides (TG) levels - ≥ 1.7 mmol/L
- 2) Reduced high-density lipoproteins (HDL cholesterol) - ≤ 1.03 mmol/L for males and ≤ 1.29 mmol/L for females
- 3) Raised blood pressure (BP) – systolic BP ≥ 130 mmHg or diastolic BP ≥ 85 mmHg
- 4) Raised fasting plasma glucose (FPG) ≥ 5.6 mmol/L.

It is known that elevated UA levels (>327 $\mu\text{mol/L}$) and hyperuricemia (>387 $\mu\text{mol/L}$) are associated with many components of the metabolic syndrome [33].

Uric acid and obesity

During the last few years, obesity has reached epidemic proportions not only in developed countries but also in developing countries [40]. Obesity is strongly influenced by urbanization

[41] [42] especially in black South Africans [43], but also by a higher socio-economic status [44] [45]. The adoption of a Westernized lifestyle has subjected the black population to a variety of physiological changes [46]. According to Kruger *et al.* [43], black South African women have higher obesity levels compared to white South African women.

Obesity is characterized by an increase in body fat and determined using the body mass index (BMI): $BMI = \text{Weight (kg)} / (\text{Height (m)})^2$ [47]. The World Health Organization has established certain cut-off points for classifying certain obesity levels in adults.

Table 2. Classification of body mass index according to the World Health Organization.

Classification	BMI (kg/m ²)	Risk for co-morbidities
Normal range	18.5 – 24.9	Average
Overweight	≥25.0	Average
Preobese	25.0 – 29.9	Increased
Obesity Class I	30.0 – 34.9	Moderate
Obesity Class II	35.0 – 39.9	Severe
Obesity Class III	≥ 40.0	Very severe

World Health Organization, 2000 [48]

Obesity is a risk factor relating to cardiovascular events, but not all obese individuals are at equal risk for developing obesity-related cardiovascular and metabolic diseases [49] [50].

One must take into account an even more relevant part of obesity: namely fat distribution. There are two patterns of fat distribution. One is peripheral obesity – where the fat depots are mainly subcutaneously in the gluteal region [51], and the second and perhaps the most important one, is abdominal obesity [47]. Intra-abdominal obesity, more commonly known as visceral obesity, is characterized mostly by depots of adipose tissue around the abdominal area and the gastrointestinal organs [34] [51]. The difference between these two is important since metabolic and cardiovascular complications are directly related to abdominal adipose tissue depots and not peripheral obesity [50-52].

According to a study done by Matsuura *et al.* [34], it was found that high levels of UA often accompany obesity, especially abdominal obesity [52]. Although the specific role of obesity in elevated UA levels is not entirely clear, it is speculated that adipose tissue plays an important role in the production of UA as well as inhibiting UA excretion.

Uric acid and insulin resistance

In insulin resistance the tissues have a diminished ability to respond to the action of insulin [53] and are usually the precursor for type 2 diabetes [54]. To compensate for the resistance, the pancreas secretes even more insulin, and over time, the excess insulin secretion leads to a drop in insulin production as a result of exhaustion of the pancreatic β -cells. The type 2 diabetic may then become insulin dependent.

Several studies reveal that high levels of plasma insulin often accompany elevated levels of UA [38] [55]. According to Waring *et al.* [5], insulin increases UA levels by acting on the renal

handling, inhibiting the excretion of UA via the proximal tubules. This mechanism might explain the association between hyperuricemia and hyperinsulinemia.

Uric acid as an anti-oxidant

In the previous paragraphs the detrimental effects of UA have been highlighted. However, there is another characteristic of UA that has not been reviewed: UA as an antioxidant. UA constitutes a great deal of the antioxidant capacity in the blood [56]. In vitro studies have demonstrated undeniably the important antioxidant ability of UA by binding with biological active oxidants [57].

An antioxidant is an enzyme or other organic molecule that can counteract the damaging effects of active oxidants in tissues [58]. Although the term technically refers to molecules that react with oxygen, it is often applied to any molecules that protect cells or organs against the damaging effects of oxidative stress [59]. Oxidative stress is a term used to describe the level of damage in a cell, tissue or organ caused by the reactive oxygen species (ROS) [60].

Oxidative stress occurs when the balance between oxidation and redox reactions is altered either by an overproduction of ROS or as a result of a deficiency in antioxidants [60] [61]. The role of oxidative stress in the development of several cardiovascular diseases has been the topic of several discussions and research studies during the last few years [62-64]. ROS causes endothelial dysfunction, as well as VSMC proliferation, which both result in the development of atherosclerosis [65] [66].

Although UA has profoundly beneficial properties, in certain physiological conditions it can easily be converted to a pro-oxidant [26] [67], producing ROS instead of scavenging ROS

[26]. In the early stages of atherosclerosis UA acts as an antioxidant, however, in the later, more developed stages of atherosclerosis, the characteristics of the antioxidant shift to take on characteristics of a pro-oxidant [26] [68].

Plasminogen activator inhibitor-1

Plasminogen activator inhibitor-1 (PAI-1) belongs to the family of serine protease inhibitors, and has a molecular mass of 50 000 Dalton [69]. It is secreted by a variety of cells, which include adipocytes, VSMC, hepatocytes, and endothelial cells [70]. PAI-1 is an important factor in blood coagulation by acting as a physiological inhibitor of tissue plasminogen activator (t-PA) and urokinase plasminogen activator (u-PA), maintaining blood coagulation homeostasis. The t-Pa and u-Pa both play an essential part of the fibrinolysis process [71]. The t-PA plays an important part in fibrin homeostasis, cleaving the inactive plasminogen, and so releasing active plasmin, which in turn is responsible for lysis of fibrin clots [72]. While u-PA, on the other hand, is involved in cell migration, tissue remodeling [73] and proteolysis [74].

There are several factors that profoundly influence the circulating PAI-1 concentrations in the blood. These factors include obesity [75], insulin and glucose concentrations [76] and Ang II [28] (Fig. 2), which will be discussed respectively.

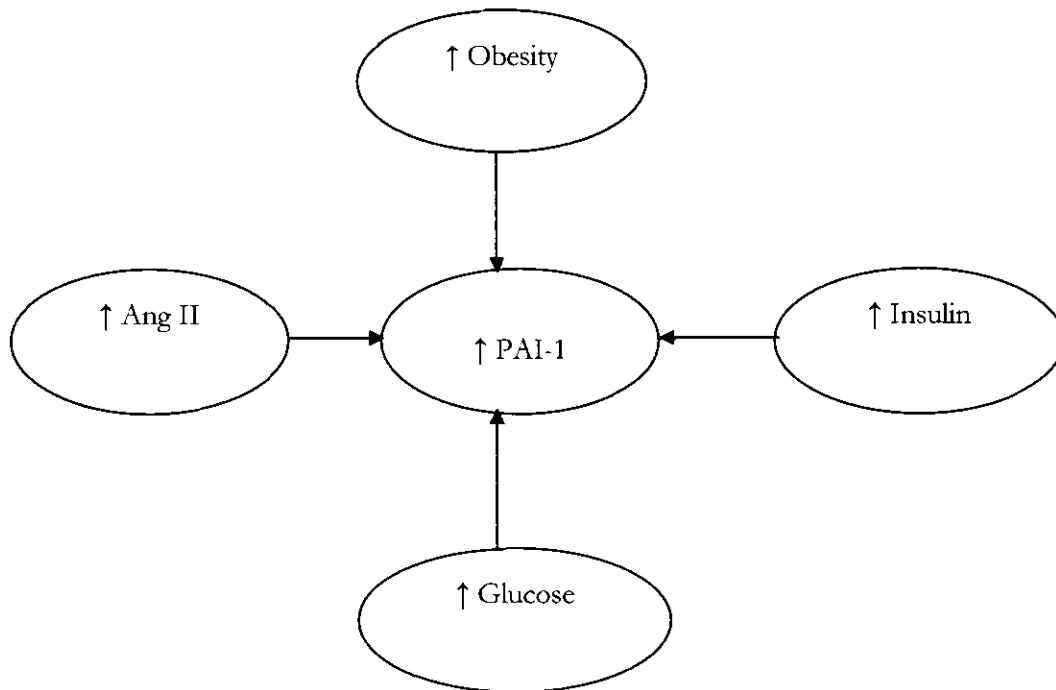


Fig. 2. Factors known to increase plasminogen activator inhibitor-1 (PAI-1) levels

For many years researchers believed that adipocytes act only as a reservoir for unused energy. It then became clear that this tissue acts as an endocrine organ secreting a variety of bioactive compounds (known as adipokines) [77] such as leptin, interleukin-6, proinflammatory cytokines and adiponectin [78]. Obesity appears to contribute significantly to the circulating PAI-1 levels [79], since obese animals and people have higher than normal PAI-1 levels [80]. Several clinical studies conducted revealed that surgical removal of fat mass or weight loss caused a significant reduction in PAI-1. According to a study conducted by Giltay *et al.* [81], it was found that visceral adipose tissue has a high capacity to produce PAI-1, independent of insulin levels and triglycerides.

The theory that insulin may play a role in the elevation of PAI-1 is derived from observations that type 2 diabetics with hyperinsulinemia often display impaired fibrinolysis [82] [83]). This impaired fibrinolysis is a possible result of the elevated levels of PAI-1. Patients with type 2 diabetes have a much higher risk for developing atherosclerosis than those who are non-diabetic [84]. It is believed that insulin *per se* causes PAI-1 levels to rise [76] by stimulating the biosynthesis of PAI-1 from adipose tissue [84]. The specific mechanism involved is not yet fully understood.

PAI-1 levels are also regulated by the RAS system [28], which acts as a regulator of blood pressure [28] [86]. Ang II has also been shown to increase the expression and secretion of PAI-1 [87].

PAI-1 and atherosclerosis

The development of atherosclerosis is a slow and progressive process. It is a disease that affects the arteries and is characterized by a build-up of lipids, cholesterol and other cellular debris within the arterial intima [88]. This plaque build-up in the arteries leads to vascular remodeling, impaired blood flow and diminished oxygen and nutrient supply to target organs. Atherosclerosis is often accompanied by vascular impairments such as endothelial dysfunction and vascular inflammation [89], as well as elevated levels of PAI-1 [88].

According to a study done by DeYoung *et al.* [90] it was found that PAI-1 might cause an increase in cell migration as well as an increase in cell proliferation, leading to the formation of neo-intima and atherosclerotic lesions. By binding with plasminogen activators, forming an inactive complex, PAI-1 diminishes the fibrinolytic process [91] adding a burden of thrombus

formation [71]. The existence of a thrombus within the arteries can accelerate the development of atherosclerosis by exposing the blood clot to more coagulation factors [71].

Ethnic differences in uric acid and PAI-1 levels

Associations between UA, PAI-1 and cardiovascular diseases have been reported [1] [75] [92-94], but few studies have been conducted in a South African population group. Most studies involved population groups from the United States. Several studies conducted in other countries found inconsistent results linking UA with any cardiovascular event. According to Watanabe *et al.* [8], the black African-American population is at higher risk for the development of hyperuricemia. Since African-American and black people from South Africa differ physically and genetically, it is difficult to extrapolate the data to South Africans [95]. Because of limited existing data, it is needed to conduct a comparative study to evaluate the levels of uric acid in African as well as Caucasian groups. It is well known that there is a difference in cardiovascular risks between different ethnic groups [94] [96] and one can expect to find different physiological effects in these groups, contributing to cardiovascular diseases.

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CHAPTER 2

THE ASSOCIATION OF URIC ACID AND PLASMINOGEN ACTIVATOR INHIBITOR-1 (PAI-1) WITH CARDIOVASCULAR FUNCTION IN SOUTH AFRICAN WOMEN: THE POWIRS-STUDY

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INSTRUCTION FOR AUTHORS: *Journal of Hypertension*

- An abstract of not more than 250 words should follow the title
- The abstract should be followed by a list of 3-10 key words or short phrases which will assist the cross-indexing of the article
- The article should be structured into the following sections headed Introduction, Methods, Results, and Discussion.
- References should be numbered consecutively in the order in which they first appear in the text and should be assigned Arabic numerals, which should be in brackets, e.g. [17]. The first seven or fewer authors should be listed; in the case of eight or more authors, list only the first six names, followed by et al.

Lip GY, Blann AD. Does hypertension confer a prothrombotic state? Virchow's triad revisited. *Circulation* 2000; 101:218-220.

- Journal names should be abbreviated as in the Index Medicus.
- Tables and figures should be assigned a numerical number, e.g. Table 1 and Fig 2.
- Scientific measures should be expressed in SI units, with blood pressure in mmHg.
- Margins should be not less than 3 cm.
- Double spacing should be used throughout the manuscripts
- Page numbers should be placed in the top right hand corner of each page.

ABSTRACT

Background: Elevated levels of uric acid (UA) and plasminogen activator inhibitor-1 (PAI-1) are often associated with cardiovascular dysfunction and the metabolic syndrome. There are no data concerning elevated levels of UA and PAI-1 in African and Caucasian women from South Africa. Since African women are more susceptible to hypertension than the Caucasian population, it is hypothesized that they will have higher levels of UA and PAI-1 than Caucasian women. We also aim to determine whether UA and PAI-1 levels are associated with cardiovascular dysfunction and the components of the metabolic syndrome within each ethnic group.

Methods: Women from African (N=102) and Caucasian (N=115) descent were recruited and their UA and PAI-1 levels measured. Comparisons between the two groups were performed as well as correlations within each ethnic group to determine correlations between UA, PAI-1 and cardiovascular variables as well as components of the metabolic syndrome.

Results: The African women had significant lower levels of UA ($p<0.01$) and PAI-1 ($p<0.01$) compared to the Caucasian women for lean, overweight and obese women despite their significantly higher blood pressure. After adjusting for components of the metabolic syndrome (waist circumference, body mass index, fasting glucose and fasting insulin levels), all significant correlations between UA and PAI-1 with cardiovascular variables disappeared. Both UA and PAI-1 strongly correlated with waist circumference, an indicator of abdominal obesity. When comparing the waist circumference between the two

ethnic groups, it is clear that the Caucasian women had a greater waist circumference than the African women, hence a higher prevalence of abdominal obesity.

Conclusion: Despite their high prevalence of hypertension, the African women had lower UA and PAI-1 levels, possibly because of their lower waist circumference compared to the Caucasian group. Although the Caucasian women showed a better cardiovascular profile compared to the African women, their elevated UA and PAI-1 levels might at a later stage lead to noticeable cardiovascular dysfunction.

INTRODUCTION

Several epidemiological studies have shown that the African population is seen as a high risk group regarding the prevalence of hypertension [1-3]. According to data from the THUSA study conducted in the North-West Province of South Africa, up to 34.8 % of the African population had a systolic blood pressure above 140 mmHg, while 26.9 % had a diastolic blood pressure above 80 mmHg [4]. Hypertension on its own is a serious health risk, but is often associated with obesity [5].

Obesity has evolved into one of the greatest health concerns worldwide and a South African demographic study conducted in 1998 revealed that 30.5 % of black women over the age of 15 years are obese [6]. In the North West Province 25.2% of African women are overweight and 28.6% are obese, resulting in more than half (53.8%) of African women being over the normal body mass index of 25 kg/m² [7]. The same study revealed that African women have higher obesity levels compared to their Caucasian counterparts.

The clustering of hypertension with obesity and insulin resistance is commonly known as the metabolic syndrome [8]. There are however, other factors such as hyperuricemia (uric acid (UA) levels $\geq 387 \mu\text{mol/L}$) and impaired fibrinolysis (elevated levels of plasminogen activator inhibitor-1 (PAI-1)) that are associated with the metabolic syndrome and its components [8-11], but are often overlooked and considered trivial.

It is still a controversial issue whether UA should be considered a risk factor leading to cardiovascular dysfunction [12-14] or merely a risk marker associated with existing cardiovascular dysfunction [15]. Although UA is formed through the breakdown of purines,

it is speculated that adipocytes, especially visceral adipocytes, also contribute to the production of UA [16]. An increase in adipose mass will therefore, lead to an increase in UA production.

Adipose tissue is not only involved in the production of UA, but also acts as an endocrine organ, by secreting a variety of biological active compounds known as adipokines [17]. One such an adipokine is PAI-1 [18-20]. PAI-1 acts as an antagonist of plasminogen activators [21], which forms an essential part in the fibrinolytic process. Abnormally high levels of PAI-1 will cause impairment of the fibrinolytic process [22], augmenting the risk of thrombus formation.

UA and PAI-1 might both be associated with obesity-related cardiovascular dysfunction as well as some components of the metabolic syndrome. It is, therefore, important to investigate possible mechanisms linking UA and PAI-1 with cardiovascular dysfunction.

UA is still under the spotlight concerning its association with cardiovascular dysfunction and there are a few possible mechanisms that might explain this association. UA is an end product of purine metabolism with serum concentrations ranging from 120 $\mu\text{mol/L}$ to as high as 420 $\mu\text{mol/L}$ [23]. Elevated serum levels of UA are not always the result of over production; it can result due to a decrease in excretion as well [24]. Insulin, for example, is known to elevate UA levels by decreasing the excretion of UA via the proximal tubules [10] [25] [26]. Hyperinsulinemia is, therefore, often associated with high levels of UA.

UA might stimulate the renin-angiotensin system [27], resulting in increased levels of Angiotensin II (Ang II). Ang II is a potent vasoconstrictor [28], which increases blood

pressure through binding on angiotensin receptors [29]. According to an experimental study conducted by Mazzali *et al* [27], it was found that UA causes arteriolopathy, independent of blood pressure. They also found that the development of the lesions in the renal arterioles could be blocked by using antagonists of angiotensin receptors, leading them to think that Ang II could play a role in the development of the remodelling of the vasculature, resulting in hypertension.

Another possible mechanism proposes that UA stimulates cell proliferation within the arterial wall [25], resulting in accelerated atherosclerosis. By binding on an anion exchanger/transporter, UA enters the vascular smooth muscle cells (VSMC) and activates a cascade of reactions [26]. These reactions include the activation of mitogen activated protein (MAP) kinase, which indirectly leads to an increase in thromboxane 2 and cyclooxygenase-2 activities [25]. An increase in thromboxane 2 will lead to an increase in monocyte chemoattractant protein-1 (MCP-1), resulting in inflammation [25] [26]. On the other hand, an increase in cyclooxygenase-2 will result in an increase in platelet derived growth factor (PDGF), leading to cell proliferation [25] [26].

As previously mentioned, elevated levels of PAI-1 are often associated with the atherothrombotic state observed in diabetes/insulin resistance [21] [22] [30] [31-33] and obesity [18] [34], but are usually considered trivial. Although the exact mechanism is not fully understood, it is speculated that insulin might activate the MAP-kinase pathways which in return stimulate PAI-1 release from cells [33].

PAI-1 plays a key role in the fibrinolytic process [35] [36] and increased levels will augment the risk of thrombus development and atherosclerosis [37] by inhibiting the conversion of plasminogen to active plasmin [38] and promoting fibrin deposition.

Due to the high prevalence of hypertension in African women [1-3], the aim of this study was to determine whether African women have higher levels of UA and PAI-1 when compared to Caucasian women. A second aim was to determine whether UA and PAI-1 levels are associated with cardiovascular parameters linked with cardiovascular dysfunction and the components of the metabolic syndrome within each ethnic group.

METHODOLOGY

Experimental group

The study was a case-case-control study conducted in the Potchefstroom district of the North West Province, South Africa. The POWIRS study (Profiles of Obese Women with the Insulin Resistance Syndrome) consisted of two parts following exactly the same methodology: POWIRS I involved 102 urban African women and POWIRS II involved 115 Caucasian women. The subjects had to be apparently healthy women between the ages of 20 and 55 years. According to the original research question of this study [39], the women were recruited according to the body mass index categories described in the guidelines of the Report of the World Health Organization Consultation on Obesity (1997) [40] (Figure 1), and paired into lean, overweight and obese groups.

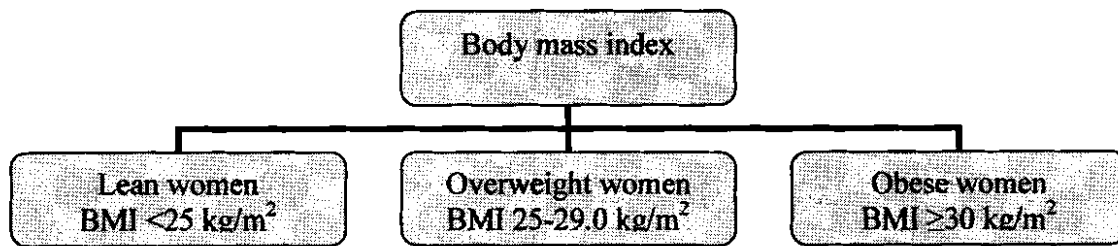


Fig. 1. Group division of subjects according to body mass indexes.

The exclusion criteria were pregnant and lactating women, oral temperatures above 37°C and HIV-positive subjects. Subjects' HIV status was determined three months prior to the study, however, their status can not be guaranteed.

Assistance was available to provide any relevant information in each subject's home language. All subjects were informed about the outcome and procedures of the study beforehand. All subjects signed an informed consent form. The study was approved by the Ethics Committee of the North-West University (Potchefstroom Campus) and complies with the Declaration of Helsinki revised in 2004 [41].

Experimental procedure

During a period of six weeks, subjects were transported to the Metabolic Unit Facility on the Campus of the University. The facility consisted of 10 single bedrooms, two bathrooms, a living room and a kitchen. The subjects reported to the facility at 16:30 each afternoon. On arrival, the women were introduced to the experimental procedure and set-up. The procedure was explained and each was allocated to her own room. Each subject received a light meal at 20:00, which excluded alcohol and caffeine, and went to sleep before 23:00.

Cardiovascular measurements

The following morning the subjects were required to stay in a lying position (Fowlers) while blood pressure measurements were taken using the Finometer device. The Finometer device was connected to the left arm and left middle finger of the subject and measurements were recorded continuously for at least seven minutes. After a recording of two minutes, a return-to-flow calibration was performed. This is an adjustment that is performed to adjust the brachial arterial pressure of each specific subject with the finger pressure. Highest precision readings were obtained after this calibration.

The Finometer device computed all cardiovascular variables online and stored the data in result files on a hard disk. The following cardiovascular variables were computed and stored: systolic blood pressure (SBP), diastolic blood pressure (DBP), heart rate (HR), stroke volume (SV), cardiac output (CO), total peripheral resistance (TPR) and arterial compliance (C_w) [42].

The vascular unloading technique of Peñáz together with the Physiocal criteria of Wesseling provided reliable, non-invasive and continuous estimates of blood pressure which are useable especially in comparative studies [43] [44]. This technique is, therefore an alternative to the invasive intra-arterial measurements without the risks and the ethical questions inherent to invasive measurement. Since the pressure waveform is available continuously, computations provide further information on the dynamics of the cardiovascular system, similar to intra-arterial measurements [45] [46].

Anthropometric measurements

Anthropometric measurements were taken according to standard methods stipulated by Norton and Olds (1996) [47]. Height measurements were taken using a stadiometer (Invicta, IP 1465, UK) and measurements were taken to the nearest 0.1 cm. Body mass measurements were taken up to the nearest 0.1 kg, using a calibrated electronic scale (Precision Health Scale, A&D Company, Japan). The waist circumference was also taken in triplicate to obtain a reliable mean value.

Biochemical analyses

A fasting blood sample was taken from the antebrachial vein using a sterile butterfly infusion set and syringes. Plasma and serum samples were prepared according to standard methods and stored at -82°C until analyses were performed.

Serum lipids and UA (uric acid) were measured on a Vitros DT60 II Chemistry system with Vitros DT slides. PAI-1 (plasminogen activator inhibitor-1) was measured with an indirect enzymatic method (Spectrolyse pL, Bipoool Umeå, Sweden). High sensitivity C-reactive protein (hs-CRP) was analysed with a high sensitivity C-Reactive Protein Kit from Immage® Immunochemistry systems (Beckman Coulter, Inc.). Serum leptin was determined with [¹²⁵I]IRMA kit (Diagnostic Systems Laboratories, Inc.). Plasma glucose was measured by the hexokinase method. Analysis of insulin levels was performed by enzyme immunoassay (BioSource EUROPE S.A. Belgium).

Questionnaire

Dietary protein intake was derived from results obtained by a registered dietician from a validated Quantitative Food Frequency Questionnaire [39].

Statistical analysis

Statistical analyses were performed using Statistica version 7.1 (Statsoft, Inc., 2005). Statistical results are presented as means and 95% confidence intervals. An independent t-test and analysis of covariance (ANCOVA) were used for comparison of variables between groups to determine significant differences. A multiple analysis of covariance (MANCOVA) was performed to compare variables between the groups, whilst adjusting for certain variables. The experimental groups were sub-divided into uric acid tertiles whereafter statistical analyses were performed using only the 1st and 3rd tertiles. Partial correlations were performed to determine associations between the dependent variables and the cardiovascular variables as well as components of the metabolic syndrome. A forward stepwise multiple regression analysis was performed separately in the African and Caucasian women using UA and PAI-1 respectively as the dependent variables and the following as independent variables: age, BMI, waist circumference, MAP, TPR, C_w, HDL, triglycerides, hsCRP, fasting glucose, fasting insulin, leptin and total protein intake. In the regression model where either UA or PAI-1 was not the dependent variable, it was added to the list of independent variables. Variables that were not normally distributed were logarithmically transformed. A P-value of ≤ 0.05 was considered as statistically significant and a P-value of ≤ 0.01 was considered as highly statistically significant.

RESULTS

The subject characteristics and parameters of each respective study group are summarized in Table 1.

Table 1. Subject characteristics

Characteristics	African women (n=102)	Caucasian women (n=115)	P-value
Age (yr)	31.25 [29.56; 32.95]	31.34 [29.64; 33.03]	0.94
BMI (kg/m ²)	27.98 [26.74; 29.23]	28.48 [27.16; 29.80]	0.60
WC (cm)	81.62 [79.02; 84.22]	86.00 [83.24; 88.76]	0.02
SBP (mmHg)	129.82 [125.97; 133.66]	125.41 [123.25; 127.57]	0.04
DBP (mmHg)	77.68 [75.58; 79.78]	72.47 [70.81; 74.14]	<0.01
MAP (mmHg)	100.03 [97.47; 102.59]	93.34 [91.54; 95.14]	<0.01
TPR (mmHg.s/mL)	1.10 [1.04; 1.15]	0.84 [0.80; 0.88]	<0.01
C _w (mL/mmHg)	1.85 [1.79; 1.91]	2.29 [2.21; 2.36]	<0.01
HDL (mmol/L)	1.25 [1.19; 1.32]	1.21 [1.15; 1.26]	0.29
Triglycerides (mmol/L)	0.71 [0.63; 0.80]	1.28 [1.15; 1.26]	<0.01
Uric acid (μmol/L)	294.21 [278.20; 310.21]	331.91 [319.39; 344.44]	<0.01
PAI-1 (units/mL)	5.97 [5.05; 6.89]	12.89 [11.30; 14.49]	<0.01
Leptin (ng/mL)	57.59 [51.63; 63.55]	51.37 [45.27; 57.47]	0.15
hsCRP (mg/L)	4.59 [3.17; 6.01]	3.27 [2.56; 3.98]	0.09
Fasting glucose (mmol/L)	5.18 [4.95; 5.42]	5.04 [4.96; 5.11]	0.22
Fasting insulin (pmol/L)	92.94 [84.75; 101.13]	92.66 [86.45; 98.87]	0.96
HOMA-IR indexes	3.08 [2.77; 3.39]	3.04 [2.80; 3.28]	0.82
Total protein intake	60.67 [56.87; 64.47]	96.68 [90.45; 102.91]	<0.01

NOTE. Values are expressed as the mean [±95 % confidence intervals];

BMI, body mass index; WC, waist circumference; SBP, systolic blood pressure; DBP, diastolic blood pressure; TPR, total peripheral resistance; C_w, Windkessel compliance; HDL, high-density lipoprotein cholesterol; PAI-1, plasminogen activator inhibitor 1; hsCRP, high sensitivity C-reactive protein; HOMA-IR, homeostasis model assessment – insulin resistance.

As expected the African women had significantly higher blood pressure (SBP, DBP and MAP) concomitant with a significantly higher TPR and significantly lower C_w compared to the Caucasian women. Despite their higher blood pressure the African women have surprisingly significantly lower levels of UA and PAI-1. The Caucasian women had significantly higher triglyceride levels, waist circumference and protein intake.

The groups were then divided into lean, overweight and obese, and after adjusting for age and waist circumference (which are both known to increase UA levels), the lean, overweight and obese Caucasian women had significantly higher UA levels when compared to the lean, overweight and obese African women (Fig. 2). Waist circumference was compared between the two ethnic groups for each level of obesity. There were significant differences for both the overweight and obese groups, but not for the lean group (Table 2)

Table 2. Waist circumference difference for each level of obesity

Obesity level	<u>Waist circumference (cm)</u>		P-value
	African women	Caucasian women	
Lean	70.23	71.21	0.49
Overweight	79.73	86.16	<0.01
Obese	93.93	100.31	0.01

The PAI-1 values showed a similar trend and again the Caucasian women showed significantly higher levels even after adjusting for age, waist circumference, fasting glucose and insulin levels – factors known to increase PAI-1 levels (Fig. 3).

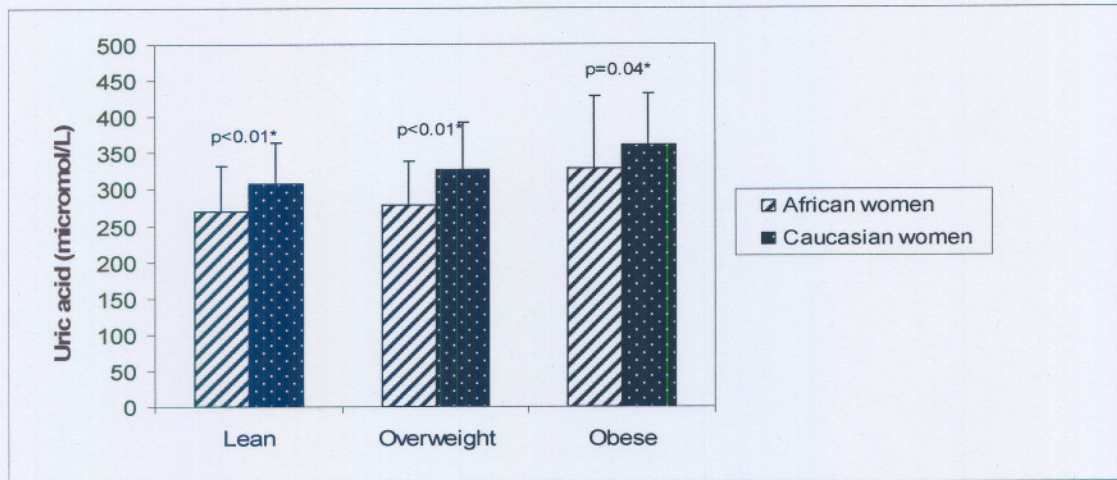


Fig. 2. Uric acid levels of African and Caucasian women for the different obesity levels after adjusting for age and waist circumference (values are mean \pm standard deviation).

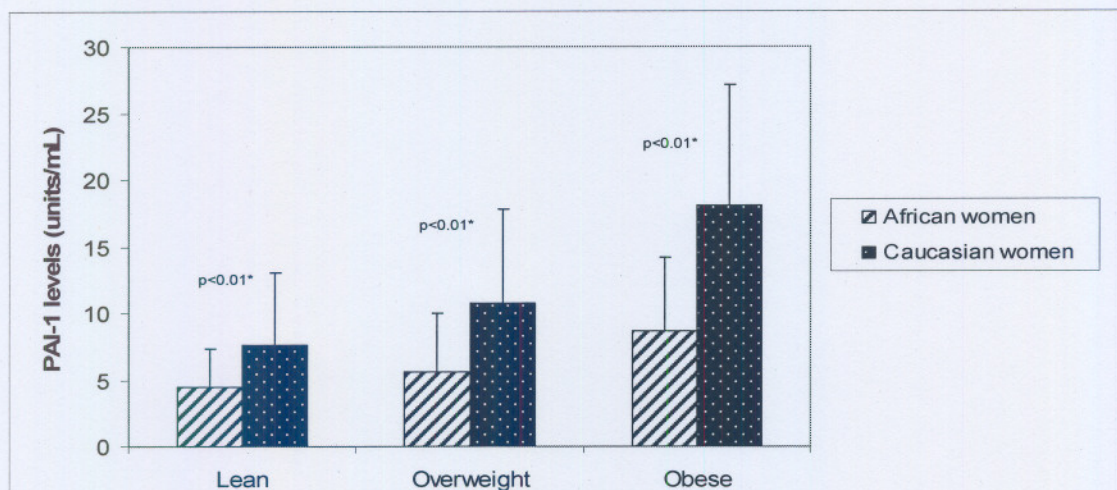


Fig. 3. PAI-1 levels of African and Caucasian women for the different obesity levels after adjusting for age, waist circumference, fasting glucose and insulin levels (values are mean \pm standard deviation).

After the groups were re-divided into tertiles using the UA and PAI-1 concentrations, the first and third tertiles were compared using an independent t-test. Table 3 and Table 4 show the difference in the cardiovascular variables and the variables associated with the metabolic syndrome.

Table 3. Differences in variables for the first and third uric acid tertiles.

Indep variables	<u>African women</u>			<u>Caucasian women</u>		
	1 st tertile	3 rd tertile	P-value	1 st tertile	3 rd tertile	P-value
	($\leq 256 \mu\text{mol/L}$)	($\geq 305 \mu\text{mol/L}$)		($\leq 298 \mu\text{mol/L}$)	($\geq 357 \mu\text{mol/L}$)	
SBP	124.77	136.28	0.03	123.19	127.01	0.14
DBP	75.16	81.55	0.01	70.90	74.46	0.09
MAP	96.74	104.70	0.01	91.22	95.11	0.09
TPR	1.07	1.11	0.61	0.88	0.81	0.13
C _w	1.88	1.83	0.60	2.23	2.39	0.07
BMI	25.90	31.31	<0.01	26.03	31.36	<0.01
WC	77.43	89.32	<0.01	80.22	93.23	<0.01
PAI-1	5.87	6.51	0.55	9.91	17.14	<0.01
Fasting insulin	84.66	113.27	0.01	84.59	108.97	<0.01
HOMA-IR	2.79	3.89	0.01	2.76	3.59	0.01
Leptin	49.76	73.08	<0.01	38.03	66.59	<0.01

SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; TPR, total peripheral resistance; C_w, Windkessel compliance; BMI, body mass index; WC, waist circumference; PAI-1, plasminogen activator inhibitor-1; HOMA-IR, homeostasis model assessment – insulin resistance.

Table 4. Differences in variables for the first and third PAI-1 tertiles.

Indep variables	<u>African women</u>			<u>Caucasian women</u>		
	1 st tertile	3 rd tertile	P-value	1 st tertile	3 rd tertile	P-value
	(≤3.19 units/mL)	(≥7.25 units/mL)		(≤8.45 units/mL)	(≥16.12 units/mL)	
SBP	125.00	132.37	0.17	123.55	128.66	0.04
DBP	75.33	78.31	0.28	69.57	75.85	<0.01
MAP	96.80	101.13	0.21	90.17	96.97	<0.01
TPR	1.13	1.04	0.17	0.87	0.80	0.17
C _w	1.86	1.87	0.94	2.15	2.45	<0.01
BMI	26.17	29.88	0.02	24.17	33.78	<0.01
WC	77.38	86.67	<0.01	75.35	98.68	<0.01
HDL	1.27	1.29	0.81	1.33	1.05	<0.01
Triglycerides	0.69	0.85	0.14	1.05	1.57	<0.01
Uric acid	298.74	309.00	0.61	306.89	364.85	<0.01
hsCRP	4.29	4.20	0.94	2.58	5.00	<0.01
Fasting glucose	4.91	5.59	0.04	4.88	5.20	<0.01
Fasting insulin	81.41	102.76	0.01	74.46	119.68	<0.01
HOMA-IR	2.58	3.58	< 0.01	2.34	4.07	<0.01
Leptin	49.17	67.81	0.01	29.40	71.43	<0.01

SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; TPR, total peripheral resistance; C_w, Windkessel compliance; BMI, body mass index; WC, waist circumference; HDL, high-density lipoproteins; hsCRP, high sensitivity C-reactive protein; HOMA-IR, homeostasis model assessment-insulin resistance.

Table 3 shows that there is a noticeable increase in the blood pressure from the lower UA levels to the higher UA levels although this is only seen in the African women and not in the Caucasian women. Both subject groups showed a significant increase in insulin, HOMA and leptin levels from the 1st tertile to the 3rd tertile. It is interesting to note that there is a significant difference in PAI-1 levels between the 1st and 3rd tertiles, however,

this is only seen in the Caucasian women and not in the African women who show no difference in PAI-1 levels between the two tertiles.

Table 4 shows that with an increase in PAI-1 levels, there is a significant increase in BMI, waist circumference, glucose and insulin levels, and HOMA-IR as well as leptin levels for both subject groups. There is a significant increase in SBP, DBP, MAP, triglycerides, UA and hsCRP, noticeable only in the Caucasian women and not in the African women.

Correlation coefficients were determined for both UA and PAI-1 with the variables listed in Table 1, within each ethnic group (Table 5 and 6).

Table 5. Correlation coefficients for uric acid.

<u>Indep variable:</u>	<u>African women</u>		<u>Caucasian women</u>	
	Correlation coefficient	P-value	Correlation coefficient	P-value
SBP	0.15	0.14	0.14	0.13
DBP	0.18	0.07	0.13	0.17
MAP	0.15	0.12	0.17	0.07
TPR	-0.05	0.64	-0.22	0.02
C _w	0.04	0.71	0.12	0.20
BMI	0.36	<0.01	0.34	<0.01
WC	0.40	<0.01	0.37	<0.01
PAI-1	0.09	0.38	0.38	<0.01
HDL	-0.06	0.58	-0.05	0.63
Triglycerides	0.43	<0.01	0.29	<0.01
hsCRP	0.19	0.06	0.28	<0.01
Fasting glucose	0.14	0.16	0.09	0.32
Fasting insulin	0.35	<0.01	0.33	<0.01

HOMA-IR	0.35	<0.01	0.30	<0.01
Leptin	0.39	<0.01	0.32	<0.01

SBP, systolic blood pressure; DBP, diastolic blood pressure; TPR, total peripheral resistance; C_w, Windkessel compliance; BMI, body mass index; WC, waist circumference; PAI-1, plasminogen activator inhibitor-1; HDL, high-density lipoproteins; hsCRP, high sensitivity C-reactive protein; HOMA-IR, homeostasis model assessment-insulin resistance.

Table 6. Correlation coefficients for PAI-1.

<u>Indep variable:</u>	<u>African women</u>		<u>Caucasian women</u>	
	Correlation coefficient	P-value	Correlation coefficient	P-value
SBP	0.12	0.21	0.23	<0.01
DBP	0.09	0.39	0.25	<0.01
MAP	0.11	0.23	0.29	<0.01
TPR	-0.19	0.06	-0.22	0.02
C _w	0.04	0.71	0.31	<0.01
BMI	0.34	<0.01	0.60	<0.01
WC	0.37	<0.01	0.68	<0.01
Uric acid	0.08	0.38	0.38	<0.01
HDL	0.01	0.29	-0.30	<0.01
Triglycerides	0.23	0.02	0.39	<0.01
hsCRP	0.07	0.50	0.37	<0.01
Fasting glucose	0.30	<0.01	0.43	<0.01
Fasting insulin	0.23	0.02	0.59	<0.01
HOMA-IR	0.28	<0.01	0.60	<0.01
Leptin	0.31	<0.01	0.53	<0.01

SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; TPR, total peripheral resistance; C_w, Windkessel compliance; BMI, body mass index; WC, waist circumference; PAI-1, plasminogen activator inhibitor-1; HDL, high-density lipoproteins; hsCRP, high sensitivity C-reactive protein; HOMA-IR, homeostasis model assessment-insulin resistance.

After adjusting for age, BMI and waist circumference all significant correlations between UA and the variables listed in Table 5 disappeared for both ethnic groups, leaving only a slightly positive correlation between UA and triglycerides ($r=0.27$) in the African women and a weak positive correlation between UA and PAI-1 ($r=0.20$) in the Caucasian women.

All significant correlations with PAI-1 and the variables listed in Table 6 disappeared after adjusting for age, BMI, waist circumference, fasting glucose and insulin levels (all of which are known to increase PAI-1 levels), except for a weak positive correlation with HDL ($r=0.21$) in the African women and a weak negative correlation with C_w ($r=-0.22$) in the Caucasian women.

Forward stepwise multiple regression analyses were performed in each ethnic group to determine the strongest contributor for UA and PAI-1, respectively (Table 7 and Table 8). The strongest contributors are shown in *italics*.

Table 7. Forward stepwise multiple regression analysis with uric acid as the dependent variable

Independent variable	Beta	Partial R^2	Cumulative R^2	P-value
<u>African women:</u>				
R^2 adjusted = 0.259				
<i>Triglycerides</i>	0.327	0.187	0.187	<0.01
Leptin	0.172	0.061	0.249	0.10
Insulin	0.193	0.018	0.267	0.06
Age	0.144	0.014	0.281	0.13
Glucose	0.141	0.015	0.296	0.16

Caucasian women:**R² adjusted = 0.220**

PAI-1	0.256	0.145	0.145	0.03
Triglycerides	0.132	0.023	0.168	0.19
Fasting glucose	0.236	0.017	0.185	0.03
BMI	0.171	0.025	0.209	0.19
Total protein	0.148	0.014	0.223	0.13
Fasting insulin	0.257	0.016	0.239	0.04
TPR	-0.243	0.018	0.257	0.04
C _w	-0.168	0.015	0.272	0.20
HDL	0.114	0.010	0.282	0.24

PAI, plasminogen activator inhibitor-1; BMI, body mass index; TPR, total peripheral resistance; C_w, Windkessel compliance; HDL, high-density lipoproteins

Table 8. Multiple regression analysis with PAI-1 as the dependent variable.

Independent variable	Beta	Partial R ²	Cumulative R ²	P-value
African women:				
R² adjusted = 0.228				
WC	0.362	0.137	0.137	0.01
HDL	0.128	0.031	0.168	0.17
Fasting glucose	0.188	0.031	0.199	0.07
TPR	-0.316	0.019	0.218	0.02
hsCRP	-0.284	0.022	0.240	0.02
C _w	-0.250	0.025	0.265	0.08
Leptin	0.211	0.016	0.281	0.11
Uric acid	-0.110	0.010	0.290	0.27

Caucasian women:**R² adjusted = 0.529**

WC	0.701	0.469	0.469	<0.01
Fasting insulin	0.178	0.039	0.508	0.06
Uric acid	0.132	0.013	0.521	0.07
hsCRP	-0.081	0.008	0.530	0.35
C _w	-0.221	0.007	0.536	0.03
Age	-0.186	0.013	0.549	0.04
Fasting glucose	0.124	0.009	0.558	0.14

WC, waist circumference; HDL, high-density lipoproteins; TPR, total peripheral resistance; hsCRP, high sensitivity C-reactive protein; C_w, Windkessel compliance.

The multiple regression analysis for UA showed that triglycerides are the strongest contributor for UA in the African women, but in the case of the Caucasian women, PAI-1 is the strongest contributor for UA (Table 6). However, the multiple regression analysis for PAI-1 showed that in both ethnic groups, waist circumference was the strongest contributor for PAI-1 levels, although to a much lesser extent in the African women.

DISCUSSION

Elevated levels of UA and PAI-1 are associated with cardiovascular dysfunction [48] [49], but few studies have been conducted in developing countries such as South Africa. To the knowledge of the researchers, no studies have been conducted to investigate this matter in African women from South Africa.

Elevated levels of UA are often seen in patients with hypertension [15] [48] [50] [51]. Because of the high prevalence of hypertension in the African population [1-3] it is hypothesised that African women will have higher levels of UA compared to Caucasian women.

Data from this study corresponds with previous findings that African women have higher blood pressure [1-3] compared to Caucasian women. However, despite the African women's higher blood pressure, their UA levels were significantly lower compared to that of the Caucasian women. This is in contrast with other literature that found the African population to be a high risk group for elevated UA levels [25] [52] or that the African population and Caucasian population have the same UA levels [48].

A partial explanation for the lower levels of UA in the African women might be their fat distribution. Subcutaneous fat is not considered as important in the development of cardiovascular dysfunction as abdominal obesity [53] [54] and it is known that UA is more commonly associated with abdominal obesity than subcutaneous obesity [55] [56]. The waist circumference can be used as a reliable indication of abdominal obesity [53] [57]. It is clear that the Caucasian group had a higher waist circumference than the African group (Table 2), indicating that the Caucasian women from this study had a higher prevalence of abdominal obesity, hence the higher levels of UA. This is only valid when the total ethnic groups are compared. However, when the waist circumference for lean, overweight and obese groups was compared between the ethnic groups, there were significant differences noticeable only between the overweight and obese groups, and not in the lean group. Despite no difference in waist circumference for the lean group, a significant difference in

the UA levels existed. Apart from waist circumference, there are, therefore, also other factors that determine the circulating UA levels.

One such a factor is protein intake. It is known that a protein (purine) rich diet will increase UA levels [58] [59] and according to this study, the Caucasian women had a significantly higher protein intake compared to the African women (Table 1). But even after adjusting for protein intake there were still significant differences in UA levels between the groups. The multiple regression analyses performed within each ethnic group also revealed no contribution from protein intake to UA levels. It is, however, not entirely clear from this study which other factors also contributed to the ethnic difference and it is, therefore, recommended that further in-depth studies be conducted.

When the blood pressure from the first and third UA tertiles of each ethnic group was compared (Table 4) only the African women showed a significant difference in blood pressure. However, it is clear from the partial correlations in both ethnic groups that UA was not associated with any cardiovascular variables or with any components associated with the metabolic syndrome. The significant difference in the blood pressure between the first and third UA tertiles in the African women might, therefore, be explained by other factors such as an increased sympathetic activity [60] [61], but that is beyond the scope of this study.

An interesting finding from this study is the significant difference in PAI-1 levels between the African and Caucasian women. Despite their state of hypercoagulability and elevated levels of fibrinogen [62], the African women had significantly lower levels of PAI-1 (Table 1 and Figure 2). Again waist circumference (abdominal obesity) can possibly be held partially responsible for the difference in PAI-1 levels. Studies have shown that adipocytes,

especially abdominal adipocytes, contain high amounts of PAI-1 mRNA [63] [64], making adipocytes one of the major sources of PAI-1 [33]. The latter is supported by a multiple regression analysis (Table 7) showing that waist circumference is the main contributor to PAI-1 levels in both ethnic groups, although to a much lesser degree in the African women.

Since there was no significant correlation between PAI-1 and any of the variables listed in Table 1 (whilst adjusting for age, BMI, waist circumference, fasting insulin and fasting glucose), it can be speculated that PAI-1 acts as a risk marker for future cardiovascular dysfunction such as atherosclerosis [65]. This might, therefore, place the Caucasian women in a high risk category for future development of atherosclerosis.

This data also show that there is a significant increase in PAI-1 levels with an increase in UA levels, but it is only seen in the Caucasian women (Table 2). The explanation for this is not clear from this study, but it can be speculated that because of uric acid's antioxidant properties [66], it is secreted as a counter protective mechanism against the atherogenic properties of PAI-1, and this might possibly be why no cardiovascular dysfunction is present in the Caucasian women, despite their high levels of both uric acid and PAI-1.

A limitation of this study is that there are not reliable Angiotensin II data available for both ethnic groups and the association of Angiotensin II with uric acid and PAI-1 levels could not therefore, be determined. Another limitation of this study is the fact that blood samples were taken at \pm 06:00 in the morning and PAI-1 has a diurnal circadian rhythm [67] which peaks in the early morning. Blood sampling for both groups was done at the same time which makes it reliable for comparison.

The study group was relatively young and apparently healthy with a low degree of cardiovascular dysfunction. It is, therefore, recommended that a future follow-up study be

performed to determine cardiovascular dysfunction and its association with UA and PAI-1 in Caucasian women.

Even though ethnic groups may have the same body mass index, their fat distribution might differ resulting in a different adipokine secretion profile. Waist circumference (abdominal obesity) seems to be the strongest associated with elevated levels of uric acid and PAI-1. Despite their higher levels of uric acid and PAI-1, the cardiovascular profile of Caucasian women seems not to be affected detrimentally. However, it could turn out that with increased aging, the detrimental effects will be more noticeable. On the other hand, the African women with lower levels of uric acid and PAI-1 had higher blood pressure, even though no significant partial correlations were obtained between cardiovascular parameters and uric acid or PAI-1. It is, therefore, concluded that PAI-1 and uric acid are not associated with cardiovascular dysfunction in young African women aged 20 – 55 years and that other factors might therefore be responsible for their higher blood pressure.

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CHAPTER 3

General findings and conclusions

INTRODUCTION

In this chapter a summary of the main findings from the article reported in this dissertation will be given. The results will be discussed, interpreted, elucidated and compared to the relevant literature in Chapter 1. Conclusions will be drawn and recommendations will be made to researchers investigating uric acid and plasminogen activator inhibitor-1 in African and Caucasian women.

SUMMARY OF MAIN FINDINGS

The significant findings of this article reported in this dissertation were:

The association of uric acid and plasminogen activator inhibitor-1 (PAI-1) with cardiovascular function in South African women: The POWIRS study (Chapter 2)

The aim of the study was to compare uric acid (UA) and plasminogen activator inhibitor-1 (PAI-1) levels between African and Caucasian women and to determine whether UA and PAI-1 are directly associated with cardiovascular dysfunction and some components of the metabolic syndrome. It was hypothesized that because of the high prevalence of hypertension in the African population, the African women in the study group will have higher levels of UA and PAI-1. A second hypothesis was that there is a positive association between UA and PAI-1 with cardiovascular dysfunction and components of the metabolic syndrome.

Despite their higher blood pressure, the African women showed significantly lower levels of UA and PAI-1 compared to the Caucasian women. The first hypothesis is, therefore rejected. After adjusting for age, body mass index, waist circumference, fasting insulin and glucose levels, there were no positive associations for UA and PAI-1 with cardiovascular variables such as systolic blood pressure (SBP), diastolic blood pressure (DBP), total peripheral resistance (TPR) and Windkessel compliance (C_w). Both UA and PAI-1 showed strong correlations with waist circumference and fasting insulin and glucose levels, all components of the metabolic syndrome.

COMPARISON OF FINDINGS WITH THE LITERATURE

When the results from this study are compared to existing literature and results regarding other ethnic populations, it is evident that certain findings confirmed those found in the literature. Confirming findings of this study are that the African women had higher blood pressure compared to the Caucasian women [1-3] as well as that both UA and PAI-1 positively correlated with abdominal obesity [4] [5].

Contradictory findings from this study were that the UA levels were significantly higher in the Caucasian women compared to the African women, while Hochberg [6] and Watanabe *et al* [7] identified the African population as a high risk group for elevated levels of UA and the development of gout. Another contradictory finding was that there was no positive association between UA and cardiovascular dysfunction (whilst adjusting for age, body mass index, waist circumference, fasting glucose and insulin levels), since several literature studies show a positive association between UA and cardiovascular dysfunction [8-10].

There are, however, several findings in the literature that are not evident in this study. They include the positive association between UA and angiotensin II [11]. On the other hand, there are several findings from this study that are not evident in the literature. This includes the positive correlation between UA and PAI-1 in the Caucasian women.

Novel findings from this study that add to available literature are the results that show that Caucasian women had higher levels of PAI-1 compared to the African women and that there was no difference in PAI-1 levels between the 1st and 3rd UA tertiles of the African women.

CHANCE AND CONFOUNDING

Before the main findings of this study are discussed, it is important to reflect critically on some important factors that may have affected the results. There are some methodological issues that could have caused weaknesses in the study and, therefore, might have influenced the different outcomes.

The number of subjects included in this study could be questioned. A forced availability sample of subjects were used (N=217) and although it was more than what is required by a statistical power analysis (N=15), the whole South African population cannot be represented by this study group since this group was recruited from the Potchefstroom district in the North West Province of South Africa.

Concerning the results, the possibility of chance should be taken into account. By using partial correlations and forward stepwise regression analyses, statistics indicated that one out of twenty significant correlations may be because of chance.

CONFOUNDERS

Although one of the inclusion criteria for the study was that the subjects should be "apparently healthy", their health is not a certainty. Other confounding factors such as alcohol intake, diet, smoking, physical activity level, HIV status and socio-economic status could have influenced the results.

DISCUSSION OF MAIN FINDINGS

It is known that ethnic differences exist in the prevalence of certain cardiovascular diseases [12]. The African population is more susceptible to the development of hypertension compared to the Caucasian population [3]. Hypertension is often associated with components of the metabolic syndrome, such as obesity [13] [14] and insulin resistance [15] [16]. However, the association of hypertension with other factors such as elevated levels of UA and PAI-1 is still uncertain.

The main focus of this study was to investigate whether UA and PAI-1 were independently associated with the high prevalence of hypertension in African women. Although the findings cannot be generalized to the whole African female population of South Africa, they serve as a foundation for future in-depth studies.

The increased blood pressure was observed in the African women [1-3], however, they had significantly lower levels of UA and PAI-1 compared to the Caucasian women. The elevated levels of UA and PAI-1 in the Caucasian women showed no significant correlations with any cardiovascular dysfunctions. Since UA and PAI-1 both correlate very strong with obesity [17] [18], it may be suggested that the Caucasian women have a different fat distribution compared to the African women.

CONCLUSION

Since the African women had higher blood pressure with lower levels of UA and PAI-1 it can be speculated that uric acid and PAI-1 are not independently associated with cardiovascular dysfunction in African women aged 25 – 55 years.

RECOMMENDATIONS

The following recommendations are proposed for future studies:

- Although it is clear from this study that there are no correlations between UA and cardiovascular dysfunction, one must keep in mind that the study group was relatively young (mean age: 31 years). For future studies it is recommended that a follow-up study should be done to clarify the vascular effects of uric acid in older populations.
- When studying the cardiovascular effects of UA, it should also be kept in mind that UA possesses anti-oxidant properties. It is, therefore, recommended that these properties should be investigated.

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- The positive associations between UA and PAI-1 in Caucasian women should be further investigated.

 - The effect of UA on angiotensin II should also be assessed in both African and Caucasian women.

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