Fibulin-1 as a marker of cardiovascular disease in HIV-infected black South Africans: a prospective study

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Let the morning bring me word of your unfailing love, for I have put my trust in you. Show me the way I should go, for to you I lift up my soul.

[Psalm 143:8]

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AUTHORS’ CONTRIBUTIONS

The contributions of each researcher involved in this study are the following:

| STUDENT | Mrs Anéél Pretorius (BSc Honours Physiology) was responsible for all literature searches, the statistical analysis and interpretation of the results, as well as the planning and writing of the manuscript. |
|SUPERVISOR| Prof JM van Rooyen (DSc), as supervisor of the concept and design of the study, contributed to the collection of data for the PURE study, assisted in the initial planning of the manuscript, and supervised the analysis and writing process. |
|CO-SUPERVISOR| Prof HW Huisman (PhD), as co-supervisor, contributed to the collection of the data and planning of the PURE study. He also supervised the planning and writing of the manuscript. |
|CO-SUPERVISOR| Dr CMT Fourie (PhD), also as co-supervisor, managed the 2008 PURE data collection and contributed to data collection in both 2005 and 2008, assisted in statistical analysis and supervised the writing of the manuscript. |

The following is a statement from the co-authors, confirming their individual role in the study and giving their permission that the manuscript may form part of this dissertation.

I, Anéél Pretorius, hereby declare that the aforementioned is representative of my actual contribution and I hereby give my consent that this manuscript may be published as part of the mini-dissertation for the Master of Science degree in Physiology.

Mrs A Pretorius

The abovementioned statements confirm the individual roles of the three co-authors respectively and Prof JM van Rooyen, Prof HW Huisman and Dr CMT Fourie hereby give permission that this manuscript may form part of the MSc of Mrs Anéél Pretorius.
SUMMARY

TITLE: Fibulin-1 as a marker of cardiovascular disease in HIV-infected black South Africans: a prospective study

BACKGROUND: There is a high prevalence of the human immunodeficiency virus (HIV) infection in South Africa and this chronic infection promotes vascular inflammation, leading to vascular damage in infected individuals. The extracellular matrix is a highly adaptive and dynamic structure that is influenced by mechanical stress, inflammation and oxidative stress and it has been suggested that changes contribute to arterial stiffness. Fibulin-1 is a fibrinogen-binding plasma protein and is part of a small group of extracellular matrix proteins (including fibronectin, laminin and von Willebrand factor) that are present in the blood at relatively high levels. Changes in fibulin-1 levels have consequences to vascular structural integrity and maintaining the integrity of the extracellular matrix in the blood vessel wall seems critical in the prevention of cardiovascular disease.

OBJECTIVE: The objective of this study was to determine the association of fibulin-1 with markers of vascular function in HIV-infected black South-Africans in the baseline study of 2005 and follow-up study in 2008.

METHODOLOGY: This substudy is embedded in the larger international Prospective Urban and Rural Epidemiology (PURE) study. The PURE study is a prospective study that addresses questions regarding the cause and development of cardiovascular risk factors and disease within populations, particularly of low- and middle-income countries, including South Africa. The South African leg of the PURE study was performed in the North-West Province, where a total of 2 010 participants (1 004 urban and 1 006 rural) were randomly recruited from a rural
and urban setting and screened during the baseline phase in 2005. For this substudy, the HIV-infected participants (N=300) of the study population were individually matched with HIV-uninfected participants (N=300) at the baseline phase (2005). The participants were matched according to age, gender, body mass index (BMI) and locality (urban and rural), and were followed up in 2008. Anthropometric and cardiovascular measurements were determined. The OMRON HEM-757 (Omron, Kyoto, Japan) apparatus was used to determine systolic and diastolic blood pressure. The pulse pressure (PP) was subsequently calculated by the difference in systolic blood pressure and diastolic blood pressure. The carotid-radial pulse wave velocity (cr-PWV) was determined with the Complior SP Acquisition system (Artech Medical, Pantin, France). The lipid profile and inflammatory markers were also determined. Independent t-tests were used to compare cardiovascular variables between the HIV-infected and HIV-uninfected participants in the baseline study. Dependent t-tests were used to compare the baseline and follow-up measurements of cardiovascular variables within HIV-infected and HIV-uninfected participants. The percentage change in all the groups was also determined over a period of three years. Pearson and partial correlations were performed to explore unadjusted and adjusted associations between change of fibulin-1 and cardiovascular variables in each group. P-values of ≤ 0.05 were regarded as statistically significant.

**RESULTS:** At baseline, as well as after three years, the fibulin-1 levels were significantly higher in HIV-infected, compared to HIV-uninfected, South Africans. Percentage change in fibulin-1 is associated with percentage change in triglycerides (TG) to high-density lipoprotein cholesterol ratio (TG/HDL-C) in HIV-infected participants, but not in HIV-uninfected participants. A significant positive correlation was seen between percentage change in fibulin-1 and soluble urokinase-type plasminogen activator receptor (suPAR) levels in the HIV-uninfected group, but no positive correlation was found in suPAR levels in the unadjusted correlations. In the baseline study (2005), as well as the follow-up (2008), the HIV-infected
participants had lower HDL-C and higher soluble forms of intercellular adhesion molecule-1 (sICAM-1), vascular cell adhesion molecule-1 (sVCAM-1) and suPAR levels in comparison with their HIV-uninfected control group. The sICAM-1 and sVCAM-1 levels were only determined in 2005. At baseline, the cr-PWV was significantly higher in the HIV-infected group (11.26 m/s vs. 10.68 m/s, p=0.03) in comparison with the HIV-uninfected group. However, in the follow-up study, no significant difference was found in the cr-PWV values between the HIV-infected and HIV-uninfected participants. There were no significant differences in percentage change in fibulin-1, TC/HDL-C ratio, TG/HDL-C ratio, cr-PWV and PP between the HIV-infected and HIV-uninfected participants over the period of three years.

DISCUSSION: The high suPAR levels and low HDL-C levels suggest the possibility that the HIV-infected participants could be more prone to develop vascular damage, which could result in further extracellular matrix remodelling. The latter is seen as the link between infection, inflammation, thrombotic activity and vascular dysfunction. The increased cr-PWV in the HIV-infected group at baseline supports the above possibility. Although no significant associations were found between fibulin-1 and inflammatory markers (namely sICAM-1, sVCAM-1, interleukin-6 [IL-6], plasminogen activator inhibitor-1 [PAI-1] or PWV), the association of the percentage change in fibulin-1 with the change in TG/HDL-C ratio suggests that TG/HDL-C ratio may contribute to probable vascular changes in HIV-infected participants and that the HIV-infected participants may be more at risk to develop cardiovascular disease in comparison with the HIV-uninfected participants.
OPSOMMING

TITEL: Fibulin-1 as 'n merker van kardiovaskulêre siekte in MIV-geïnfecteerde swart Suid-Afrikaners: 'n opvolgstudie

AGTERGROND: Daar is 'n hoë voorkoms van die menslike immuniteitsgebrek-virus (MIV)-infeksie in Suid-Afrika en hierdie chroniese infeksie bevorder vaskulêre inflammasie, wat tot vaskulêre skade in geïnfecteerde individue lei. Die ekstrasellulêre matriks is 'n hoog aanpasbare en dinamiese struktuur wat deur mecaniese stres, inflammasie en oksidatiewe stres beïnvloed word en daar is aanduidings dat veranderinge tot arteriële styfheid kan bydra. Fibulin-1 is 'n fibrinegeen-bindende plasmaproteïen en is deel van 'n klein groepie ekstrasellulêre matriksproterene (insluitend fibronektien, laminien en von Willebrand-faktor) wat in relatiewe hoë vlakke in die bloed teenwoordig is. Veranderinge in fibulin-1-vlakke hou gevolge in vir vaskulêre strukturele integriteit en dit wil voorkom asof die handhawing van die ekstrasellulêre matriks in die bloedvatwand se integriteit van kritieke belang is in die voorkoming van kardiovaskulêre siekte.

DOEL: Die doel van hierdie studie was om die assosiasie tussen fibulin-1 en merkers van vaskulêre funksie in MIV-geïnfecteerde swart Suid-Afrikaners te bepaal tydens die basislynstudie van 2005 en 'n opvolgstudie in 2008.

METODOLOGIE: Hierdie substudie vorm deel van die groter internasionale Prospective Urban and Rural Epidemiology (PURE)-studie. Die PURE-studie is 'n opvolgstudie wat vrae aanpak aangaande die oorsaak en ontwikkeling van kardiovaskulêre risikofaktore en siekte in bevolkings, veral van laer- en middel-inkomste lande, insluitend Suid-Afrika. Die Suid-Afrikaanse been van die PURE-studie is in die Noordwesprovincie uitgevoer, waar 'n totaal van 2 010 deelnemers (1 004 stedelik en 1 006 landelik) ewekansig in 'n stedelike en landelike
gebied gewerf en in 2005 tydens die basislynfase gesif is. Vir hierdie substudie is die MIV-geïnfecteerde deelnemers \((N = 300)\) van die studiebevolking individueel tydens die basislynfase (2005) met MIV-ongeïnfecteerde deelnemers \((N = 300)\) gepas. Die deelnemers is volgens ouderdom, geslag, liggaamsmassa-indeks en gebied (stedelik en landelik) gepas en in 2008 opgevolg. Antropometriese en kardiovaskulêre metings is bepaal. Die OMRON HEM-757 (Omron, Kyoto, Japan)-apparaat is gebruik om die sistoliese en diastoliese bloeddruk te bepaal. Die polsdruk is vervolgens deur die verskil in sistoliese en diastoliese bloeddruk bereken. Die karotis-radialis-polsgolfnelheid (cr-PWV) is deur die Complior SP Acquisitionstelsel (Artech Medical, Pantin, Frankryk) bepaal. Die lipiedprofiel en inflammasiemerkers is ook bepaal. Tydens die basislynfase is onafhanklike t-toetse gebruik om die kardiovaskulêre veranderlikes tussen die MIV-geïnfecteerde en MIV-ongeïnfecteerde deelnemers te vergelyk. Afhanklike t-toetse is gebruik om die basislyn- en opvolgmetings van die kardiovaskulêre veranderlikes tussen MIV-geïnfecteerde en MIV-ongeïnfecteerde deelnemers te vergelyk. Die persentasie verandering in al die groepe is ook oor 'n tydperk van drie jaar bepaal. Die Pearson en parsiële korrelasies is uitgevoer om onaangepaste en aangepaste assosiasies tussen die verandering van fibulin-1- en kardiovaskulêre veranderlikes in elke groep te ondersoek. P-waardes van \( \leq 0.05 \) is as statisties betekenisvol beskou.

RESULTATE: Tydens die basislynfase, sowel as drie jaar later, was die fibulin-1-vlakke in MIV-geïnfecteerde Suid-Afrikaners betekenisvol hoër as in MIV-ongeïnfecteerde Suid-Afrikaners. Die persentasie verandering in fibulin-1 word met die persentasie verandering in trigliseride-tot-hoëdigtheid-lipoproteïen-cholesterol \((TG/HDL-C)\) in MIV-geïnfecteerde deelnemers geassocieer, maar nie in MIV-ongeïnfecteerde deelnemers nie. 'n Betekenisvolle positiewe korrelasie is tussen die persentasie verandering in fibulin-1 en oplosbare urokinase-tipe plasminogeen-geaktiveerde reseptor \((suPAR)\)-vlakke in die MIV-ongeïnfecteerde groep opgemerk, maar geen positiewe korrelasie is in suPAR-vlakke in die onaangepaste korrelasies
gevind nie. Tydens die basislynfase (2005), sowel as die opvolgstudie in 2008, het die MIV-geïnfekteerde deelnemers laer HDL-C en hoër oplosbare vorms van intersellulêre adhesiemolekule-1 (sICAM-1), vaskulêre sel-adhesiemolekule-1 (sVCAM-1) en suPAR-vlakke getoon in vergelyking met hul MIV-ongeïnfekteerde kontrolegroep. Die sICAM-1- en sVCAM-1-vlakke is slegs in 2005 bepaal. Tydens die basislynfase was die cr-PWV aansienlik hoër in die MIV-geïnfekteerde groep (11.26 m/s vs.10.68 m/s, p = 0.03) as in die MIV-ongeïnfekteerde groep. In die opvolgstudie is daar egter geen betekenisvolle verskille in die cr-PWV-waardes gevind tussen die MIV-geïnfekteerde en MIV-ongeïnfekteerde deelnemers nie. Oor die tydperk van drie jaar was daar geen betekenisvolle verskille in persentasie verandering in fibulin-1, TC/HDL-C ratio, TG/HDL-C ratio, cr-PWV en PD tussen die MIV-geïnfekteerde en MIV-ongeïnfekteerde deelnemers nie.

BESPREKING: Die hoë suPAR- en lae HDL-C-vlakke dui op die moontlikheid dat die MIV-geïnfekteerde deelnemers meer geneig kan wees om vaskulêre skade te ontwikkel, wat weer tot verdere ekstrasellulêre matriksmodellering kan lei. Laasgenoemde word as die skakel gesien tussen infeksie, inflammasie, trombotiese aktiwiteit en vaskulêre disfunksie. Die verhoogde cr-PWV in die MIV-geïnfekteerde groep tydens die basislynfase ondersteun bogenoemde moontlikheid. Alhoewel daar geen betekenisvolle assosiasies gevind is tussen fibulin-1 en merkers van vaskulêre funksie (naamlik sICAM-1, sVCAM-1, interleukin-6 [IL-6], plasminogeen-geaktiveerde inhibeerder-1 [PAI-1] of PWV) nie, dui die assosiasie van die persentasie verandering in fibulin-1 met die verandering in TG/HDL-C daarop dat die TG/HDL-C kan bydra tot waarskynlike vaskulêre veranderinge in MIV-geïnfekteerde deelnemers en dat die MIV-geïnfekteerde deelnemers moontlik 'n groter risiko vir die ontwikkeling van kardiovaskulêre siekte het in vergelyking met die MIV-ongeïnfekteerde deelnemers.
PREFACE

The study forms part of the programme for the degree Master of Science in Physiology.

Chapter 1 contains a literature overview of all the variables that are applicable to this study with motivation, aims and hypotheses included. In Chapter 2, the peer-reviewed journal, Journal of Inflammation, is considered for submission of the manuscript. Chapter 3 is a basic, conclusive chapter on the study results and their implications, as well as recommendations for future research.

OUTLINE OF THE STUDY

This study is divided into three chapters which consist of the following information:

• Chapter 1 contains the general introduction, an overview of published data, the questions arising from the literature, the motivation for and objectives of the study, as well as the hypotheses.

• Chapter 2 contains the manuscript of the study, entitled Fibulin-1 as a marker of cardiovascular disease in HIV-infected black South Africans: a prospective study.

• In Chapter 3, the summarised findings and limitations of the study are discussed.

• At the end of Chapters 1 and 2, the relevant references are consistent with the guidelines for publishing in the aforementioned journal.
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<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>AIDS</td>
<td>Acquired immune deficiency syndrome</td>
</tr>
<tr>
<td>ANCOVA</td>
<td>Analysis of covariance</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
</tr>
<tr>
<td>BHS/AAMI</td>
<td>British Hypertension Society/Advancement of Medical Instrumentation</td>
</tr>
<tr>
<td>BMI</td>
<td>Body mass index</td>
</tr>
<tr>
<td>BP</td>
<td>Blood pressure</td>
</tr>
<tr>
<td>CAM</td>
<td>Cell adhesion molecules</td>
</tr>
<tr>
<td>CI</td>
<td>Confidence intervals</td>
</tr>
<tr>
<td>CRP</td>
<td>C-reactive protein</td>
</tr>
<tr>
<td>CVD</td>
<td>Cardiovascular diseases</td>
</tr>
<tr>
<td>DBP</td>
<td>Diastolic blood pressure</td>
</tr>
<tr>
<td>HDL-C</td>
<td>High-density lipoprotein cholesterol</td>
</tr>
<tr>
<td>HIV</td>
<td>Human immunodeficiency virus</td>
</tr>
<tr>
<td>ICAM</td>
<td>Intracellular adhesion molecules</td>
</tr>
<tr>
<td>IDF</td>
<td>International Diabetes Federation</td>
</tr>
<tr>
<td>IL-6</td>
<td>Interleukin-6</td>
</tr>
<tr>
<td>IMT</td>
<td>Intima-media thickness</td>
</tr>
<tr>
<td>MAP</td>
<td>Mean arterial pressure</td>
</tr>
<tr>
<td>N</td>
<td>Number of subjects</td>
</tr>
<tr>
<td>PAI-1</td>
<td>Plasminogen activator inhibitor-1</td>
</tr>
<tr>
<td>PP</td>
<td>Pulse pressure</td>
</tr>
<tr>
<td>PURE study</td>
<td>Prospective Urban and Rural Epidemiology study</td>
</tr>
<tr>
<td>PWV</td>
<td>Pulse wave velocity</td>
</tr>
<tr>
<td>SBP</td>
<td>Systolic blood pressure</td>
</tr>
<tr>
<td>suPAR</td>
<td>Soluble urokinase plasminogen activator receptor</td>
</tr>
<tr>
<td>TC</td>
<td>Total cholesterol</td>
</tr>
<tr>
<td>TG</td>
<td>Triglycerides</td>
</tr>
<tr>
<td>TC/HDL-C ratio</td>
<td>Total cholesterol/high-density lipoprotein cholesterol</td>
</tr>
<tr>
<td>TG/HDL-C ratio</td>
<td>Triglycerides/high-density lipoprotein cholesterol</td>
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<tr>
<td>vCAM</td>
<td>Vascular adhesion molecules</td>
</tr>
</tbody>
</table>
1.1 General introduction

Fibulin-1 is a protein that has been found in association with extracellular matrix (ECM) structures such as connective tissue fibres and basement membrane that surround vascular smooth muscle [1-3]. This protein accumulates in the arterial wall and in plasma, and appears to be a factor that is associated with arterial extracellular matrix changes [4]. It functions not only as a structural component, but provides the scaffolding for cells and tissues, and plays an essential role in tissue morphogenesis that affects cell adhesion, migration and cell growth. It is also a modulator for various cellular processes, such as differentiation and angiogenesis [5].

The fibulin-1 gene produces two variants (C and D). Two additional variants (A and B) exist, but at very low levels and the functions of the A and B variants are yet to be determined [6]. Fibulin-1C is required to regulate cell shape, extracellular matrix formation, wound healing and cell adhesion [7]. Overexpression of fibulin-1D reduces tumour formation, whereas the ratio of fibulin-1C to fibulin-1D is increased in ovarian carcinoma [8]. Fibulin-1 is associated with vascular damage and inflammation [1-3], which could lead to vascular dysfunction. This has not been studied in HIV-infected individuals in the North-West Province of South Africa yet.
There is a high prevalence of the human immunodeficiency virus (HIV) infection in South Africa and this places a heavy burden on the health system. The chronic infection of HIV-infected individuals promotes vascular inflammation, leading to vascular damage [9-11] (fig.1). HIV infection may therefore be a precursor for a growing threat of non-communicable diseases such as chronic vascular disease (CVD). Increased levels of inflammatory markers such as C-reactive protein (CRP), interleukin-6 (IL-6) [10] and increased cell adhesion molecules, namely intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule (VCAM-1) [12], have been reported in the HIV-infected population [13,14] (fig.1). Biological markers such as cell adhesion molecules (CAM) give an indication regarding the development of endothelial dysfunction and, eventually, vascular dysfunction [14] (fig.1).
In the case of vascular injury, the inflammatory response is mainly mediated by monocytes, macrophages and T-lymphocytes [15]. These cells are then attracted to the endothelium via adhesion molecules and migrate through the layer of endothelial cells to infiltrate the vessel wall [12]. Once these monocytes and T-lymphocytes have invaded the vascular ECM and become activated, they secrete several substances, including cytokines, growth factors, chemokines and matrix metalloproteinases (MMPs) [16,17] (fig.1). Fibulin-1 influences the structural and functional properties of the ECM [18]. Findings have indicated that fibulin-1 acts as a cofactor for the matrix metalloproteinases and is increased in atherosclerotic lesions [18,19].

MMPs are a group of zinc-dependent endopeptidases that degrade a variety of components of the ECM. Their activities in the vascular tissues contribute to vascular remodelling that is associated with CVD [20]. Vascular remodelling involves the reorganisation and degradation of the ECM scaffold, and hyperplasia or hypertrophy of vascular smooth muscle cells (VSMCs), thus contributing to vascular stiffness [21-23]. An important consequence of long-term increase in MMP activity is increased arterial stiffness [21-23]. This change reduces vessel compliance and distensibility, and increases arterial pulse pressure (PP) and pulse wave velocity (PWV). MMPs' degradation of arterial elastin [21], increased collagen [24] and fibrinonectin [25,26] leads to a decrease in vascular function. Changes in fibulin-1 levels have consequences to vascular structural integrity, while maintaining the integrity of the ECM in the blood vessel wall seems critical in preventing cardiovascular disease [18, 27] (fig.1).

Godyna et al. found that fibulin-1 can bind to fibrinogen and can be incorporated into fibrin clots. This finding has prompted investigation into the potential role of fibulin-1 as a prothrombotic agent [28]. Following vascular injury and inflammation, fibulin-1 presents in the extracellular matrix of the vessel wall, interacts with plasma fibrinogen and promotes platelet adhesion, which then leads to the formation of a platelet plug [28]. Platelets interact with exposed subendothelial matrices after vascular injury [28] (fig.1). The ability of fibulin-1
to bind to ECM proteins such as elastin [29] and fibrinonectin [25] may provide a means for fibrinogen to bind to subendothelial ECM and contribute to thrombus formation [28] (fig.1).

### 1.2 Literature overview

#### 1.2.1 The human immunodeficiency virus (HIV)

Millions of people have died from the acquired human immunodeficiency virus (HIV) [30]. In 2010, with an estimated 5.6 million people being HIV infected, South Africa continued to have the world's largest HIV epidemic [30]. Acquired immunodeficiency syndrome (AIDS) is characterised by the progressive destruction of a person's immune system and is the last and most serious stage of HIV infection [31]. It is generally accepted that HIV causes AIDS and this infection accounts for about 20% of all deaths and disability-adjusted life years lost in Africa, which makes it the largest single component of the continent's disease burden [32].

There are two known species of HIV that infect humans, namely HIV-1 and HIV-2 [33]. HIV-1 can be divided into two groups: HIV-1 group M (major) and HIV-1 group O (outlier) [34,35]. The major cause of AIDS is, however, HIV-1 group M. Over the years, HIV-1 sequences have diverged substantially and can be classified into subtypes A-J [34,36]. HIV-1 is easily transmitted, virulent and responsible for HIV infections throughout the world, especially in sub-Saharan Africa [34,37]. Subtype C is the most prevalent in sub-Saharan Africa and accounts for 55-60% of all HIV infections worldwide [38,39]. HIV-2 is primarily confined to West Africa [33].

The HIV infection is associated with vascular damage, leading to subclinical inflammation and increased cardiovascular risk [11,13,19]. In HIV infection, the endothelium is under the combined influence of a viral load that injures or activates the endothelium [40], causing functional changes to the endothelium that resemble inflammation and may lead to endothelial dysfunction [41] (fig.1).
The mechanisms underlying the association between inflammation and endothelial dysfunction are complex and multifaceted [42]. In HIV-infected patients, the endothelium could be activated either directly by HIV or by a leukocyte-mediated inflammatory cascade that is triggered by the HIV infection. This could lead to endothelial dysfunction, accelerated atherosclerosis and increased coagulation, which could result in thrombosis [41]. HIV infection is characterised by a profound inflammatory response which may trigger the synthesis of pro-inflammatory cytokines, including tumour necrosis factor (TNF), interleukin-1 (IL-1), IL-6 and CRP [43]. This may contribute to endothelial dysfunction [12], vascular inflammation, atherogenesis and a prothrombotic state [10,41] (fig.1).

1.2.2 HIV infection and endothelial dysfunction

The association between HIV and endothelial dysfunction is an area of rapidly growing interest. Endothelial dysfunction, the most plausible link between infection, inflammation and atherosclerosis, has been investigated since the beginning of the HIV epidemic (fig.1). Endothelium dysfunction is an early marker of atherosclerosis, which is associated with an increased risk of cardiovascular events [44]. A dysfunctional endothelium has been reported in HIV-infected patients [45,46].

The endothelium plays a fundamental role in the dynamic regulation of the circulation and is involved in important homeostatic mechanisms such as vascular tone regulation, non-thrombotic vascular surface and immunomodulation [47]. The endothelium is a cell layer that regulates the exchange of water and small molecules, as well as coagulation and fibrinolysis [48]. As a result of its position between blood and the vascular wall, the endothelium is constantly exposed to potentially noxious circulating agents such as cholesterol and infective agents [41] (fig.2).

In HIV infection, the endothelium is under the combined influence of a viral load, increased concentration of circulating antigens and immune reconstruction [40,49]. This causes
profound functional changes of the endothelium, resulting in chronic arterial inflammation and injury, which in turn promote dysfunction of the endothelium, atherosclerosis and thrombosis [40,41] (fig.2).

During endothelial dysfunction, the endothelial cells release increased levels of endothelin-1, angiotensin II, plasminogen activator inhibitor-1 (PAI-1) and von Willebrand factor [12,14,49, 50]. Normally, tissue factor is not present on the endothelial cell surface. During endothelial dysfunction, however, tissue factor becomes expressed as thrombin production [12] (fig.2). High levels of the soluble adhesion molecules, tissue plasminogen activator (t-PA) and plasminogen activator inhibitor (PAI-1) represent early markers of the development of vascular dysfunction [14,49]. Increased levels of soluble adhesion molecules were documented in different stages of HIV [51-53].

When pro-inflammatory cytokines such as tumour necrosis factor-alpha (TNF-a) or IL-6 are activated, the expression of adhesion molecules such as ICAM-1, VCAM-1 and E-selectin increases, leading to the migration of inflammatory cells to the subendothelium [12] (fig.2). Plaque formation is caused by the accumulation of inflammatory cells [54]. These adhesion molecules stimulate monocyte adherence and increased levels of selectins that promote expression of monocytes to the endothelial surface [55] (fig.2) The increased numbers of inflammatory cells make plaque more vulnerable to rupture, leading to myocardial infarction or endothelial dysfunction [56-58].
Figure 2: Development of atherosclerosis and its thrombotic complications

EC: endothelial cells, adhesion molecule; MCP-1: monocytes chemoattractant protein-1; MMPs: matrix metalloproteinases; NO: nitric oxide; PGI₂: prostacyclin; SMC: vascular smooth muscle cells; and TF: tissue factor [44].

1.2.3 The extracellular matrix (ECM) and fibulin-1

The extracellular matrix (ECM) contains collagen IV, laminin and small amounts of structural proteins such as fibronectin and fibulin [2]; it forms a complex, three-dimensional network among the cells of different tissues in an organ-specific manner [59]. The ECM was initially considered to be an inert, space-filling material that provided only mechanical strength to tissues and organs. Today, the ECM is considered to be a highly adaptive and dynamic structure that plays a fundamental role in myocardial ventricular remodelling, as it is regulated by neurohormonal activation, inflammation, oxidative stress and mechanical stress [60]. Ultimately, this can lead to a change in the collagen and fibulin-1 levels in the ECM [61].

Fibulin-1 is a fibrinogen-binding blood protein and is part of a small group of proteins, including fibronectin, laminin and von Willebrand factor that are present in the blood at relatively high levels [6]. Studies have showed that fibulin-1 is widely expressed in the
intercellular components of the connective tissues, matrix fibers and basement membranes [2]. A major function of fibulin-1 is the regulation of cell motility and guidance [62]. Based on findings from in vitro studies, fibulin-1 can suppress the motility (that is the migration velocity and persistence time) of many types of cancer cells and also the activity of other ECM proteins, including fibronectin [63], which is one of its principal binding proteins [64]. Fibulin-1 appears to be a factor that is associated with arterial extracellular matrix changes, which could lead to increased vascular stiffness and the development of cardiovascular diseases [62]. Cangemi et al. were able to demonstrate that increased plasma fibulin-1 concentrations were predictive of overall cardiovascular mortality in type 2 diabetes mellitus [4]. They found that changes in arterial fibulin-1 concentrations in relation to elastic fibres in diabetes may relate to findings from other studies that show signs of reduced intimal elastin content in diabetic arteries, leading to vascular dysfunction [65-67]. Therefore, maintaining the integrity of the ECM in the blood vessel wall seems critical in preventing cardiovascular disease [4].

1.2.4 Matrix metalloproteinases (MMPs)

Matrix metalloproteinases (MMPs) are involved in the degrading of most of the ECM components in the connective tissue [20]. MMPs mediate a large variety of biological reactions such as the regulation of vascular function [68], leukocyte activation [68] and platelet function, as well as pathological processes such as cancer, atherosclerosis and other inflammatory disorders [69].

Findings have indicated that fibulin-1 acts as a cofactor for the MMPs and is increased in atherosclerotic lesions [19]. The ECM turnover is altered in arterial tissue, as is evidenced by increased concentrations of the MMP metallopeptidase 2 (MMP-2) [70,71], a proteinase that cleaves elastin, which is associated with vascular stiffness [72].

Four MMPs, including MMP-1, MMP-2, MMP-3 and MMP-9, have been identified in human platelets [20]. In resting platelets, these enzymes are stored in the latent form. Tissue inhibitors of metalloproteinases (TIMPs), a group of endogenous inhibitors, play an important
role as mediators of tissue remodelling, as well as in the regulation of MMP activity [73,74]. All MMPs can be inhibited by different TIMPs. These TIMPs bind to the active site of MMPs and block the access to ECM substrates [61].

Platelets are small cell elements that are produced by fragmentation of large mother cell megakaryocytes. After vascular injury, platelets adhere to the damaged portion of the vascular wall, initiating a set of reactions that lead via platelet aggregation to the formation of a haemostatic plug or occlusive thrombus [28]. The mechanisms of MMPs’ interactions with platelets are still being explored and further research is needed.

1.2.5 Vascular wall and lipids
HIV infection, independent of the use of antiretroviral therapy (ART), may increase the risk for atherosclerotic CVD via adverse changes in blood lipids, inflammation and thrombotic activity [75]. High levels of low-density lipoprotein cholesterol (LDL-C) and triglycerides (TG), and low levels of high-density lipoprotein cholesterol (HDL-C) are known to increase the risk of CVD. These factors can possibly be one of the initiating factors in the formation of atherosclerosis, which ultimately leads to CVD [11,75-77].

Like most lipids, cholesterol circulates the plasma as part of various lipoprotein complexes. These include very low-density lipoproteins (VLDL), low-density lipoproteins (LDL) and high-density lipoproteins (HDL) [78]. LDL is the main cholesterol carrier and delivers cholesterol to the cells [78]. HDL-C serves as an acceptor for cholesterol from the tissue and transports the cholesterol back to the liver [79]. Low levels of HDL-C are also viewed as a risk factor for CVD [78]. Studies show that each 1% increase in HDL-C was associated with a 2-3% reduction in CVD risk [80]. HDL-C possesses several anti-inflammatory and antithrombotic properties that may protect against injury to endothelial surfaces [81]. The ratio between LDL-C and HDL-C is viewed as an accurate predictor of CVD; the lower the ratio, the lower the risk [82].
LDL is widely known as 'bad' cholesterol [44]. Elevated levels of LDL are associated with cardiovascular disease because of an increased deposition of cholesterol on the arterial walls, leading to atherosclerosis [83]. An increase in LDL is associated with an increase in plasma viscosity [83]. HDL is widely known as 'good' cholesterol and is associated with the removal of excess LDL. An increase in HDL is associated with a decrease in plasma viscosity [78,79].

Research shows that triglyceride-rich lipoproteins produce typical atherosclerotic changes. TG-rich lipoproteins show an increased movement into the intima [84]. The result is the formation of fatty streaks, which are a key factor in the formation of atherosclerosis [84]. There is thus reason to believe that TG-rich lipoproteins have an initiating effect on atherosclerosis. HIV-infected people exhibit high levels of TG and low-density lipoprotein, and low levels of HDL-C [13,85-87]. The link between HIV and metabolic derangements remains unclear and more research is needed to determine the underlying causes of the change in the lipid profile in HIV infection [75].

1.2.6 Pro-inflammatory markers

1.2.6.1 C-reactive protein (CRP)

C-reactive protein (CRP) represents an extensively studied pro-inflammatory molecule and is an acute phase reactant, synthesised primarily in hepatocytes and secreted by the liver. CRP is seen as a robust clinical marker because of its analytical stability, reproducible results and high sensitivity assays [88,89]. In healthy individuals, the concentrations of CRP are low in plasma [90]. In response to injury, infection and inflammation, the levels can rise dramatically to approximately 300 mg/l after 48 hours and can decrease just as rapidly with the resolution of the condition [91]. Therefore, CRP concentrations serve as a sensitive marker of systemic inflammation and are also associated with cardiovascular risk factors and cardiovascular and non-cardiovascular causes of death [92].
CRP is mainly produced in the liver in response to IL-6 and has thus been thought of as an inactive downstream, bystander marker of the inflammatory cascade [93]. IL-6 is a cytokine and CRP concentrations have shown to be a direct indicator of IL-6 levels in humans in vivo [93]. A high serum concentration level of high-sensitivity C-reactive protein (hs-CRP) is seen as an independent risk factor and predictor of CVD [94], and is thought to induce ICAM and VCAM secretion [94]. It is, therefore, very important that CRP should be screened for in an effort to identify patients who are at high risk of cardiovascular events more efficiently [95,96].

According to Masia et al., the effect of CRP in HIV-infected persons remains unknown [97]. The increased levels of CRP in HIV-infected participants [85,98] probably relate to altered fat distribution and metabolic abnormalities [77,98]. Van Wijk et al. reason that increased levels of CRP are most likely caused by the chronic immune activation that is associated with the HIV infection [98].

1.2.6.2 Interleukin-6 (IL-6)

Interleukin-6 (IL-6) is an immune protein in the hematopoietin family and is released in response to infection and inflammation. Its functions range from key roles in acute-phase protein induction to B- and T-cell growth and differentiation [99]. IL-6 can have direct effects on cells, mediate the effects of other cytokines, be co-agonistic or antagonistic in conjunction with other cytokines, and interact with glucocorticoids [99].

From the literature, it is evident that IL-6, CRP and tumour necrosis factor-a (TNF-a) are involved in the pathobiology of CVD, indicating that low-grade systemic inflammation plays a key role in this condition [92,94]. In most recent studies, cardiorespiratory fitness has been shown to have a complimentary effect on CRP and IL-6 levels [100,101]. Enhanced fitness may have an anti-inflammatory role with improved insulin resistance that may be the mechanism for lowering CVD risk and type 2 diabetes mellitus [101,102]. IL-6 is a cytokine
that is produced in the adipose tissue of healthy humans. It is released into the circulation and activates the production of CRP in the liver [93].

1.2.6.3 Soluble urokinase plasminogen activator receptor (suPAR)

Soluble urokinase plasminogen activator receptor (suPAR) is a known inflammatory marker and a strong predictor of immunologic failure and mortality in HIV-infected patients who are not receiving highly active antiretroviral therapy (HAART) [103]. SuPAR is a novel biomarker and stable plasma protein [104] that is expressed predominantly by leukocytes [105] and associated with inflammation and progression of disease [104].

SuPAR is present in plasma and red blood cells [106,107] in various concentrations, depending on the ‘activation’ level of the immune system, since higher activation increases serum suPAR levels [108,109]. An elevated suPAR level has been associated with poor clinical outcomes in patients who are suffering from infectious diseases [109] such as HIV infection [110], tuberculosis [111] and several cancers [110].

In patients who are receiving HAART, suPAR demonstrated its potential as a treatment efficacy marker when its levels decreased with effective therapy [112]; it therefore has potential clinical benefits. However, Anderson et al. found that suPAR remained elevated in some HIV-infected patients, independently of the HAART’s effects on it, which reflected a possible low-grade pro-inflammatory state [110].

Anderson et al. also concluded that suPAR may reflect the metabolic status of the HIV-infected patients on HAART and linked dysmetabolism with low-grade inflammation [110], which was similar to the findings of Kolb et al. They suggested that suPAR is a potential marker of dysmetabolism in HIV-infected patients on stable HAART [113]. It is not clear whether the overall outcome that is associated with increased blood levels of suPAR in HIV-infected patients is caused by a direct association between HIV and the components of the
suPAR system or whether it is simply caused by the effect of mirror inflammation [112]. However, blood levels of suPAR were linked with inflammation and immune activation [109,112,114].

SuPAR reflects the immune and pro-inflammatory status of patients that is caused by HIV and tuberculosis [110]. However, recent studies have shown that suPAR is related to cardiovascular function [112]. The cardiovascular health of the black South African population is a major health concern, as this group suffers mostly from hypertension and stroke, leading to an alarming increase in cardiovascular morbidity and mortality [115]. SuPAR may be able to contribute to the early detection and prevention of cardiovascular diseases.

1.2.7 Intercellular and vascular cell adhesion molecules (ICAM and VCAM)

Intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) have molecular structures that resemble immunoglobulins and are markers of endothelial dysfunction [116]. These molecules are expressed on leukocytes and platelets and facilitate endothelial adhesion of circulating leukocytes. Upon activation by pro-inflammatory cytokines such as IL-6, they facilitate endothelial expression of adhesion molecules and the migration of inflammatory cells to the subendothelium, leading to the development of vascular remodelling [12]. While IL-6 is an early stimulator of the inflammatory process and CRP is produced in response to IL-6 secretion, CRP is thought to induce ICAM and VCAM secretion [88]. These adhesion molecules indicate vascular endothelial injury and dysfunction [9,50,117].

In pathological studies, ICAM-1 and VCAM-1 have been detected in atherosclerotic plaques and have been found to be upregulated in arterial endothelial cells in lesion-prone areas [118]. Interindividual variations in plasma concentration of soluble intercellular adhesion molecule-1 (sICAM-1) and soluble vascular cell adhesion molecule-1 (sVCAM-1) have been
demonstrated. Plasma levels may also be influenced by race, for example people of African origin have lower levels of sICAM-1 and sVCAM-1 [12]. Higher plasma levels of VCAM-1 and ICAM-1 are seen in HIV-infected people [13,50,119,120].

The finding of increased sICAM-1 concentrations in HIV-infected patients [121] pointed to the occurrence of endothelial damage as measurable by soluble adhesion molecule plasma levels. On the other hand, higher concentrations of sICAM-1 were seen in AIDS patients with acute opportunistic infections than in HIV-infected patients without acute infections, both values being higher than the values of HIV-uninfected patients [122].

A correlation between ICAM-1 concentrations and the progression of disease, as well as the reduction of CD4+ cell count, was also reported. Increased leucocyte adherence to the aortic endothelium of HIV-infected patients, and increased VCAM-1 levels and E-selectin plasma concentrations add further experimental evidence of endothelial cell involvement in the AIDS syndrome [123,124]. An association between concentrations of ICAM-1, as well as concentrations of VCAM-1 and E-selectin, and future cardiac events could also be shown in apparently healthy people by the prospective Atherosclerosis Risk in Communities (ARIC) study [125]. Altogether, these data raise the possibility that soluble CAM can serve as a molecular biomarker for the early diagnosis of atherosclerosis, but this possibility requires further evaluation on other large populations.

1.2.7.1 Pulse wave velocity (PWV) as measurement of arterial stiffness

Aortic pulse wave velocity (PWV) is a non-invasive measurement of arterial stiffness and is associated with end-organ changes such as increased ventricular stress (caused by afterload) and arterial intima-media thickening [98,126]. Aortic PWV is also an independent predictor of cardiovascular mortality [127,128]. Acute inflammation leads to an increase in arterial stiffness and faster wave reflections [98]. From the literature, it is evident that CRP,
as a marker of inflammation, is associated with stiffness of medium, muscular-type arteries as expressed by the carotid-radial PWV (cr-PWV) in HIV-infected participants [129].

Arterial stiffness and faster wave reflections are markers of cardiovascular disease and independent predictors of cardiovascular risk [127]. The effect of HIV infection per se on aortic stiffness and wave reflections has not been defined clearly [98].

1.2.8 Prothrombotic factors

1.2.8.1 Fibrinogen

Fibrinogen is a plasma protein and biomarker of inflammation and its degradation products have been associated with microvascular leakage. Fibrinogen, which has a computed molecular weight of 340 kDa, is a soluble glycoprotein that is synthesised in the liver and found in the plasma in 'usual' concentrations of 1.5 to 4.5 g/l [130]. Currently, the recommended optimal range for fibrinogen is 2-3 g/l [130]. Many cardiovascular disorders are accompanied by an increased blood content of this high molecular weight plasma adhesion protein [26,95].

Fibrinogen plays an important role in blood coagulation [33]. When elevated, it identifies individuals who have a high risk for developing cardiovascular diseases [131] that involve inflammatory processes such as hypertension [132], diabetes and stroke [133]. Synthesis of fibrinogen involves other inflammatory mediators such as IL-6 [131] which, like fibrinogen, are associated with an elevation of blood pressure [132]. Because of its role in platelet aggregation, plasma viscosity and fibrin formation, fibrinogen is a haemostatic risk factor for CVD [134,135].

Fibulin-1 can bind to fibrinogen and can be incorporated into fibrin clots, leading to vascular dysfunction [136]. Following vascular injury and inflammation, fibulin-1 presents in the extracellular matrix of the vessel wall, interacts with plasma fibrinogen and promotes platelet adhesion, leading to the formation of a platelet plug [28]. Importantly, epidemiological studies
have showed high plasma levels of fibrinogen as a risk factor for atherosclerosis progression and cardiovascular diseases, including stroke, coronary disease and peripheral arterial occlusive disease [132-134]. Kannel et al. concluded that fibrinogen and CRP determination may be useful tools to identify individuals at risk of thrombotic complications [26]. Hsue et al. found that fibrinogen levels were increased in HIV-infected participants [85].

The precise role of fibrinogen in CVD pathology is not completely clear. As an acute phase reactant, it might be a marker of inflammation which probably plays a role in CVD, since it seems as if a combination of inflammatory and thrombotic processes contributes to the development of CVD [137].

1.2.8.2 Plasminogen activator inhibitor-1 (PAI-1)

Plasminogen activator inhibitor-I (PAI-1) belongs to the family of serpin protease inhibitors [138] and is secreted by a variety of cells [139]. It is seen as a coagulation marker [167] and elevated levels are associated with endothelial dysfunction and CVD [166]. In normal human plasma, PAI-1 (a protein with a molecular weight of 52 kDa) levels can range from 0.5-1.5 nmol/l [138]. By acting as a physiological inhibitor of tissue plasminogen activator (t-PA) and urokinase plasminogen activator (u-PA) to maintain the homeostasis of the blood coagulation process, PAI-1 is an important factor in blood coagulation [140].

An abnormal level of PAI-1 will disrupt the homeostasis, resulting in increased risk of thrombus formation, myocardial infarction and associated vascular diseases [141]. High plasma levels of PAI-1 may be associated with the development of atherosclerosis [141], where the high levels promote atherosclerotic plaque formation by decreasing the capacity to degrade fibrin, thus enhancing the chance for damaging thrombosis to develop plaque rupture [142]. Therefore, high levels of PAI-1 reduce fibrinolytic potential, thereby increasing the risk of CVD [142].
1.2.9 Cardiovascular disease (CVD) in South Africa

Cardiovascular disease (CVD) is the most widespread disease in the Western world [143,144], and a leading cause of morbidity and mortality worldwide [145,146]. Risk factors for CVD, both major and minor, include high blood pressure, type 2 diabetes, cancer, chronic lung disease and depression [147]. Neurological stroke and coronary artery disease already account for more than a third of deaths in people older than 65 years in South Africa [148]. CVD is also the product of a pathogenic process that is associated with the development of atherosclerotic plaque in the arteries [41].

Atherosclerosis consists of the formation of fibro-fatty and fibrous lesions, preceded and accompanied by inflammation [149]. It often takes years to become clinically apparent [149]. Atherosclerosis is a multi-factorial process that is associated with genetic, environmental and lifestyle factors [149]. Arterial wall damage results from the many complex interactions between noxious stimuli and the healing responses of the arterial wall. The mechanisms of the process of atherosclerosis are also not completely clear [149].

Hypertension affects a major proportion of the South African population with a high prevalence in the urban (56%) as well as the rural areas (20-23%) in South Africa, including the North-West Province [115,148]. Cardiovascular disease is becoming the major cause of mortality among the black African population in South Africa [150,151]. Recently, it was indicated that black Africans are more frequently diagnosed with heart failure than any of the other ethnic groups [148]. This might be due to the increase in urbanisation during the last decade, which resulted in non-communicable diseases to become more prevalent in this population group [150].
1.2.11 Motivation for the study

Fibulin-1 is associated with vascular damage and inflammation, which could lead to vascular dysfunction and has not been studied in HIV-infected individuals in the North-West Province of South Africa. The HIV infection rate is high in South Africa and a leading cause of morbidity and mortality worldwide [30]. HIV infection may lead to vascular damage and vascular inflammation. It may also increase the risk of CVD [13,50] and is associated with endothelial dysfunction [12,50], accelerated atherosclerosis [85] and coagulatory disorders [152]. Increased levels of inflammatory markers CRP and IL-6, as well as increased cell adhesion molecules, have been reported in the HIV-infected population [13,14]. This increased concentration of circulating antigens causes profound functional changes of the endothelium which, in turn, activate and promote ECM remodelling, leading to vascular dysfunction. Once the vascular ECM is activated, MMPs are secreted. MMPs contribute to vascular remodelling that is associated with CVD [20]. Vascular remodelling involves the degradation and reorganisation of the ECM scaffold, and hypertrophy or hyperplasia of vascular smooth muscle cells (VSMCs), thus contributing to thickened vessel wall and vascular stiffness [21-23].

Following vascular injury and inflammation, fibulin-1 presents in the extracellular matrix of the vessel wall, interacts with plasma fibrinogen and promotes platelet adhesion, leading to the formation of a platelet plug [28]. After vascular injury, platelets interact with exposed subendothelial matrices [28]. The ability of fibulin-1 to bind to ECM proteins such as fibrinoneectin [25] and elastin [29] may provide a means for fibrinogen to bind to subendothelial ECM and contribute to the formation of thrombus. These complications could become a serious health problem by increasing the prevalence of non-communicable diseases in South Africa. Although South Africa is the country with the highest HIV infection rate in the world, literature (especially longitudinal data concerning the HIV-infected black South Africans and the influence on CVD) is lacking to a great extent.
1.2.12 Questions arising from the literature

- Will fibulin-1 be higher in HIV-infected South Africans in comparison with HIV-uninfected South Africans?
- Will fibulin-1 be associated with markers of vascular dysfunction such as blood pressure, sICAM-1, sVCAM-1, IL-6, PAI-1, PWV and PP in HIV-infected individuals?

1.2.13 Outline of the study

![Diagram](image)

Figure 3: The outline of the PURE study’s substudy

This substudy is embedded in the larger international Prospective Urban and Rural Epidemiology (PURE) study. The overarching PURE study is a prospective study that addresses questions regarding the cause and development of cardiovascular risk factors and disease within populations, particularly of low- and middle-income countries, including South Africa. The South African leg of the PURE study was performed in the North-West Province, where a total of 2 010 participants (1 004 urban and 1 006 rural) were randomly recruited from a rural and urban setting and screened during the baseline phase in 2005.
The inclusion criteria were volunteers over the age of 35 years with no self-reported diseases.

For this substudy, the 300 newly identified HIV-infected participants (men and women) of the baseline PURE study population were individually matched with 300 HIV-uninfected participants (case-control design) according to age, gender, body mass index (BMI) and locality (urban and rural). After three years, 294 participants (N=154 HIV-uninfected and N=140 HIV-infected) were followed up. The test participants and control group were studied simultaneously. Of the HIV-uninfected group, 146 participants were lost to follow-up and in the HIV-infected group, 160 participants were lost to follow-up. The loss of participants could possibly be due to deaths or relocation. In fig. 3, an outline is given of the substudy of PURE in which HIV participants were employed.

1.2.14 Objectives of the study

Firstly, the objectives of this study were to determine whether fibulin-1 levels are elevated in HIV-infected South Africans in comparison with HIV-uninfected South Africans at baseline and after three years. Secondly, the objectives were to determine whether fibulin-1 is associated with markers of vascular function in the HIV-infected participants.

1.2.15 Hypotheses

The proposed hypotheses are the following:

- Fibulin-1 will be higher in HIV-infected South Africans in comparison with HIV-uninfected South Africans at baseline and after three years.
- Fibulin-1 is associated with markers of vascular function such as blood pressure, sICAM-1, sVCAM-1, IL-6, PAI-1 PWV and PP in HIV-infected individuals.
1.2.16 References


30. UNAIDS Sub-Saharan Africa. Aids epidemic update: Regional Summary.


60. Sackner-Bernstein JD: **The myocardial matrix and the development and progression of ventricular remodeling.** *Curr Cardiol Rep* 2000, **2**:112-119.

61. Hutchinson KR, Stewart JA, Lucchesi PA: **Extracellular matrix remodeling during the progression of volume overload-induced heart failure.** *J Mol Cell Cardiol* 2010, **48**:564-569.


66. Tanno T, Yoshinaga K, Sato T: **Alteration of elastin in aorta from diabetics.** *Atherosclerosis* 1993, **101**:129-134.


105. Plesner T, Behrendt N, Ploug M: Structure, function and expression on blood and bone marrow cells of the urokinase-type plasminogen activator receptor, uPAR. *Stem Cells 1997, 15:398-408.*


between low grade inflammation and arterial stiffness in patients with essential hypertension. *J Hypertens* 2006, **24**:2231-2238.


140. Schneider DJ: *Diabetes, PAI-1 and atherogenesis*. 


151. Hinderliter AL, Blumenthal JA, Waugh R, Chilukuri M, Sherwood A: Ethnic differences in left ventricular structure: relations to hemodynamics and diurnal blood


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    - References with correct punctuation should be styled as follows for journals: Koonin EV, Altschul SF, Bork P. **BRCA1 protein products: functional motifs. Nat Genet** 1996, **13**:266-267.
Fibulin-1 as a marker of cardiovascular disease in HIV-infected black South Africans: a prospective study

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Abstract

Background: Human immunodeficiency virus (HIV) infection causes functional changes to the endothelium that resemble inflammation, which may lead to endothelial dysfunction. The high Soluble urokinase plasminogen activator receptor (suPAR) and low high density lipoprotein cholesterol (HDL-C) levels suggest the possibility that the HIV-infected participants are more prone to develop vascular damage. This may result in further extracellular matrix remodelling, which is seen as the link between infection, inflammation and vascular dysfunction. Changes in fibulin-1 levels have consequences to vascular structural integrity; maintaining the integrity of the extracellular matrix in the blood vessel wall seems critical in the prevention of cardiovascular disease.

Methods: The 300 newly diagnosed HIV-infected participants were compared to 300 uninfected controls of the same age, gender, body mass index and locality. After three years, 294 participants (N=154 HIV-uninfected and N=140 HIV-infected) were followed up. Comparisons were made and associations determined by independent t-tests and analysis of covariance. Dependent t-tests were used to compare baseline and follow-up measurements of cardiovascular variables within HIV-infected and HIV-uninfected participants. The percentage change was determined in all the groups over a period of three years. Fasting lipids, serum glucose and C-reactive protein were determined with the Konelab in 2005 and the Beckman Coulter in 2008. Low-density lipoprotein-cholesterol (LDL-C) was calculated by the Friedewald formula. Fibulin-1 concentration was determined with sandwich immunoassay.

Results: The HIV-infected participants exhibited higher fibulin-1 levels, as well as high Intracellular adhesion molecules-1 (sICAM-1), Vascular adhesion molecules-1 (sVCAM-1) and suPAR levels and low HDL-C levels in comparison with their uninfected control group. No significant associations were found between fibulin-1 and markers of vascular function, namely sICAM-1, sVCAM-1, IL-6, fibrinogen, suPAR, pulse pressure or pulse wave velocity. Percentage change in fibulin-1 is associated with percentage change triglyceride/high-
density lipoprotein-cholesterol ratio (TG/HDL-C) in HIV-infected participants and in the entire group. No associations were found between percentage change fibulin-1 TG/HDL-C ratio in the HIV-uninfected group.

**Conclusions:** Fibulin-1 levels were significantly higher in HIV-infected participants in comparison with HIV-uninfected participants at baseline and after three years. The association of the percentage change in fibulin-1 and TG/HDL-C ratio suggested that vascular changes were present in HIV-infected participants and that they might be more at risk to develop cardiovascular disease, opposed to the HIV-uninfected participants.

**Keywords:** Fibulin-1, HIV, vascular dysfunction, inflammation, CVD
Background

Fibulin-1 is a fibrinogen-binding plasma protein and is part of a small group of extracellular matrix (ECM) proteins, including von Willebrand factor and fibronectin that are present in the blood at relatively high levels [1]. The protein fibulin-1 has been found in structures such as connective tissue fibres and basement membrane [2,3]; it maintains the structural integrity against mechanical strain over a lifetime [3]. Changes in Fibulin-1 is associated with vascular damage and inflammation [3], which could lead to vascular dysfunction and has not been studied in HIV-infected individuals in the North-West Province of South Africa.

The ECM is a highly adaptive and dynamic structure that is influenced by mechanical stress, inflammation and oxidative stress [4]. It has been suggested that changes in the ECM could contribute to arterial stiffness, which could lead to CVD [5]. Changes in fibulin-1 levels have consequences to vascular structural integrity and maintaining the integrity of the ECM in the blood vessel wall, and may be critical in preventing cardiovascular disease [6].

The HIV infection rate is high in South Africa. HIV infection may lead to vascular damage and vascular inflammation [7]; it may also increase the risk of CVD [8] and is associated with endothelial dysfunction [9], accelerated atherosclerosis [10] and coagulatory disorders [11]. In HIV infection, the endothelium is under the combined influence of a viral load that activates or injures the endothelium [12], causing functional changes to the endothelium [12] that resemble inflammation [13] and this may lead to endothelial dysfunction. Increased levels of CRP, IL-6 [14] and increased CAM (ICAM-1 and VCAM-1) [7,9,14,15] have been reported in the HIV-infected population [8].

Although South Africa is the country with the highest number of people living with HIV infection in the world, literature concerning the HIV-infected black South Africans and the influence of fibulin-1 on ECM is non-existent. The high suPAR levels and low HDL-C levels suggest the possibility that the HIV-infected participants are more prone to develop vascular
damage, which may result in further ECM remodelling; this is seen as the link between infection, inflammation, thrombotic activity and vascular dysfunction [1]. Therefore, the aim of this study was firstly to determine the fibulin-1 levels in HIV-infected South Africans in comparison with HIV-uninfected South Africans at baseline and after three years. Secondly, the aim was to determine the association between fibulin-1 and markers of cardiovascular dysfunction and percentage change of variables over a period of three years, and to determine associations between the cardiovascular variables and fibulin-1 within HIV-infected and HIV-uninfected participants.

Methods

Study design and participants

This substudy is embedded in the larger international PURE study. The overarching PURE study is a prospective study that will track changes in risk factors and chronic diseases by using periodic, standardised data collection in urban and rural areas of 17 developing countries in transition, including South Africa. The South African leg of the PURE study was performed in the North-West Province, where a total of 2 010 participants (1 004 urban and 1 006 rural) were randomly recruited from a rural and urban setting and screened during the baseline phase in 2005. For this substudy, the 300 newly identified HIV-infected participants (men and women) of the baseline PURE study population were individually matched with 300 HIV-uninfected participants (case-control design) according to age, gender, BMI and locality (urban and rural). After three years, 294 participants (N=154 HIV-uninfected and N=140 HIV-infected) were followed up. The test participants and control group were studied simultaneously. Of the HIV-uninfected group, 146 participants were lost to follow-up and in the HIV-infected group, 160 participants were lost to follow-up. The loss of participants could possibly be due to deaths or relocation.
Ethical considerations

After all procedures had been explained to them in their home language, each participant gave written, informed permission to participate in the study. This study was approved by the Ethics Review Board of the North-West University and the study protocol conformed to the ethical guidelines of the Declaration of Helsinki (2008) for investigation of human participants.

Experimental protocol

For the data collection, participants arrived each morning at the research locality of the rural or urban areas at 08:00. They were introduced to the setup and after the procedures had been explained, they signed the informed consent forms and received HIV pre-counselling that was given by trained counsellors. The HIV-infected participants were referred to the local clinic or hospital if necessary. Feedback on the HIV status and cardiovascular variables of the participants were also given during individual post-counselling. In the course of the morning, demographic and lifestyle questionnaires were completed with the help of the specially trained field workers in each subject’s home language. Lifestyle data included self-reported current tobacco and alcohol use, as well as medical history.

Anthropometric measurements

Waist circumference, height and weight were measured by using standardised procedures. Body height, weight, hip and waist circumference were measured (Precision Health Scale, A & O Company, Japan; Invicta Stadiometer, IP 1465, UK; Holtain unstretchable metal tape).

Cardiovascular measurements

The OMRON HEM-757 (Omron, Kyoto, Japan) apparatus, which is a British Hypertension Society/Advancement of Medical Instrumentation (BHS/AAMI) -validated apparatus, was used to determine heart rate, systolic blood pressure (SBP) and diastolic blood pressure
(DBP) with the cuff on the left upper arm in the sitting position. Suitable cuffs were used for obese participants.

PP was subsequently calculated by the difference in SBP and DBP. The cr-PWV was measured with the Complior SP Acquisition system (Artech Medical, Pantin, France) on the left side of each participant in the supine position. An increase in cr-PWV gives an indication of arterial stiffness and the development of atherosclerosis.

Biochemical analyses

All participants were asked to fast for a minimum time of eight hours. For the baseline study, fasting lipids (total cholesterol, HDL-C and TG), serum glucose and serum hs-CRP were determined with the Konelab autoanalyser (Thermo Fisher Scientific Oy, Vantaa, Finland); for the study in 2008, the above mentioned were determined with the Beckman Coulter DxC 800 Synchron ® Clinical System (Beckman Coulter Inc., CA). Serum concentrations of hs-IL-6 were measured by using human ELISAs (Quantikine® HS ELISA, R&D Systems, Minneapolis, USA).

The suPAR levels were measured by using the suPARnostic ® ELISA kit (ViroGates, Copenhagen, Denmark). Concentrations of sICAM-1 and sVCAM-1 were assessed by sandwich ELISAs (human sICAM-1 and human sVCAM-1 assay, IBL, Hamburg, Germany); sICAM-1 and sVCAM-1 were only determined in 2005. LDL-C was calculated by using the Friedewald formula \[ \text{LDL} = \text{TC} - \text{HDL} - (0.45 \times \text{TG}) \].

Fibulin-1 was determined by using a two-antibody sandwich ELISA immunoassay [3]. Microtiter cells were coated overnight with 3/tg/mi mouse anti-fibulin monoclonal 5D12/H7 IgG in 0.1 M sodium carbonate buffer with a pH of 9.5. The chromogenic substrate p-nitrophenyl phosphate (Sigma Chemical Co.) was used to measure cell-bound enzymatic activity. The concentration of the fibulin-1 standard was determined by the Bradford assay.
The quantitative determination of fibrinogen in plasma was performed by the Multifibren U-test (Dade Behring), a modification of the Clauss method on the Dade Behring BCS coagulation analyser. The quantification of PAI-1 activity was performed by a chromogenic assay kit, Spectrolyse®/pL PAI-1 (Trinity Biotech plc, Bray Co, Ireland).

The HIV status was determined with rapid tests directly after blood sampling in accordance with the protocol of the National Department of Health of South Africa. Serum was used for testing with the First Response test and was repeated with the Pareeshak card test to confirm the results.

Statistical analyses

Statistica software v10.0 (StatSoft, Inc., Tulsa, OK, USA) was used for statistical analysis. Normal distribution of the variables was tested prior to any statistical analysis. Variables that did not fulfill these criteria (hs-CRP, HDL-C, TG, hs-IL6, sICAM-1, sVCAM-1, PAI-1 and fibrinogen) were normalised by logarithmic transformation before analysis, reporting the geometric mean and the 5th and 95th percentile intervals. Independent t-tests were performed to compare means between HIV-infected and HIV-uninfected Africans in the baseline study. Chi-square tests were used to compare proportions. An ANCOVA was performed to compare the characteristics of the continuous variables of the HIV-infected and HIV-uninfected groups whilst adjusting for age, BMI and gender. Additionally, cr-PWV was adjusted for mean arterial pressure. Dependent t-tests were used to compare the baseline and follow-up measurements of cardiovascular variables within HIV-infected and HIV-uninfected participants. The percentage change in all the groups was also determined over a period of three years. Pearson and partial correlations were performed to explore adjusted and unadjusted associations between change of fibulin-1 and cardiovascular variables in each group. P-values of ≤0.05 were considered statistically significant.
Results

The characteristics of the HIV-infected and HIV-uninfected participants in the baseline study (2005) are compared in Table 1. The HIV-infected and HIV-uninfected participants did not differ with respect to age, BMI or WC. The fibulin-1 levels were significantly higher in the HIV-infected group in comparison with the HIV-uninfected group (83.86 µg/ml vs. 73.20 µg/ml; p = 0.003). The PP (p = 0.02), total cholesterol (TC) (p < 0.001), fibrinogen (p = 0.004), HDL-C (p < 0.001), glucose (p = 0.05) and TG (p < 0.001) were all significantly lower in the HIV-infected participants. The SBP of the HIV-infected participants was borderline significantly lower (p = 0.07) in comparison with the HIV-uninfected. The cr-PWV (p = 0.03), TC/HDL-C ratio (p < 0.001), TG/HDL-C ratio (p < 0.001), sICAM-1 (p = 0.005), sVCAM-1 (p < 0.001) and suPAR (p = 0.01) were significantly higher in the HIV-infected group. There were more tobacco users in the HIV-infected group in comparison with the HIV-uninfected group (45.7% vs. 40.26%, p<0.001). The self-reported use of alcohol was higher in HIV-uninfected groups in comparison with HIV-infected groups (29.22% vs. 26.43%, p<0.001).

Table 2 displays the adjusted differences between the HIV-uninfected and HIV-infected participants in the baseline study of 2005 and the follow-up in 2008. Adjustments were made for age, gender and BMI. Additionally, cr-PWV was also adjusted for mean arterial pressure. The cholesterol was significantly higher in the HIV-uninfected group in 2005 (p < 0.001) and in 2008 (p = 0.04). In the HIV-infected group, the TG levels (0.82, p < 0.001) were lower in the baseline study, but higher (1.05, p = 0.03) in the follow-up study. At baseline (2005), the higher levels of fibulin-1 (p = 0.002), sICAM-1 (p = 0.004), sVCAM-1 (p < 0.001), suPAR (p = 0.01) and fibrinogen (p = 0.003) in the infected group remained significant. Data is not shown in Table 2. Fibulin-1 and suPAR levels differed in 2008. In the HIV-infected group, the fibulin-1 levels (80.79 p=0.04) were lower in the follow-up study, but higher (83.71 p=0.002) in the baseline study. The suPAR levels (4.03 P=0.02) were lower in the baseline study, but higher (4.49 p=0.004) in the follow-up study. The significant higher
cr-PWV at baseline \( (p = 0.005) \) disappeared in the HIV-infected group and no differences were found during the follow-up study \( (p = 0.51) \). TC/HDL-C ratio and TG/HDL-C ratio levels were all higher in the HIV-infected participants in comparison with the HIV-uninfected participants in the baseline and follow-up study.

Pearson and partial correlations were used to explore adjusted and unadjusted associations, but no significant associations were found between fibulin-1 and markers of vascular function, namely sICAM-1, sVCAM-1, PP, IL-6, PAI-1 or PWV. Dependent t-tests were used to compare the baseline and follow-up measurements of cardiovascular variables within HIV-infected and HIV-uninfected participants, but no significant differences were found. The researchers, therefore, proceeded to calculate the percentage change within all groups over a period of three years \( (2005-2008) \).

In Table 3, percentage change in the cardiometabolic profile of HIV-infected and HIV-uninfected participants over a period of three years is shown. There were no significant differences in percentage change in fibulin-1, TC/HDL-C ratio, TG/HDL-C ratio, cr-PWV and PP between the HIV-infected and HIV-uninfected participants over the period of three years.

Table 4 shows unadjusted and adjusted correlations of the changes in cardiometabolic variables with percentage change in fibulin-1 within all the groups over a period of three years. In the single regression analyses, a significant positive correlation exists between percentage change in fibulin-1 and TG/HDL-C ratio in the entire group \( (r = 0.19, p = 0.001) \) and a borderline positive correlation in the HIV-infected participants \( (r = 0.24, p = 0.006) \). After the adjustments had been made for age, BMI and gender, the association between change in fibulin-1 and TG/HDL-C ratio in the HIV-infected participants \( (r = 0.200, p = 0.009) \) remained significant. A significant positive correlation \( (r = 0.17, p = 0.04) \) was seen between percentage change in fibulin-1 and suPAR in the HIV-uninfected group, but no positive correlation was found with suPAR in the unadjusted correlations.
Table 1: Characteristics of the HIV-infected and HIV-uninfected African participants in the baseline study

<table>
<thead>
<tr>
<th>Variables</th>
<th>HIV uninfected ((N = 154)) 2005</th>
<th>HIV infected ((N = 140)) 2005</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>45.29 ± 8.26</td>
<td>44.31 ± 7.01</td>
<td>0.28</td>
</tr>
<tr>
<td>BMI ((\text{kg/m}^2))</td>
<td>23.46 ± 5.75</td>
<td>22.86 ± 5.68</td>
<td>0.37</td>
</tr>
<tr>
<td>WC (cm)</td>
<td>76.69 ± 9.94</td>
<td>75.17 ± 10.81</td>
<td>0.31</td>
</tr>
<tr>
<td><strong>Cardiovascular measurements</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>129.90 ± 22.99</td>
<td>125.34 ± 20.33</td>
<td>0.07</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>86.33 ± 14.72</td>
<td>85.12 ± 13.62</td>
<td>0.47</td>
</tr>
<tr>
<td>PP (mmHg)</td>
<td>44.01 ± 14.31</td>
<td>40.36 ± 11.37</td>
<td>0.02</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>101 ± 16.76</td>
<td>98.58 ± 15.44</td>
<td>0.21</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>73.07 ± 14.60</td>
<td>75.36 ± 13.94</td>
<td>0.18</td>
</tr>
<tr>
<td>cr-PWV (m/s)</td>
<td>10.68 ± 2.38</td>
<td>11.26 ± 2.17</td>
<td>0.03</td>
</tr>
<tr>
<td><strong>Biochemical analyses</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fibulin-1 ((\mu\text{g/m}\text{f}))</td>
<td>73.20±25.16</td>
<td>83.86±31.79</td>
<td>0.003</td>
</tr>
<tr>
<td>sICAM-1 ((\text{ng/mol}))</td>
<td>405.91 (98.41-1344.78)</td>
<td>524.54 (180.35-1573.15)</td>
<td>0.005</td>
</tr>
<tr>
<td>sVCAM-1 ((\text{ng/mol}))</td>
<td>379.09 (18.91-2245.82)</td>
<td>753.88 (55.29-2990.76)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>suPAR ((\text{ng/ml}))</td>
<td>3.61 ± 1.28</td>
<td>4.04 ± 1.60</td>
<td>0.01</td>
</tr>
<tr>
<td>CRP ((\text{mg/l}))</td>
<td>2.22 (0.24-30.30)</td>
<td>2.67 (0.31-40.91)</td>
<td>0.29</td>
</tr>
<tr>
<td>IL-6 ((\text{pg/ml}))</td>
<td>3.97 (1.15-20.35)</td>
<td>4.10 (1.23-2.09)</td>
<td>0.72</td>
</tr>
<tr>
<td>Fibrinogen ((\text{g/l}))</td>
<td>3.16 (1.40-1.74)</td>
<td>2.63 (1.30-7.00)</td>
<td>0.004</td>
</tr>
<tr>
<td>PAI-1 ((\text{IU/ml}))</td>
<td>1.22 ± 16.14</td>
<td>1.56 ± 13.18</td>
<td>0.44</td>
</tr>
<tr>
<td>Chol ((\text{mmol/l}))</td>
<td>5.07 ± 1.37</td>
<td>4.53 ± 1.28</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TG ((\text{mmol/l}))</td>
<td>1.08 (0.53-2.40)</td>
<td>0.82 (0.35-1.78)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LDL-C ((\text{mmol/l}))</td>
<td>2.82 ± 1.22</td>
<td>2.67 ± 1.01</td>
<td>0.26</td>
</tr>
<tr>
<td>HDL-C ((\text{mmol/l}))</td>
<td>1.59 (0.82-3.19)</td>
<td>1.17 (0.56-2.30)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Glucose ((\text{mmol/l}))</td>
<td>5.59 ± 1.02</td>
<td>5.21 ± 1.21</td>
<td>0.05</td>
</tr>
<tr>
<td>TC/HDL-C ratio</td>
<td>3.67 ± 1.43</td>
<td>3.97 ± 1.53</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TG/HDL-C ratio</td>
<td>0.90 ± 1.37</td>
<td>1.24 ± 1.11</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Lifestyle</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tobacco users N (%)</td>
<td>62 (40.26)</td>
<td>64 (45.71)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Alcohol users N (%)</td>
<td>45 (29.22)</td>
<td>37 (26.43)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Data are expressed as arithmetic mean ± standard deviation, geometric mean (5th-95th percentiles) or percentage of N. N: number of participants; BMI: body mass index; WC: waist circumference; SBP: systolic blood pressure; DBP: diastolic blood pressure; PP: pulse pressure; MAP: mean arterial pressure; HR: heart rate; cr-PWV: carotid-radialis pulse wave velocity; TG: triglyceride; LDL-C: low-density lipoprotein cholesterol; HDL-C: high-density lipoprotein cholesterol; TC/HDL-C ratio: total cholesterol/high-density lipoprotein cholesterol ratio; TG/HDL-C ratio: triglyceride/high-density lipoprotein cholesterol ratio; CRP: C-reactive protein; IL-6: interleukin-6; sICAM-1: serum intercellular adhesion molecule-1; sVCAM-1: serum vascular cell adhesion molecule-1; PAI-1: plasminogen activator inhibitor-1; suPAR: soluble urokinase plasminogen activator receptor. All P-values were obtained with the independent t-test, except for tobacco and alcohol users, where the Chi-square test was used. P-values of ≤0.05 are regarded as significant.
Table 2: Adjusted analysis (ANCOVA) in HIV-infected and HIV-uninfected participants in the baseline and follow-up study

<table>
<thead>
<tr>
<th>Variables</th>
<th>HIV uninfected 2005 (N = 154)</th>
<th>HIV infected 2005 (N = 140)</th>
<th>P-value</th>
<th>HIV uninfected 2008 (N = 154)</th>
<th>HIV infected 2008 (N = 140)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>WC (cm)</td>
<td>76.09 ± 0.39</td>
<td>75.78 ± 0.41</td>
<td>0.59</td>
<td>77.61 ± 0.56</td>
<td>76.66 ± 0.59</td>
<td>0.24</td>
</tr>
<tr>
<td>Cardiovascular</td>
<td></td>
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<tr>
<td>measurements</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>129.42 ± 1.71</td>
<td>125.82 ± 1.79</td>
<td>0.15</td>
<td>133.54 ± 1.55</td>
<td>129.96 ± 1.62</td>
<td>0.11</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>86.12 ± 1.14</td>
<td>85.32 ± 1.19</td>
<td>0.63</td>
<td>88.38 ± 0.98</td>
<td>87.04 ± 1.03</td>
<td>0.35</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>100.71 ± 1.28</td>
<td>98.87 ± 1.34</td>
<td>0.33</td>
<td>103.43 ± 1.12</td>
<td>101.35 ± 1.16</td>
<td>0.2</td>
</tr>
<tr>
<td>PP (mmHg)</td>
<td>43.75 ± 1.00</td>
<td>40.63 ± 1.05</td>
<td>0.03</td>
<td>45.17 ± 0.97</td>
<td>42.92 ± 1.01</td>
<td>0.11</td>
</tr>
<tr>
<td>cr-PWV (m/s)</td>
<td>10.60 ± 0.17</td>
<td>11.28 ± 0.17</td>
<td>0.005</td>
<td>11.21 ± 0.17</td>
<td>11.38 ± 0.18</td>
<td>0.51</td>
</tr>
<tr>
<td>Biochemical analyses</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fibulin-1 (µg/ml) ^1</td>
<td>73.36 ± 2.25</td>
<td>83.71 ± 2.37</td>
<td>0.002</td>
<td>74.43 ± 2.16</td>
<td>80.79 ± 2.23</td>
<td>0.04</td>
</tr>
<tr>
<td>suPAR (ng/ml)</td>
<td>3.62 ± 0.12</td>
<td>4.03 ± 0.12</td>
<td>0.02</td>
<td>3.71 ± 0.19</td>
<td>4.49 ± 0.19</td>
<td>0.004</td>
</tr>
<tr>
<td>CRP (mg/l)</td>
<td>2.18 (1.73 - 2.74)</td>
<td>2.72 (2.13 - 3.45)</td>
<td>0.2</td>
<td>2.60 (2.09 - 3.21)</td>
<td>3.08 (2.46 - 3.84)</td>
<td>0.28</td>
</tr>
<tr>
<td>Chol (mmol/l)</td>
<td>5.04 ± 0.11</td>
<td>4.53 ± 0.11</td>
<td>&lt;0.001</td>
<td>4.23 ± 0.09</td>
<td>3.94 ± 0.10</td>
<td>0.04</td>
</tr>
<tr>
<td>TG (mmol/l)</td>
<td>1.08 (1.00 - 1.16)</td>
<td>0.82 (0.76 - 0.89)</td>
<td>&lt;0.001</td>
<td>0.92 (0.84 - 1.00)</td>
<td>1.05 (0.95 - 1.15)</td>
<td>0.03</td>
</tr>
<tr>
<td>LDL-C (mmol/l)</td>
<td>2.80 ± 0.09</td>
<td>2.69 ± 0.09</td>
<td>0.38</td>
<td>2.53 ± 0.07</td>
<td>2.51 ± 0.08</td>
<td>0.87</td>
</tr>
<tr>
<td>HDL-C (mmol/l)</td>
<td>1.58 (1.48 - 1.69)</td>
<td>1.16 (1.09 - 1.25)</td>
<td>&lt;0.001</td>
<td>1.08 (1.00 - 1.16)</td>
<td>0.82 (0.76 - 0.89)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Glucose (mmol/l)</td>
<td>5.55 ± 0.09</td>
<td>5.31 ± 0.09</td>
<td>0.06</td>
<td>4.59 ± 0.07</td>
<td>4.58 ± 0.08</td>
<td>0.96</td>
</tr>
<tr>
<td>TC/HDL-C ratio</td>
<td>3.35 ± 0.12</td>
<td>3.99 ± 0.12</td>
<td>&lt;0.001</td>
<td>4.15 ± 0.19</td>
<td>4.92 ± 0.20</td>
<td>0.005</td>
</tr>
<tr>
<td>TG/HDL-C ratio</td>
<td>0.88 ± 0.10</td>
<td>1.25 ± 0.11</td>
<td>0.01</td>
<td>1.16 ± 0.12</td>
<td>1.68 ± 0.13</td>
<td>0.003</td>
</tr>
</tbody>
</table>

Data are expressed as arithmetic mean ± standard deviation, geometric mean (95% confidence interval) or percentage of N. N: number of participants; SBP: systolic blood pressure; DBP: diastolic blood pressure; PP: pulse pressure; TC/HDL-C ratio: total cholesterol/high-density lipoprotein cholesterol ratio; TG/HDL-C ratio: triglyceride/high-density lipoprotein cholesterol ratio; HDL-C: high-density lipoprotein cholesterol; LDL: low-density lipoprotein cholesterol; CRP: C-reactive protein; IL-6: interleukin-6; suPAR: soluble urokinase plasminogen activator receptor. All P-values were obtained with ANCOVA, except for tobacco and alcohol users, where the Chi-square test was used. Adjustments were made for age, gender and body mass index. Additionally, PWV was also adjusted for mean arterial pressure and heart rate. P-values of ≤0.05 are regarded as significant.
Table 3: Percentage change of variables within the HIV-infected and HIV-uninfected groups over a period of three years (2005-2008)

<table>
<thead>
<tr>
<th>Variables</th>
<th>HIV uninfected</th>
<th>HIV infected</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(N = 154)</td>
<td>(N = 140)</td>
<td></td>
</tr>
<tr>
<td><strong>Cardiovascular measures</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% change SBP</td>
<td>5.41 (3.37- 7.45)</td>
<td>3.61 (1.47- 5.75)</td>
<td>0.23</td>
</tr>
<tr>
<td>% change DBP</td>
<td>4.46 (2.52- 6.40)</td>
<td>2.94 (0.91- 4.98)</td>
<td>0.29</td>
</tr>
<tr>
<td>% change PP</td>
<td>12.48 (7.67- 17.28)</td>
<td>7.06 (2.02- 12.10)</td>
<td>0.13</td>
</tr>
<tr>
<td>% change MAP</td>
<td>4.57 (2.73- 6.41)</td>
<td>3.07 (1.14- 5.00)</td>
<td>0.27</td>
</tr>
<tr>
<td>% change cr-PWV</td>
<td>7.88 (3.50- 12.26)</td>
<td>5.71 (1.10- 10.32)</td>
<td>0.50</td>
</tr>
<tr>
<td><strong>Biochemical analyses</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% change TC/HDL-C ratio</td>
<td>25.54 (16.18- 34.90)</td>
<td>34.59 (24.87- 44.31)</td>
<td>0.19</td>
</tr>
<tr>
<td>% change TG/HDL-C ratio</td>
<td>49.33 (31.25- 67.41)</td>
<td>67.32 (48.65- 85.98)</td>
<td>0.18</td>
</tr>
<tr>
<td>% change glucose</td>
<td>-12.08 (-15.47- -8.69)</td>
<td>-11.72 (-15.25- -8.20)</td>
<td>0.89</td>
</tr>
<tr>
<td>% change fibulin-1</td>
<td>-4.73 (-9.76- 0.29)</td>
<td>-5.04 (-10.25- 0.18)</td>
<td>0.94</td>
</tr>
</tbody>
</table>

Data are expressed as percentage change in mean (5th-95th percentiles). N: number of participants; SBP: systolic blood pressure; DBP: diastolic blood pressure; PP: pulse pressure; MAP: mean arterial pressure; cr-PWV: carotid-radialis pulse wave velocity; TC/HDL-C ratio: total cholesterol/high-density lipoprotein cholesterol ratio; TG/HDL-C ratio: triglyceride/high-density lipoprotein cholesterol ratio. All P-values were obtained with ANCOVA, adjusted for pre-values. P-values of ≤0.05 are regarded as significant.
Table 4: Unadjusted and adjusted correlations of the percentage change of variables within the entire group of HIV-infected and HIV-uninfected participants over a period of three years (2005-2008)

<table>
<thead>
<tr>
<th>Variables</th>
<th>Entire group (N = 294)</th>
<th>HIV uninfected (N = 154)</th>
<th>HIV infected (N = 140)</th>
</tr>
</thead>
<tbody>
<tr>
<td>% change fibulin-1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% change PP</td>
<td>r= -0.03 P= 0.58</td>
<td>r= -0.04 P=0.64</td>
<td>r= -0.04 P= 0.69</td>
</tr>
<tr>
<td>% change cr-PWV</td>
<td>r= -0.06 P= 0.31</td>
<td>r= -0.06 P= 0.51</td>
<td>r= -0.11 P= 0.22</td>
</tr>
<tr>
<td>% change TC/HDL-C ratio</td>
<td>r = 0.10 P= 0.10</td>
<td>r = 0.12 P= 0.14</td>
<td>r = 0.16 P= 0.06</td>
</tr>
<tr>
<td>% change TG/HDL-C ratio</td>
<td>r = <strong>0.19 P= 0.001</strong></td>
<td>r = 0.15 P= 0.08</td>
<td>r= <strong>0.24 P= 0.006</strong></td>
</tr>
<tr>
<td>% change suPAR</td>
<td>r= 0.11 P= 0.08</td>
<td>r= 0.15 P= 0.08</td>
<td>r= 0.13 P= 0.14</td>
</tr>
</tbody>
</table>

Adjustments: age, body mass index and gender. Additionally, cr-PWV was also adjusted for mean arterial pressure and heart rate.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Unadjusted correlations</th>
</tr>
</thead>
<tbody>
<tr>
<td>% change PP</td>
<td>r= -0.03 P= 0.57</td>
</tr>
<tr>
<td>% change cr-PWV</td>
<td>r= -0.06 P= 0.35</td>
</tr>
<tr>
<td>% change TC/HDL-C ratio</td>
<td>r = 0.010 P= 0.11</td>
</tr>
<tr>
<td>% change TG/HDL-C ratio</td>
<td>r = <strong>0.20 P= 0.001</strong></td>
</tr>
<tr>
<td>% change suPAR</td>
<td>r= 0.11 P= 0.06</td>
</tr>
</tbody>
</table>

N: number of participants; PP: pulse pressure; cr-PWV: carotid-radialis pulse wave velocity; TC/HDL: total cholesterol/high-density lipoprotein cholesterol; TG/HDL-C: triglyceride/high-density lipoprotein cholesterol; suPAR: soluble urokinase plasminogen activator receptor. Significant (p < 0.05) correlation coefficients are indicated in bold. P-values of ≤0.05 are regarded as significant.
Discussion

The aim of this study was firstly to determine the fibulin-1 levels in HIV-infected and HIV-uninfected South Africans at baseline (2005) and after three years. The fibulin-1 levels were found to be significantly higher in the HIV-infected participants in comparison with the HIV-uninfected participants. Percentage change in fibulin-1 levels is associated with percentage change TG/HDL-C ratio in HIV-infected participants and in the entire group. However, no associations were found between percentage change fibulin-1 and TG/HDL-C ratio in the HIV-uninfected group.

At baseline, the cr-PWV was significantly higher in the HIV-infected group (11.26 m/s$^1$ vs. 10.68 m/s$^1$, $p = 0.03$) and the PP was significantly lower in the HIV-infected group (40.36 mmHg vs. 44.01 mmHg, $p = 0.02$) in comparison with the HIV-uninfected group. However, in the follow-up study, no significant difference in the cr-PWV or PP values between the HIV-infected and HIV-uninfected participants were found, suggesting that no vascular deterioration was detected.

The ICAM and VCAM levels were significantly increased in HIV-infected participants, but were only measured in 2005. Increased levels of cell adhesion molecules have been reported in the HIV-infected population [7,15,19], indicating endothelial injury. It was found that HIV infection induced the expression of the adhesion molecules and this could be a possible mechanism by which HIV infection contributes to vascular dysfunction [11,12].

Vascular remodelling involves degradation and reorganisation of the ECM scaffold, contributing to thickened vessel wall and vascular stiffness [20,21]. Following vascular injury and inflammation, fibulin-1 presents in the ECM of the vessel wall, interacts with plasma fibrinogen and promotes platelet adhesion, leading to the formation of a platelet plug [10].
Because of its role in platelet aggregation and fibrin formation [35], the occurrence of high plasma levels of fibrinogen is seen as a risk factor for CVD [31]. Godyna et al. found that fibulin-1 can bind to fibrinogen and can be incorporated into fibrin clots, leading to vascular dysfunction [37]. Studies which investigated the prothrombotic state in HIV-infected populations showed increased levels of fibrinogen [10,41,42]. However, fibrinogen, the coagulation and inflammation marker, was significantly lower in the HIV-infected participants at baseline, which indicates no sign of a prothrombotic state. These findings are in agreement with the findings of James et al., who found that HIV infection was not associated with fibrinogen concentration in Africans [43]. The short duration of the HIV infection might explain the lack of an increased coagulation that was found in the HIV-infected participants.

Associations between fibulin-1 and the cardiovascular variables were expected by the researchers, but the result of the study is not consistent with the findings from the literature. Claudia Cangemi et al. found correlations between plasma fibulin-1 and markers of arterial stiffness, namely the carotid compliance and PP [3]. They also found associations between plasma fibulin-1 concentrations and signs of myocardial dysfunction, but did not find any correlation between lipids and plasma fibulin-1 [3]. In contrast to the latter findings of Cangemi, no significant associations were found between fibulin-1 and markers of vascular function, namely sICAM-1, sVCAM-1, IL-6, fibrinogen, suPAR, PP or PWV. However, an association between percentage change of fibulin-1 and TG/HDL-C ratio were found, which suggests that vascular changes are present in HIV-infected participants and that they might be more at risk to develop cardiovascular disease, opposed to the HIV-uninfected participants, as the TG/HDL-C ratio is a good marker of cardiovascular risk [45].

It is well known that the lipid profile of HIV-infected people changes, and that these changes include increased levels of TG and decreased levels of HDL-C [7,23-26]. These factors can possibly be one of the initiating factors in the formation of arteriosclerosis, which ultimately leads to CVD [27,28]. Research shows that triglyceride-rich lipoproteins produce an
increased movement into the intima, leading to the formation of fatty streaks. These streaks ultimately lead to arteriosclerotic lesions, which then lead to vascular dysfunction [27-30]. Change in fibulin-1 might influence the structural and functional properties of the ECM in atherosclerotic lesions. Recent findings indicate that fibulin-1 acts as a cofactor for MMPs, whose expression is augmented in atherosclerotic lesions [34]. Argraves et al. reveal that there is prominent fibulin-1 deposition within atherosclerotic lesions and clots. These findings point to the possible roles for fibulin-1 in processes that are associated with the progression of thrombotic complications, which may lead to vascular dysfunction [1].

The vascular dysfunction that has been noticed in the HIV-infected participants in 2005 may possibly be linked to dyslipidemia, CAMs and suPAR levels. The high inflammatory markers, high suPAR levels and low HDL-C levels suggest the possibility that the HIV-infected participants are more prone to develop vascular damage. This may result in further ECM remodelling, which is seen as the link between infection, inflammation, thrombotic activity and vascular dysfunction [31,32]. At baseline study, TG levels were lower in HIV-infected participants in comparison with HIV-uninfected participants, but higher after three years. As observed in previous studies [33], the TG/HDL-C and TC/HDL-C ratio, which is closely linked to lipid disorders, was higher in the HIV-infected participants. At baseline and follow-up study, the P values remained significant for both of the ratios. An elevated suPAR level reflects an inflammatory and immune system activation, leading to infectious diseases [20] such as HIV infection [21,22]. Low-grade inflammation is a chronic subclinical inflammatory state which may contribute to the development of cardiovascular disease [23] through its association with atherosclerosis [23]. The immune and pro-inflammatory status of HIV-infected patients is reflected by the circulating suPAR levels [24]. The results showed an increase in suPAR levels after three years in the HIV-infected group, indicating inflammation [44].
To the researchers' knowledge, this is the first study that has investigated the associations between fibulin-1 and CVD in HIV-infected black South Africans. Although previous studies suggested that ECM plays a role in vascular dysfunction, leading to CVD [39,40], the results of this study is not consistent with the findings of the aforementioned study. No significant associations were found between fibulin-1 and markers of vascular function, namely sICAM-1, sVCAM-1, IL-6, PAI-1 or PWV. Significant associations with TG/HDL ratio were found in the entire group and in HIV-infected participants in the unadjusted and adjusted correlations, which was not seen in the uninfected group. This could not be confirmed with the forward stepwise regression analysis therefore the relationship between TG/HDL ratio and fibulin-1 is not independent of co-factors. This study adds to the idea that a link exists between HIV infection, fibulin-1 and the damaging effect of dyslipidemia on the endothelium.

A limitation of this study is that the duration of the HIV infection is unknown, because the participants were newly identified as being HIV infected in the baseline study. The IL-6, sICAM-1, sVCAM-1, fibrinogen and PAI-1 were only determined in the baseline study. The results did not infer causality. The participants commenced ARV treatment between 2005-2008, it is uncertain how long the duration of treatment was and therefore not adjusted for.

In conclusion, the HIV-infected participants exhibited higher fibulin-1 levels, as well as high sICAM-1, sVCAM-1 and suPAR levels, and low HDL-C levels in comparison with their uninfected control group. Although no significant associations were found between fibulin-1 and markers of vascular function, namely sICAM-1, sVCAM-1, IL-6, PAI-1 or PWV, the association of the percentage change in fibulin-1 with change in TG/HDL-C suggests that TG/HDL-C may contribute to probable vascular changes in HIV-infected participants and that the HIV-infected participants may be more at risk to develop cardiovascular disease in comparison with the HIV-uninfected participants.
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Declaration of conflict of interest

The authors declare that they have no conflict of interests.
References


3.1 Introduction
In Chapter 3, a summary of the main findings is given, with a brief discussion of the aim, results and conclusion of this substudy of the PURE study. Weaknesses in the study are depicted, with recommendations to negate the influence of such weaknesses in future with regard to fibulin-1 and the development of cardiovascular disease in HIV-infected black South Africans.

3.2 Summary of main findings
The aim of this study was to determine the association of fibulin-1 with variables of vascular function in HIV-infected black South Africans. The hypotheses that were proposed are the following:

- Fibulin-1 will be higher in HIV-infected South Africans compared to HIV-uninfected South Africans at baseline and after three years.
- Fibulin-1 is associated with markers of vascular function such as blood pressure, sICAM-1, sVCAM-1, IL-6, PAI-1, PWV and PP in HIV-infected individuals

The significant findings from the article, *Fibulin-1 as a marker of cardiovascular disease in HIV-infected black South Africans: a prospective study*, were the following:

At the baseline study, the HIV-infected participants had lower HDL-C and higher fibulin-1, sICAM-1, sVCAM-1, TC/HDL-C ratio, TG/HDL-C ratio and suPAR levels in comparison with their HIV-uninfected control group. The high suPAR and low HDL-C levels suggested the possibility that the HIV-infected participants are more prone to develop vascular damage that may result in further ECM remodelling, which is seen as the link between infection, inflammation, thrombotic activity and vascular dysfunction (Figure 1, p18).

The protein fibulin-1 has been found in association with ECM structures that surround vascular smooth muscle. Fibulin-1 accumulates in the arterial wall and change in fibulin-1 might influence the structural and functional properties of the ECM in atherosclerotic lesions.
Recent findings indicate that fibulin-1 is a cofactor for MMPs whose expression is augmented in atherosclerotic lesions. This findings point to the possible role of fibulin-1 in processes that is associated with the progression of thrombotic complications that may lead to vascular dysfunction.

Following vascular injury and inflammation, fibulin-1 presents in the ECM of the vessel wall, interacts with plasma fibrinogen and promotes platelet adhesion, leading to the formation of a platelet plug. Godyna et al. found that fibulin-1 can bind to fibrinogen and can be incorporated into fibrin clots. This prompted an investigation into the potential role of fibulin-1 as a prothrombotic agent that leads to vascular dysfunction. Studies which investigated the prothrombotic state in HIV-infected populations showed increased levels of fibrinogen. However, the fibrinogen levels were significantly lower in the HIV-infected participants at baseline, which indicated no signs of a prothrombotic state. However, these findings are in agreement with the findings of James et al., who found that HIV infection was not associated with fibrinogen concentration in Africans. No link was found between HIV, fibulin, fibrinogen and coagulation in the HIV-1 infected participants.

Associations between fibulin-1 and the cardiovascular variables were expected, but the results of the study were not consistent with the findings in literature. Cangemi et al. found correlations between plasma fibulin-1 and markers of arterial stiffness, namely the carotid compliance and pulse pressure. They also found associations between plasma fibulin-1 concentrations and signs of myocardial dysfunction, but did not find any correlation between lipids and plasma fibulin-1. In contrast, no significant associations were found between fibulin-1 and markers of vascular function, namely sICAM-1, sVCAM-1, IL-6, PAI-1 or pulse wave velocity. An association between change in fibulin-1 and TG/HDL-C ratio was found, though, which suggests that vascular changes are present in HIV-infected participants and that they might be more at risk to develop cardiovascular disease than the HIV-uninfected participants, as an increase in the TG/HDL-C ratio is a good marker of cardiovascular risk.
In the unadjusted correlations, the associations in the HIV-infected participants between fibulin-1 and TG/HDL-C ratio were highly significant \( (r = 0.24, p = 0.006) \). After adjustments for age, BMI and gender had been applied (Table 4), the association between percentage change in TG/HDL-C ratio and fibulin-1 remained significant \( (r = 0.200, p = 0.009) \). This association could not be confirmed with the forward stepwise regression analysis.

Although no significant associations were found between fibulin-1 and markers of vascular function, namely sICAM-1, sVCAM-1, IL-6, PAI-1 or pulse wave velocity, the association of the percentage change in fibulin-1 with change in TG/HDL-C ratio suggests that TG/HDL-C ratio may contribute to probable vascular changes in HIV-infected participants and that the HIV-infected participants may be more at risk to develop cardiovascular disease in comparison with the HIV-uninfected participants.

3.3 Strengths and weaknesses
In 2005, a prospective study design were applied and carefully matched to the control subjects according to age, gender, BMI and locality.

The study is unique, especially in these aspects:

The participants were unaware of their infected status and have, therefore, never received any ARV treatment (2005). They commenced treatment after 2005 and a weakness is that no adjustments were made for ARV's taken with the follow up 2005-2008. The data from this study are limited to the HIV-infected black South Africans in the North-West Province of South Africa.

3.4 Confounding
Adjustments were made for age, BMI and gender as confounders. The cr-PWV was adjusted additionally for mean arterial pressure and heart rate to provide credible results. Due to the study design (prospective), the results could not infer causality.
3.5 Recommendations for future research

1. Education on cardiovascular disease and the risk involved, as well as promotion of a healthier lifestyle, is needed amongst the HIV-infected population of South Africa.

2. The influence of the antiretroviral therapy on vascular function and vascular deterioration that was seen in the HIV-infected population needs to be studied further.

3. Future and longer term follow-up studies are recommended to address the metabolic and cardiovascular complications of the first-line treatment of the roll-out programme that specifically account for ethnic heterogeneity and HIV-1 subtype variants.

3.6 Conclusion

The HIV-infected participants exhibited higher fibulin-1 levels, inflammation and vascular damage at baseline and after three years. The association of percentage change in fibulin-1 and TG/HDL-C ratio suggested that vascular changes are probably present in HIV-infected participants and that they might be more at risk to develop cardiovascular disease in comparison with the HIV-uninfected participants.