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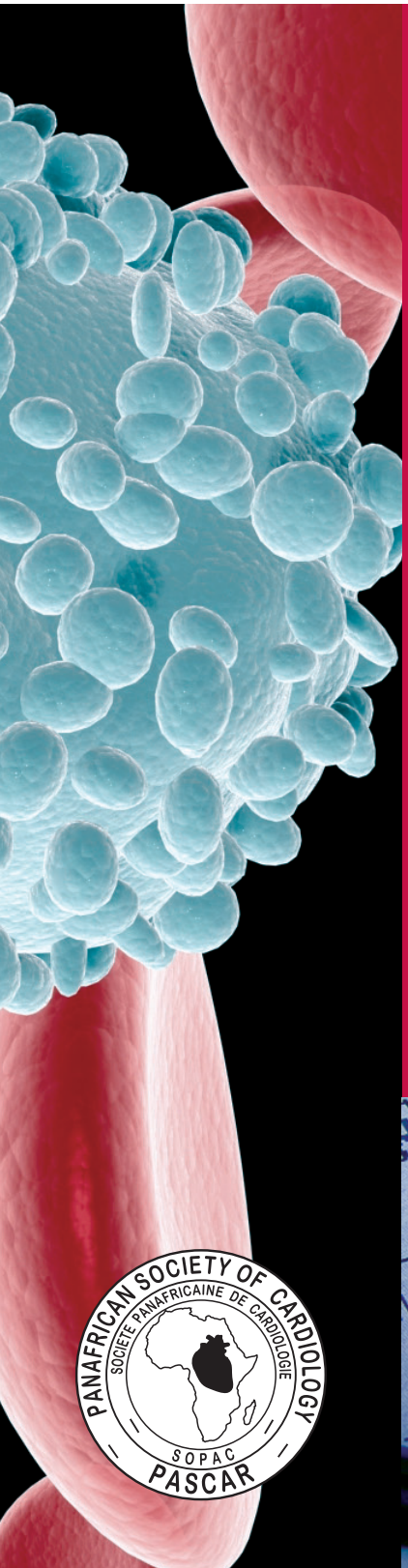

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FINANCIAL & PRODUCTION CO-ORDINATOR
ELSABÉ BURMEISTER
Tel: 021 976 8129
Fax: 086 664 4202
Cell: 082 775 6808
e-mail: elsabe@clinicscardive.com

PRODUCTION EDITOR
SHAUNA GERMISHUIZEN
Tel: 021 785 7178
Cell: 083 460 8535
e-mail: shauna@clinicscardive.com

CONTENT MANAGER
MICHAEL MEADON (Design Connection)
Tel: 021 976 8129
Fax: 0866 557 149
e-mail: michael@clinicscardive.com

GAUTENG CONTRIBUTOR
PETER WAGENAAR
Cell 082 413 9954
e-mail: skylark65@myconnection.co.za

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Postal address: PO Box 1013, Durbanville, RSA, 7551

Tel: 021 976 8129
Fax: 0866 644 202
Int.: +27 21 976 8129

e-mail: info@clinicscardive.com

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From the Editor's Desk

As always, contributors continue to provide an eclectic array of submissions to CVJA, which I hope provide something of interest to readers from diverse backgrounds with varying interests.

As an official journal of PASCAR, it is a pleasure to be able to publish the results of the PASCAR and World Heart Federation Cardiovascular Diseases Scorecard project for Cameroon, prepared by Dzudie and colleagues (page 103). The objective of the scorecard is to create a clear picture of the current state of cardiovascular disease prevention, control and management, accompanied by similar information on related non-communicable diseases in 12 African countries. The authors have successfully achieved the aims of the project for Cameroon and the article should be a useful asset for researchers, clinicians and those planning health resources, both in that country and the continent. Similar scorecard reports for other countries are eagerly awaited.

Emet and co-workers (page 75) report on a non-invasive technique based on the surface ECG measurement of the P-wave. They consider that heterogeneity in atrial conduction, seen as a variation in P-wave duration between differently orientated surface electrocardiogram (ECG) leads, called P-wave dispersion (PwD), is an easily calculated ECG parameter that can be used to predict increased atrial strain and indicates a poor prognosis in patients with pulmonary arterial hypertension (PAH). They report their experience in 32 patients and a similar number of healthy controls. They conclude that PwD can easily be calculated from a surface ECG to estimate the functional status and prognosis of the patient with PAH. Multiple other factors may influence P-wave duration, including left-sided heart disease, excluded in this series, and this non-invasive technique requires validation in a larger cohort before it can be accepted for widespread application.

Norman, Woodiwiss and others (page 91) remind us that chronic kidney disease (CKD) is a major public health problem and not only progresses to end-stage renal disease, but also predicts cardiovascular events beyond conventional risk factors. In a community-based study of participants over 16 years of age in an urban setting, where obesity was common, they investigated risk factors for the development of CKD. Information such as they provide is important if implementation of preventative measures may be able to slow the progression to CKD. This is particularly important in Africa where resources for renal replacement therapy are severely limited.

Opinion has it that consumption of rooibos tea confers health benefits. To my knowledge there are no properly conducted clinical trials supporting this opinion but it is a view held very strongly by some. In a series of elegant experiments in an animal model, Smit-van Schalkwyk and co-workers (page 81) show rooibos co-treatment exerted beneficial vascular effects in nicotine-exposed rats, and that this was associated with increased antioxidant enzyme activity. However it is a long way from animal experimentation to clinical application and there is a need for a properly conducted clinical trial to explore the alleged health benefits of rooibos. Until that is conducted and published, rooibos will remain, in the opinion of some, a pleasant, unusually flavoured and refreshing drink.

Lionel Opie was arguably one of the foremost cardiovascular researchers on the continent and it is fitting that we publish the excellent tribute from Ntusi (page 80).

I am overwhelmed, not only by the scale and severity of the devastating COVID-19 pandemic, but also by the flood of information and literature that accompanies it. Much of this has been rushed into publication and it is difficult to evaluate prior to careful review and commentary from learned colleagues knowledgeable in these areas, which are new to many of us, myself included. It also seems that clinical trials of treatments that are unlikely candidates for cure are being planned or are underway. Inevitably the knowledge that a medicine is in trial is often misinterpreted by both medical and non-medical persons as meaning that the medicine has value in a particular circumstance. As we all know, well-planned, adequately powered, randomised, double-blind, placebo-controlled trials remain the gold standard and the results of such trials should be awaited before embracing potentially harmful treatments of unproven benefit.

Pat Commerford
Editor-in-Chief

Cardiovascular Topics

Endothelial dysfunction in HIV-positive patients with acute coronary syndromes

Ahmed Vachiat, Therese Dix-Peek, Raquel Duarte, Pravin Manga

Abstract

Aim: This study investigated endothelial function in HIV-positive patients with acute coronary syndrome (ACS). Flow-mediated dilatation, pulse-wave velocity, carotid intima–media thickness and endothelial biomarkers were used to non-invasively investigate endothelial dysfunction.

Methods: Twenty HIV-positive patients with ACS (HIV+/ACS) were compared to 20 HIV-negative patients with ACS (HIV-/ACS) and 20 HIV-positive patients without ACS (HIV+/no ACS).

Results: Endothelial function measured by flow-mediated dilatation (FMD) was similar in both the HIV+/ACS (5.2; IQR 1.4–13.4%) and HIV-/ACS groups (3.7; IQR 2.3–4.4%) ($p = 0.78$). Arterial stiffness, measured by pulse-wave velocity (PWV) was low in all three cohorts. Carotid intima–media thickness (CIMT) was also low in all three cohorts. The vascular cellular adhesion molecule-1 (VCAM-1) levels in HIV-positive patients with and without ACS were significantly higher than in the HIV-/ACS cohort ($p = 0.033$ and 0.024, respectively).

Conclusion: Non-invasive investigations such as FMD, CIMT and PWV did not identify patients with HIV who were at high risk of ACS. Endothelial biomarkers may be more useful markers to identify HIV-positive patients who have endothelial dysfunction and increased risk of ACS.

Keywords: HIV, acute coronary syndromes, endothelial dysfunction, flow-mediated dilatation, pulse-wave velocity, carotid intima–media thickness

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Division of Cardiology, Department of Medicine, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa
Ahmed Vachiat, MB BCh, MMed, FCP (SA), Cert Cardiology (SA), PhD, ahmedvachiat@gmail.com
Pravin Manga, MB BCh, FCP (SA), PhD

Molecular Biology Laboratory, Department of Internal Medicine, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa
Therese Dix-Peek, MSc
Raquel Duarte, PhD

There are approximately 37 million people living with human immunodeficiency virus (HIV) worldwide, of whom 70% live in sub-Saharan Africa.[1,2] Increased life expectancy globally as a result of better access to combination antiretroviral therapy (cART) and high levels of traditional cardiovascular disease risk factors have increased the prevalence of ischaemic heart disease (IHD) in this population.[3,4] Developed countries generally have an older HIV-positive population with a higher IHD risk profile compared to a younger HIV-positive population in the developing world with a lower IHD risk profile.[5] Developed nations have substantial data on IHD in HIV-positive populations while there is a paucity of data from developing regions.

The endothelium lines the internal surface of blood vessels and is responsible for vascular homeostasis, such as maintenance of vascular tone and non-thrombotic vascular surfaces, as well as immunomodulation. With the onset of endothelial dysfunction, the vasculature is predisposed to vasoconstriction, leukocyte adherence, platelet activation, pro-oxidation, thrombosis, impaired coagulation and vascular inflammation.[6] Endothelial dysfunction has therefore been identified as a key step in promoting atherogenesis, and is well described to be an early predictor of future cardiovascular events in patients both with and without established cardiovascular disease.[7-9]

Endothelial function can be measured in many different ways. The more common technique and one that is well validated is the non-invasive measurement of endothelial function, which relies on high-resolution ultrasound of the brachial artery.[6] Another approach is by measuring endothelial biomarkers.

Endothelial biomarkers, such as cellular adhesion molecules, are either present on the surface of endothelial cells or are expressed on endothelial cells in response to certain stimuli. Endothelial biomarkers include the selectins (E-selectin, P-selectin, L-selectin), vascular adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1), which are involved in leukocyte rolling, adhesion and trans-endothelial migration into sub-intimal spaces.[6] There is evidence that these cellular adhesion molecules can be considered reliable biomarkers for the development and severity of atherosclerosis and could add to the predictive value of the classical risk factors for IHD in HIV-negative populations.[6,10] There are insufficient data that this can be applied in HIV-positive patients, particularly those with acute coronary syndromes (ACS).

Endothelial function has been studied in HIV-positive patients since the onset of the epidemic.[11] There appears to be an intricate interplay between endothelial function and inflammation, as markers such as VCAM-1 and ICAM-1 are elevated in patients early after HIV infection.[12] The mechanism of endothelial

dysfunction and promotion of atherosclerosis in HIV-infected individuals is complex but is thought to be related to a number of factors including direct involvement of HIV, endothelial activation and vascular inflammation.[13-15]

Endothelial dysfunction is regarded as a link between infection, inflammation and atherosclerosis, and there are recent data suggesting the presence of endothelial dysfunction in untreated HIV-positive patients.[16] Furthermore, following cART, it has been shown that there is a fall in markers of endothelial activation such as monocyte chemo-attractant protein, P-selectin and VCAM-1.[12]

Although endothelial function has been studied in numerous studies in the past, there is a paucity of data on endothelial function in HIV-positive patients presenting with ACS.[17] Therefore we aimed, firstly, to assess the presence and degree of endothelial dysfunction in HIV-positive patients presenting with ACS by using flow-mediated dilatation (FMD) and endothelial biomarkers. Our secondary aim was to assess carotid intima–media thickness (CIMT) in these patients as a surrogate marker of atherosclerosis.

Methods

This was a prospective study of 60 patients at a large urban public hospital in Johannesburg, South Africa, recruited over a three-year period (July 2012 to July 2015). Twenty HIV-positive patients presenting with ACS (HIV+/ACS) were compared to 20 HIV-negative patients with ACS (HIV-/ACS) and 20 HIV-positive patients without ACS (HIV+/no ACS).

Inclusion criteria included age ≥ 18 years and HIV-positive patients presenting with ACS. Risk factors for coronary artery disease (CAD) that were studied included age, smoking, hypertension, diabetes and family history of premature CAD (men ≥ 55 years, women ≥ 65 years of age). Exclusion criteria included prior myocardial infarction, life-threatening disease that prevented a two-year follow up, and known carotid artery disease. Ethical approval for the study was obtained from the local institutional body (M111143).

The HIV+/ACS and HIV-/ACS patients were matched for age and gender. The HIV+/no ACS patients could only be matched for gender as cART-naïve patients presenting at our HIV clinic were found to be much younger patients. CIMT thickness was measured in all three groups using an 11-Mhz transducer (Phillips iE33 ultrasound machine) with the patient in the supine position with the head tilted 30 degrees to the left for the right carotid artery assessment and then 30 degrees to the right for the left carotid artery. The carotid artery bifurcation was visualised and the software automatically measured carotid artery intima thickness of the distal wall 10 mm from the bifurcation bulb.

Endothelial function was measured non-invasively using brachial FMD according to standardised guidelines.[18] The maximum diameter of the brachial artery was measured at rest. A blood pressure cuff was then inflated to at least 50 mmHg above the systolic pressure to occlude arterial inflow for five minutes. The brachial artery diameter was again measured two minutes after cuff release when the maximum dilation of the vessel usually occurs.

Applanation tonometry of the radial artery converts the radial pulse wave into an aortic pulse wave. Pulse-wave velocity (PWV) was measured from sequential waveform measurements at the carotid and femoral sites. The distance that the pulse wave travels was determined as the difference between the distance from the femoral sampling site to the suprasternal notch, and the distance from the carotid sampling site to the suprasternal notch.

Endothelial biomarkers were measured in all 60 patients and included interleukin (IL)-1β, IL-1Ra, IL-6, tumour necrosis factor alpha (TNF-α), monocyte chemotactic protein-1 (MCP-1), plasminogen activator inhibitor-1 (PAI-1), E-selectin, P-selectin, ICAM-1 and VCAM-1. Following blood collection in EDTA tubes, specimens were centrifuged for 20 minutes at 4°C and 1 000 × *g*. The plasma was decanted into 1.5-ml microcentrifuge tubes and stored at –70°C for later analysis.

IL-1β, IL-1Ra, IL-6, MCP-1 and TNF-α concentrations were quantified using the Bio-Plex Pro™ human cytokine standard 27 (Plex Bio-Rad Laboratories, Hercules, CA) as per manufacturer's instructions. The plasma was diluted four-fold for these assays. E-selectin and P-selectin plasma levels were measured using the Human Magnetic Luminex assay (R&D Systems, Minneapolis, MN) as per manufacturer's recommendations. Plasma was diluted two-fold to measure E-selectin and P-selectin levels. The serum concentrations for VCAM-1, ICAM-1 and PAI-1 were analysed in sera diluted 40-fold using the Milliplex human sepsis magnetic panel 1 (Merck Millipore, Billerica, MA) as per manufacturer's instructions. Samples were analysed using the Bio-Plex 200 system (Bio-Rad) and concentrations were determined using the 5-PL method using Bio-Plex Manager 5.0 software.

Statistical analysis

The χ^2 test was used to assess the relationship between categorical variables and groups. The Fisher's exact test was used where the requirements for the χ^2 test could not be met. The relationship between continuous variables and groups was assessed with one-way analysis of variance (ANOVA) for the three groups and the unpaired *t*-test for two groups. *Post hoc* tests for ANOVA were conducted using the Tukey–Kramer adjustment for multiple comparisons. Where the data did not meet the assumptions of these tests, a non-parametric alternative, the Kruskal–Wallis test was used for three groups, and the Wilcoxon rank sum test for two groups. Paired comparisons between continuous variables were carried out with the paired *t*-test or the Wilcoxon matched-pairs test. Data analyses were carried out using SAS version 9.4 for Windows. A 5% level of significance was used.

Results

The HIV+/ACS patients had a mean age of 51.1 years (± 8.1) and 13 were male (65%). The mean age of 36.0 years (± 6.8) in the HIV+/no ACS group was significantly lower than that of the HIV+/ACS group [51.1 years (± 8.1)] and the HIV-/ACS group [52.3 years (± 9.0)] ($p < 0.0001$). The proportion of males in each group ranged between 50 and 80%, but the differences were not statistically significant ($p = 0.14$) (Table 1).

Ten (50%) of the HIV+/ACS group were on cART and none was on protease inhibitors. Seven (35%) of the patients in the HIV+/ACS group were newly diagnosed with HIV. There were 15 (75%) hospital admissions with ST-segment elevation myocardial infarction (STEMI) (eight anterior, seven inferior), three (15%) with non-ST segment elevation myocardial infarction and two patients with unstable angina (10%). The typical presentation

Table 1. Patient demographics, risk factors and clinical investigations

		HIV+/ACS (n = *20*)	*HIV-/ACS* (n = *20*)	*HIV+/no ACS* (n = *20*)	p-*value*
Age (years)#		51.1 (8.1)	52.3 (9)	36 (6.8)	< 0.0001
Race (black), *n* (%)		17 (75)	7 (35)	20 (100)	< 0.0001
Male, *n* (%)		13 (65)	16 (80)	10 (50)	0.14
Risk factors, *n* (%)					
Smoking		11 (55)	10 (50)	1 (5)	0.0012
Hypertension		6 (30)	7 (35)	0	0.0006
Diabetes		2 (10)	9 (45)	0	0.0006
Dyslipidaemia		2 (10)	10 (50)	0	0.0002
Family history		1 (5)	2 (10)	0	0.31
CIMT# (mm)		0.66 (0.16)	0.70 (0.06)	0.50 (0.00)	0.0005[1,3] 0.0001[2,3]
PWV# (m/s)		4.1 (1.1)	4.6 (1.0)	3.6 (0.6)	0.12
Laboratory	Normal values				
Haemoglobin# (g/dl)	14.3–18.3	12.9 (2.6)	15.0 (1.7)	11.4 (2.1)	0.0086,[1,2] < 0.0001[2,3]
Creatinine* (µmol/l)	64–104	74 (65–90)	86 (75–99)	63 (48–71)	0.017,[1,2] 0.0003[1,3]
Total cholesterol# mean (mmol/l)	< 4.5	4.0 (0.9)	5.2 (1.2)	3.1 (0.8)	0.019,[1,3] 0.0011[2,3]
Triglycerides* (mmol/l)	< 1.7	1.2 (0.95–1.45)	1.3 (0.95–1.95)	1.2 (0.90–1.40)	0.27
HDL# (mmol/l)	> 1.0 male > 1.3 female	1.05 (0.29)	1.03 (0.24)	0.94 (0.27)	0.44
LDL# (mmol/l)	< 2.5	2.3 (0.7)	3.5 (1.0)	1.6 (0.6)	0.028,[1,3] < 0.0001[2,3]
CD4* (cells/mm³)		301 (205–417)	N/A	143 (19–198)	0.0020

#Mean ± SD, *median (IQR).
CIMT = carotid intima–media thickness, PWV = pulse-wave velocity, LDL = low-density lipoprotein, HDL = high-density lipoprotein, CD4 = cluster of differentiation.

in the HIV+/ACS group was a young patient with STEMI involving the left anterior descending artery, which was the most common artery involved (60%), followed by the right coronary artery (35%) and the left circumflex artery (20%).

Risk factors in the HIV+/ACS group included smoking in 11 (55%), hypertension in six (30%), diabetes in two (10%), dyslipidaemia in two (10%), and one (5%) patient had a family history of IHD (Fig. 1). The prevalence of diabetes and dyslipidaemia was higher in the HIV-/ACS group compared to the HIV+/ACS and the HIV+/no ACS groups (p = 0.0006 and 0.0002, respectively). The prevalence of smoking and hypertension was lower in the HIV+/no ACS group compared to the other HIV+/ACS and the HIV-/ACS groups (p = 0.0012 and 0.0006, respectively) (Fig. 1). Low-density lipoprotein (LDL) levels were no different in ACS patients whether they were HIV positive or negative. HIV-positive patients without ACS had significantly lower LDL levels.

Endothelial function was measured using FMD in all three groups. The median percentage difference in FMD between baseline (before blood pressure cuff inflation) and post blood pressure cuff deflation was significantly higher for the HIV+/no ACS group (14.3; IQR 6.7–20.6%) compared to the HIV+/ACS group (5.2; IQR 1.4–13.4%) and the HIV-/ACS group (3.7; IQR 2.3–4.4%) (p = 0.044 and 0.0016, respectively) (Fig. 2).

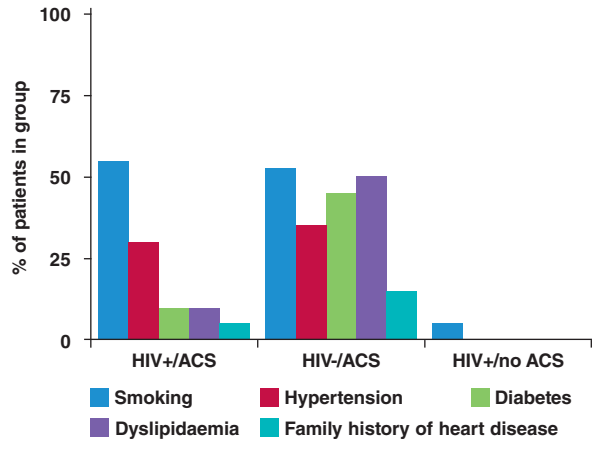

Fig. 1. Risk-factor profile. There were more smokers but there were fewer traditional risk factors (hypertension, diabetes, dyslipidaemia and family history of ischaemic heart disease) in the HIV+/ACS cohort compared to the HIV-/ACS and HIV+/no ACS cohorts.

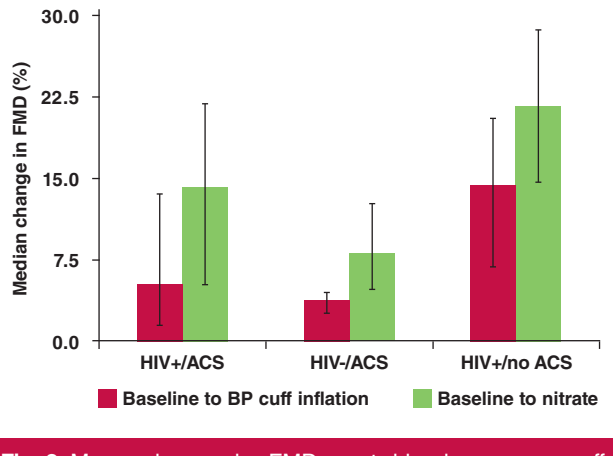

Fig. 2. Mean change in FMD post blood pressure cuff inflation. HIV+/ACS patients had a similar change from baseline of brachial flow-mediated dilatation compared to the HIV-/ACS patients (p = 0.78). The HIV+/no ACS group had the most vasoreactivity but they were significantly younger (p = 0.0001).

Table 2. Endothelial biomarkers

Median levels (interquartile ranges)	*HIV+/ACS*[1]	*HIV-/ACS*[2]	*HIV+/no ACS*[3]	p-*value for H0: no between-group differences*	p-*value for* post hoc *group differences*
VCAM-1 (ng/ml)	402 (292–609)	287 (257–412)	503 (292–822)	0.025	0.033[1,2] 0.024[2,3]
ICAM-1 (ng/ml)	172 (135–211)	167 (129–193)	225 (199–293)	0.0009	0.0079[1,3] 0.0010[2,3]
IL-6 (pg/ml)	5.9 (3.9–12.4)	10.7 (4.8–50)	3.8 (1.9–5.3)	0.0053	0.033[1,3] 0.0050[2,3]
E-selectin (ng/ml)	24.3 (19.1–30.6)	27.8 (21.5–42.2)	47.1 (31.8–65.8)	0.0005	0.0007[1,2] 0.012[2,3]
IL-1β (pg/ml)	0.36 (0.27–0.48)	0.32 (0.28–0.41)	0.38 (0.30–0.51)	0.29	
IL-1Ra (pg/ml)	224 (173–266)	215 (120–258)	181 (125–258)	0.75	
MCP-1 (pg/ml)	26.5 (15.0–41.2)	22.4 (18.0–33.3)	22.4 (16.5–27.4)	0.70	
TNF-α (pg/ml)	2.5 (1.4–4.4)	2.0 (1.6–4.4)	2.5 (2.0–4.8)	0.60	
P-selectin (ng/ml)	25.8 (20.3–32.9)	20.8 (19.3–24.6)	23.5 (19.6–29.7)	0.12	
PAI-1 (ng/ml)	67.6 (36.7–149.8)	85.6 (34.7–138.4)	66.2 (35.6–91.7)	0.59	

There were significant differences in the following endothelial biomarkers; VCAM-1, ICAM-1, IL-6 and E-selectin. The median VCAM-1 levels in HIV-positive patients with and without ACS were significantly higher than in the HIV-/ACS cohort. However, median ICAM-1 levels were significantly higher in HIV-positive patients without ACS than in the HIV+/ACS and HIV-/ACS cohorts. The HIV+/no ACS group had significantly lower median levels of IL-6 than both the HIV+/ACS and the HIV-/ACS groups. The median E-selectin levels in the HIV+/ACS patients and HIV-/ACS patients were significantly lower than that of the HIV+/no ACS group. There was no significant difference in median IL-1β, IL-1Ra, MCP-1, TNF-α, P-selectin and PAI-1 levels between the groups.
VCAM-1 = vascular cellular adhesion molecule 1, ICAM-1 = intercellular adhesion molecule 1, IL = interleukin, MCP-1 = monocyte chemotactic protein 1, TNF-α = tumour necrosis factor alpha, PAI-1 = plasminogen activator inhibitor 1.

Patients without ACS had the most vasoreactivity compared to the patients with ACS.

The mean PWV in the HIV+/ACS group was 4.1 m/s (SD 1.1), 4.6 m/s (SD 1.1) in the HIV-/ACS group and 3.9 m/s (SD 1.1) in the HIV+/no ACS group. These values were all low with no significant differences between the three groups ($p = 0.12$) (Table 1).

CIMT was measured in all three groups. The mean CIMT of the two groups with ACS was not different. The CIMT in the group without ACS (0.50; ± 0.08 mm) was marginally but significantly lower than the CIMT of both the HIV+/ACS group (0.66; ± 0.16 mm) and the HIV-/ACS group (0.70; ± 0.06 mm) ($p = 0.0005$ and $p < 0.0001$, respectively) (Table 1).

There were significant differences in the endothelial biomarkers VCAM-1, ICAM-1, IL-6 and E-selectin in the three groups (Table 2). The median VCAM-1 levels in HIV-positive patients with and without ACS were significantly higher than in the HIV-/ACS cohort (402 and 503 vs 287 ng/ml) ($p = 0.033$ and 0.024, respectively). Median ICAM-1 levels on the other hand, were significantly higher in only the HIV-positive patients without ACS compared to the other two cohorts ($p = 0.0079$ and 0.0010, respectively). The HIV+/no ACS group had significantly lower median levels of IL-6 than both the HIV+/ACS and the HIV-/ACS groups ($p = 0.033$ and 0.0050, respectively). The median E-selectin levels in HIV+/ACS patients and HIV-/ACS patients were significantly lower than that of the HIV+/no ACS group ($p = 0.0007$ and 0.012, respectively). There was no significant difference in median IL-1β, IL-1Ra, MCP-1, TNF-α, P-selectin and PAI-1 levels between the groups.

Discussion

Studies suggest that an overall 1.5–2.0-fold increased risk of acute myocardial infarction is conferred by HIV infection.[19,20] Common to most studies is that the mean age at presentation of ACS in HIV-infected patients is a decade younger than the general population, with a mean age of 50 years.[5,21,22] This is similar to our cohort where the mean age was 51 years.

IHD in HIV-positive patients presenting with ACS have different risk-factor profiles in developing and developed regions. Traditional risk factors such as hypertension, diabetes and dyslipidaemia are more common in developed regions. The current study had fewer patients with hypertension, diabetes, dyslipidaemia and family history of IHD in HIV-positive patients with ACS.

In developing countries smoking appears to be the dominant risk factor with prevalence rates of 24.4% in HIV-positive males.[5,13,23] More than half of the HIV+/ACS patients in our cohort were smokers with fewer other traditional risk factors. Furthermore, there is evidence that smoking contributes to endothelial dysfunction.[24] These findings make smoking cessation an important modifiable risk factor for the prevention of IHD in HIV-positive patients.

Mechanisms for the development of atherosclerosis in HIV are multifactorial and include chronic inflammation and immune activation. These in turn may also lead to endothelial dysfunction, further contributing to the pathogenesis of atherosclerosis.[13,19,25] Endothelial dysfunction is associated with increased levels of reactive oxygen species and decreased nitric oxide levels and hence decreased vascular reactivity.[6] HIV itself has been linked to endothelial dysfunction.[13,26] This is supported by findings of a significant improvement in FMD in patients in the first 24 weeks after initiation of cART, suggesting that suppression of HIV viraemia leads to improved endothelial function.[27]

The HIV-1 envelope protein gp120 and the regulatory protein Tat are associated with endothelial cell apoptosis and increased cellular adhesion molecules (ICAM-1, E-selectin). Furthermore, a prothrombotic state [increase of von Willebrand factor, PAI-1 and tissue plasminogen activator (t-PA)] have been implicated in the pathogenesis of atherosclerosis in HIV-positive patients.[28] Telomere length and CDKN2A expression were both consistent with increased biological ageing in HIV-infected individuals.[29] Anti-retroviral therapies may be linked to CAD, specifically protease inhibitors, however the risk of CAD associated with anti-retroviral therapy is small compared with the impressive reductions in all-cause mortality with cART.[30]

Endothelial dysfunction is well known to be associated with atherosclerosis in HIV-negative populations.[31,32] It has also been described in HIV-positive patients without ACS.[26,33,34] In our study, HIV+/ACS patients had almost the same degree of endothelial dysfunction as the control group of ACS patients without HIV. Although it would appear that endothelial dysfunction was not more prevalent in the HIV-positive group with ACS, it must be borne in mind that the latter group had significantly fewer coronary risk factors such as hypertension, diabetes, dyslipidaemia and family history of IHD compared to the HIV-negative patients with ACS, suggesting that HIV infection itself contributed to endothelial dysfunction. HIV-positive patients without ACS had the highest brachial artery vasoreactivity compared to the patients with ACS. This is an interesting finding but is most likely a reflection of the much younger age of this group compared to the other two groups.

In HIV-positive patients, endothelial activation may lead to structural and functional vascular changes. Exposure to long-term sub-clinical inflammation is related to accelerated stiffening of large arteries. Peripheral waveform analysis using PWV has been shown to provide a non-invasive method of measuring 'global' endothelial function.[35] Increasing levels of arterial stiffness are correlated with higher PWV. The value for stiff vessels is a PWV > 10 m/s and this value has been found to be an independent marker of end-organ damage.[36,37] In our study we found uniformly low values (< 5 m/s) for PWV. This finding, we believe, is largely an age-related effect. This is supported by Fourie *et al*. who recently reported increasing PWV velocities only after the age of 50 years in HIV-positive, cART-naïve patients.[16]

By studying ACS patients who were HIV positive and comparing them to ACS patients who were HIV negative, we were able to assess endothelial activation in both groups of patients. In developing countries, high levels of endothelial markers such as IL-6, TNF-α, PAI-1 and sCD14 have been reported in HIV-positive compared with HIV-negative patients.[38-41] Studies of endothelial function from developing nations have demonstrated that in recently seroconverted Kenyan women, endothelial biomarkers (VCAM-1 and ICAM-1) were significantly elevated in these patients early after HIV infection.[12]

In a recent study from South Africa, HIV-positive patients were found to have higher levels of adhesion molecules compared to HIV-negative patients, with an odds ratio of 3.9 (2.2–7.0) for ICAM-1 and 16.2 (7.5–35) for VCAM-1.[42] Furthermore, the same investigators reported that ICAM-1 and VCAM-1 were elevated in both treated and cART-naïve patients, with the odds being greater for the never-treated group.[36]

Another study from South Africa, which assessed endothelial biomarkers in ACS patients who were HIV positive, also found significantly elevated VCAM-1 levels in HIV-positive patients with ACS compared to control patients who were either HIV negative or HIV positive without ACS.[5] Similarly, in our cohort of HIV+/ACS patients, we found significantly higher levels of VCAM-1 compared to HIV-negative patients, findings that are in concert with previous reported results.

VCAM-1 is a member of the immunoglobulin super-family and is involved in cellular adhesion and transmigration of leucocytes through endothelial cells, and is thought to a play a role in the development of atherosclerosis.[34] With the stimulation of endothelial cells by inflammatory cytokines there is increased expression of VCAM-1 and this is associated with an increased predictive value for future cardiovascular events.[43] Therefore VCAM-1 may be an informative biomarker for predicting the risk of HIV disease progression, morbidity and mortality.[12]

The pro-inflammatory cytokine, IL-6, induces expression of adhesion molecules such as VCAM-1 and ICAM-1 and may be seen as an early modulator of leukocyte trafficking in the vascular wall.[44] However, we did not find significantly elevated levels of IL-6 in HIV-positive and HIV-negative patients with ACS. Lack of a significant difference in IL-6 and other markers of endothelial dysfunction in HIV-infected and non-infected controls have also been previously reported.[5,36]

Non-invasive surrogate tools such as CIMT and coronary computer tomography angiography indicate an increased prevalence of sub-clinical atherosclerosis in HIV-positive compared to HIV-negative patients.[45-47] A meta-analysis of 13 observational studies suggests a trend towards increased CIMT in HIV-infected patients.[45] As early as childhood, HIV-infected children receiving cART were found to have increased CIMT, suggesting that IHD risk may already be heightened in HIV-infected patients at a young age.[48]

CIMT has been shown to decrease with cART and less CIMT progression was associated with suppressed viral load at baseline.[49] In our cohort, CIMT measurements were unexpectedly lower in the HIV+/ACS compared to the HIV-/ACS patients. The HIV+/no ACS patients also had low CIMT measurements. One possible explanation for the finding of lower CIMT in HIV+/ACS patients is the relative lack of traditional risk factors for atherosclerosis, such as hypertension, diabetes and low LDL levels in the HIV+/ACS group. It has been shown that the presence of high cardiovascular risk-factor profiles in HIV-positive patients is associated with increased CIMT.[50]

This study has the following limitations. First, the study is a single-centre study with a small sample size. Given the nature of the study, it took almost three years to recruit 20 HIV-positive patients presenting with ACS to our centre. With the small sample size we were not able to perform multivariate analyses. Second, given the type of the clinical presentation of patients in the study we were unable to completely match case–control patients for age, gender and cardiovascular risk factors. Although the HIV+/no ACS group were gender matched, they could not be matched for age as the majority of HIV+/no ACS patients, who were cART-naïve, presenting at the HIV clinic were young. Lastly, HIV+/no ACS patients in the study were newly diagnosed and therefore the duration of infection was not known.

Conclusion

Endothelial dysfunction as assessed by brachial FMD was similar in HIV-positive patients with ACS compared to HIV-negative patients with ACS. Endothelial biomarkers such as VCAM-1 and ICAM-1 were significantly raised in HIV-positive patients compared to HIV-negative patients. However, VCAM-1 was the only endothelial marker that was significantly raised in HIV-positive patients with ACS compared to HIV-negative patients with ACS. Our cohort of HIV-positive patients with ACS had impaired FMD but near-normal CIMT and PWV measurements. Given that FMD, CIMT and PWV were similar in HIV-positive and HIV-negative patients with ACS, the use of endothelial biomarkers may provide a more promising modality to investigate endothelial activation and subsequent dysfunction.

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One-off DNA test could predict heart attack risk in childhood

People at high risk of a heart attack in adulthood could be spotted much earlier in life with a reasonably inexpensive, one-off DNA test, according to research funded, among others, by the British and Australian heart foundations.

An international team led by researchers from the University of Leicester, University of Cambridge and the Baker Heart and Diabetes Institute in Australia used UK Biobank data to develop and test a powerful scoring system, called a genomic risk score (GRS), which can identify people who are at risk of developing coronary heart disease prematurely because of their genetic make-up.

Genetic factors have long been known to be major contributors of someone's risk of developing coronary heart disease – the leading cause of heart attacks. Currently, to identify those at risk, doctors use scores based on lifestyle and clinical conditions associated with coronary heart disease, such as cholesterol level, blood pressure, diabetes and smoking. But these scores are imprecise, age-dependent and miss a large proportion of people who appear 'healthy', but will still develop the disease.

The 'big-data' GRS technique takes into account 1.7 million genetic variants in a person's DNA to calculate their underlying genetic risk for coronary heart disease. The team analysed genomic data of nearly half a million people, aged between 40 and 69 years, from the UK Biobank research project. This included over 22 000 people who had coronary heart disease.

The GRS was better at predicting someone's risk of developing heart disease than each of the classic risk factors for coronary heart disease alone. The ability of the GRS to predict coronary heart disease was also largely independent of these known risk factors. This showed that the genes that increase the risk of coronary heart disease don't simply work by elevating blood pressure or cholesterol, for example.

People with a genomic risk score in the top 20% of the population were over four times more likely to develop coronary heart disease than someone with a genomic risk score in the bottom 20%. In fact, men who appeared healthy by current NHS health check standards but had a high GRS were just as likely to develop coronary heart disease as someone with a low GRS and two conventional risk factors such as high cholesterol or high blood pressure. These findings help to explain why people with healthy lifestyles and no conventional risk factors can still be struck by a devastating heart attack.

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Does acid reflux precipitate ischaemia in subjects with acute coronary syndrome?

Sunil K George, Boikhutso Tlou, Somalingum Ponnusamy, Datshana P Naidoo

Abstract

Aim: It has been postulated that gastro-oesophageal reflux disease (GORD) may trigger coronary ischaemia through viscerocardiac reflex vasoconstriction in subjects with ischaemic heart disease (IHD). Our aim was to estimate the prevalence of GORD in subjects with IHD who present with acute coronary syndrome (ACS) and to determine whether GORD may serve as a trigger for ischaemic events.

Methods: Twenty patients with isolated reflux oesophagitis and 39 with acute coronary syndrome (ACS with concomitant GORD) were studied. Twenty-two subjects comprising normal volunteers and those who were admitted for minor surgical trauma were used as normal controls. All subjects underwent oesophago-gastroduodenal endoscopy (EGD) and acid instillation with hydrochloric acid (0.1 M), as well as nuclear imaging (sestaMIBI) with technetium^{99}. Ischaemia was detected by ST depression using ECG monitoring for one hour during and immediately after EGD.

Results: Of the 111 subjects with ACS, 39 (35.1%) had erosive GORD and comprised the study group. Subjects with ACS had more incidence of diabetes ($p = 0.001$), hypertension ($p = 0.002$), a history of smoking ($p = 0.006$) and elevated serum triglyceride levels ($p = 0.008$) compared to the GORD group. Risk-factor clustering in the form of the metabolic syndrome was more common in ACS subjects (44 vs 5%; $p = 0.008$). ST depression was documented in 8/39 (20.5%) patients in the ACS group and 5/20 (25%) in the GORD group ($p = 0.958$). Reversible perfusion defects on sestaMIBI scan were seen in 35.6% of the ACS subjects.

Conclusion: Although GORD is common in subjects with ACS, we have not been able to show that GORD may serve as a trigger for ischaemia in these subjects.

Keywords: reflux oesophagitis, ischaemia, chest pain

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Department of Cardiology, Nelson R Mandela School of Medicine, University of KwaZulu-Natal, Durban, South Africa
Sunil K George, MD, FCP (SA)
Somalingum Ponnusamy, MB ChB, FCP (SA), Cert Cardiol (Physicians) (SA)
Datshana P Naidoo, MD, FRCP, naidood@ukzn.ac.za

Discipline of Public Health Medicine, School of Nursing and Public Health, University of KwaZulu-Natal, Durban, South Africa
Boikhutso Tlou, BSc, PhD

Chest pain is one of the most frequent complaints in the emergency department and demands careful evaluation in order to determine the aetiology and institute appropriate care. Of all the chest pain syndromes, gastro-oesophageal reflux disease (GORD) is perhaps the most common, with prevalences ranging from two to 10% in Europe and 7% in America.[1] Significant co-morbidity in the form of obesity often co-exists, with its associated complications such as erosive oesophagitis[1-6] that frequently presents with heartburn and must be differentiated from cardiac ischaemia or myocardial infarction.

The epidemiology of GORD therefore requires further study in the ischaemic heart disease (IHD) population, including those presenting with acute chest pain.[7] We examined the prevalence of GORD in subjects with acute coronary syndrome (ACS) and attempted to show whether GORD could precipitate ischaemia in these subjects.

Methods

Patients admitted to the coronary care unit (CCU) with a diagnosis of ACS were screened for the study. ACS was defined according to the criteria of Braunwald.[8] Patients who were stable and pain free for at least three days were studied. Patients who were acutely ill or unstable, and those with renal impairment, left bundle branch block or known peptic ulceration were excluded.

After obtaining informed consent, the subject was examined, bloods were sampled and the baseline electrocardiograph (ECG) was recorded. Parameters recorded included weight measured to the nearest 0.5 kg, and waist and hip circumferences as well as height according to standard guidelines.[9] Risk factors were identified and categorised according to the presence/absence of the metabolic syndrome using the harmonised criteria.[10]

For the endoscopic procedure, after an overnight fast, subjects underwent oesophago-gastroduodenal endoscopy (EGD) and acid instillation. With the endoscope positioned just proximal to the esophago-gastric junction, a volume of 60 ml of 0.1 M hydrochloric acid was administered over five minutes.[11] The acid concentration was prepared by adding 5 ml of concentrated acid to 495 ml of deionised water. All EGD procedures were performed in the gastrointestinal (GI) unit by the author (SG) using a fibre-optic instrument (Olympus Evis 2000, Tokyo, Japan). The Los Angeles method was used to classify reflux oesophagitis (Table 1).[12]

During endoscopy, an electrocardiographic Holter recording was performed and 0.1 M acid was instilled via endoscopy. The Holter recording was performed for an hour using a three-channel recorder (Schiller MT101 Baar, Switzerland). The diagnosis of ischaemia was inferred if at least 1 mm ST depression was observed during, and/or in the hour after acid installation. Ventricular arrhythmias were graded according to Lown's criteria,[13] i.e. grade 0: no ventricular premature depolarisations, grade 1: < 30 ventricular extrasystoles per

Table 1. The Los Angeles classification of oesophagitis (Armstrong *et al*. Gastroenterology 1996).

Grade A	One (or more) mucosal break no longer than 5 mm that does not extend between the tops of two mucosal folds
Grade B	One (or more) mucosal break more than 5 mm long that does not extend between the tops of two mucosal folds
Grade C	One (or more) mucosal break that is continuous between the tops of two or more mucosal folds but which involves less than 75% of the oesophageal circumference
Grade D	One (or more) mucosal break that involves at least 75% of the oesophageal circumference

hour, grade 2: > 30 extrasystoles per hour, grade 3: multiform ventricular extrasystoles, grade 4a: two consecutive ventricular extrasystoles, and grade 4b: three or more consecutive ventricular extrasystoles.

Shortly before discharge from hospital, a technetium (^{99m}Tc) methoxyisobutylisonitrile (sestaMIBI) test was performed using a two-day protocol in which ^{99m}Tc sestaMIBI was administered at rest (day 1) and at peak stress (day 2). Exercise stress and/or coronary vasodilator pharmacological stress using dipyridamole was used. Atropine was given at peak stress if the heart rate was less than 120 beats per minute. Imaging was performed using a Siemens e.cam (Munich, Germany) one-hour post injection. Attenuation correction was done in all overweight patients. SestaMIBI processing was done using 4DM SPECT software. Analysis was done using Siemens Axiom Artis software (Munich, Germany).

^{99m}Tc sestaMIBI findings were reported as normal, areas of infarction, or ischaemia. A scan was defined as normal when there was complete uptake of the radioisotope at rest, with no change post stress. Ischaemia was defined as an area of absent or reduced uptake on stress that shows normalisation during rest (i.e. reversibility). An infarct was defined as an area of absent or reduced uptake on stress that remained fixed at rest (i.e. no reversibility).

Two groups of subjects were included in the study to assess the effects of acid installation independent of concomitant ischaemia. Patients referred to the gastrointestinal unit at Addington Hospital with heartburn, who were diagnosed with erosive reflux oesophagitis at EGD, comprised the GORD group (Fig. 1). Subjects in whom the endoscopy was normal were also selected as normal control subjects. All subjects underwent acid instillation and Holter recording.

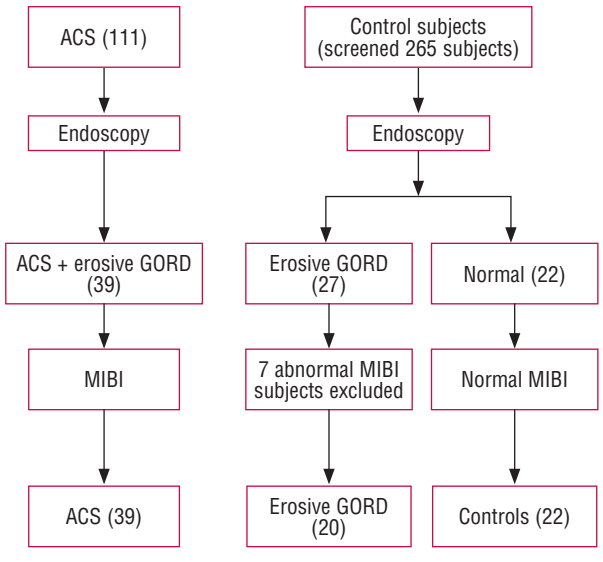

Fig. 1. Selection of subjects for the study. All subjects underwent endoscopy and sestaMIBI scanning.

Informed consent was obtained from all individuals in the study and approval was granted by the bio-ethics committee of the Faculty of Health Sciences, Nelson R Mandela School of Medicine, University of KwaZulu-Natal.

Statistical analysis

Data analysis was conducted using SPSS (Statistical Packages for the Social Sciences) software (version 23). A p-value < 0.05 was deemed as statistically significant. A descriptive statistical analysis of the data (means and percentages) was initially conducted prior to inferential analysis. Proportions were used to estimate the prevalence of GORD in subjects with ACS. Difference in the proportions of ischaemia/infarction between study and control groups was analysed using the Pearson chi-squared test as well as determining whether the presence of GORD could trigger ischaemic events. Logistic regression was used to assess the odds of developing ST changes after acid instillation. Means for the groups were compared using one-way analysis of variance, followed by the Tukey *post hoc* test.

Results

A total of 376 patients underwent consecutive endoscopy. The 111 subjects with ACS were admitted to the CCU. They were stable and underwent endoscopy to determine the presence of oesophagitis (Fig. 1). Of these ACS subjects, 39 had grade A reflux oesophagitis and constituted the ACS study group.

Of the 265 patients with dyspepsia, 27 had GORD with grade A reflux oesophagitis. Seven of these subjects had either reversible (ischaemic) or fixed (infarct) changes on the sestaMIBI scan and were excluded, leaving 20 subjects with isolated GORD. None of the controls showed any reversible (ischaemic) or fixed (infarct) changes on the sestaMIBI scan, indicating they were also free of significant coronary artery disease.

There were 30 males and nine females (mean age 52 and 51 years, respectively) in the ACS group. These 39 subjects comprised 35 (89.7%) with ST-elevation myocardial infarction (MI) (45.7% were in the inferior territory, 25.7% anterior and 28.6% lateral) and four (10.3%) subjects with non-ST-elevation MI.

There was no significant difference in the age distribution between ACS subjects and those with isolated GORD. There was no difference in body mass index between the controls and ACS subjects ($p = 0.974$) but waist measurements were lower in the control subjects (ACS vs control $p = 0.003$; GORD vs control $p = 0.002$) (Table 2).

As expected, risk-factor analysis revealed that incidence of diabetes mellitus, hypertension and smoking were more frequent in the ACS group. Control subjects were free of hypertension, hypercholesterolaemia and diabetes. Plasma glucose level was elevated in male subjects in both the ACS and GORD groups, but was normal in the controls ($p < 0.001$). Risk-factor clustering in the form of the metabolic syndrome was present in 17/39 (44%) in the ACS group, 1/20 (5%) in the GORD group and none in the control group.

Table 2. Demographic data and baseline risk-factor profile

	ACS			*GORD*			*Control*			p-*value*		
	F (n = *9*)	*M* (n = *30*)	*Total* (n = *39*)	*F* (n = *15*)	*M* (n = *5*)	*Total* (n = *20*)	*F* (n = *14*)	*M* (n = *8*)	*Total* (n = *22*)	*ACS vs GORD*	*GORD vs control*	*ACS vs control*
Age	52	51	52 ± 9	49	49	49 ± 12	48	44	46 ± 12	0.651	0.002	0.266
Waist (cm)	93.3	93.7	94.0 ± 1.9	94.9	99.5	96.0 ± 1.8	85.7	78.9	83.5 ± 9.1	0.702	0.030	0.002
Waist/hip	0.93	1.0	0.9 ± 0.1	0.9	0.9	0.90	0.8	0.9	0.90	0.037	0.030	< 0.001
BMI (kg/m^2)	26.1	24.2	24.7 ± 4.0	29.3	25.8	28.4 ± 5.5	26.3	21.0	24.4 ± 4.5	0.009	0.014	0.974
Diabetes	6 (15.4)	11 (28.2)	17 (43.6)	1 (5.0)	1 (5.0)	2 (10)	0	0	0	0.001	0.597	0.031
Hypertension	5 (13.0)	12 (30.1)	17 (43.6)	1 (5.0)	0	1 (5)	0	0	0	0.002	0.294	< 0.001
History of smoking	3 (7.7)	17 (43.6)	20 (51.2)	3 (15.0)	3 (15.0)	6 (30.0)	2 (9.0)	3 (13.6)	5 (22.7)	0.006	0.133	< 0.001
SBP (mmHg)	122	123	123 ±15.7	117	123	117 ± 11.7	117	113	120 ± 11.0	0.198	0.661	0.706
DBP (mmHg)	74	74	74 ± 9.3	71	75	65 ± 6.0	70	69	64 ± 5.0	< 0.001	0.917	< 0.001
Plasma glucose (mmol/l)	5.8	8.7	7.9 ± 3.4	4.3	5.2	4.5 ± 0.61	4.3	4.3	4.3 ± 0.4	< 0.001	0.972	< 0.001
Serum cholesterol (mmol/l)	6.9	5.0	5.9 ± 2.5	5.1	4.9	5.1 ± 0.9	5.0	4.0	4.3 ± 0.5	0.296	0.325	0.010
Serum HDL-C (mmol/l)	1.0	1.0	0.9 ± 0.2	1.1	1.1	1.1 ± 0.2	1.3	1.6	1.4 ± 0.6	0.068	0.087	< 0.001
Serum LDL-C (mmol/l)	2.6	3.0	3.0 ± 1.2	2.3	2.2	2.2 ± 0.7	2	1.6	1.8 ± 0.5	0.176	0.121	0.028
Serum triglycerides (mmol/l)	2.2	1.7	2.0 ± 0.3	1.4	1.2	1.4 ± 0.2	1.0	1.0	1.0 ± 0.9	0.008	0.119	< 0.001

ACS, acute coronary syndrome; GORD, gastro-oesophageal disease; F, female; M, male; SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol. Figures in brackets denote percentages.

All subjects had grade A oesophagitis in both the ACS and the GORD group. There was no chest pain on acid instillation among the subjects with isolated GORD and the controls. Mild retrosternal chest pain developed in two subjects in the ACS group but this was short lived and did not require nitroglycerin, nor was it associated with ECG changes.

During acid installation, ECG recording showed 8/39 (20.5%) subjects in the ACS group developed ST depression indicative of ischaemia, compared to 5/20 (25%) patients with GORD, and none of the controls (Fig. 2). There was no difference in the prevalence of ST depression occurring with acid instillation (Table 3) in the ACS compared to the GORD patients (p = 0.958). The odds ratio of developing ST changes after acid installation in ACS compared to GORD patients was 1.06 (95% CI: 0.3166–3.5411, p = 0.9261).

Baseline characteristics were similar in subjects with and without ST depression. Significant ventricular arrhythmias (Lown grade 4) were recorded in one subject in the ACS group during acid instillation compared to none in the GORD subjects (Table 4) (p = 0.001). This patient did not have ST changes indicative of ischaemia.

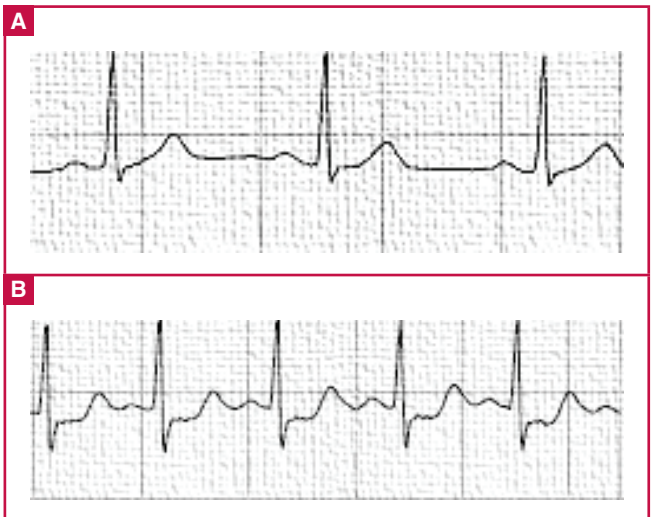

Fig. 2. ST changes induced by acid instillation in an ACS patient. Normal baseline ECG (A) followed by horizontal ST depression (B). There was no accompanying chest pain.

Discussion

It is estimated that about 30% of subjects undergoing coronary angiography in the USA have normal epicardial coronary arteries and in these individuals, oesophageal diseases may account for the symptoms in 18 to 56%.[14] Chest pain arising from the oesophagus may be indistinguishable from angina pectoris, since patients may present with similar symptoms and only subtle differences.

The clinical history does not always enable a physician to distinguish accurately between cardiac and oesophageal causes of chest pain. Symptoms suggestive of cardiac ischaemia are typically effort-induced pain that radiates to the arm and is

Table 3. ST changes after acid instillation

ST depression	*ACS* (*n* = *39*)	*GORD* (*n* = *20*)	*Control* (*n* = *22*)	*ACS vs GORD p-value*
Female, *n* (%)	3 (7.7)	4 (20)	0	
Male, *n* (%)	5 (12.8)	1 (5)	0	
Total, *n* (%)	8 (20.5)	5 (25)	0	0.958

Compared to the controls, ST changes were more frequently recorded in ACS (p = 0.010) and GORD (p = 0.014) subjects. No differences were observed in ST changes between ACS and GORD (p = 0.958) subjects.
ACS, acute coronary syndrome; GORD, gastro-oesopheageal reflux disease.

Table 4. Prevalence of ventricular arrhythmias (Lown grade)

Grade	*ACS* (n = *39*)	*GORD* (n = *20*)	*Control* (n = *22*)	p-*value*
0, *n* (%)	11/39 (28.2)	15/20 (75)	14/22 (64)	
1, *n* (%)	21/39 (53.8)	4/20 (20)	8/22) (36)	
2, *n* (%)	3/39 (7.7)	1/20 (5)	0	
3, *n* (%)	3/39 (7.7)	0	0	< 0.001
4, *n* (%)	1/39 (2.6)	0	0	0.001

Significant Lown grade arrhythmias. Lown 3 ($p < 0.001$) and Lown 4 ($p = 0.001$) were more frequent in ACS compared to GORD subjects and controls after acid instillation.
ACS, acute coronary syndrome; GORD, gastro-oesopheageal reflux disease.

relieved by rest and nitroglycerin. However, it is documented that relief with sublingual nitrogycerin is not limited to a pain of coronary origin,[15] making it difficult for the clinician to distinguish between the two conditions when symptoms overlap. While it may complicate symptomatology in subjects with acute coronary syndrome,[16] there is increasing evidence that GORD may also precipitate cardiovascular events,[17] particularly since both conditions share the same nerve plexuses for their innervation.[18]

The problem is compounded by the fact that gastro-oesophageal reflux may occur during exercise and cause exertional chest pain that mimics angina pectoris. This phenomenon has been described even during treadmill testing.[19] Since the prevalence of cardiac and oesophageal disease increases as patients age, these ailments may co-exist and interact to precipitate ischaemia. It has been shown that as many as 50% of patients with cardiac pain have symptoms of oesophageal disease.[20]

This study showed a 35.1% prevalence of erosive GORD in patients with recent ACS, which is in keeping with other studies that have shown prevalence rates varying from 39 to 53%.[21-25] Only one study by Battaglia *et al*. has yielded a much lower prevalence of 15%, the reasons for which are not clear, possibly due to a small study sample.[26]

Studies using pH monitoring have documented consistently similar prevalence rates for GORD in patients with IHD.[22,24,27] In a study of 51 patients with coronary artery disease, Rosztóczy *et al*. reported a 45% prevalence of GORD using pH monitoring and manometry.[27] Similarly, Svensson *et al*. found a prevalence of 42% on manometry.[25] Myocardial perfusion imaging and oesophageal scintigraphy have also shown a 39% prevalence of both oesophageal dysfunction and IHD.[23] Whatever the modality used to detect GORD, it is apparent that at least one-third of subjects with IHD have concomitant GORD.

In this study we determined whether GORD could precipitate ischaemia and used acid instillation during EGD as a surrogate for acid reflux.[24] In our study, 20.5% of patients with ACS + GORD developed ST depression on ECG monitoring shortly (within five minutes) after acid instillation. Two studies similar to ours have observed that eight[17] and 27%[27] of subjects with GORD and co-existing IHD developed ST changes after acid installation. In our study, we documented similar ST changes in the group with isolated GORD who did not have coronary disease, as demonstrated on sestaMIBI scanning, and were therefore unable to conclude that these changes were indicative of ischaemia.

Lam *et al.* looked at 30 patients with angiographic evidence of IHD admitted to the CCU with angina.[28] Their 24-hour ECG and pH recordings showed that chest pain was preceded by a drop in pH in only one patient. Based on such findings, Lam *et al.*[28] and Valori[29] are also doubtful of the existence of the link between GORD and ischaemic heart disease. Several authors have suggested that the development of ST changes and the documentation of ischaemia in these subjects during acid installation is probably coincidental,[28-31] whereas other researchers have postulated the existence of an oesophago-cardiac reflex resulting in 'linked angina'[11,17,27] precipitated by acid reflux.

The mechanisms for the development of ST changes in subjects with GORD and IHD have not been clearly established and are possibly due to a combination of factors. In Rosztóczy's study of 51 patients, ST changes occurred in patients with epicardial and microvascular disease, as well as in those with a negative cardiological evaluation. This suggests that the infarct territory is not a factor in the development of ST changes.[27] Recently Hui *et al.*[32] suggested that oesophageal pain could result in myocardial ischaemia via an adrenergic stimulus, resulting in increased myocardial oxygen demand. Alternatively, he postulated that oesophageal pain could trigger the oesophago-cardiac reflex, resulting in coronary vasoconstriction and decreased myocardial oxygen supply.

The explanation for ischaemic ST changes developing in our subjects with isolated GORD cannot be explained, since these subjects did not have coronary disease, as demonstrated on sestaMIBI scans. The possibility that the ECG changes could have been induced by the procedure itself is unlikely, since no such ST changes were documented in the normal control group who did not have GORD or IHD.

Chauhan *et al*. proposed a possible mechanism for ST changes developing in subjects without IHD on the basis of microvascular vasoconstriction, as demonstrated by a reduction in coronary blood flow following acid instillation in subjects with syndrome X and normal coronary arteries.[11] They suggested that these microvascular changes could be mediated via the same neural reflex or the release of vasoactive substances following acid installation. Since ST changes on acid installation occurred in subjects with coronary syndrome X but not in transplant recipients (denervated hearts), Chauhan and co-workers concluded that ST changes were due to a viscerocardiac reflex.

A significant finding in our study was the high prevalence of the metabolic syndrome in the ACS patients, and to some extent in the isolated GORD subjects, indicating that a few patients with GORD were also insulin resistant. The association of microvascular disease with syndrome X and the metabolic syndrome[33] might explain the development of ischaemic ST changes via the oesophago-cardiac reflex, which is thought to increase microvascular resistance, potentially resulting in myocardial ischaemia.[11] Microvascular disease is an established cause of myocardial ischaemia[33-35] in the absence of epicardial disease. Although our findings may indicate the presence of underlying microvascular disease in association with the metabolic syndrome,[33] which we documented in both the GORD and ACS subjects,[11] a more plausible explanation could be that the ST changes documented during acid installation were a false-positive finding, unrelated to the presence of ischaemia,[36] or a reflex phenomenon that does not necessarily indicate ischaemia.[11,17,27]

An advantage of our study is that oesophageal assessment was done using fibre-optic endoscopy and only patients with erosive oesophagitis were selected for the study. A limitation of earlier studies was that endoscopy was not performed to assess the oesophageal lesion.[11,37,38] Evidence of erosive oesophagitis

on endoscopy provides macroscopic evidence of oesophagitis that is graded by internationally accepted criteria.[12] According to Saltissi[39] and Rosztóczy *et al.*,[27] ST changes are more likely to develop in a diseased oesophagus (e.g. with GORD) due to pre-sensitisation of pain receptors.

A possible limitation of our study is related to the selection of GORD subjects, all of whom had grade A oesophagitis. It is thought that severe oesophagitis associated with ulceration (grade C onwards), tissue damage and inflammation might[40] have been a more potent trigger[41] stimulating the viscero-cardiac reflex resulting in ST changes. Furthermore, it may also be argued that oesophageal acid instillation is non-physiological, with effects distinctly different from symptoms experienced in oesophageal reflux disease. Single, short episodes of acid installation might not have produced significant ST changes of ischaemia since the acid infusion in our study was for a duration of five minutes. Over a 24-hour period, multiple episodes of reflux might occur, possibly reaching the threshold for triggering ischaemia.[41] Higher rates of acid-induced chest pain (25 and 68%) have been reported in studies by Davies *et al.*[37] and Tougas *et al.*[41] where infusion was for 10 and 20 minutes, respectively, and associated with a reduction in the threshold for angina.

There are other limitations that need to be considered in evaluating the results of this study. The first consideration relates to the sample size. The study recruited 39 subjects with ACS, of whom 14 had reversible ischaemia on sestaMIBI. Since the development of ST changes is more likely to occur in subjects with reversible ischaemia in comparison to subjects with completed infarcts (and no residual ischaemia), a larger sample of subjects with reversible ischaemia would have been more appropriate. Furthermore, ECG monitoring was only conducted for a period of one hour after acid instillation.

This study has clinical implications in the approach to subjects with retrosternal chest pain symptoms. Reliance on the chest pain characteristics is often difficult in subjects with dual pathology (GORD plus IHD), often rendering symptoms atypical. In non-acute subjects, non-invasive testing with sestaMIBI is helpful for the detection of coronary artery disease and requires treatment on an individual basis. The development of ST changes in subjects with recent ACS and a history of GORD is much more imminent and has immediate prognostic implications for the management of significant ischaemia and should be addressed on an individual basis. Such patients need immediate endoscopy to detect and stage GORD in order to evaluate the risk of bleeding in subjects receiving antiplatelet therapy.

In subjects with GORD the presence of cardiovascular risk factors in association with ST changes should alert the clinician to co-existing coronary artery disease. In the absence of epicardial coronary disease, the possibility of microvascular disease should be considered in subjects with the metabolic syndrome and their risk factors addressed. Proton pump inhibitors should be considered as part of the treatment[4,22,32,33,42,43] in patients where myocardial ischaemia co-exists with GORD as they are more prone to cardiovascular events.

Conclusion

We have shown that at least one-third of our ACS patients had concomitant GORD, which was probably related to the high prevalence of obesity in these subjects. While we are unable to conclude that GORD may serve as a trigger for ischaemia in subjects with ACS, the high prevalence of the metabolic syndrome in these subjects suggests the possibility of underlying microvascular disease.

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Sevoflurane- and propofol-based regimens show comparable effect on oxygenation in patients undergoing cardiac valve replacement with cardiopulmonary bypass

Zhen Luo, Xiaozhen Wei, Yunxia Zuo, Guizhi Du

Abstract

Background: Our study aimed to compare the effects of sevoflurane- and propofol-based anaesthetic regimens on oxygenation during the early period of cardiopulmonary bypass (CPB) in patients undergoing cardiac valve-replacement surgery.

Methods: Patients undergoing mechanical mitral, aortic or double valve replacement were enrolled and randomly divided into two groups: the sevoflurane-based anaesthetic regimen group consisted of patients who received 1–3% sevoflurane inhalation during anaesthesia maintenance and the propofol-based anaesthetic regimen group consisted of patients who received 6–10 mg/kg/h of propofol infusion during anaesthesia maintenance. The partial pressure of oxygen/fraction of inspired oxygen (PaO_2/FiO_2), respiratory mechanics and haemodynamics were recorded during CPB.

Results: Forty-two patients met the eligibility criteria for the study. The groups did not differ in terms of clinical and demographic characteristics, and pre- and intra-operative features. Changes in oxygenation were mild (mean PaO_2/FiO_2 from 358 ± 82 to 471 ± 106 mmHg) within one hour of CPB in our patients. There were no differences in PaO_2/FiO_2, respiratory mechanics and haemodynamics between the sevoflurane and propofol groups.

Conclusion: In patients undergoing cardiac valve replacement with CPB, lung injury was mild, and sevoflurane- and propofol-based anaesthetic regimens showed similar effect on oxygenation, respiratory mechanics and haemodynamics during the early stage of CPB.

Keywords: oxygenation, sevoflurane, propofol, cardiac valve replacement, cardiopulmonary bypass

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Department of Anaesthesiology, West China Hospital, Sichuan University, Chengdu, China
Guizhi Du, MD, PhD, du_guizhi@yahoo.com
Yunxia Zuo, MD, 769574913@qq.com
Zhen Luo, MD
Xiaozhen Wei, MD

During cardiopulmonary bypass (CPB), the lung is subjected to ischaemia/reperfusion injury and systemic inflammatory response syndrome.[1] Pulmonary complications with variable severity are common after cardiac surgery with CPB. However, with improvement in membrane oxygenation and the development of extracorporeal circulation, the incidence of lung injury has declined.[2]

Fast-track cardiac care has been advocated in recent years, including a complex intervention of several components of care during cardiac anaesthesia and in the postoperative period. It has been demonstrated that fast-track anaesthetic techniques for cardiac surgery contribute to a shorter intensive care unit (ICU) stay.[3]

Experimental evidence has documented that propofol, a widely used intravenous drug for fast-track anaesthetic regimens, can improve lung function in endotoxin-induced lung injury.[4] Volatile anaesthetics are frequently employed in cardiothoracic surgery, however, the effects of inhalational anaesthetic agents on pulmonary oxygenation remain controversial.

Recent studies have demonstrated that sevoflurane or isoflurane could impair oxygenation in oleic acid-induced lung injury in dogs.[5,6] However, in endotoxin-induced lung injury in rats, sevoflurane improved oxygenation compared to propofol.[7] Furthermore, in thoracic aortic occlusion-induced lung injury in pigs, sevoflurane and propofol showed a similar effect on oxygenation.[8] These results indicate that the effects of sevoflurane on oxygenation vary with different lung injury models.

The purpose of this study was to evaluate the severity of the insult on lung oxygenation and the effects of sevoflurane- and propofol-based anaesthetic regimens on oxygenation during the early stage of CPB in patients undergoing cardiac valve replacement surgery. We hypothesised that lung injury would not be severe and a sevoflurane-based anaesthetic regimen could not impair oxygenation compared to a propofol-based regimen during the early period of CPB.

Methods

This prospective, randomised study was approved by the local institutional ethics committee. Written informed consent was obtained from every patient. The study was conducted according to the Declaration of Helsinki.

Patients undergoing mechanical mitral, aortic or double valve replacement (ASA III) were screened for eligibility. Exclusion criteria included patients with relevant pulmonary disorders such as pulmonary oedema, pneumonia, bronchial asthma, acute respiratory distress syndrome (ARDS), those with pre-operative pulmonary therapy or pre-operative detected pathological lung-function tests [vital capacity (VC), forced expiratory volume in one second (FEV_1) and blood gas analysis], and those with an ejection fraction of less than 30%, significant hepatic disease (alanine

aminotransferase or aspartate aminotransferase > 150 IU/l), renal failure (creatinine > 200 µmol/l), or history of seizure, and stroke.

Patients were randomly divided into two groups using sealed, opaque assignment envelopes as follows: the sevoflurane group, a sevoflurane-based anaesthetic regimen, and the propofol group, a propofol-based anaesthetic regimen. In both groups, anaesthesia was induced with midazolam (0.05 mg/kg) and sufentanil (1 µg/kg). Anaesthesia was maintained with sufentanil (1 µg/kg/h) combined with a continuous intravenous infusion of propofol (6–10 mg/kg/h) in the propofol group, or with 1–3% sevoflurane in the sevoflurane group, based on bispectral index monitoring (maintained at 40–60).

Tracheal intubation was facilitated by administration of 0.15 mg/kg cisatracurium besylate. After endotracheal intubation, patients were mechanically ventilated on a volume-controlled mode with fraction of inspired oxygen (FiO_2) of 0.5, inspiratory:expiratory ratio (I:E) of 1:2, extrinsic positive end-expiratory pressure ($PEEP_e$) of 0 cm H_2O, frequency of 10–12 breaths/min and tidal volume (TV) of 8 ml/kg. To keep arterial blood gases within the physiological range, the respiratory rate (RR) was adjusted with the guidance of end-tidal CO_2 monitoring and intermittent arterial blood gas analyses.

Standard CPB was established with aortic and both vena caval cannulations. The priming solution contained Ringer's lactate solution, 6% HAES–steril (130/0.4), sodium bicarbonate, mannitol and heparin with a target of 24–25% haematocrit.[9] During CPB, systemic hypothermia of 28–30°C and a pump flow of 2.4–2.5 l/min/m^2 were applied. Patients were transferred to ICU after surgery.

Haemodynamics and respiratory mechanics were recorded at baseline, before CPB, at 15 min after declamping, and at five, 30 and 60 min after cessation of CPB. The partial pressure of oxygen/fraction of inspired oxygen (PaO_2/FiO_2) was calculated and recorded at baseline, 15 min after declamping, and at five, 30 and 60 min after cessation of CPB.

Statistical analysis

Results are expressed as mean ± SD. Data of haemodynamics, respiratory mechanics and PaO_2/FiO_2 were analysed using repeated-measures ANOVA. Differences in clinical characteristics and parameters at each time point between the groups were analysed by the independent samples *t*-test for continuous variables or chi-squared test for categorical variables. Statistical analysis was performed with the SPSS software package (version 18; SPSS Inc, Chicago, IL). Significance was assumed at $p < 0.05$.

Table 1. Demographic, pre- and intra-operative data of the patients

Variables	*S group* (n = *21*)	*P group* (n = *21*)	p-*value*
Age (years)	44.6 ± 9.2	45.0 ± 10.4	0.909
Gender (M/F)	15/6	14/7	0.739
Weight (kg)	54.5 ± 6.4	54.9 ± 9.1	0.859
NYHA classification (*n*)			
Class II	5	4	0.707
Class III	16	17	
CPB time (min)	106 ± 28	97 ± 33	0.433
Time of cross-clamping (min)	63 ± 21	58 ± 26	0.340

Data are presented as mean ± standard deviation or number as appropriate. S: sevoflurane; P: propfol; NYHA: New York Heart Association; CPB: cardio-pulmonary bypass.

Table 2. Haemodynamic variables of the study groups

Variables	*Baseline*	*Pre-CPB*	*15 min after declamping*	*5 min post-CPB*	*30 min post-CPB*	*60 min post-CPB*
HR, beats/min						
Sevoflurane	73 ± 24	88 ± 20	93 ± 20	94 ± 19	92 ± 17	87 ± 15
Propofol	73 ± 19	93 ± 17	98 ± 16	101 ± 7	86 ± 13	85 ± 15
MAP, mmHg						
Sevoflurane	71 ± 12	62 ± 9	56 ± 5	63 ± 5	66 ± 8	68 ± 8
Propofol	75 ± 9	58 ± 10	60 ± 8	64 ± 10	69 ± 6	74 ± 6
CVP, mmHg						
Sevoflurane	6.4 ± 6.1	6.6 ± 4.3	5.2 ± 5.0	10.2 ± 3.1	10.1 ± 2.9	9.4 ± 2.2
Propofol	5.0 ± 3.9	6.2 ± 3.2	6.2 ± 3.9	8.7 ± 4.0	9.5 ± 4.0	10.6 ± 3.6

Data are presented as mean ± standard deviation. HR: heart rate; MAP: mean arterial pressure; CVP: central venous pressure; CPB: cardiopulmonary bypass.

Results

Patient clinical and demographic characteristics, and pre- and intra-operative features are listed in Table 1. Forty-two patients were enrolled in the study, with 21 in each group. The sevoflurane group included 15 males and six females weighing 54.5 ± 1.4 kg, with a mean age of 44.6 ± 2.0 years, and the propofol group comprised 14 males and seven females weighing 54.9 ± 2.4 kg, with a mean age of 45.0 ± 2.7 years ($p = 0.739$, 0.859 and 0.909, respectively, by chi-squared test and independent samples *t*-test). With regard to NYHA classification, there were five class II patients in the sevoflurane group and four in the propofol group, and 16 class III patients in the sevoflurane group and 17 in the propofol group ($p = 0.707$ by chi-squared test). The intra-operative characteristics such as CPB time and time of cross-clamping were not different between the two groups ($p = 0.433$ and 0.340, respectively, by independent samples *t*-test).

Changes in haemodynamic variables are shown in Table 2. There were no differences in heart rate (73 ± 24 vs 73 ± 19 beats/min, $p = 0.962$ by independent samples *t*-test), mean arterial pressure (71 ± 12 vs 75 ± 9 mmHg, $p = 0.258$ by independent samples *t*-test) and central venous pressure (CVP) (6.4 ± 6.1 vs 5.0 ± 3.9 mmHg, $p = 0.485$ by independent samples *t*-test) between the sevoflurane and propofol groups at baseline. These haemodynamic variables were similar in both groups before CPB, at 15 min after declamping, and five, 30 and 60 min post-CPB ($p = 0.787$, 0.179, and 0.720, respectively, by repeated-measures ANOVA).

Changes in respiratory mechanics are shown in Table 3. At

Table 3. Mechanical variables of the study groups

Variables	*Baseline*	*Pre-CPB*	*15 min after declamping*	*5 min post-CPB*	*30 min post-CPB*	*60 min post-CPB*
PIP, cm H_2O						
Sevoflurane	16.6 ± 3.3	16.9 ± 3.0	18.9 ± 4.2	17.0 ± 3.8	16.0 ± 2.9	17.0 ± 2.7
Propofol	16.3 ± 2.7	16.6 ± 2.1	16.8 ± 2.2	16.0 ± 2.6	15.5 ± 2.1	16.5 ± 1.9
mPaw, cm H_2O						
Sevoflurane	6.2 ± 1.3	6.2 ± 1.0	6.5 ± 0.8	6.4 ± 1.2	5.7 ± 1.7	6.4 ± 1.6
Propofol	5.8 ± 0.7	5.8 ± 0.5	6.3 ± 0.7	5.9 ± 0.8	5.6 ± 0.5	5.8 ± 0.7
iPEEP, cm H_2O						
Sevoflurane	2.7 ± 0.9	2.6 ± 1.0	2.9 ± 0.9	3.0 ± 0.9	3.0 ± 0.9	3.1 ± 1.0
Propofol	2.5 ± 0.5	2.6 ± 0.5	2.8 ± 0.5	2.5 ± 0.5	2.5 ± 0.5	2.6 ± 0.5
DLC, ml/cm H_2O						
Sevoflurane	39.6 ± 6.6	39.1 ± 4.9	33.6 ± 7.4	40.3 ± 6.0	41.4 ± 4.7	37.7 ± 6.3
Propofol	41.0 ± 7.9	41.7 ± 6.3	41.6 ± 5.4	41.4 ± 5.8	41.2 ± 7.3	38.3 ± 6.9

Data are presented as mean ± standard deviation. TV: tidal volume; PIP: peak inspiratory pressure; mPaw: mean airway pressure; iPEEP: intrinsic positive end-expiratory pressure; DLC: dynamic lung compliance.

baseline, peak inspiratory pressure (PIP), mean airway pressure (mPaw), intrinsic positive end-expiratory pressure (iPEEP) and dynamic lung complicance (DLC) were not different between the sevoflurane and propofol groups (p = 0.795, 0.445, 0.608 and 0.486, respectively, by independent samples t-test). These mechanical variables were similar in each group before CPB, at 15 min after declamping, and five, 30 and 60 min post-CPB (p = 0.625, 0.561, 0.326 and 0.342, respectively, by repeated measures ANOVA).

As shown in Fig. 1, PaO_2/FiO_2 was not different between the sevoflurane and propofol groups at baseline (423 ± 90 vs 459 ± 57 mmHg, p = 0.242 by independent samples t-test). There was also no difference in PaO_2/FiO_2 between the groups at 15 min after declamping (411 ± 125 vs 471 ± 106 mmHg), and five (454 ± 52 vs 454 ± 32 mmHg), 30 (440 ± 76 vs 457 ± 31 mmHg) and 60 min (358 ± 82 vs 360 ± 97 mmHg) post-CPB (p = 0.477 by repeated-measures ANOVA).

Discussion

Our study showed that there were no differences in PaO_2/FiO_2, respiratory mechanics and haemodynamics during CPB in patients undergoing cardiac valve replacement when a sevoflurane- or propofol-based anaesthetic regimen was applied. This is the first investigation to evaluate the difference in oxygenation between an inhaled and intravenous anaesthetic regimen in cardiac surgery with CPB.

The results of this study showed that the oxygenation index of PaO_2/FiO_2 was not significantly decreased (> 400 mmHg at 15 min after declamping, and at five and 30 min post-CPB, and ~ 360 mmHg at 60 min post-CPB) compared with the respective baselines in the sevoflurane- and propofol-based groups, indicating that lung injury was mild during the early period of CPB in our patients undergoing cardiac valve replacement surgery.

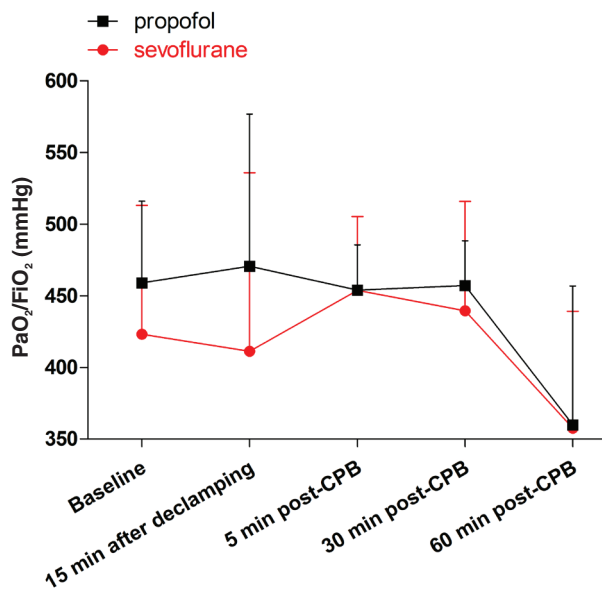

Fig. 1. Changes in PaO_2/FiO_2 at baseline, 15 min after declamping, and five, 30 and 60 min post-CPB. Red indicates the sevoflurane group, black indicates the propofol group (p = 0.477 by repeated-measures ANOVA).

Volatile anaesthetics are frequently employed in cardiothoracic surgery. Early clinical investigations showed during one-lung ventilation (OLV) there was no difference in oxygenation when sevoflurane or propofol was administered in patients undergoing open thoracic surgery.[10,11] This is consistent with our results of a similar effect on oxygenation by sevoflurane- and propofol-based anaesthesia in cardiac valve replacement surgery. However, in another OLV by Cho,[12] desflurane impaired arterial oxygenation compared with propofol anaesthesia in patients with thoracoscopic surgery. The discrepancy regarding the effects on oxygenation by volatile anaesthetics and propofol during OLV in thoracic surgical patients may be ascribed to different volatile anaesthetics (sevoflurane vs desflurane) and thoracic surgical manner (with or without chest opened).

In animal studies, controversy exists regarding the effects of inhalational anaesthetic agents on oxygenation when compared to intravenous anaesthetic propofol. Voigtsberger,[7] Schläpfer[13] and Kellner[14] demonstrated that sevoflurane administration led to a better oxygenation compared to propofol administration in a rat model of lipopolysaccharide (LPS)-induced mild acute lung injury (ALI) (mean PaO_2/FiO_2 ~ 400–500 mmHg after two or three hours of LPS insult). However, in a recent study, the authors found there was no difference in oxygenation between isoflurane- and propofol-based anaesthetic regimens in a dog model of OLV,[15] which is consistent with the finding by Karci *et al.*[16] that sevoflurane and propofol showed comparable effects on PaO_2 in a rat model of OLV.

In our oleic acid-induced canine severe ALI model (mean PaO_2/FiO_2 < 200 mmHg), although the oxygenation was worse in sevoflurane-sedated dogs compared with propofol-sedated dogs during a six-hour mechanical ventilation,[5] possibly via sevoflurane-induced pulmonary vasodilation and its inhibition of hypoxic pulmonary vasoconstriction (HPV),[17] no difference was found in oxygenation between seveflurane and propofol at five and six hours following mechanical ventilation.[5] Different models and subjects may account for literature discrepancies in terms of the effects of sevoflurane compared to propofol on oxygenation in animal experiments.

Our study has limitations. A one-hour observation period after CPB with sevoflurane- or propofol-based cardiac anaesthesia may be too short. Our results reflect only the early time effect on oxygenation by both anaesthetic regimens during CPB. The long-term effect of sevoflurane- or propofol-based anaesthesia on gas exchange deserves further investigation in patients undergoing cardiac surgery with CPB.

Conclusion

In patients undergoing cardiac valve replacement with CPB, the changes in PaO_2/FiO_2 and lung injury were mild, and sevoflurane- or propofol-based anaesthesia showed a similar effect on oxygenation, respiratory mechanics and haemodynamics during the early stage of CPB. Both sevoflurane- and propofol-based regimens can be used in cardiac anaesthesia.

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… continued from page 64

Crucially, the GRS can be measured at any age, including childhood, as DNA does not change. This means that those at high risk can be identified much earlier than is possible through current methods and can be targeted for prevention with lifestyle changes and, where necessary, medicines. The GRS is also a one-time test and with the cost of genotyping to calculate the GRS now less than £40 GBP ($50 USD) it is within the capability of many health services to provide.

Senior author Professor Sir Nilesh Samani, professor of cardiology in the University of Leicester's department of cardiovascular sciences and medical director at the British Heart Foundation said: 'At the moment we assess people for their risk of coronary heart disease in their 40s through NHS health checks. But we know this is imprecise and also that coronary heart disease starts much earlier, several decades before symptoms develop. Therefore if we are going to do true prevention, we need to identify those at increased risk much earlier.

'This study shows that the GRS can now identify such individuals. Applying it could provide a most cost-effective way of preventing the enormous burden of coronary heart disease, by helping doctors select patients who would most benefit from interventions and avoiding unnecessary screening and treatments for those unlikely to benefit.'

Lead author Dr Michael Inouye of the Baker Heart and Diabetes Institute and University of Cambridge said: 'The completion of the first human genome was only 15 years ago. Today, the combination of data science and massive-scale genomic cohorts has now greatly expanded the potential of healthcare.

'While genetics is not destiny for coronary heart disease, advances in genomic prediction have brought the long history of heart disease risk screening to a critical juncture, where we may now be able to predict, plan for, and possibly avoid a disease with substantial morbidity and mortality.'

This study was supported by funding from the British Heart Foundation, National Health and Medical Research Council (NHMRC, Australia), the Victorian Government and the Australian Heart Foundation. It was supported in Leicester by the National Institute for Health Research (NIHR), Leicester Biomedical Centre – a partnership between Leicester's Hospitals, the University of Leicester and Loughborough University.

Source: Medical Brief 2019

An easy method for monitoring patients with pulmonary hypertension: P-wave dispersion

Arif Oguzhan Cimen, Samim Emet

Abstract

Background: Pulmonary arterial hypertension (PAH) is a haemodynamic and pathophysiological condition with restricted flow through the pulmonary arterial circulation. In pulmonary hypertension, right ventricular hypertrophy and diastolic dysfunction can lead to an increase in atrial strain, fibrosis and dilation, which cause inhomogeneous atrial conduction. Interlead variation in P-wave duration is called P-wave dispersion (PwD), which is an electrocardiographic parameter that can be used to predict atrial arrhythmias. Our aim was to investigate the relationship between PwD, functional capacity, and invasive and non-invasive haemodynamic parameters of patients diagnosed with PAH.

Methods: Between 2015 and 2017 we enrolled 33 patients admitted to our in-patient clinic and diagnosed with PAH, and 32 healthy individuals for the control group. Details of these patients at the time of diagnosis were analysed, including gender, age, physical examination, electrocardiogram (ECG), echocardiography, six-minute walk test distance (6MWD), haemodynamic parameters and blood tests for biochemical markers that are correlated with clinical severity. Statistical analyses were performed using SPSS version 20.0 (SPSS Inc, Chicago, Illinois, USA). Statistical significance was taken as $p < 0.05$.

Results: In the forward stepwise multiple linear regression analysis, PwD and mean pulmonary artery pressure determined by right heart catheterisation were independently related to the functional capacity tested by the 6MWD ($p < 0.02$ and $p < 0.01$, respectively).

Conclusion: PwD can easily be calculated from a surface ECG to indirectly estimate the functional status and prognosis of the patient with PAH.

Keywords: P-wave dispersion, functional status, haemodynamic parameters, pulmonary artery hypertension

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Pulmonary arterial hypertension (PAH) is a haemodynamic and pathophysiological condition with restricted flow through the pulmonary arterial circulation, resulting in increased pulmonary vascular resistance (PVR) and ultimately, right-sided heart failure (HF).[1] Pre-capillary PAH is defined as an increase in mean pulmonary arterial pressure (mPAP) ≥ 25 mmHg and PVR > 3.0 Wood units (WU) without significant elevation of the pulmonary capillary wedge pressure (PCWP) (PCWP ≤ 15 mmHg) at rest as assessed by right heart catheterisation (RHC).[2] Right ventricular (RV) function is a major determinant of functional capacity and prognosis in PAH.[3]

In PAH, RV hypertrophy and diastolic dysfunction can lead to an increase in atrial strain, fibrosis and dilation. This is called right heart reverse remodelling (RHRR). RHRR and RV failure are major determinants of symptoms and reduced survival time in PAH.[4] These changes result in inhomogeneous atrial conduction.

Heterogeneity in atrial conduction can be seen as a variation in P-wave duration between differently orientated surface electrocardiogram (ECG) leads. Interlead variation in P-wave duration is called P-wave dispersion (PwD)[5] and is an easily calculated ECG parameter that can be used to predict increased atrial strain caused by RHRR linked to atrial arrhythmias. This can indicate a poor prognosis in PAH patients.[5]

Our aim was to investigate the relationship between PwD, functional capacity, and invasive and non-invasive haemodynamic parameters of patients diagnosed with PAH.

Methods

We enrolled 33 patients (26 females and seven males, mean age of 48.6 ± 2.6 years), who were admitted to our in-patient clinic between 2015 and 2017 and diagnosed with PAH according to the European Society of Cardiology/European Respiratory Society (ESC/ERS) guidelines for the diagnosis of PAH,[2] and 32 healthy individuals for the control group. Written informed consent was obtained. The study complied with the Declaration of Helsinki and the local ethics committee approved trial protocol (number: 78, date: 12.01.2015 and 03).

The inclusion criteria were:

- patients diagnosed with group 1 PAH according to the ESC/ERS guidelines, including idiopathic PAH
- patients diagnosed with group 3 PAH according to the ESC/ERS guidelines, including lung diseases and/or hypoxia
- patients diagnosed with group 4 PAH according to the ESC/ERS guidelines, including chronic thromboembolic pulmonary hypertension and other pulmonary artery obstructions
- patients diagnosed with group 5 PAH according to the ESC/ERS guidelines, including unclear and/or multifactorial mechanisms.

The exclusion criteria were:

- patients with PAH due to left-sided heart diseases (PAH group 2 patients)
- patients with coincident cardiac diseases (hypertension, coronary artery disease, diabetes, renal failure), left bundle branch block, significant arrhythmias, including atrial fibrillation,

Department of Cardiology, Medical Faculty, Bahcesehir University, Istanbul, Turkey
Arif Oguzhan Cimen, MD

Department of Cardiology, Medical Faculty, Istanbul University, Istanbul, Turkey
Samim Emet, MD, samim.emet@istanbul.edu.tr

Wolff–Parkinson–White syndrome, supraventricular tachycardia, atrioventricular block and pacemaker rhythm
- patients with serum electrolyte imbalances and receiving antiarrhythmic drugs were excluded due to their possible effects on ECG measurements.

Details of these patients at the time of diagnosis were analysed, including gender, age, physical examination, echocardiography, six-minute walk test distance (6MWD), haemodynamic parameters and blood tests for biochemical markers that are correlated with clinical severity.

All standard 12-lead ECGs were recorded with a speed of 25 mm/s and a 10-mm/mV gain on the same day before RHC and the diagnosis of PAH. PwD was measured as the difference between the maximum and minimum P-wave duration recorded from multiple different surface ECG leads[6] (Fig. 1). The time in milliseconds was measured on digitised 12-lead ECG recordings using the on-screen digital caliper software Cardio Calipers version 3.3 (Iconico, Inc, New York, NY). Results were taken as the average of two readings. The patients' ECGs were interpreted by a cardiologist who was blinded to their clinical characteristics.

Transthoracic echocardiography (TTE) was performed on a Vivid 7 (GE, Horten, Norway) system with transducer frequencies appropriate to patient size. All quantitative measures were performed by a cardiologist who was blinded to the clinical characteristics of the patients. All measurements were done in accordance with the current guidelines.[7,8] RV and right atrial (RA) end-diastolic transverse dimension were recorded from the apical four-chamber view. Systolic pulmonary artery pressure was calculated from the tricuspid regurgitation Doppler using the modified Bernoulli equation. Tricuspid annular-plane systolic excursion (TAPSE) was measured using M-mode from the lateral tricuspid annulus. All healthy individuals in the control group underwent TTE for the same measurements as the patient group.

RHC, performed for all patients, was determined as elevated systolic pulmonary artery pressure according to echocardiography. All patients were diagnosed by right-sided heart catheterisation according to standard criteria: mPAP ≥ 25 mmHg and PVR > 3 WU at rest in the presence of a normal PCWP (≤ 15 mm Hg). Heart rate and systemic blood pressure were measured just before RHC. RA, RV, pulmonary artery and pulmonary capillary wedge pressures were measured via a catheter passed through a sheath placed in the femoral vein.

Cardiac output (CO) was determined by the Fick method, using oxygen consumption. Cardiac index (CI) was calculated from the formula:

$$\text{CI (l/min/m}^2\text{)} = \frac{\text{CO (l/min)}}{\text{body surface (m}^2\text{)}}$$

PVR was calculated as:

$$\text{PVR (WU)} = \frac{\text{mPAP (mmHg)} - \text{PCWP}}{\text{CO (l/min)}}$$

All parameters were calculated as the mean value of three different measurements, according to current recommendations.[9] After baseline haemodynamics were obtained, vasoreactivity was assessed with either inhaled nitric oxide or iloprost.

Statistical analysis

The research data used to support the findings of this study were supplied by the corresponding author under license and so cannot be made freely available. Requests for access to these data should be made to the corresponding author.

Continuous variables with parametric distribution are expressed as mean ± standard deviation. Categorical data are expressed as frequencies and their differences were analysed using the chi-squared test. Variables were investigated using visual (histograms, probability plots) and analytical methods

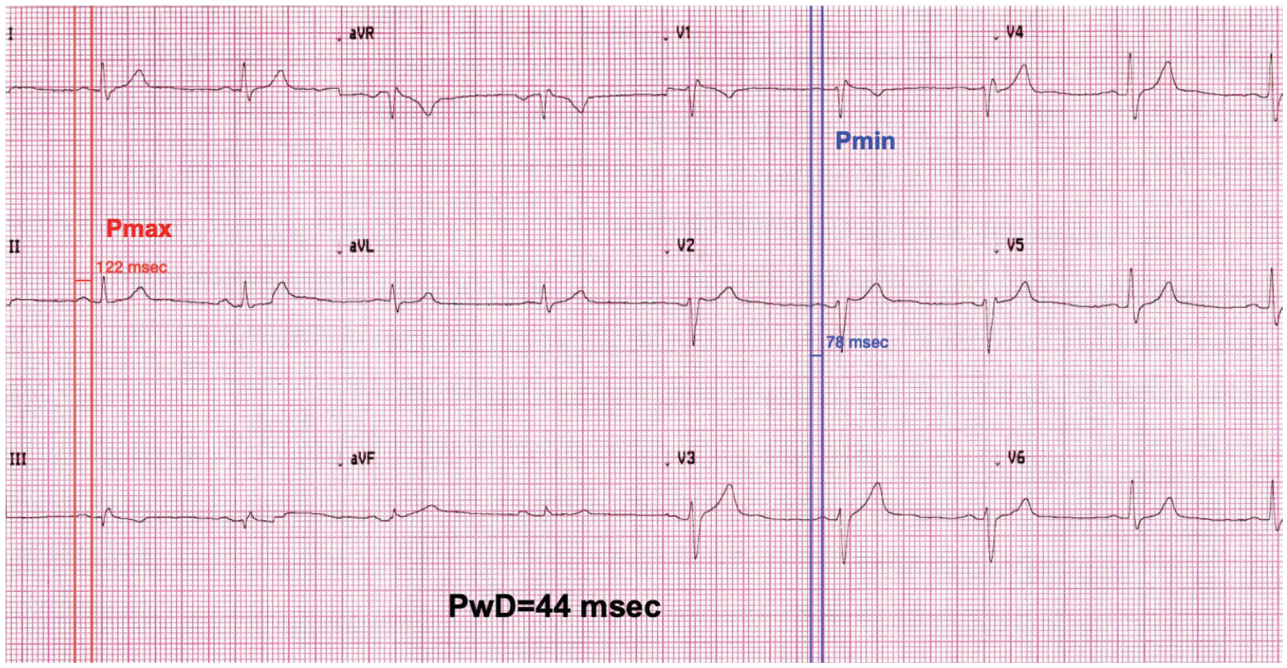

Fig. 1. PwD measurement in a sample ECG. DII derivation showing a maximum P-wave duration of 122 ms and VI derivation showing a minimum P-wave duration of 78 ms. PwD was calculated as 44 ms.

(Kolmogorov–Smirnov/Shapiro–Wilk's test) to determine whether or not they were normally distributed. Parameters that were normally distributed, and correlation coefficients and their significance were calculated using the Pearson's test.

A multiple linear regression model was used to identify independent predictors of the 6MWD for the functional capacity of PAH patients. A 5% type I error level was used to infer statistical significance. Statistical analyses were performed using SPSS version 20.0 (SPSS Inc, Chicago, Illinois, USA). Statistical significance was taken as $p < 0.05$.

Results

A total of 33 patients were enrolled into the study. The baseline clinical, haemodynamic, echocardiographic and ECG parameters of patients are shown in Table 1. The mean age was 47.9 ± 15.08 years, and the female gender was dominant with a rate of 78%.

Systolic PAP on echocardiography was 82 ± 24.57 mmHg, while mPAP determined by RHC, was 52.29 ± 24.33 mmHg. Maximum P-wave duration, calculated by surface ECG, was 112.129 ± 36.03 ms, while minimum P-wave duration was 52.729 ± 16.44 ms. Mean P-wave dispersion was 59.399 ± 28.93 ms. TTE measurements of the control group are also given in Table 1.

The correlations between PwD, mPAP calculated from RHC and other haemodynamic and echocardiographic parameters are shown in Table 2. There was a strong negative correlation between PwD and 6MWD ($r = –0.63$, $p < 0.001$) (Table 2, Fig. 2), and also between mPAP calculated from RHC and 6MWD ($r = –0.79$, $p < 0.001$) (Fig. 3). There was a positive correlation between PwD and mPAP calculated from RHC ($r = 0.34$, $p = 0.04$) (Table 2).

In the multiple linear regression analysis, PwD and mPAP determined by RHC were independently related to functional capacity tested by 6MWD (Tables 3, 4). The multiple linear regression analysis yielded a formula to predict 6MWD:

6MWD = 578.2 (as a constant) – 1.37 × PwD – 3.03 × mPAP by RHC

There was a significant correlation between PwD, RV size and systolic PAP measured by echocardiography in the patient population ($r = 0.51$, $p < 0.01$; $r = 0.36$, $p = 0.04$, respectively) (Table 5). There was no correlation between PwD, TAPSE, and RA and RV size in the control group ($r = –0.22$, $p = 0.22$; $r = –0.09$, $p = 0.60$; $r = –0.30$, $p = 0.09$, respectively) (Table 6).

Discussion

To the best of our knowledge, this study is the first investigating the relationship between PwD, functional capacity and haemodynamic parameters in PAH. PwD and mPAP obtained

Table 1. Baseline demographic, haemodynamic, echocardiographic and electrocardiographic parameters of the patients and control group

Variables	*Min, Max*	*Mean ± SD*
Age (year)	24, 74	47.9 ± 15.08
Cath meanPre (mmHg)	25, 109	52.21± 24.33
Cath sysPre (mmHg)	39, 153	80.12 ± 34.08
Cath diastPre (mmHg)	12, 67	30.75 ± 15.49
6MWD (m)	170, 530	339.39 ± 104.63
sPAP echo (mmHg)	45, 120	82 ± 24.57
TAPSE (mm)	13, 21	16.06 ± 1.93
ProBNP (pg/ml)	99, 3141	791.6 ± 787.8
PVR (Wood)	4.3, 26.7	10.46 ± 5.72
CI (l/min/m^2)	1.7, 2.6	2.15 ± 0.26
RA size (cm)	2.8, 5.4	4.78 ± 5.37
RV size (cm)	2.6, 4.7	3.26 ± 0.53
P_{max} (ms)	80, 200	112.12 ± 36.03
P_{min} (ms)	40, 80	52.72 ± 16.44
PwD (ms)	20, 120	59.39 ± 28.93
P_{max} (control) (ms)	60, 100	77.5 ± 13.19
P_{min} (control) (ms)	40, 80	52.5 ± 17.41
PwD (control) (ms)	0, 40	25 ± 12.44
RV size (control) (cm)	2.2, 2.8	2.46 ± 0.16
RA size (control) (cm)	2.6, 3.3	2.96 ± 0.18
TAPSE (control) (mm)	16, 23	18.87 ± 1.8

Cath meanPre, mean catheter pressure of pulmonary artery; Cath sysPre, systolic catheter pressure of pulmonary artery; Cath diastPre, diastolic catheter pressure of pulmonary artery; 6MWD, six-minute walk distance; sPAP echo, systolic pulmonary arterial pressure measured with echocardiography; TAPSE, tricuspid annular-plane systolic excursion; ProBNP, N-terminal brain natriuretic peptide; PVR, pulmonary vascular resistance; CI, cardiac index; RA, right atrial; RV, right ventricular; P_{max}, maximum P-wave duration; P_{min}, minimum P-wave duration; PwD, P-wave dispersion; P_{max} (control), maximum P-wave duration of the control group; P_{min} control, minimum P-wave duration of the control group; PwD (control), P-wave dispersion of the control group; RV size (control), right ventricular size of the control group; RA size (control), right atrial size of the control group; TAPSE (control), tricuspid annular-plane systolic excursion of the control group.

Table 2. Correlations between the 6MWD, mean catheter pressure of pulmonary artery, P-wave dispersion and other haemodynamic and echocardiographic parameters

	Correlations							
	Cath meanPre	*TAPSE*	*Pro BNP*	*PVR*	*RV size*	*PwD*	*6MWD*	*sPAP echo*
Cath meanPre								
r	1	–0.85	0.67	0.93	0.62	0.34	–0.79	0.85
p		< 0.001	< 0.001	< 0.001	< 0.001	0.04	< 0.001	< 0.001
6MWD								
r	–0.79	0.68	–0.61	–0.63	–0.65	–0.63	1	–0.74
p	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

Cath meanPre, mean catheter pressure of pulmonary artery; 6MWD, six-minute walk distance; TAPSE, tricuspid annular-plane systolic excursion; ProBNP, N-terminal brain natriuretic peptide; PVR, pulmonary vascular resistance; RV, right ventricular; PwD, P-wave dispersion; sPAP echo, systolic pulmonary arterial pressure measured with echocardiography.

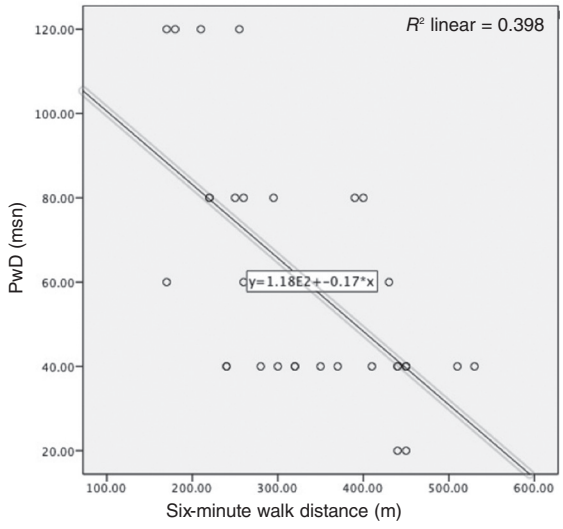

Fig. 2. Correlations between the six-minute walk distance and P-wave dispersion in scatterplots.

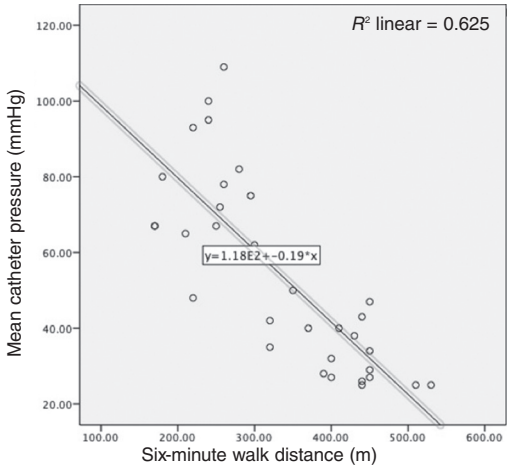

Fig. 3. Correlations between the six-minute walk distance and mean catheter pressure of the pulmonary artery in scatterplots.

Table 3. Multiple linear regression model to identify independent predictors of 6MWD test for functional capacity of PAH patients

		Unstandardised coefficients		*Standardised coefficients*	
Model		*B*	*Std error*	*Beta*	*p-value*
1	(Constant)	694.18	261.15		0.01
	TAPSE	–5.33	9.81	–0.09	0.59
	ProBNP	–0.007	0.02	–0.05	0.73
	PwD	–1.09	0.44	–0.30	0.02
	RV size	–13.72	25.29	–0.07	0.59
	PVR	8.73	6.06	0.41	0.16
	CI	2.61	87.58	0.007	0.97
	sPAP echo	–0.68	0.84	–0.16	0.42
	Age	0.51	0.91	0.07	0.57
	MeanPAPcath	–4.15	1.48	–0.92	0.01
2	(Constant)	699.25	194.06		
	TAPSE	–5.28	9.43	–0.09	
	ProBNP	–0.007	0.02	–0.05	
	PwD	–1.09	0.40	–0.30	
	RV size	–13.54	24.01	–0.06	
	PVR	8.66	5.46	0.41	
	sPAP echo	–0.69	0.80	–0.16	
	Age	0.53	0.78	0.07	
	MeanPAPcath	–4.15	1.45	–0.92	
3	(Constant)	692.31	189.47		
	TAPSE	–4.95	9.21	–0.09	0.59
	PwD	–1.12	0.39	–0.31	< 0.01
	RV size	–13.09	23.54	–0.06	0.58
	PVR	8.12	5.15	0.38	0.12
	sPAP echo	–0.79	0.73	–0.18	0.29
	Age	0.64	0.7	0.09	0.37
	MeanPAPcath	–4.06	1.4	–0.9	< 0.01
4	(Constant)	599	74.61		< 0.001
	PwD	–1.11	0.38	–0.31	< 0.01
	RV size	–13.6	23.18	–0.06	0.56
	PVR	8.11	5.07	0.38	0.12
	sPAP echo	–0.75	0.72	–0.17	0.30
	Age	0.56	0.68	0.07	0.41
	MeanPAPcath	–3.76	1.26	–0.83	< 0.01

MeanPAPcath, mean catheter pressure of pulmonary artery; 6MWD, six-minute walk distance; sPAP echo, systolic pulmonary arterial pressure measured with echocardiography; TAPSE, tricuspid annular-plane systolic excursion; ProBNP, N-terminal brain natriuretic peptide; PVR, pulmonary vascular resistance; CI, cardiac index; RV, right ventricular; PwD, P-wave dispersion.

Table 4. Multiple linear regression model to identify independent predictors of 6MWD test for functional capacity of PAH patients

		Unstandardised coefficients		*Standardised coefficients*	
Model		*B*	*Std error*	*Beta*	*p-value*
5	(Constant)	565.46	47.33		< 0.001
	PwD	–1.2	0.34	–0.33	< 0.01
	PVR	7.88	4.99	0.37	0.12
	sPAP echo	–0.78	0.71	–0.18	0.27
	Age	0.62	0.66	0.08	0.36
	MeanPAPcath	–3.83	1.24	–0.85	< 0.01
6	(Constant)	596.5	33.55		< 0.001
	PwD	–1.16	0.34	–0.32	< 0.01
	PVR	7.79	4.98	0.37	0.129
	sPAP echo	–0.64	0.69	–0.15	0.36
	MeanPAPcath	–4.1	1.2	–0.91	< 0.01
7	(Constant)	574.83	24.06		< 0.001
	PwD	–1.2	0.34	–0.33	< 0.01
	PVR	7.20	4.93	0.03	0.15
	MeanPAPcath	–4.54	1.108	–1.008	< 0.001
8	(Constant)	578.2	24.42		< 0.001
	PwD	–1.37	0.32	–0.38	< 0.001
	MeanPAPcath	–3.03	0.41	–0.67	< 0.001

MeanPAPcath, mean catheter pressure of pulmonary artery; 6MWD, six-minute walk distance; sPAP echo, systolic pulmonary arterial pressure measured with echocardiography; PVR, pulmonary vascular resistance; PwD, P-wave dispersion.

by RHC were found to be independent predictors of functional capacity in PAH patients.

PwD has been used in an extensive list of clinical conditions. P-wave duration and PwD reveal prolongation of the intra- and inter-atrial conduction time and inhomogeneous propagation of sinus impulses, which are well-known electrophysiological characteristics in patients with atrial arrhythmias and especially paroxysmal atrial fibrillation.[5] PwD has been used in the assessment of risk for atrial fibrillation in patients without obvious heart disease, coronary artery disease, hypertension, valvular heart diseases, heart failure, congenital heart diseases, rheumatological diseases and various clinical situations.[5] PwD has been demonstrated to be a sensitive and specific ECG predictor of atrial fibrillation in various clinical settings.[5] In our study, we found a strong correlation between PwD, mPAP and functional status in PAH patients.

Factors reflecting RV function as assessed by RHC, including

Table 5. Correlation between echocardiographic and ECG parameters in the patient population

		TAPSE	RA size	RV size	sPAP echo
PwD	r	–0.31	0.05	0.51	0.36
	p	0.07	0.74	< 0.01	0.04

TAPSE, tricuspid annular-plane systolic excursion; RA, right atrial; RV, right ventricular; sPAP echo, systolic pulmonary arterial pressure measured with echocardiography; PwD, P-wave dispersion.

Table 6. Correlation between echocardiographic and ECG parameters in the control group

		TAPSE	*RA size*	*RV size*
PwD	r	–0.22	–0.09	–0.30
	p	0.22	0.60	0.09

TAPSE, tricuspid annular-plane systolic excursion; RA, right atrial; RV, right ventricular; PwD, P-wave dispersion.

mPAP, cardiac output and cardiac index, have been shown to be significant predictors of survival time in PAH. In addition, a range of other factors that reflect RV structure and function, including echocardiographic parameters, exercise capacity and serum biomarkers, have been shown to be of prognostic value.[10-12] PwD is an easily calculated ECG parameter that has been found to have a strong correlation with the haemodynamic parameters shown to be of prognostic value. Our study also showed that PwD is an independent predictor of functional status in PAH patients.

The 6MWD is currently the only exercise end-point accepted by the Food and Drug Administration and European Agency for the Evaluation of Medicinal Products for studies evaluating treatment effects in PAH.[13] It is a good indicator of prognosis[11] and has been shown to decrease in proportion to the severity of World Health Organisation functional class, and to correlate with cardiac output, total pulmonary resistance[14] and changes in PVR.[15] A 6MWD ≥ 500 m should be the goal of therapy, while a 6MWD ≤ 300 m should prompt intensification of treatment.[13] Since we have a formula, we can easily calculate the 6MWD to estimate the adequacy of treatment and prognosis of the patient.

In the setting of PAH and RV failure, when RV end-diastolic volumes and pressures increase, increased RV wall stress leads to reduced RV stroke volume. Elevated RV end-diastolic volumes also cause tricuspid annular dilatation, which worsens tricuspid valve insufficiency and increases atrial wall tension. Mercurio *et al*. demonstrated that during the course of PAH, atrial fibrillation is a predictor of a poor prognosis.[16] PwD is a good predictor of atrial fibrillation.[5]

Although there has been significant development in our understanding of PAH and its management over the years, there is a need for further studies. In particular, despite its importance, the right ventricle has been less well studied, as have the processes underlying pulmonary vascular remodelling and strategies to modify it. There is still much to learn about the assessment of right heart function, and we have not identified ideal alternate markers for PAH. Catheterisation remains the gold standard, but is invasive and complex. Evidence is accumulating for additional measurements for predicting prognosis of PAH patients. PwD is a more robust method of predicting prognosis in patients and may be a directive to treatment.

Our study has some limitations. One is the small number of patients because of the exclusion criteria of our study. However, it was important for the correct patient selection to exclude compounding factors and strengthen the study. In addition, our study did not include follow up of patients to reveal their prognosis. Finally, the correlations between echocardiography and ECG (P-wave dispersion) were in some cases very low. P-wave dispersion reflects the 'invasive state' of patients.

Conclusion

To the best of our knowledge, our study is the first investigating the relationship between PwD, functional capacity and haemodynamic parameters in PAH. PwD and mPAP obtained by RHC were found to be independent predictors of functional capacity in PAH patients. PwD is easily calculated from surface ECG to indirectly estimate the invasive status and prognosis of patients.

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In Memoriam

Lionel Henry Opie (6 May 1933 – 20 February 2020)

Lionel Henry Opie was born in the small town of Hanover in the Karoo in 1933 and was the only son of Dr William Henry Opie and Mrs Marie Opie.

Inspired by his father's example to study medicine, Lionel graduated in medicine from the University of Cape Town (UCT) in 1955 with 1st class honours and the final year gold medal. Following his internship at Groote Schuur Hospital, he successfully applied for the Rhodes scholarship and read for a DPhil at the University of Oxford from 1957, graduating in 1959 with a dissertation titled *The Physiology of Artificial Respiration*. Immediately after leaving Oxford, he spent two years at Harvard Medical School as the Samuel Levine Fellow in Cardiology, doing research on myocardial metabolism.

He graduated with an MD from UCT in 1961 for his thesis titled *Myocardial Intermediary Metabolism*. Lionel returned to London to undertake further basic science research under the supervision of Nobel prize winners, Professor Sir Hans Krebs (of Krebs cycle fame) and Professor Sir Ernst Chain (who had shared the Nobel prize with Fleming and Florey for the discovery of penicillin).

Following his stint in the laboratory, Lionel was appointed as a consultant in medicine at the Royal Postgraduate Medical School in London in 1969. From 1970 to 1986 he was co-founder and co-editor of the *Journal of Cellular and Molecular Cardiology* with Richard Bing. Later, Lionel would establish two new journals with the help of Carol, his wife.

Lionel returned to Cape Town in 1971 and established his laboratory, working on basic research in ischaemic heart disease and cardioprotection. His initial research funding was made possible through the generous donation by Christiaan Barnard from the proceeds of his best-selling book, *One Life*. In 1976, the South African Medical Research Council awarded Lionel a unit and funded this for 22 years until 1998.

Lionel Opie

As a scientist, Lionel had several key contributions and (1) worked out the key metabolic derangements of carbohydrate and fatty acid metabolism of the ischaemic heart, (2) demonstrated how acute myocardial infarction induces acute adrenergic stimulation, which increases circulating free fatty acids, further damaging the heart and inhibiting glucose uptake, and (3) determined the role of beta-blockers in treating acute coronary syndromes. These concepts had significant clinical implications, and the treatment of acute coronary syndromes with beta-blockers is now routine therapy worldwide and has saved millions of lives.

Division of Cardiology, Department of Medicine, University of Cape Town and Groote Schuur Hospital; Hatter Institute for Cardiovascular Research in Africa, Faculty of Health Sciences, University of Cape Town; Cape Universities Body Imaging Centre, Faculty of Health Sciences, University of Cape Town, Cape Town, South Africa
Ntobeko AB Ntusi, MB ChB, DPhil, MD, ntobeko.ntusi@uct.ac.za

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Rooibos (*Aspalathus linearis*) protects against nicotine-induced vascular injury and oxidative stress in Wistar rats

Michelle Smit-van Schalkwyk, Shantal Windvogel, Hans Strijdom

Abstract

Background: Rooibos (*Aspalathus linearis*) is an indigenous South African plant, traditionally used by the local population as a remedy against several ailments. More recently, rooibos was shown to exhibit potent antioxidant properties, attributed to its polyphenols. We assessed whether treatment with fermented rooibos (RF), unfermented rooibos (RUF) and melatonin (Mel), a well-documented antioxidant included for comparison, could counter the harmful vascular and pro-oxidant effects of nicotine.

Methods: Vascular function, antioxidant enzyme activity and lipid peroxidation were assessed in male adult rats treated with nicotine (5 mg/kg body weight/day) and 2% RF, 2% RUF or 4% Mel co-administration. Nitric oxide (NO) production and cell viability were measured in nicotine-exposed rat aortic endothelial cells (AECs) pre-treated with RF (0.015 mg/ml).

Results: Vascular studies showed that co-administration with RF or Mel exerted anti-contractile and pro-relaxation responses in aortic rings, and increased hepatic superoxide dismutase and catalase activity in nicotine-exposed animals. Co-treatment with Mel additionally decreased lipid peroxidation in nicotine-exposed rats. RUF exerted anti-contractile responses in aortic rings of nicotine-treated animals, while in nicotine-exposed AECs, RF pre-treatment increased intracellular NO levels.

Conclusion: For the first time, we have shown that rooibos co-treatment exerted beneficial vascular effects in nicotine-exposed rats, and that this was associated with increased antioxidant enzyme activity.

Keywords: nicotine, *Aspalathus linearis*, rooibos, melatonin, endothelial dysfunction

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Tobacco smoking is one of the most important risk factors for the development of cardiovascular disease and is responsible for approximately 12% (6.2 million) of all deaths globally.[1] It is estimated that over five million people are current or ex tobacco users and that over 600 000 non-smokers die from exposure to second-hand smoke.[2] Nicotine, the addictive substance in tobacco, is associated with the development of endothelial dysfunction (ED) through oxidative stress. ED is an early, reversible precursor of atherosclerosis.[3] In turn, atherosclerosis is the underlying pathology for many cardiovascular diseases, often resulting in myocardial infarction and stroke.[4]

Nitric oxide (NO) plays an important role in protection against the onset and progression of cardiovascular disease. The ability of the endothelium to synthesise and release NO is essential in regulating haemostasis, vessel tone, blood pressure and vascular remodelling.[5] Furthermore, reactive oxygen species (ROS) and the resultant oxidative stress are important mediators of the pathological manifestations of ED. ROS reduce or eliminate the protective abilities of NO, which in turn could lead to ED.[6]

Experimental and clinical data indicate that exposure to nicotine increases oxidative stress and has the potential to induce ED.[7] While endogenous mechanisms such as antioxidant enzymes as well as non-enzymatic defences exist to combat the deleterious effects of oxidative stress, they might not offer sufficient protection against the ROS produced during nicotine exposure.[8,9]

Early endothelial changes such as ED are reversible,[10] rendering it clinically relevant to identify possible treatment modalities such as anti-oxidant therapy, which could counter the harmful effects of increased ROS production, and hence restore the release of endothelioprotective NO. Protecting the endothelium will result in reduced or delayed atherogenesis, which lowers the risk of cardiovascular mortality. Such therapies may include dietary supplementation with natural plant products or chemically synthesised versions of endogenous molecules with known antioxidant capacity.

Rooibos (*Aspalathus linearis*) is an indigenous South African plant that is popularly consumed as a beverage and is known to possess bio-active properties.[11] Rooibos boasts a unique flavonoid content and contains various dihydrochalcones, including aspalathin, a C-linked dihydrochalcone glucoside,[12,13] and aspalanin, a cyclic dihydrochalcone,[14] which are both unique to *Aspalathus linearis*.

Both unfermented (green) and fermented (red) forms of rooibos are commercially available. Green rooibos is immediately dried after the cutting phase, whereas non-enzymatic oxidative degradation of aspalathin results in the characteristic red-brown fermented form.[15] Rooibos has been shown to exert potent anti-oxidant, immune-modulating and chemo-protective actions, with the additional benefit of having minimal adverse effects.[16] In addition, rooibos possesses cardioprotective properties, including the improvement of dyslipidaemia and redox status in human study participants,[17] as well as being able to exert protective effects on cultured cardiomyocytes from diabetic rats.[18]

Centre for Cardio-metabolic Research in Africa, Division of Medical Physiology, Biomedical Sciences, Faculty of Medicine and Health Sciences, Stellenbosch University, Tygerberg, South Africa
Michelle Smit-van Schalkwyk, PhD, michelle.smitvanschalkwyk@gmail.com
Shantal Windvogel, PhD
Hans Strijdom, PhD

A seven-week rooibos treatment protocol was shown to protect against ischaemia/reperfusion injury in isolated perfused rat hearts.[19] Furthermore, in a rat model of chronic rooibos consumption, no adverse effects were found.[20] However, despite these promising results, studies into the cardioprotective effects of rooibos, both fermented and unfermented, remain limited, with investigations into the effects of rooibos on the vascular endothelium, in particular, lacking.

In addition to controlling circadian rhythms, the hormone melatonin has been shown to be a versatile biological signalling molecule,[21] involved in many physiological processes in humans and animals, including blood pressure control and the scavenging of free radicals.[22,23] In addition to the pineal gland, melatonin is secreted from a variety of organs (regarded as non-endocrine organs) and tissues, including the retina, Harder's glands, gastroenteric mucous membrane, megakaryocytes, platelets, lymphocytes, bone marrow and the skin, but at lower and varying rates.[24,25] Under experimental conditions, chronic melatonin administration was demonstrated to be cardioprotective, which can be attributed to its free-radical scavenging and antioxidant properties.[26,27] Melatonin has also been suggested to be atheroprotective and may slow the progression of atherosclerotic development.[28] Melatonin has been shown to act as a vasoconstrictor in the caudal artery and a vasorelaxant in the mesenteric artery and aorta.[25] In addition, melatonin treatment has not been associated with any toxic effects.[29]

In view of the above, this study aimed to address a considerable knowledge gap related to the putative beneficial effects of rooibos on nicotine-induced vascular injury and oxidative stress. It is of particular interest and importance to investigate whether medicinal plants such as rooibos may protect the vascular endothelium by countering the harmful effects of increased ROS production associated with nicotine exposure and restoring the release of NO. Melatonin was included in the study as it is known to be a potent antioxidant and cardioprotective molecule, hence, it served as a control for rooibos.

Methods

Ethics approval was received from Stellenbosch University; project number SU-ACUM12-00041. Experiments were conducted according to the Revised South African National Standard for the Care and Use of Animals for Scientific Purposes (South African Bureau of Standards, SANS 10386, 2008).

A total of 90 adult male Wistar rats, weighing between 220 and 310 g at the start of the study, were housed in the central animal facility of the Faculty of Medicine and Health Sciences, Stellenbosch University. Animals were housed at room temperature (23°C ± 2°C) under normal 12-hour light and 12-hour dark cycles with free access to rat chow and fluids, and allowed to adapt to laboratory conditions for seven days prior to the start of treatment. Animals were randomly assigned to treatment groups of 10 rats per group in order to prevent bias, and individually caged in order to monitor fluid intake. The experimental rats were weighed daily.

Nicotine [(-)-nicotine, Sigma-Aldrich, St. Louis, MO, USA] was dissolved in sterile 0.9% physiological saline and injected subcutaneously. Physiological saline (0.9%) served as the vehicle control for nicotine and was also injected subcutaneously. Rooibos (2% fermented and unfermented) was a gift from the Promec Unit of the South African Medical Research Council and was prepared according to a standard laboratory protocol.[30] Rooibos solution served as the drinking fluid in the cages housing the rats assigned to the rooibos experimental groups. Melatonin (Sigma-Aldrich, St. Louis, MO, USA) was dissolved in 1 ml absolute ethanol and then added to the drinking water at a final concentration of 0.05% (v/v) ethanol with melatonin, as previously described.[27] Fresh melatonin preparations were supplied on a daily basis and rat fluid intake was monitored daily to ensure that the correct concentration of melatonin was received. The melatonin solution served as the drinking fluid in the cages housing the rats assigned to the melatonin experimental groups. See Table 1 for the treatment groups, as well as their abbreviations, used in the remainder of the text.

At the end of the six-week treatment period, the rats were fasted overnight and euthanised with an overdose of sodium pentobarbital (160 mg/kg) by means of intra-peritoneal injection. Blood was collected and allowed to clot on ice for 30 minutes, after which it was centrifuged at 1 200 g for 10 minutes at 4°C and the serum was aspirated. Liver tissue was excised, rinsed in saline solution, blotted dry and snap frozen in liquid nitrogen. Serum and snap-frozen liver tissue were then stored at –80°C for subsequent analysis. The aorta was excised and immediately used for vascular contraction/relaxation studies.

Biochemical analysis of rooibos

The soluble solid content of the rooibos preparation was determined gravimetrically (six repetitions each) after drying 1-ml aliquots at 70°C for 24 hours, and these were subsequently placed in a desiccator for 24 hours. Total polyphenol content and analysis for known flavonoid compounds were determined by the ARC Infruitec-Nietvoorbij, Post-Harvest Wine Technology Division, Stellenbosch, South Africa. The total polyphenol content was determined using the Folin-Ciocalteu's phenol reagent, as described by Arthur *et al*.[31] The absorbance was read at 765 nm and expressed as mg gallic equivalents per mg soluble solids.

Analysis for known flavonoid compounds was determined according to an established HPLC method.[32] Flavonol content was spectrophotometrically determined using a Spectronic® 20 GenesysTM photospectrometer (Spectronic Instruments, Leeds, UK) at 360 nm, according to a standard protocol,[33] utilising quercetin as standard. Both quercetin and rooibos were diluted in 95% ethanol.

Table 1. Treatment groups and abbreviations

Group	*Abbreviation*
Control groups (n = 10/group)	
Saline vehicle control	Veh control
Drinking control (tap water)	Water control
Melatonin 4 mg/kg bw/day	Mel
2% fermented rooibos	RF
2% unfermented rooibos	RUF
Treatment groups (n = 10/group)	
Nicotine 5 mg/kg bw/day	Nicotine
Nicotine 5 mg/kg bw/day + melatonin 4 mg/kg bw/day	NMel
Nicotine 5 mg/kg bw/day + 2% fermented rooibos	NRF
Nicotine 5 mg/kg bw/day + 2% unfermented rooibos	NRUF

bw: body weight.

Flavanol content was determined at 640 nm using a Spectronic® 20 GenesysTM photospectrometer (Spectronic Instruments, Leeds, UK) according to a standard protocol,[33] using the 4-(dimethylamino)-cinnamaldehyde (DAC) reaction. DAC and rooibos were dissolved in HCl-MeOH (1:3). Catechin was dissolved in methanol to prepare a 0.05% solution and this served as standard for the flavanol determinations. For both flavonol and flavanol determinations, the optimal dilution factor of rooibos was determined and subsequent analysis was performed in triplicate.[33]

Ex vivo investigations: aortic ring isometric tension studies

The thoracic aorta was excised and immediately placed in ice-cold Krebs Henseleit buffer (KHB, composition in mM: NaCl 119, $NaHCO_3$ 25, KCl 4.75, KH_2PO_4 1.2, $MgSO_4.7H_2O$ 0.6, Na_2SO_4 0.6, $CaCl_2.H_2O$ 1.25 and glucose 10). All connective tissue and perivascular fat were removed and the aorta was cut into 3–4-mm segments and mounted in a 25-ml organ bath containing oxygenated (95% O_2 and 5% CO_2) KHB and maintained at 37°C. The rings were equilibrated for 30 minutes under a resting tension of 1.5 g. The tension (in grams of tension) of the aortic ring was recorded with an isometric force transducer (TRI202PAD, Panlab, ICornellà, BCN, Spain) and the data were analysed with LabChart 7 software (Dunedin, New Zealand).

Following the initial equilibration period, aortic rings were exposed to a first round of contraction (100 nM phenylephrine; Phe) (Sigma-Aldrich, St Louis, MO, USA) and relaxation (10 µM acetylcholine; ACh) (Sigma-Aldrich, St Louis, MO, USA) in order to establish the functionality of the endothelium. Following wash-out of the Phe and ACh, the aortic rings were equilibrated for a further 30 minutes.

The contractile response of the aortic rings was determined at cumulative concentrations of Phe (100 nM – 1 µM). After each addition of Phe, a plateau response was reached before the addition of the next dose. At the end of the plateau phase of the final Phe concentration (1 µM), the rings were subjected to cumulative concentrations of ACh (30 nM – 10 µM) to induce relaxation. The final concentration of ACh resulted in maximum percentage relaxation of contraction and was the endpoint of the experiment. The relaxation responses to ACh were expressed as a percentage of the contraction caused by the final Phe concentration (1 µM).

Antioxidant enzyme activity

The activities of superoxide dismutase (SOD) and catalase (CAT) were determined in liver tissue. Liver tissue homogenates were prepared in phosphate buffer-containing microcentrifuge tubes using the Bullet Blender 24 and 0.5-mm zirconium oxide beads (Next Advance, NY, USA). The supernatant was collected after centrifugation at 12 000 rpm for 20 minutes and aliquots were stored at –80°C until the day of analysis.

SOD activity was determined using a commercially available superoxide dismutase assay kit (Cayman Chemical Company, Ann Arbor, MI, USA), which measured total SOD (Cu/Zn and Mn) of mammalian tissue. One unit (U) of SOD was defined as the amount of enzyme needed to exhibit 50% dismutation of the superoxide free radical. Tetrazolium salt was used for the detection of superoxide radicals, and bovine erythrocyte SOD (Cu/Zn) served as standard.

The protocol by Ellerby and Bredesen was adapted for use in a 96-well plate to determine CAT activity.[34] In a 96-well clear UV plate, 5 µl diluted sample and 170 µl buffer (50 mM potassium phosphate, pH 7.0) were added, where after 0.1% H_2O_2 was added to initiate the reaction. The linear decline in absorbance was monitored every 30 seconds at 240 nm for five minutes in a FLUOstar Omega Microplate Reader (BMG Labtech, Offenburg, Germany). CAT activity (µmole/min/µg protein) was determined using the molar extinction coefficient of 43.6/M/cm.

Lipid peroxidation

Thiobarbituric acid reactive substances (TBARS) were measured by spectrophotometric methods using a Labsystems multiscan MS analyser (AEC Amersham Co, South Africa) according to a method described previously.[34] Serum samples (200 µl) were mixed with 10 µl butylated hydroxytoluene (BHT) (Fluka Chemie, Switzerland) in ethanol (85%) (Merck Chemicals, South Africa) and orthophosphoric acid (15 mol/l) (Sigma-Aldrich, St Louis, MO, USA) buffer at pH 3.6 and vortexed. Thiobarbituric acid (TBA) (Sigma-Aldrich, St Louis, MO, USA) reagent (25 µl) was added and vortexed again. After incubation at 90°C for 45 minutes in a water bath, the reaction was terminated by placing the tubes on ice.

TBARS were extracted with n-butanol, saturated NaCl (50 µl) was added and the mixture was centrifuged at 12 000 rpm for one minute. Absorbance was read at 532 nm and values were expressed in µmol/l of serum.

Supplementary *in vitro* investigations

Adult rat aortic endothelial cell (AEC) cultures were purchased from VEC Technologies (Rensselear, New York, USA) and received in culture. Cell cultures were maintained in a standard tissue culture incubator (Forma Series II, Thermo Electron Corporation, Waltham, MA, USA) at an atmospheric composition of 21% O_2, 5% CO_2, 40–60% humidity, and temperature was maintained at 37°C. The endothelial cell growth medium (EGM-2, Clonetics, Cambrex Bio Science, Walkersville, USA) was supplemented with 10% FBS (Highveld Biological, Lyndhurst, South Africa) and standard endothelial growth factors [vascular endothelial growth factor (VEGF), human epidermal growth factor (hEGF), long-chain human insulin-like growth factor (R3-IGF-1), human fibroblastic growth factor (hFGF), hydrocortisone, antibiotics (gentamicin and amphotericin B) and ascorbic acid] according to the manufacturer's instructions. Cells were grown to confluency, as determined by microscopic evaluation and passaging to the next generation was performed in a 1:2 ratio.

Cells grown to confluency were exposed to 100 µM for 24 hours. Nicotine was diluted with phosphate-buffered saline (PBS). RF was freeze dried in a FreeZone6 (Labconco, Kansas City, MO, USA) freeze drier to remove the aqueous fraction. Freeze-dried RF was made up to a 20 mg/ml stock solution in cell culture medium and further diluted in cell culture medium to a concentration of 0.015 mg/ml. Cells were co-treated with nicotine and RF. In all cases, cells were examined for NO production and necrosis.

Table 2. Soluble solid, total polyphenolic, flavonol and flavanol content of 2% fermented and 2% unfermented rooibos

Variables	*2% RF*	*2% RUF*	*NRF*	*NRUF*
Soluble solids (mg/ml)	3.50 ± 0.22	4.60 ± 0.40#		
Total phenolic content (mg gallic acid/mg soluble solids)	0.16 ± 0.01	0.23 ± 0.03#		
Daily total phenolic intake (mg gallic acid equivalents/day/100 g bw)	5.17 ± 0.28	9.43 ± 0.46#	4.86 ± 0.31	8.07 ± 0.26#
Flavonol content (mg quercetin equivalents/mg soluble solids)	0.36 ± 0.02@	0.18 ± 0.02		
Daily flavonol intake (mg quercetin equivalents/ day/100 g bw)	1.11 ± 0.06@	0.74 ± 0.04	1.04 ± 0.07@	0.63 ± 0.02
Flavanol content (mg catechin equivalents/mg soluble solids)	0.05 ± 0.00	0.10 ± 0.01#		
Daily flavanol intake (mg catechin equivalents/ day/100 g bw)	0.10 ± 0.01	0.37 ± 0.02#	0.09 ± 0.01	0.32 ± 0.01#

bw: body weight; NRF: nicotine 5 mg/kg bw/day + 2% RF co-treatment; NRUF: nicotine 5 mg/kg bw/day + 2% RUF co-treatment.
#$p < 0.05$ vs 2% RF treatment groups; @$p < 0.05$ vs 2% RUF treatment groups; $n = 5–6$.

Flow cytometric analysis: NO production was measured by 4,5-diaminofluorescin-2 diacetate (DAF-2/DA) fluorescence (Calbiochem, San Diego, CA, USA) according to a previously established protocol.[36,37] Diethylamine NONOate diethylammonium salt (DEA/NO) served as positive control. Propidium iodide (PI, Sigma-Aldrich, St Louis, MO, USA) was used to determine necrosis,[38] and osmotic stress-induced cell injury served as a positive control.

Statistical analysis

All data are expressed as mean ± standard error of the mean (SEM). When comparisons between two groups were made, an unpaired *t*-test was performed. For multiple comparisons, the ANOVA (two-way where appropriate), followed by the Bonferroni correction, was applied. A *p*-value < 0.05 was considered significant. All data were analysed using GraphPad Prism® 5 software (GraphPad Software, San Diego, CA, USA). All aortic ring isometric tension data are expressed as the percentage contraction from a resting tension of 1.5 g or percentage relaxation of maximum contraction, respectively. For *in vitro* investigations, controls were adjusted to 100% and values are expressed as a percentage of the controls.

Results

Biochemical analysis of rooibos

RUF had a significantly higher soluble solid content and total polyphenolic content compared to RF, while the daily total phenolic intake of the RUF treatment groups (2% RUF, and 2% RUF and 5 mg/kg bw/day nicotine co-treatment) was also significantly higher than that of the RF treatment groups (2% RF, and 2% RF and 5 mg/kg bw/day nicotine co-treatment) (Table 2).

RF had a significantly higher flavonol content than RUF. The daily flavonol intake of the RF treatment groups was also significantly higher than that of the RUF treatment groups (Table 2), while RUF had a significantly higher flavanol content than RF. The daily flavanol intake of the RUF treatment groups was significantly higher than that of the RF treatment groups (Table 2). Values of known flavonoid compounds, as determined by HPLC analysis, are given in Table 3.

Ex vivo investigations: aortic ring isometric tension studies

The vascular function of all treatment groups was assessed by means of aortic ring isometric tension studies. The experimental protocol consisted of cumulative additions of Phe and ACh to test the functionality of the endothelium. Aortic rings from the nicotine-treated rats showed a significant pro-contractile response to Phe administration when compared to the saline vehicle control (Fig. 1A), with E_{max} values of 131.3 ± 17.33% (nicotine) vs 102.9 ± 4.99% (vehicle control), but Phe had no significant effect on relaxation (Fig. 1B). Aortic rings from Mel-treated rats (E_{max} value of 78.06 ± 7.39%) showed a significant anti-contractile response to Phe administration when compared to the water control, RF and RUF treatment groups (Fig. 2A) (E_{max} values of 110.9 ± 10.64, 112.9 ± 9.67 and 108.3 ± 8.11%, respectively). Aortic rings from Mel, RF and RUF treatment rats (E_{max} values of 86.62 ± 4.5, 70.84 ± 6.91 and 79.94 ± 7.01%, respectively) showed a significant pro-relaxation response to ACh administration when compared to the water control group (E_{max} value of 63.28 ± 4.03%) (Fig. 2B).

Aortic rings from NMel, NRF and NRUF treatment rats (E_{max} values of 84.64 ± 6.67, 109.2 ± 9.87 and 110.2 ± 6.29%, respectively) showed a significant anti-contractile response to Phe administration when compared to the nicotine-treated group (E_{max} value of 131.3 ± 17.33%). Additionally, aortic rings from NMel-treated rats also showed a significant anti-contractile response to Phe administration when compared to the NRF- and NRUF-treated groups (Fig. 3A). Aortic rings from NMel- and NRF-treated rats (E_{max} values of 93.11 ± 3.28 and 89.60 ± 5.96%, respectively) showed a significant pro-relaxation response

Table 3. HPLC quantification of flavonoids in 2% fermented and 2% unfermented rooibos consumed by rats

	2% fermented rooibos		*2% unfermented rooibos*	
Flavonoid compounds	*% of soluble solids*	*Daily intake (mg/100 g bw)*	*% of soluble solids*	*Daily intake (mg/100 g bw)*
Phenylpyruvic acid-2-O-glucoside (PPAG)	0.391 ± 0.03	0.124 ± 0.01	0.361 ± 0.04	0.148 ± 0.01
Aspalathin	0.221 ± 0.01	0.070 ± 0.01	8.907 ± 1.05	3.645 ± 0.18
Nothofagin	0.051 ± 0.01	0.016 ± 0.00	1.311 ± 0.15	0.537 ± 0.03
Isoorientin	0.933 ± 0.06	0.295 ± 0.02	1.478 ± 0.17	0.605 ± 0.03
Orientin	0.842 ± 0.05	0.266 ± 0.01	1.132 ± 0.13	0.463 ± 0.02
Ferulic acid	0.055 ± 0.01	0.017 ± 0.00	not detected	
Quercetin-3-robinobioside	0.562 ± 0.04	0.178 ± 0.01	0.395 ± 0.05	0.162 ± 0.01
Vitexin	0.165 ± 0.01	0.052 ± 0.01	0.168 ± 0.02	0.069 ± 0.01
Hyperoside	0.156 ± 0.01	0.050 ± 0.01	0.066 ± 0.01	0.027 ± 0.01
Rutin	0.047 ± 0.01	0.015 ± 0.00	0.313 ± 0.04	0.128 ± 0.01
Isovitexin	0.168 ± 0.01	0.053 ± 0.01	0.224 ± 0.03	0.091 ± 0.01
Isoquercitrin	0.106 ± 0.01	0.034 ± 0.01	0.099 ± 0.01	0.041 ± 0.01
Luteolin-7-glucoside	0.024 ± 0.01	0.008 ± 0.00	0.031 ± 0.01	0.013 ± 0.00

bw: body weight; $n = 5–6$.

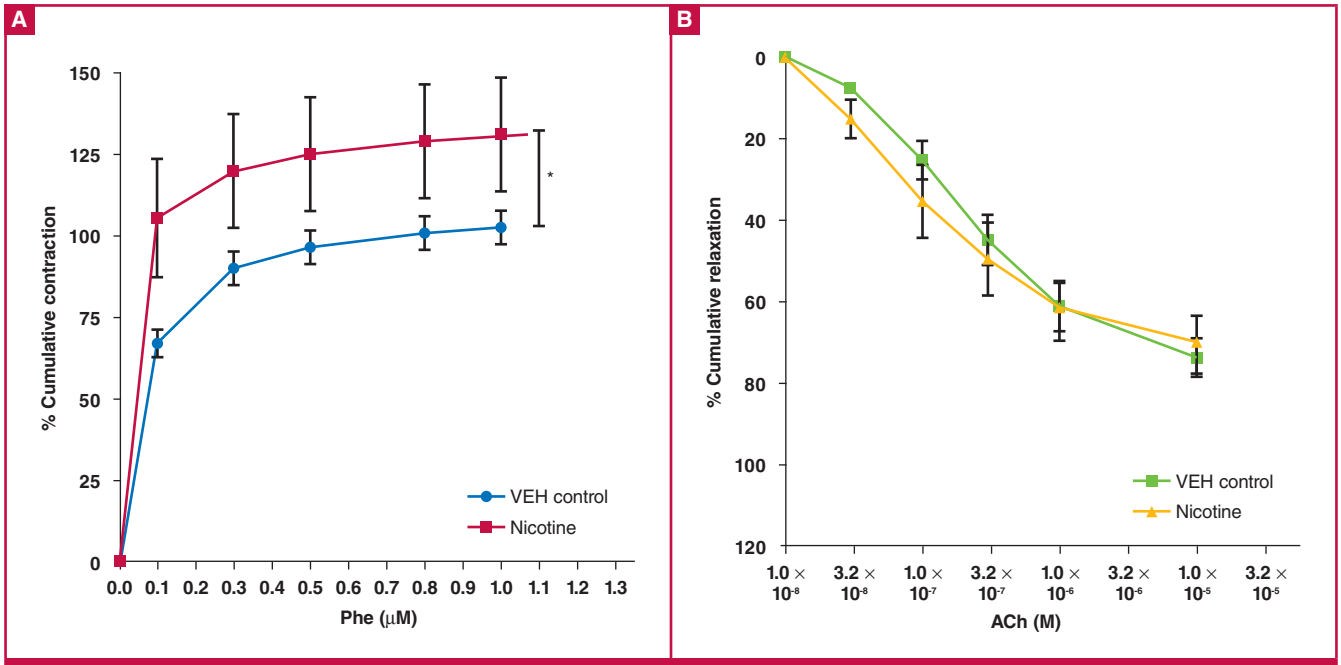

Fig. 1. (A) Contractile responses of aortic rings harvested from the veh control and nicotine-treated rats following cumulative Phe administration (*$p < 0.05$ nicotine vs veh control). (B) relaxation response of aortic rings harvested from veh control and nicotine treated rats following cumulative ACh administration.

to ACh administration when compared to the nicotine- and NRUF-treated groups (E_{max} values of 69.8 ± 6.02 and 70.55 ± 6.49%, respectively) (Fig. 3B).

Antioxidant enzyme activity

Nicotine has a high affinity for the liver[39] and is also metabolised by the liver.[40] It has previously been demonstrated that nicotine treatment resulted in a decrease in SOD[41,42] and CAT[42] activity in the liver, compared to untreated controls. Our results indicate that SOD activity in liver tissue homogenates was significantly increased in the veh control, RF, NMel and NRF groups compared to the nicotine-treated group. SOD activity was also increased in the RF and RUF groups compared to the water control. Additionally, SOD activity in the RF group was increased when compared to the Mel group (Table 4). CAT

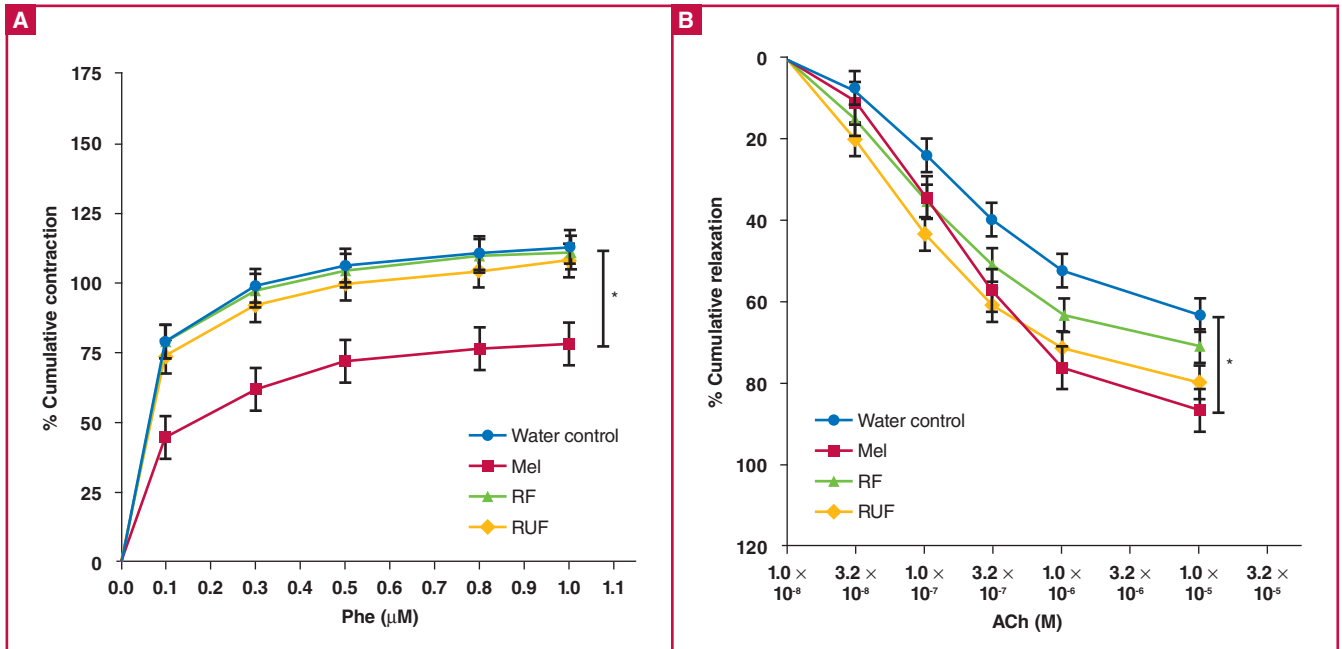

Fig. 2. (A) Contractile responses of aortic rings from Mel, RF, RUF and water control animals following cumulative Phe administration (*$p < 0.05$ Mel vs RF, RUF and water control). (B) Relaxation response of aortic rings harvested from Mel, RF, RUF and water control-treated rats following cumulative ACh administration. (*$p < 0.05$ water control vs Mel, RF and RUF).

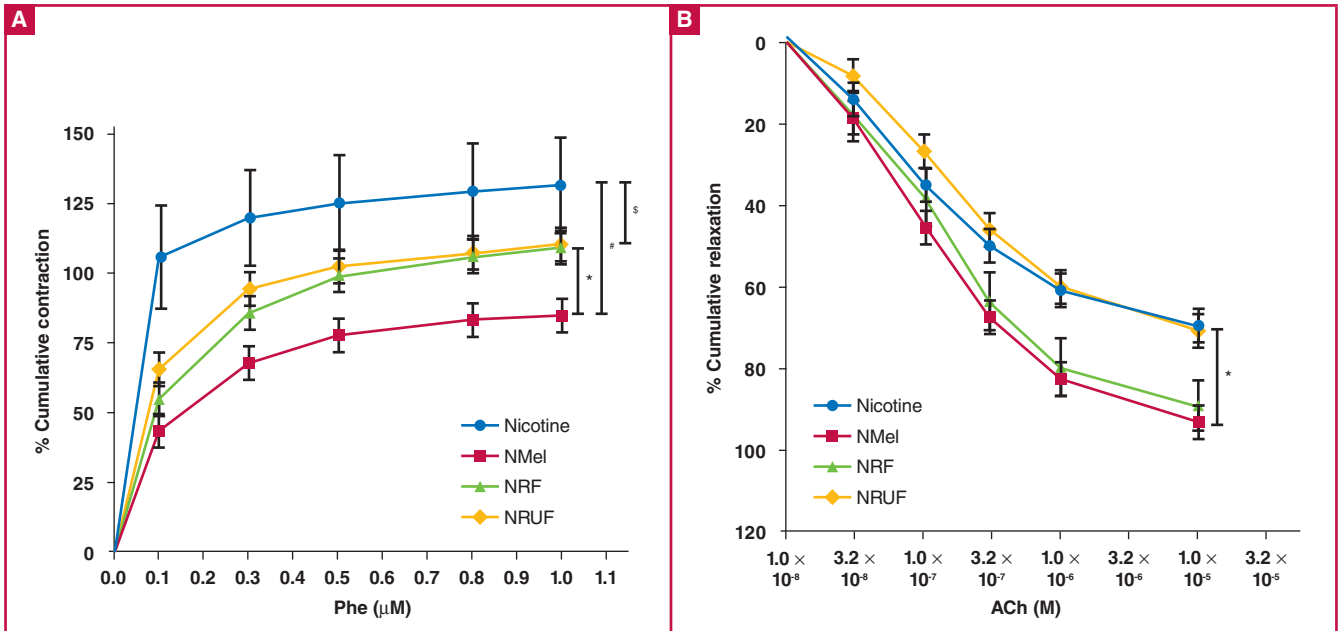

Fig. 3. (A) Contractile responses of aortic rings harvested from nicotine, NMel, NRF and NRUF-treated rats following cumulative Phe administration (*$p < 0.05$ NMel vs NRF and NRUF; #$p < 0.05$ NMel vs nicotine; $$p < 0.05$ NRF, NRUF vs nicotine). (B) Relaxation response of aortic rings harvested from nicotine, NMel, NRF and NRUF-treated rats following cumulative ACh administration (*$p < 0.05$ NMel and NRF vs nicotine and NRUF).

activity in liver tissue homogenates was significantly increased in the veh control, Mel, NMel and NRF groups compared to the nicotine-treated group. CAT activity was also increased in the veh control group compared to the water control (Table 4).

Lipid peroxidation

TBARS levels in serum of the nicotine-treated group were significantly increased when compared to the RF, veh control, water control, RUF, Mel and NMel treatment groups. TBARS levels were also significantly increased in the NRF- and NRUF-treated groups compared to the RF treatment group (Table 5).

Supplementary *in vitro* investigations

Based on the effects of RF on nicotine-induced vascular changes in the *in vivo* investigations, RF was selected for performing additional *in vitro* investigations. According to separate dose–response experiments for the NO production and necrosis investigations (data not shown), nicotine was used at a concentration of 100 μM and RF at a concentration of 0.015 mg/ml. Nicotine at a concentration of 100 μM over a treatment period of 24 hours resulted in significant reduction in NO production, as indicated by DAF-2/DA fluorescence (Fig. 4), and an increase in necrosis, as indicated by PI fluorescence (Fig. 5), when compared to controls.

AECs were pre-treated for one hour with 0.015 mg/ml RF, followed by the addition of 100 μM nicotine for a further 24 hours. Pre-treatment with 0.015 mg/ml RF was associated with a modest but significant increase in NO production in nicotine-injured cells compared to cells treated with nicotine

Table 4. Effects of melatonin and rooibos (fermented and unfermented) treatment on SOD activity (U/mg protein) and CAT activity (μmole/min/μg) in liver tissue homogenates of all treatment groups

Treatment group	*SOD activity (U/mg protein)*	*CAT activity (μmole/min/μg)*
Veh control	155.3 ± 6.7*	868.3 ± 138.4*#
Water control	135.5 ± 5.0	427.2 ± 51.4
Nicotine	121.4 ± 14.7	295.8 ± 76.7
Mel	133.4 ± 10.4	597.7 ± 98.2*
RF	192 ± 21.1*#$	597.5 ± 111.7
RUF	182.7 ± 20.4#	515.6 ± 111.9
NMel	180.8 ± 9.0*	565.8 ± 87*
NRF	160.7 ± 6.4*	727.3 ± 158.6*
NRUF	164.7 ± 16.6	191.9 ± 27.6

Values are mean ± SEM of 9–10 rats per group.
*Significantly different compared to nicotine treatment group ($p < 0.05$); #significantly different compared to water control group ($p < 0.05$); $significantly different compared to Mel treatment group ($p < 0.05$).

Table 5. Effects of melatonin and rooibos treatment on lipid peroxidation in serum of all treatment groups

Treatment groups	*TBARS (μmol MDA equivalents/l)*
Veh control	2.907 ± 0.2
Water control	2.997 ± 0.3
Nicotine	4.615 ± 0.3#$&@!*
Mel	2.829 ± 0.9
RF	2.472 ± 0.3
RUF	3.350 ± 0.3
NMel	3.411 ± 0.3
NRF	3.772 ± 0.3#
NRUF	3.707 ± 0.1#

Values are mean ± SEM of 10 rats per group.
#Significantly different compared to RF ($p < 0.05$); $significantly different compared to veh control ($p < 0.05$); &significantly different compared to water control ($p < 0.05$); @significantly different compared to RUF ($p < 0.05$); !significantly different compared to Mel ($p < 0.05$); *significantly different compared to NMel ($p < 0.05$).

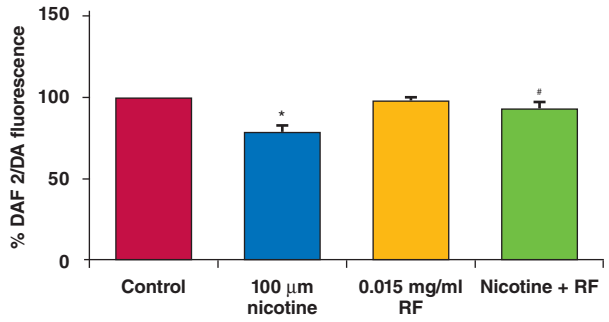

Fig. 4. The effects of RF pre-treatment on NO-production as measured by DAF-2/DA fluorescence. $^{*}p < 0.05$ vs control; $^{\$}p < 0.05$ vs 100 µM nicotine (n = 6– 8 per group).

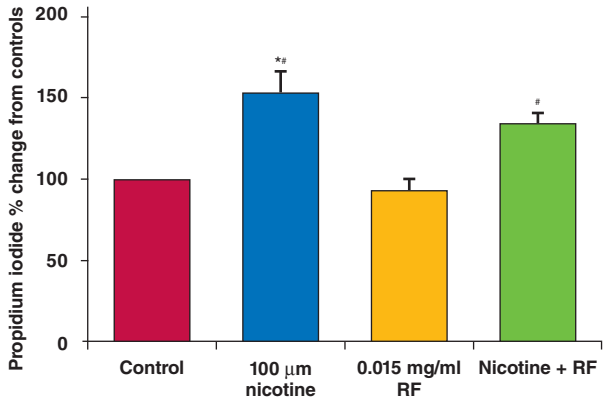

Fig. 5. The effects of RF pre-treatment on cell viability. Percentage change in necrosis indicated by PI fluorescence. $^{*}p < 0.05$ compared to control. $^{\#}p < 0.05$ compared to 0.015 mg/ml RF; (n = 6–8 per group).

only, as indicated by DAF-2/DA fluorescence (Fig. 4). However, pre-treatment with 0.015 mg/ml RF was not able to significantly reduce necrosis in nicotine-injured cells, as indicated by PI fluorescence (Fig. 5).

Discussion

To the best of our knowledge, this is the first study to investigate the effects of rooibos, both fermented and unfermented, in a rat model of nicotine-induced vascular changes and oxidative stress. The protective effects of RF and RUF were compared to the known beneficial effects of the potent antioxidant and free-radical scavenger, melatonin.

Exposure to 5 mg/kg bw/day nicotine over a six-week treatment period resulted in increased vascular contractility in aortic rings and a reduction in antioxidant enzyme activity (SOD and CAT) in liver tissue. Lipid peroxidation, as indicated by TBARS levels, was increased in serum samples of nicotine-exposed rats, therefore indicating that nicotine increases oxidative stress. The harmful vascular endothelial effects of nicotine were further characterised in a model of cultured rat AECs, where nicotine treatment (100 nM; 24 hours) was associated with reduced NO production and reduced cell viability.

In vascular studies, when RF (2%) and melatonin (4 mg/kg bw/day) were co-administered with nicotine, the harmful pro-contractile effects observed in aortic rings from rats treated with nicotine only were attenuated. Additionally, endothelium-dependent vasorelaxation was significantly enhanced in groups co-treated with RF and melatonin. The effects of RUF were limited to reducing contractility in aortic rings of nicotine-treated animals. Furthermore, co-administration of RF and melatonin with nicotine resulted in increased SOD and CAT activity in liver tissue of rats compared to those treated with nicotine only, whereas co-administration of RUF with nicotine did not result in any significant increase in SOD or CAT activity. Co-treatment with melatonin additionally decreased lipid peroxidation. In the nicotine-injured AECs, pre-treatment with RF (0.015 mg/ml) significantly increased NO production.

Nicotine-induced vascular changes and oxidative stress have previously been demonstrated by others. Nicotine exposure resulted in pro-contractile responses in the aortic rings of rats, where aortic rings were challenged with Phe[43] or KCl[44,45] to elicit contractile responses. Furthermore, exposure to nicotine resulted in decreased SOD activity in the liver and increased lipid peroxidation in Sprague-Dawley rats,[41] as well as decreased CAT activity, when compared to untreated controls in Wistar rats.[42] In these studies, oxidative damage, resulting in impaired integrity of the vascular endothelium, was suggested as a possible mechanism of action.[43-45]

It is, to the best of our knowledge, the first time that oral ingestion of RF over a period of six weeks has been demonstrated to improve vascular endothelial function, associated with increased activity of important antioxidant enzymes, in a rat model of nicotine-induced injury. The potential of rooibos to enhance antioxidant defences, including SOD and CAT activity, has previously been demonstrated in rat brain extracts in an immobilisation stress model,[46] while SOD levels were significantly higher in RUF-treated animals in a rat colitis model.[47]

These actions have been attributed to the flavonoid content in rooibos[46] and the potential ability of rooibos to reduce DNA damage caused by oxidative reactions.[47] Epidemiological evidence suggests that dietary-derived antioxidants have the potential for disease prevention,[48] and it has been shown that dietary polyphenols can increase endothelium-dependent NO generation by modulating cellular sensors for oxidative stress. NO is capable of reacting with $O_2^{\cdot-}$ to form peroxynitrite, which can lead to the nuclear accumulation of nuclear factor erythroid 2-related factor (Nrf2).[49] Nrf2 is a redox-sensitive transcription factor, involved in antioxidant response element (*ARE*)-dependent gene expression,[50] and under conditions of oxidative stress, Nrf2 is capable of activating *ARE*-dependent transcription of phase II and antioxidant defence enzymes, such as glutathione-*S*-transferase, GPx and heme-oxygenase-1.[51]

Although the beneficial effects of RF on vascular endothelial function and oxidative stress were comparable to those observed with melatonin, the effects of RUF treatment were more modest and limited to vascular contractility only. The difference in the effects of RF and RUF is particularly interesting, since the phytochemical content of rooibos changes considerably during the fermentation process. The main difference was in the aspalathin and nothofagin contents, which were considerably

higher in RUF. This is consistent with previous findings showing that the amount of aspalathin can decrease by 98% during fermentation.[52]

However, in the present study, the antioxidant and free-radical scavenger ferulic acid was found to be present in RF, but not RUF. Ferulic acid is a potent antioxidant and free-radical scavenger,[53] which also possesses blood pressure-lowering effects.[54] It has also been suggested that ferulic acid has multifactorial vasodilating effects, involving reduction of angiotensin II and activation of eNOS, leading to an increase in NO levels.[55] The presence of ferulic acid could therefore help to explain the modulatory capacity of RF in this experimental setting of nicotine-induced vascular injury.

The modulatory capabilities of melatonin were expected, since melatonin is a known antioxidant and free-radical scavenger and the effects of melatonin to reduce or abolish vascular injury have previously been demonstrated. Our findings support previous data by showing that melatonin was capable of decreasing contraction and enhancing relaxation in the aortas of nicotine-treated animals. The pro-relaxation action of melatonin in aortic ring studies was first demonstrated in the rabbit aorta,[56] and it has been suggested that melatonin could enhance endothelium-dependent vasodilation, which could be explained by the enhancement of the vascular NOS pathway.[57]

A four-week melatonin treatment period has previously been shown to increase SOD activity in liver tissue of nicotine-treated rats,[58] while an eight-week treatment period increased SOD activity in liver tissue in a fructose-induced model of the metabolic syndrome.[59] In a rat model of renovascular hypertension, a nine-week treatment period with melatonin led to an increase in SOD and CAT activity in kidney and heart tissue.[60]

Even though both melatonin and rooibos exerted beneficial effects on the vascular system and increased antioxidant activity in nicotine-exposed rats, it is possible that melatonin and rooibos exert their effects through different mechanisms. It is, however, possible that these mechanisms result in a restoration of vascular homeostasis and, in particular, the function of NO.

The addition of Western blotting analysis of aortic rings could provide more information on the underlying cellular mechanisms of the different treatment groups. Proteins of interest that would add value to our understanding of the underlying mechanisms include eNOS, the main enzyme responsible for vascular production of NO, and protein kinase B (PKB)/AKT, a cell growth and survival protein and upstream activator of eNOS and an important anti-apoptosis protein. Furthermore, investigating the role of p22phox, a marker of NADPH-oxidase activity, which is an important vascular source of ROS and oxidative stress, may also further elucidate the cellular mechanism involved. Proteomic analysis of aortic rings to explore large-scale protein expression patterns and differential protein regulation could greatly contribute to a better understanding and identification of novel cellular pathways and mechanisms involved in vascular injury and protection.

Limitations of the study include the absence of blood pressure measurements in the rodent model, which would have provided clinically relevant data relating to vascular function, and should be considered in future studies. In addition, *in vitro* investigations into the effect of melatonin on nicotine-injured rat AECs would have supplied valuable insights into cellular mechanisms and are worth exploring.

Conclusions

Nicotine administration resulted in significant vascular and endothelial injury, associated with increased oxidative stress and reduced antioxidant activity. In a novel finding, our data showed that rooibos, specifically RF, exerted beneficial effects on the vascular and endothelial system of nicotine-exposed rats, and increased liver antioxidant enzyme activity. The results shown with RF are similar to those observed with melatonin, whose protective actions in the cardiovascular system are well established. However, RUF did not exert beneficial effects to the same extent as RF and melatonin, and was capable of reducing contractility in aortic rings of nicotine-treated animals only.

It is plausible that both RF and Mel exerted their beneficial vascular effects through their antioxidant properties, although other mechanisms cannot be ruled out. Restoration of vascular homeostasis, underscored by eNOS activation and subsequent increased release of NO, as shown in the cultured cell experiments, may also underlie the protective actions of both rooibos and melatonin. Based on the data presented in this study, fermented rooibos may show promise as a future cost-effective therapeutic option on its own or as adjuvant therapy in combatting the harmful effects of nicotine exposure on the vasculature system, endothelium and redox status.

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…continued from page 80

His glucose hypothesis has stood the test of time, and his discovery of the role of excess cyclic AMP in fatal myocardial infarction made UCT famous. His scholarship on myocardial reperfusion proved that insulin can directly protect the myocardium from ischaemia–reperfusion injury and established the concept of preconditioning as a powerful form of cardiac protection.

At the time of his retirement, Lionel had published just under 600 articles in peer-reviewed scientific journals, 31 books on heart disease (including *Drugs for the Heart*, now in its 9th edition, and *Heart Physiology*, now in its 4th edition) and 141 book chapters. The National Research Foundation supported him for 10 years and awarded him an A1 research rating in 2008. In 2003, Lionel entered a research partnership with Professor Derek Yellon of the Hatter Institute at the University College London and established the Hatter Institute at UCT. Together, they had a prolific research collaboration and established the cardiology, diabetes and nephrology 'At The Limits' conferences, with *The Lancet* as a partner.

Lionel is remembered by many as the doyen of cardiovascular medicine and research in Africa and a global leader in the field of cardioprotection. During his illustrious career, he received many awards, including the National Order of Mapungubwe in Silver in 2005, the highest national award in South Africa. He received honorary doctorates from the Universities of Stellenbosch and Copenhagen, and election to fellowship of many prestigious medical societies in this country and globally.

He is survived by wife, Carol, and daughters, Jessica and Amelia, and grandchildren, Liam and Eva.

Impact of metabolic and inflammatory changes on glomerular function beyond conventional risk factors in an urban South Africa community with prevalent obesity

Glenda Norman, Angela J Woodiwiss, Vernice Peterson, Monica Gomes, Pinhas Sareli, Gavin R Norton

Abstract

Objectives: To determine the extent to which metabolic and inflammatory changes are associated with renal damage beyond conventional risk factors in a community sample with a high prevalence of obesity in urban South Africa.

Methods: This was a cross-sectional, community-based study in 1 010 (n = 872 without diabetes mellitus, DM) randomly selected participants over 16 years of age in an urban, developing community (Soweto, Johannesburg) with a high prevalence of obesity (41.8%). We assessed estimated glomerular filtration rate (eGFR), conventional risk factors including adiposity indices, and metabolic changes and plasma resistin concentrations (ELISA) and the homeostasis model of insulin resistance (HOMA-IR). Relationships independent of haemodynamic loads were confirmed using ambulatory blood pressure and central arterial haemodynamics.

Results: In multivariate regression models conducted in those without DM, HOMA-IR (standardised β-coefficient = –0.13 ± 0.03, $p < 0.0001$) and plasma resistin concentrations (β-coefficient = –0.10 ± 0.02, $p < 0.0001$) were second only to age, and at least as strong as systolic blood pressure (β-coefficient = –0.04 ± 0.03, $p = 0.19$) in the impact on eGFR, while alternative conventional risk factors including adiposity indices and the metabolic syndrome features contributed little to eGFR. Similar results were obtained in relationships with chronic kidney disease (CKD) and in the whole group including those with DM. Adjustments for ambulatory blood pressure or central arterial loads did not influence these relationships.

Conclusions: The impact on glomerular function of insulin resistance and inflammatory changes is well beyond modifiable conventional risk factors, including the metabolic syndrome. Targeting conventional risk factors alone is likely to result in a marked residual risk of renal damage produced by insulin resistance and inflammation.

Keywords: chronic kidney disease, resistin, insulin resistance

Cardiovascular Pathophysiology and Genomics Research Unit, School of Physiology, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa

Glenda Norman, PhD
Angela J Woodiwiss, PhD, angela.woodiwiss@wits.ac.za
Vernice Peterson, PhD
Monica Gomes, MS
Pinhas Sareli, MD, FRCP
Gavin R Norton, MD, PhD, gavin.norton@wits.ac.za

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Chronic kidney disease (CKD), as defined by reductions in estimated glomerular filtration rate (eGFR), is in most countries a major public health problem.[1,2] CKD not only progresses to end-stage renal disease, but also predicts cardiovascular events beyond conventional risk factors.[3-8] Although poor control of hypertension and diabetes mellitus accounts for a substantial portion of reductions in eGFR, decreases in eGFR may be attributed to obesity.[9-11] Importantly, several studies have demonstrated relationships between the obesity-associated metabolic change, insulin resistance,[12-15] or increased circulating concentrations of pro-inflammatory adipokines[16-29] and renal dysfunction independent of diabetes mellitus or conventional blood pressure (BP). These effects may be through direct actions on the kidney,[30-32] and as a consequence, the adverse effects of these metabolic and inflammatory changes may not be amenable to preventative strategies by targeting conventional risk factors alone.

Obesity-associated insulin resistance or adipocytokine changes may nevertheless also contribute to the development of type 2 diabetes mellitus or hypertension.[30] Hence the impact of these changes on renal function may not be distinct from these conventional risk factors.[30] Targeting conventional risk factors may therefore have major benefits to preventing metabolic or inflammatory effects on CKD. However, the extent to which insulin resistance and adipocytokine changes compared to modifiable conventional cardiovascular risk factors determine eGFR or CKD is unclear. Therefore, in a large sample of a community with prevalent obesity, we assessed the relative impact on eGFR and CKD of insulin resistance and inflammatory changes compared to that of modifiable conventional risk factors including metabolic syndrome features. To avoid the statistical limitation of performing multiple comparisons with several biomarkers, in this study we focused on the pro-inflammatory adipokine resistin, as of all the adipokines, resistin has demonstrated the most consistent and robust relationships with renal dysfunction.[16,18-26,28,29]

Methods

This study was conducted according to the principles outlined in the Helsinki Declaration. The Committee for Research on Human Subjects of the University of the Witwatersrand approved the protocol (approval numbers: M02-04-72 renewed as M07-04-69, M12-04-108 and M17-04-01). Participants gave informed, written consent.

The study design has previously been described.[33,34] Nuclear families of black African descent with siblings older than 16 years were randomly recruited from the South West Township (SOWETO) of Johannesburg, South Africa. To ensure that relationships noted were independent of haemodynamic factors that could be altered by therapy, 24-hour and aortic BP and carotid–femoral pulse-wave velocity (PWV) and index of aortic stiffness were determined.

Of the 1 010 participants studied, 896 participants had aortic PWV measurements and 688 had 24-hour ambulatory BP measurements that met with pre-specified quality-control criteria (longer than 20 hours and more than 10 and five readings for the computation of day and night means, respectively). Of the 1 010 participants, 872 did not have diabetes mellitus, and 779 of these had aortic PWV measurements and 600 had 24-hour ambulatory BP measurements.

Demographic and clinical data were obtained using a standardised questionnaire.[33,34] Regular tobacco use was defined as daily cigarette smoking, and regular alcohol consumption as one beer a day or a bottle of wine (750 ml) a week or 250 ml of spirits a week. Height, weight, waist circumference (WC) and hip circumference were measured using standard approaches and participants were identified as being overweight if their body mass index (BMI) was ≥ 25 kg/m^2 and obese if their BMI was ≥ 30 kg/m^2. Central obesity was defined as an enlarged WC (≥ 88 cm in women and ≥ 102 cm in men).

Fasting laboratory blood tests of renal function, blood glucose levels, lipid profiles and percentage glycated haemoglobin (HbA_{1c}) (Roche Diagnostics, Mannheim, Germany) were performed. Fasting plasma insulin concentrations were determined from an insulin immulite, solid-phase, two-site chemiluminescent immunometric assay (Diagnostic Products Corporation, Los Angeles, CA, USA) and insulin resistance was estimated by the homeostasis model assessment of insulin resistance (HOMA-IR) using the formula [insulin (μU/ml) × glucose (mmol/l)]/22.5.

Diabetes mellitus (DM) was defined as the use of insulin or oral hypoglycaemic agents or an HbA_{1c} value greater than 6.5%. The metabolic syndrome was defined as a combination of the presence of WC ≥ 88 cm in women and ≥ 102 cm in men, fasting blood glucose ≥ 5.6 mmol/l, triglycerides ≥ 1.7 mmol/l, high-density lipoprotein (HDL) cholesterol < 1.04 mmol/l in men and < 1.30 mmol/l in women, and systolic BP ≥ 130 mmHg or diastolic BP ≥ 85 mmHg or treatment for hypertension.

Nurse-derived conventional BP was measured after 10 minutes of rest in the seated position, as previously described,[33,34] within a half hour of obtaining blood samples and in the opposite arm to that subjected to venesection. These measurements were performed to an accuracy of 2 mmHg by a trained nurse using a mercury sphygmomanometer and an appropriate-sized cuff according to guidelines. Five consecutive BP readings were obtained 30 to 60 seconds apart. The average of the five readings was taken as the BP. Hypertension was diagnosed in those receiving antihypertensive therapy or having a conventional BP ≥ 140/90 mmHg.

Ambulatory 24-hour BP was determined using SpaceLabs monitors (model 90207; Spacelabs, Redmond, Washington, USA), as previously described.[34] The size of the cuff was the same as that used for conventional BP measurements. Monitors were programmed to measure 24-hour BP at 15-minute intervals from 06:00 to 22:00 hours and at 30-minute intervals from 22:00 to 06:00 hours. Intra-individual means of the ambulatory measurements were weighted by the time interval between successive readings.[34] The average (± SD) number of BP readings obtained was 60.7 ± 12.2 (range = 24–81) for the 24-hour period.

Central aortic haemodynamics were determined as previously described.[33,34] After participants had rested for 15 minutes in the supine position, arterial waveforms at the radial (dominant arm), carotid and femoral artery pulses were recorded by applanation tonometry. Pressure waveforms were recorded during an eight-second period using a high-fidelity SPC-301 micromanometer (Millar Instrument, Inc, Houston, Texas) interfaced with a computer employing SphygmoCor, version 9.0 software (AtCor Medical Pty, Ltd, West Ryde, New South Wales, Australia).

To determine aortic BP the pulse wave obtained from the radial tonometer recordings was calibrated by manual measurement (auscultation) of brachial BP taken immediately before the recordings. The radial pressure waveform was converted into a central (aortic) waveform using a validated generalised transfer function incorporated in SphygmoCor software. Central aortic systolic BP was derived from the aortic waveform. Aortic PWV was determined from sequential waveform measurements at the carotid and femoral sites.

The time delay in the pulse waves between the carotid and femoral sites was determined using an electrocardiograph-derived R wave as a fiducial point. Pulse transit time was taken as the average of 10 consecutive beats. The distance that the pulse wave travels was determined as the difference between the distance from the femoral sampling site to the suprasternal notch, and the distance from the carotid sampling site to the suprasternal notch. Aortic PWV was calculated as the ratio of the distance to the transit time (m/s).

Serum creatinine concentrations were measured using the Advia Chemistry systems (Siemens) with calibration traceable to isotope dilution mass spectrometry (IDMS). The four-variable Modification of Diet in Renal Disease Study (MDRD) equation and the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation were employed to estimate GFR. The ethnicity factor as recommended in African Americans when calculating the MDRD and CKD-EPI eGFR was not applied in this study as the use results in overestimation of kidney function in black Africans.[35,36]

Blood samples were centrifuged and immediately stored at –80°C. Plasma concentrations of human resistin and high-sensitivity C-reactive protein (range 0.01–50 ng/ml) (hs-CRP) concentrations were measured using enzyme-linked immunosorbent assays (Quantikine, R&D Systems Inc, Minneapolis, MN, USA). Resistin was selected as an adipocytokine with possible adverse effects on renal function beyond that of obesity *per se* and CRP as a marker of general inflammation. The resistin assay had a lower detection limit of 0.026 ng/ml and intra- and inter-assay coefficients of variation ranging from 3.8 to 5.3% and 7.8 to 9.2%, respectively. The CRP assay had a mean lower detection limit of 0.010 ng/ml and intra- and inter-assay coefficients of variation ranging from 3.8 to 8.3% and 6.0 to 7.0%, respectively.

Statistical analysis

Database management and statistical analyses were performed with SAS software, version 9.4 (SAS Institute Inc, Cary, NC, USA). Continuous data are reported as mean ± SD or SEM, or median and interquartile range. Unadjusted means and

proportions were compared by the large-sample *z*-test and the c^2 statistic, respectively. As HOMA-IR and circulating concentrations of inflammatory markers were non-normally distributed, they were transformed to improve on the distribution (Table 1). Bivariate correlations were assessed from Pearson's correlation coefficients. Independent relations were assessed from linear, logistic or stepwise regression analysis with appropriate adjustors. Probability values were further adjusted for non-independence of family members using the method of maximum likelihood as implemented by the mixed procedure as defined in the SAS package.

To determine the relative contribution of risk factors to variations in eGFR, standardised β-coefficients were determined. As DM is so strongly associated with renal dysfunction, in the primary analysis we assessed relationships in the sample that excluded those with DM. However, to compare the impact of metabolic changes with that of DM on renal function at a community level and to ensure that the exclusion of DM did not result in a selection bias, we also performed the same analysis in the whole sample, including those with DM.

Table 1. Distribution of circulating inflammatory markers or the homeostasis model of insulin resistance (HOMA-IR) before and after transformation of data (n = 1 010)

Parameter	*Skewness*	*Kurtosis*	*Shapiro–Wilk*
Untransformed			
C-reactive protein	4.22	31.2	0.64
Resistin	2.81	15.8	0.80
HOMA-IR	4.33	26.3	0.57
Logarithm of:			
C-reactive protein	–0.37	0.04	0.99
Resistin	0.12	0.52	1.00
HOMA-IR	0.36	–0.24	0.98

Results

Table 2 shows the characteristics of the non-diabetic participants in the study sample and Table 3 shows the characteristics of all participants in the study sample. More women than men participated and a high prevalence of hypertension and obesity was noted, with many hypertensives being untreated or uncontrolled; 33.9% of the non-diabetic sample had an eGFR = 60–90 ml/min/1.73 m^2 and 4.4% an eGFR = 20–60 ml/min/1.73 m^2; 34.9% of the full study sample had an eGFR = 60–90 ml/min/1.73 m^2 and 6.4% an eGFR = 20–60 ml/min/1.73 m^2.

In the non-diabetic cohort, only blood glucose, low-density lipoprotein (LDL) cholesterol concentrations and metabolic syndrome features (MDRD eGFR only) showed weak independent relationships with eGFR. No adiposity index or other obesity-associated metabolic abnormality (lipids) was independently associated with eGFR or creatinine concentrations (Table 4).

However, in multivariate regression models, independent of conventional risk factors, including metabolic syndrome

Table 2. Participant characteristics without diabetes mellitus

Characteristics	*All*	*With 24-hour BP*	*With PWV*
Number (% female)	872 (63.1)	600 (63.0)	779 (61.4)
Age (years)	41.7 ± 17.8	41.5 ± 17.5	41.2 ± 17.9
Body mass index (kg/m^2)	28.6 ± 7.7	28.1 ± 7.4	28.0 ± 7.2
% overweight/obese	23.4/38.2	24.8/35.2	23.9/35.6
Waist circumference (cm)	88.8 ± 16.2 (n = 850)	87.9 ± 15.8 (n = 584)	87.7 ± 15.6 (n = 762)
% abdominal obesity	40.6	37.3	37.6
Waist:hip ratio	0.82 ± 0.10 (n = 850)	0.82 ± 0.10 (n = 584)	0.82 ± 0.10 (n = 762)
Regular tobacco (% subjects)	15.6	15.5	16.9
Regular alcohol (% subjects)	21.3	21.8	22.1
% females postmenopausal	37.4	35.2	36.4
% hypertension	39.1	37.7	37.9
Current antihypertensive meds (%)	17.1	15.7	16.2
Glucose (mmol/l)	4.72 ± 0.86	4.74 ± 0.91	4.70 ± 0.82
LDL-C (mmol/l)	2.58 ± 0.93	2.58 ± 0.96	2.57 ± 0.94
HDL-C (mmol/l)	1.43 ± 0.42	1.43 ± 0.41	1.44 ± 0.42
Triglycerides (mmol/l)	1.13 ± 0.86	1.13 ± 0.94	1.12 ± 0.87
Metabolic syndrome (%)*	31.9/25.9/11.9/6.6	32.2/24.7/12.3/6.5	32.6/25.4/11.0/5.4
Insulin (µU/ml)	8.05 (3.86 to 15.00)	8.46 (3.94–16.45)	7.87 (3.77–14.70)
HOMA-IR	1.60 (0.75–3.07)	1.74 (0.80–3.59)	1.58 (0.72–2.99)
Resistin (ng/ml)	10.6 (7.6–15.2)	10.5 (7.5–14.6)	10.6 (7.7–15.1)
C-reactive protein (ng/ml)	3.35 (1.27–7.72)	3.06 (1.17–7.55)	3.14 (1.22–7.44)
Office SBP/DBP (mm Hg)	127 ± 22/83 ± 13	126 ± 22/83 ± 12	126 ± 22/83 ± 12
24-hour SBP/DBP (mm Hg)	117 ± 14/72 ± 10 (n = 600)	117 ± 14/72 ± 10	117 ± 14/72 ± 10
Aortic PWV (m/s)	5.95 ± 2.36 (n = 779)	6.06 ± 2.36 (n = 529)	5.95 ± 2.36
Aortic SBP (mm Hg)	119 ± 22	118 ± 22	118 ± 22
Creatinine (µmol/l)	74.0 ± 16.8	74.4 ± 16.4	73.9 ± 16.7
eGFR (MDRD) (ml/min/1.73 m^2)	96.1 ± 25.8	95.6 ± 25.4	97.0 ± 25.6
eGFR (CKD-EPI) (ml/min/1.73 m^2)	97.1 ± 21.4	96.8 ± 21.3	97.9 ± 21.4

Data shown are mean ± SD, median and interquartile range and proportions. PWV, pulse-wave velocity; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model of insulin resistance; SBP, systolic blood pressure; DBP, diastolic BP; eGFR, estimated glomerular filtration rate; MDRD, Modification of Diet in Renal Disease equation; CKD-EPI, Chronic Kidney Disease Epidemiology equation. *Metabolic syndrome (%) is percentage of individuals with one/two/three/four components of the metabolic syndrome.

Table 3. Participant characteristics

Characteristics	*All*	*With 24-hour BP*	*With PWV*
Number (% female)	1010 (63.6)	688 (62.9)	896 (62.2)
Age (years)	44.0 ± 18.2	43.9 ± 18.1	43.5 ± 18.4
Body mass index (kg/m²)	29.3 ± 7.9	28.7 ± 7.6	28.6 ± 7.4
% overweight/obese	23.5/41.8	25.4/38.2	24.3/38.8
Waist circumference (cm)	90.6 ± 16.8 (*n* = 984)	89.7 ± 16.4 (*n* = 669)	89.5 ± 16.1 (*n* = 876)
% abdominal obesity	44.9	41.9	41.8
Waist:hip ratio	0.84 ± 0.10 (*n* = 984)	0.84 ± 0.10 (*n* = 669)	0.83 ± 0.10 (*n* = 876)
Regular tobacco (% subjects)	14.7	14.8	16.1
Regular alcohol (% subjects)	19.9	20.3	20.5
% females postmenopausal	43.8	41.8	42.9
% diabetes mellitus	13.7	12.8	13.1
% glucose-lowering agents	7.5	7.0	7.3
% hypertension	44.7	43.3	43.8
Current antihypertensive meds (%)	22.9	21.1	22.1
Glucose (mmol/l)	5.26 ± 2.46	5.30 ± 2.60	5.26 ± 2.54
LDL-C (mmol/l)	2.63 ± 0.94	2.62 ± 0.97	2.63 ± 0.95
HDL-C (mmol/l)	1.40 ± 0.41	1.41 ± 0.41	1.41 ± 0.42
Triglycerides (mmol/l)	1.24 ± 1.12	1.25 ± 1.28	1.23 ± 1.15
Metabolic syndrome (%)*	28.5/25.1/13.7/11.8	29.1/24.1/14.1/11.1	29.2/24.8/13.2/10.2
Insulin (µU/ml)	8.45 (4.15–15.32)	8.92 (4.32–17.2)	8.32 (4.02–15.00)
HOMA-IR	1.80 (0.84–3.74)	1.86 (0.88–4.19)	1.74 (0.82–3.65)
Resistin (ng/ml)	10.8 (7.7–15.7)	10.5 (7.5–15.2)	10.7 (7.7–15.5)
C-reactive protein (ng/ml)	3.80 (1.42–8.44)	3.45 (1.24–8.37)	3.65 (1.31–8.06)
Office SBP/DBP (mm Hg)	128 ± 22/84 ± 13	128 ± 22/83 ± 12	128 ± 22/83 ± 12
24-hour SBP/DBP (mm Hg)	118 ± 15/72 ± 10 (*n* = 688)	118 ± 15/72 ± 10	118 ± 15/72 ± 10
Aortic PWV (m/s)	6.26 ± 2.67 (*n* = 896)	6.39 ± 2.78 (*n* = 608)	6.26 ± 2.67
Aortic SBP (mm Hg)	120 ± 22	119 ± 22	119 ± 22
Creatinine (µmol/l)	75.1 ± 22.0	75.6 ± 19.9	75.0 ± 22.3
eGFR (MDRD) (ml/min/1.73 m²)	94.3 ± 26.1	93.8 ± 25.9	95.0 ± 25.8
eGFR (CKD-EPI) (ml/min/1.73 m²)	95.0 ± 22.2	94.5 ± 22.3	95.7 ± 22.1

Data shown are mean ± SD, median and interquartile range and proportions. PWV, pulse-wave velocity; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model of insulin resistance; SBP, systolic blood pressure; DBP, diastolic BP; eGFR, estimated glomerular filtration rate; MDRD, Modification of Diet in Renal Disease equation; CKD-EPI, Chronic Kidney Disease Epidemiology equation. *Metabolic syndrome (%) is percentage of individuals with one/two/three/four components of the metabolic syndrome.

features, HOMA-IR and plasma resistin concentrations (Figs 1, 2), but not CRP concentrations (Table 5) were independently associated with creatinine concentrations or eGFR and these effects were unaffected by adjustments for adiposity indices or obesity-associated metabolic abnormalities, including metabolic syndrome features (Figs 1, 2). The independent relationships between HOMA-IR or resistin concentrations and eGFR or creatinine concentrations were noted in several subgroups of the sample, including men and women separately (Table 6), and across tertiles of adiposity indices (data not shown).

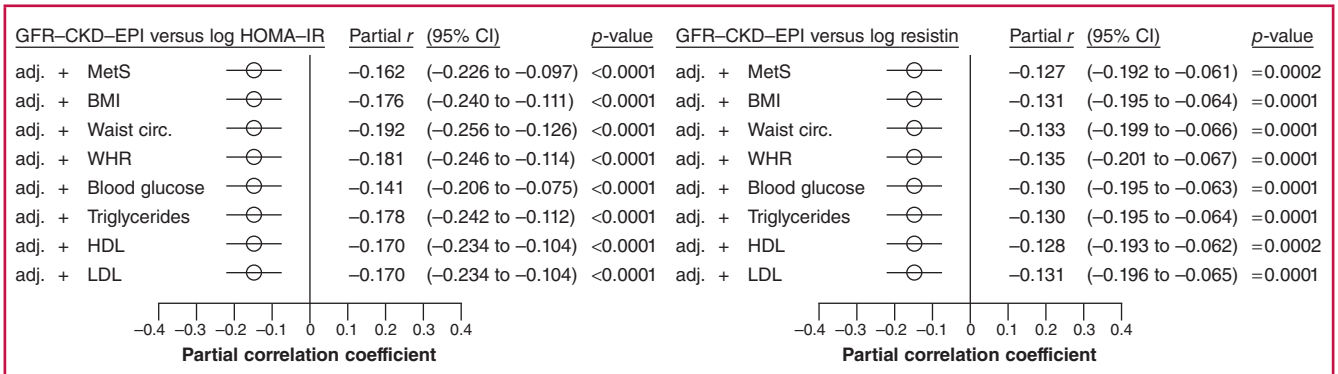

Fig. 1. Independent relationships between the homeostasis model of insulin resistance (HOMA-IR), or plasma resistin concentrations and estimated glomerular filtration rate (eGFR) beyond indices of adiposity or obesity-associated metabolic features in non-diabetic participants of a community sample (*n* = 850). Adjustments are for age, gender, conventional systolic blood pressure, regular tobacco use, regular alcohol consumption and the adiposity index or metabolic syndrome features indicated. CKD-EPI, Chronic Kidney Disease Epidemiology equation; CI, confidence interval; MetS, the metabolic syndrome; BMI, body mass index; WC, waist circumference; WHR, waist-to-hip ratio; LDL, low-density lipoprotein; HDL, high-density lipoprotein.

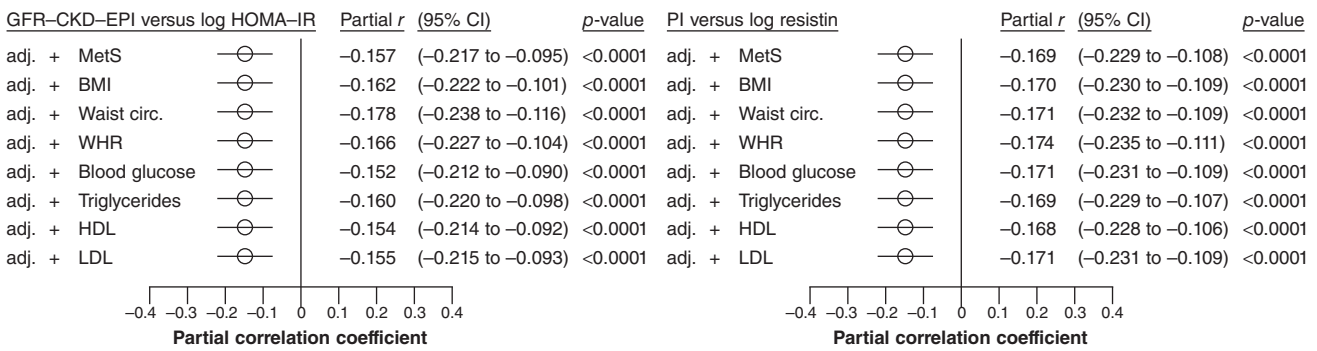

Fig. 2. Independent relationships between the homeostasis model of insulin resistance (HOMA-IR), or plasma resistin concentrations and estimated glomerular filtration rate (eGFR) beyond indices of adiposity or obesity-associated metabolic features in a community sample (n = 984). Adjustments are for age, gender, conventional systolic blood pressure, regular tobacco use, regular alcohol consumption, diabetes mellitus, HbA_{1c} and the adiposity index or metabolic syndrome features indicated. CKD-EPI, Chronic Kidney Disease Epidemiology equation; CI, confidence interval; MetS, the metabolic syndrome; BMI, body mass index; WC, waist circumference; WHR, waist-to-hip ratio; LDL, low-density lipoprotein; HDL, high-density lipoprotein.

The independent relationships between HOMA-IR and eGFR (partial r, obese = –0.181, $p < 0.005$, non-obese = –0.162, $p < 0.0005$) or creatinine concentrations (data not shown) were similar in obese versus non-obese participents. Furthermore, the independent relationships between resistin concentrations and eGFR (partial r, obese = –0.128, $p < 0.02$, non-obese = –0.149, $p < 0.001$) or creatinine concentrations (data not shown) were similar in obese versus non-obese participents. Importantly, the relationships between resistin and eGFR were as robust in unrelated participants (parents alone, partial r = –0.214, $p < 0.005$) as in related participants (parents and their children and siblings, partial r = –0.139, $p < 0.0001$). Moreover, the independent relationships between resistin concentrations and eGFR were independent of CRP concentrations (Table 7).

Table 4. Multivariate adjusted relationships between adiposity indices or obesity-related metabolic changes and estimated glomerular filtration rate in non-diabetic participants of a community sample (n = 850)

	MDRD eGFR		*CKD-EPI eGFR*	
eGFR versus	*Partial* r *(95% CI)*	p-*value*	*Partial* r *(95% CI)*	p-*value*
Body mass index	–0.035 (–0.10 to 0.03)	0.31	0.018 (–0.08 to 0.05)	0.60
Waist circumference	–0.027 (–0.09 to 0.04)	0.44	–0.009 (–0.08 to 0.06)	0.78
Waist:hip ratio	–0.019 (–0.09 to 0.05)	0.59	–0.010 (–0.08 to 0.06)	0.78
Glucose	–0.103 (–0.17 to –0.04)	< 0.01	–0.097 (–0.16 to –0.03)	< 0.01
Triglycerides	–0.004 (–0.07 to 0.06)	0.91	0.002 (–0.06 to 0.07)	0.95
HDL-C	0.045 (–0.02 to 0.11)	0.19	0.044 (–0.02 to 0.11)	0.20
LDL-C	–0.094 (–0.16 to –0.03)	< 0.01	–0.083 (–0.15 to –0.02)	< 0.05
Metabolic syndrome	–0.088 (–0.15 to –0.02)	< 0.01	–0.062 (–0.13 to 0.01)	0.07

LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; eGFR, estimated glomerular filtration rate; MDRD, Modification of Diet in Renal Disease equation; CKD-EPI, Chronic Kidney Disease Epidemiology equation. Adjustments are for age, gender, conventional systolic blood pressure, regular tobacco use and regular alcohol consumption.

In stepwise regression models, HOMA-IR and resistin concentrations were second and third only to age and at least as strong as BP or PWV in the impact (standardised β-coefficient) on eGFR and CKD in both the non-diabetic participants (Tables 8, 9) as well as in all participants (Tables 10, 11). The impact of HOMA-IR and resistin concentrations together on eGFR or CKD was markedly greater than the impact of conventional risk factors, including metabolic syndrome features, combined. The relative impact of HOMA-IR or resistin concentrations on eGFR or CKD were similar irrespective of whether conventional brachial BP, 24-hour BP, aortic BP or aortic PWV were included in the regression models in both the non-diabetic participants (Tables 8, 9) as well as in all participants (Tables 10, 11).

The independent relationships between insulin resistance or resistin concentrations and eGFR were independent of each other (Tables 8, 9, non-diabetic participants; Tables 10, 11, all participants) and of CRP concentrations (Table 7). Independent relationships between insulin resistance or resistin concentrations and creatinine concentrations or eGFR translated into comparable stepwise decreases in eGFR across octiles of HOMA-IR or resistin concentrations, as did changes in eGFR across octiles of systolic BP (Figs 3, 4).

Table 5. Multivariate-adjusted (partial r) relationships between C-reactive protein concentrations and estimated glomerular filtration rate in non-diabetic participants of a community sample and the full community sample

CRP versus	*Partial* r *(95% CI)*	p-*value*
Non-diabetic participants		
MDRD eGFR	–0.05 (–0.11–0.02)	0.17
CKD-EPI eGFR	–0.05 (–0.12–0.02)	0.16
All participants		
MDRD eGFR	–0.05 (–0.11–0.01)	0.13
CKD-EPI eGFR	–0.04 (–0.10–0.02)	0.19

CRP, C-reactive protein; eGFR, estimated glomerular filtration rate; MDRD, Modification of Diet in Renal Disease equation; CKD-EPI, Chronic Kidney Disease Epidemiology equation.
Adjustments are for age, gender, conventional systolic blood pressure, waist circumference, regular tobacco use, regular alcohol consumption, diabetes mellitus (in all participants), HbA_{1c} (in all participants) and the metabolic syndrome.

Discussion

The main findings of this study are as follows. In a large urban community sample with prevalent obesity (41.8% of sample) in South Africa, independent of confounders, insulin resistance and resistin concentrations combined produced a greater impact on creatinine concentrations, eGFR and CKD than modifiable conventional risk factors, including metabolic syndrome features or DM *per se*. These effects were noted irrespective of whether conventional BP or alternative obesity-associated haemodynamic changes (including 24-hour or aortic BP and aortic PWV) were considered.

Obesity contributes to the development and progression of CKD.[9-11] Animal-based studies suggest a distinct action of insulin resistance in contributing to these effects.[30] While a number of prior studies have demonstrated independent relationships between indices of insulin resistance and CKD,[12-14,37] these studies also show relationships that are stronger in the presence of obesity.[12,13] In addition, several of these prior studies have demonstrated that relationships between insulin resistance and glomerular function are strengthened by increasing components of the metabolic syndrome such as central obesity, hypertension and the presence of dyslipidaemia.[12,37] Therefore, while a relationship between insulin resistance and CKD is accepted, the impact of insulin resistance relative to obesity-associated conventional risk factors on eGFR is unknown.[30]

In contrast to previous findings,[12,13,37] we show that relationships between HOMA-IR and eGFR are unaffected by the extent of obesity *per se* or the presence of metabolic features (glucose or

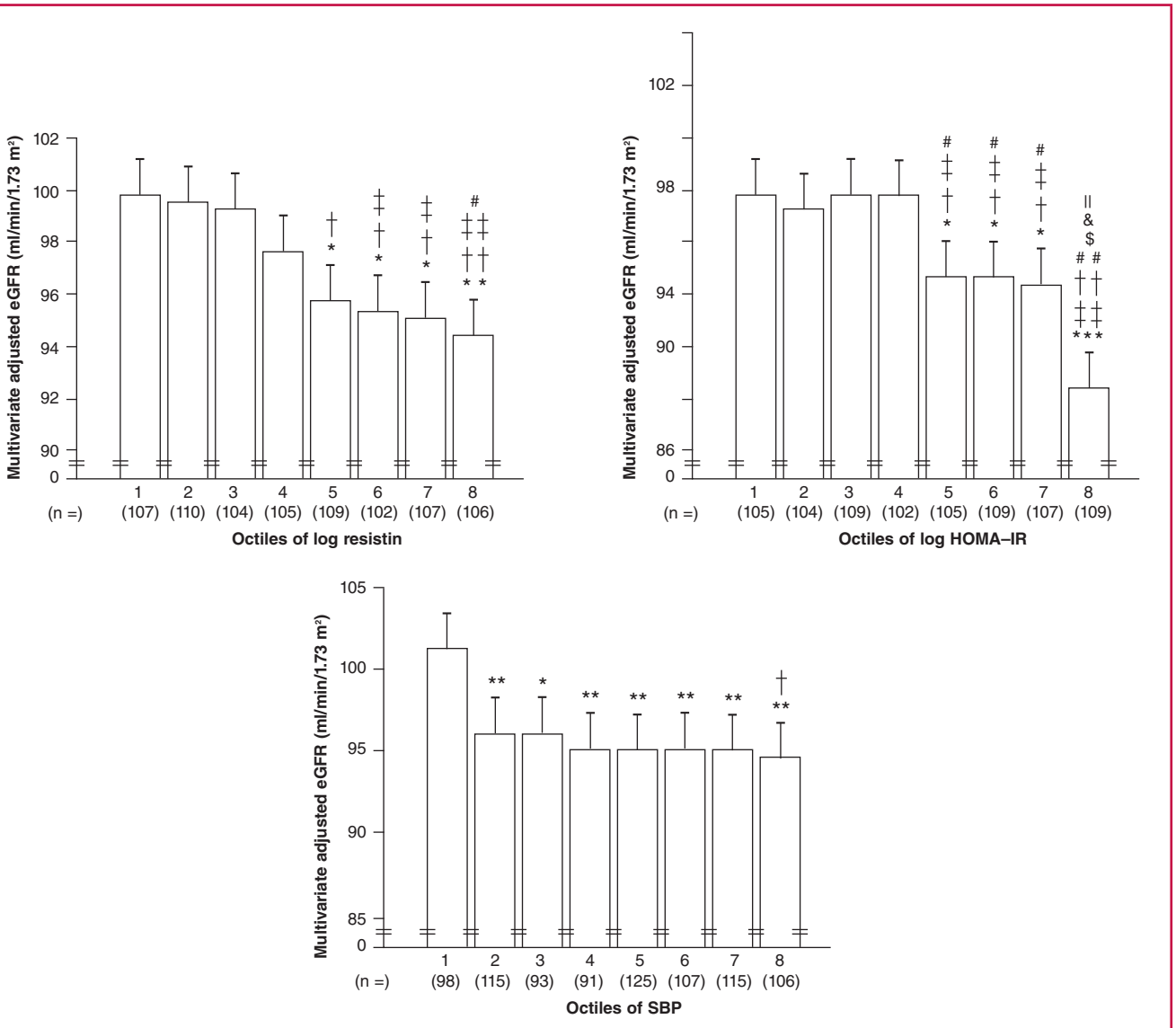

Fig. 3. Multivariate adjusted estimated glomerular filtration rates (eGFR) across octiles of the homeostasis model of insulin resistance (HOMA-IR) or plasma resistin concentrations compared to systolic blood pressure in non-diabetic participants of a community sample ($n = 850$). Adjustments are for age, gender, waist circumference, conventional systolic blood pressure (for HOMA-IR and resistin), regular tobacco use, regular alcohol consumption and metabolic syndrome features. SBP, systolic blood pressure. $^{*}p < 0.05$, $^{**}p < 0.005$, $^{***}p < 0.0001$ vs octile 1, $^{\dagger}p < 0.05$, $^{\dagger\dagger}p < 0.0001$ versus octile 2, $^{\ddagger}p < 0.05$, $^{\ddagger\ddagger}p < 0.0001$ versus octile 3, $^{\#}p < 0.05$, $^{\#\#}p < 0.0001$ versus octile 4, $^{\$}p < 0.05$ versus octile 5, $^{\&}p < 0.05$ versus octile 6, $^{\|}p < 0.05$ versus octile 7.

lipid abnormalities or the metabolic syndrome *per se*), neither of which showed strong independent correlations with eGFR. In addition, these effects were independent of BP. These data therefore support a view that the adverse renal effects of insulin resistance are distinct from that of obesity, associated metabolic features or the metabolic syndrome *per se*, and that the impact is at least as strong as that of modifiable conventional risk factors (BP).

Importantly, in combination with circulating resistin concentrations, the impact of insulin resistance is markedly stronger than the combined impact of modifiable conventional risk factors. Consequently, targeting conventional risk factors alone, including obesity and associated metabolic abnormalities, may result in a marked residual impact on the development of CKD in communities with a high prevalence of obesity. As the relationships between insulin resistance and eGFR in the present study cannot be accounted for by obesity or associated metabolic features, further studies are urgently required to identify the origins of the insulin resistance beyond obesity in the South African context.

There is also evidence to support a role for adipocytokines (obesity-associated inflammatory changes) in the development and progression of CKD.[18,21,25,26,28,29] Animal-based studies similarly suggest a distinct action of inflammatory substances in contributing to these effects.[31,32] However, clinical data are again unclear as to the relative impact of adipocytokines compared to that of conventional risk factors on renal function. While a number of prior studies have demonstrated relationships between circulating concentrations of adipocytokines and CKD,[18,21,25,26,28,29] whether these relationships are stronger in the

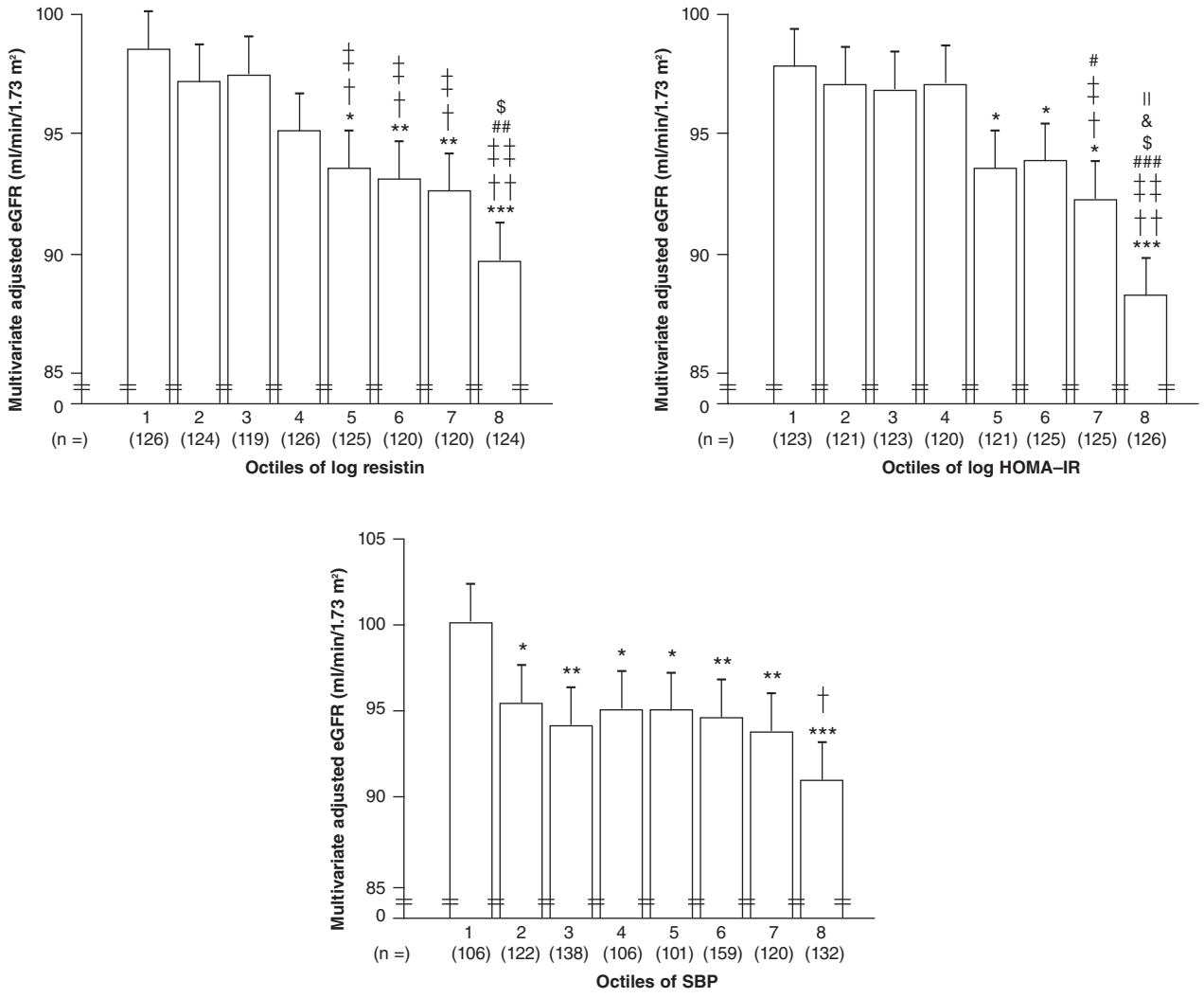

Fig. 4. Multivariate adjusted estimated glomerular filtration rates (eGFR) across octiles of the homeostasis model of insulin resistance (HOMA-IR) or plasma resistin concentrations compared to systolic blood pressure in a community sample (n = 984). Adjustments are for age, gender, waist circumference, conventional systolic blood pressure (for HOMA-IR and resistin), regular tobacco use, regular alcohol consumption, diabetes mellitus, HbA_{1c} and the metabolic syndrome. SBP, systolic blood pressure. $^{*}p < 0.05$, $^{**}p < 0.005$, $^{***}p < 0.0001$ vs octile 1, $^{\dagger}p < 0.05$, $^{\dagger\dagger}p < 0.0001$ versus octile 2, $^{\ddagger}p < 0.05$, $^{\ddagger\ddagger}p < 0.0001$ versus octile 3, $^{\#}p < 0.05$, $^{\#\#}p < 0.005$, $^{\#\#\#}p < 0.0001$ versus octile 4, $^{\$}p < 0.05$ versus octile 5, $^{\&}p < 0.05$ versus octile 6, $^{\|}p < 0.05$ versus octile 7.

Table 6. Gender-specific multivariate-adjusted (partial *r*) relationships between the homeostasis model of insulin resistance or resistin concentrations and estimated glomerular filtration rate in non-diabetic participants of a community sample and the full community sample

	Women		*Men*	
eGFR versus	*Partial* r *(95% CI)*	p-*value*	*Partial* r *(95% CI)*	p-*value*
Non-diabetic participants				
	(*n* = 550)		(*n* = 322)	
HOMA-IR	–0.177 (–0.258 to –0.093)	< 0.0001	–0.159 (–0.267 to –0.047)	< 0.005
Resistin	–0.133 (–0.216 to –0.048)	< 0.005	–0.144 (–0.252 to –0.032)	< 0.02
All participants	(*n* = 642)		(*n* = 368)	
HOMA-IR	–0.159 (–0.235 to –0.080)	< 0.0005	–0.153 (–0.255 to –0.048)	< 0.005
Resistin	–0.152 (–0.228 to –0.073)	< 0.0005	–0.202 (–0.301 to –0.098)	< 0.0005

eGFR, estimated glomerular filtration rate; HOMA-IR, homeostasis model of insulin resistance. Adjustments are for age, gender, conventional systolic blood pressure, waist circumference, regular tobacco use, regular alcohol consumption, diabetes mellitus (in all participants), HbA_{1c} (in all participants) and the metabolic syndrome.

Table 7. Impact of adjustments for C-reactive protein on multivariate adjusted relationships between resistin concentrations and estimated glomerular filtration rate in non-diabetic participants of a community sample and the full community sample

	MDRD eGFR		*CKD-EPI eGFR*	
Resistin versus	*Partial* r *(95% CI)*	p-*value*	*Partial* r *(95% CI)*	p-*value*
Non-diabetic participants				
eGFR	–0.130 (–0.196 to –0.063)	< 0.0005	–0.129 (–0.195 to –0.062)	< 0.0005
+ CRP	–0.126 (–0.192 to –0.059)	< 0.0005	–0.125 (–0.191 to –0.058)	< 0.0005
All participants				
eGFR	–0.160 (–0.221 to –0.097)	< 0.0001	–0.170 (–0.231 to –0.108)	< 0.0001
+ CRP	–0.152 (–0.214 to –0.090)	< 0.0001	–0.164 (–0.225 to –0.101)	< 0.0001

eGFR, estimated glomerular filtration rate; MDRD, Modification of Diet in Renal Disease equation; CKD-EPI, Chronic Kidney Disease Epidemiology equation. Adjustments are for age, conventional systolic blood pressure, waist circumference, regular tobacco use, regular alcohol consumption, diabetes mellitus (in all participants), HbA_{1c} (in all participants), the metabolic syndrome and C-reactive protein as indicated.

presence of obesity or obesity-associated metabolic features, and the impact of adipocytokines relative to that of conventional risk factors, have not been determined. In this regard, we show that relationships between circulating resistin concentrations and eGFR were unaffected by the extent of obesity, the presence of obesity-associated metabolic features, or the metabolic syndrome *per se* and that the relative impact of resistin concentrations on eGFR or CKD is at least as strong as modifiable conventional risk factors.

Importantly, the combined impact of HOMA-IR and circulating resistin concentrations on eGFR is substantially greater than the combined impact of modifiable conventional risk factors. As the relationships between resistin concentrations and eGFR in the present study cannot be accounted for by obesity or associated metabolic features, as with insulin resistance, further studies are urgently required to identify the origins of resistin beyond obesity in the South African context. In this regard, as resistin in humans is derived largely from circulating white blood cells, consideration must be given to the possibility of chronic infective processes, such as that produced by human immunodeficiency virus, contributing to this process.

Caution should be exercised in interpreting the results of this study. The lack of independent relationships between several adiposity indices and renal function in the present study, despite our ability to show strong independent relationships with BP and several metabolic parameters in the same sample as recently described,[38] should not be interpreted to suggest that obesity is not a cause of renal dysfunction in the population studied. Indeed, there is a large body of evidence to show that both insulin resistance and excess adiposity collectively contribute to renal damage and to support a link between inflamed adipose tissue and the development of kidney injury in obesity. Moreover, a number of studies indicate that obesity elicits renal dysfunction independent of insulin resistance.

However, there are several possible reasons why clinically employed adiposity indices failed to show relationships in the present study. First, obesity may be associated with hyperfiltration and an increased GFR. Therefore eGFR may underestimate the contribution of obesity to CKD. Nonetheless, this would not explain a marked impact of insulin resistance and resistin on eGFR, while adiposity indices failed to do so. Second, in the present study we did not assess relationships between visceral fat, which is often poorly indexed by indirect measures such as waist circumference or waist-to-hip ratio, and renal function. The fact that resistin was independently associated with eGFR and that resistin is derived from white

Table 8. Relative impact [standardised slopes (β-coefficients)] of factors accounting for variations in estimated glomerular filtration rate in non-diabetic participants of a community sample

Models with	*Brachial SBP (n = 850)*		*24-hour SBP (n = 584)*		*Aortic SBP (n = 843)*		*Aortic PWV (n = 762)*	
eGFR versus	β-*coeff ± SEM*	p-*value*	β-*coeff ± SEM*	p-*value*	β-*coeff ± SEM*	p-*value*	β-*coeff ± SEM*	p-*value*
Age	–0.66 ± 0.03	< 0.0001	–0.66 ± 0.04	< 0.0001	–0.65 ± 0.03	< 0.0001	–0.65 ± 0.04	< 0.0001
HOMA-IR	–0.13 ± 0.03	< 0.0001	–0.11 ± 0.03	< 0.001	–0.12 ± 0.03	< 0.0001	–0.13 ± 0.03	< 0.0001
Resistin	–0.10 ± 0.02	< 0.0001	–0.08 ± 0.03	< 0.005	–0.11 ± 0.02	< 0.0001	–0.11 ± 0.03	< 0.0001
Hypertension	–0.001 ± 0.033	0.99	–0.02 ± 0.04	0.55	0.002 ± 0.034	0.96	–0.01 ± 0.03	0.73
Waist circumference	0.03 ± 0.03	0.32	0.03 ± 0.04	0.42	0.03 ± 0.03	0.34	0.02 ± 0.04	0.63
Glucose	–0.03 ± 0.03	0.23	–0.05 ± 0.04	0.19	–0.04 ± 0.03	0.23	–0.04 ± 0.03	0.16
Metabolic syndrome	–0.004 ± 0.039	0.92	0.02 ± 0.05	0.61	–0.003 ± 0.039	0.94	0.04 ± 0.04	0.36
Brachial SBP	–0.04 ± 0.03	0.19	–	–	–	–	–	–
24-hour SBP	–	–	–0.04 ± 0.03	0.23	–	–	–	–
Aortic SBP	–	–	–	–	–0.05 ± 0.03	0.16	–	–
Aortic PWV	–	–	–	–	–	–	–0.07 ± 0.03	< 0.05

eGFR, estimated glomerular filtration rate; SBP, systolic blood pressure; PWV, pulse-wave velocity; β-coeff, standardised β-coefficient; HOMA-IR, homeostasis model of insulin resistance. Also included in the regression models were gender, regular tobacco use and regular alcohol consumption.

Table 9. Relative impact [standardised slopes (β-coefficients)] of factors accounting for CKD in non-diabetic participants of a community sample

Models with (n = CKD/total)	*Brachial SBP (326/850)*		*24-hour SBP (225/584)*		*Aortic SBP (322/843)*		*Aortic PWV (281/762)*	
CKD versus	β-*coeff ± SEM*	p-*value*	β-*coeff ± SEM*	p-*value*	β-*coeff ± SEM*	p-*value*	β-*coeff ± SEM*	p-*value*
Age	0.58 ± 0.04	< 0.0001	0.56 ± 0.05	< 0.0001	0.58 ± 0.04	< 0.0001	0.57 ± 0.05	< 0.0001
HOMA-IR	0.11 ± 0.03	< 0.0005	0.08 ± 0.04	< 0.05	0.11 ± 0.03	< 0.0005	0.11 ± 0.03	< 0.002
Resistin	0.06 ± 0.03	< 0.03	0.07 ± 0.03	< 0.05	0.07 ± 0.03	< 0.02	0.08 ± 0.03	< 0.02
Hypertension	0.005 ± 0.039	0.90	0.04 ± 0.04	0.31	0.01 ± 0.04	0.80	–0.01 ± 0.04	0.83
Waist circumference	–0.08 ± 0.04	< 0.05	–0.04 ± 0.05	0.35	–0.09 ± 0.04	< 0.03	–0.06 ± 0.04	0.15
Glucose	0.04 ± 0.03	0.18	0.06 ± 0.04	0.13	0.04 ± 0.03	0.18	0.05 ± 0.04	0.20
Metabolic syndrome	–0.009 ± 0.045	0.84	–0.08 ± 0.05	0.14	–0.01 ± 0.04	0.88	–0.05 ± 0.05	0.33
Brachial SBP	–0.01 ± 0.04	0.79	–	–	–	–	–	–
24-hour SBP	–	–	0.01 ± 0.04	0.72	–	–	–	–
Aortic SBP	–	–	–	–	–0.02 ± 0.04	0.63	–	–
Aortic PWV	–	–	–	–	–	–	0.04 ± 0.04	0.35

CKD, chronic kidney disease; SBP, systolic blood pressure; PWV, pulse-wave velocity; β-coeff, standardised β-coefficient; HOMA-IR, homeostasis model of insulin resistance. Also included in the regression models were gender, regular tobacco use and regular alcohol consumption. CKD was identified as eGFR values < 90 ml/min/1.73 m^2 from eGFR determined using the Chronic Kidney Disease Epidemiology equation.

blood cells, often originating from inflamed adipose tissue at a visceral level, indeed supports the notion that inflamed adipose tissue contributes to the development of kidney injury. However, clinically, direct measures of visceral fat (computer-assisted tomography or ultrasound) are not considered to be conventional risk factors and therefore a large component of the adverse effects of adiposity on renal function that are mediated by insulin resistance or resistin will go undetected.

Although relationships between either insulin resistance or circulating adipocytokine concentrations and eGFR have been demonstrated on several previous occasions,[12-14,18,21,25,26,28,29,37] these relationships have employed conventional office BP as the haemodynamic adjustor. In this regard, none of these studies has considered the possibility that adjustments for conventional office BP measurements are inadequate for discounting the haemodynamic actions of insulin resistance, inflammatory changes or obesity *per se*. In this regard, obesity effects on BP, which are likely to be induced in part by insulin resistance, are not detected using office BP measurements alone. Indeed, obesity results in masked effects on in-office BP.[39]

Moreover, both insulin resistance[40] and inflammatory adipocytokines[41] may cause increases in aortic stiffness, and through an impact on aortic impedance, may produce renal microvascular damage beyond brachial BP. Hence, it is uncertain whether relationships between insulin resistance or adipocytokines and eGFR[12-14,18,21,25,26,28,29,37] are indeed beyond the adverse haemodynamic effects of these changes. However, in the present study, relationships between HOMA-IR or resistin concentrations and eGFR were largely unaffected by adjustments for 24-hour BP, aortic BP or aortic PWV. The present study therefore suggests that the actions of insulin resistance or resistin are distinct from that of the adverse haemodynamic consequences of these alterations.

Limitations

There are several limitations to this study. It is cross-sectional in design and hence the relationships noted may not be causal, and reverse causality may account for several of the relationships noted. In this regard, the relationship between resistin concentrations and eGFR in this study may reflect a shared genetic background[42] rather than an adverse effect of resistin on kidney function. However, as demonstrated by us, the relationships are as robust in unrelated participants (parents alone) as in related participants (parents and their children and siblings), suggesting that a shared genetic background is unlikely

Table 10. Relative impact [standardised slopes (β-coefficients)] of factors accounting for variations in estimated glomerular filtration rate in a community sample

Models with	*Brachial SBP (n = 984)*		*24-hour SBP (n = 669)*		*Aortic SBP (n = 977)*		*Aortic PWV (n = 876)*	
eGFR versus	β-*coeff ± SEM*	p-*value*	β-*coeff ± SEM*	p-*value*	β-*coeff ± SEM*	p-*value*	β-*coeff ± SEM*	p-*value*
Age	–0.67 ± 0.03	< 0.0001	–0.67 ± 0.04	< 0.0001	–0.66 ± 0.03	< 0.0001	–0.64 ± 0.04	< 0.0001
HOMA-IR	–0.13 ± 0.02	< 0.0001	–0.12 ± 0.03	< 0.0001	–0.13 ± 0.02	< 0.0001	–0.14 ± 0.03	< 0.0001
Resistin	–0.12 ± 0.02	< 0.0001	–0.12 ± 0.03	< 0.0001	–0.12 ± 0.02	< 0.0001	–0.12 ± 0.02	< 0.0001
Diabetes mellitus	0.005 ± 0.030	0.87	0.009 ± 0.036	0.80	0.003 ± 0.030	0.92	–0.007 ± 0.032	0.83
Hypertension	0.02 ± 0.03	0.42	–0.02 ± 0.04	0.60	0.02 ± 0.03	0.49	–0.006 ± 0.032	0.84
HbA_{1c}	0.009 ± 0.031	0.77	–0.005 ± 0.038	0.89	0.009 ± 0.031	0.78	0.031 ± 0.033	0.34
Waist circumference	0.05 ± 0.03	0.08	0.05 ± 0.04	0.18	0.06 ± 0.03	0.07	0.04 ± 0.03	0.27
Metabolic syndrome	–0.003 ± 0.037	0.93	0.005 ± 0.044	0.90	–0.008 ± 0.037	0.84	0.015 ± 0.039	0.71
Brachial SBP	–0.09 ± 0.03	< 0.005	–	–	–	–	–	–
24-hour SBP	–	–	–0.04 ± 0.03	0.21	–	–	–	–
Aortic SBP	–	–	–	–	–0.08 ± 0.03	< 0.01	–	–
Aortic PWV	–	–	–	–	–	–	–0.09 ± 0.03	< 0.005

eGFR, estimated glomerular filtration rate; SBP, systolic blood pressure; PWV, pulse-wave velocity; β-coeff, standardised β-coefficient; HOMA-IR, homeostasis model of insulin resistance. Also included in the regression models were gender, regular tobacco use and regular alcohol consumption.

Table 11. Relative impact [standardised slopes (β-coefficients)] of factors accounting for chronic kidney disease in a community sample

Models with (n = CKD/total)	*Brachial SBP (404/984)*		*24-hour SBP (279/669)*		*Aortic SBP (400/977)*		*Aortic PWV (348/876)*	
CKD versus	β-*coeff* ± *SEM*	p-*value*	β-*coeff* ± *SEM*	p-*value*	β-*coeff* ± *SEM*	p-*value*	β-*coeff* ± *SEM*	p-*value*
Age	0.59 ± 0.04	< 0.0001	0.57 ± 0.04	< 0.0001	0.59 ± 0.04	< 0.0001	0.56 ± 0.04	< 0.0001
HOMA-IR	0.12 ± 0.03	< 0.0001	0.09 ± 0.04	< 0.02	0.12 ± 0.03	< 0.0001	0.11 ± 0.03	< 0.0005
Resistin	0.07 ± 0.03	< 0.02	0.08 ± 0.03	< 0.02	0.06 ± 0.03	< 0.05	0.07 ± 0.03	< 0.02
Diabetes mellitus	0.05 ± 0.04	0.20	0.02 ± 0.04	0.65	0.05 ± 0.04	0.20	0.04 ± 0.04	0.37
Hypertension	0.02 ± 0.04	0.62	–0.03 ± 0.04	0.46	0.01 ± 0.04	0.76	0.02 ± 0.04	0.57
HbA_{1c}	–0.03 ± 0.04	0.50	–0.03 ± 0.04	0.54	–0.03 ± 0.04	0.47	–0.03 ± 0.04	0.48
Waist circumference	–0.06 ± 0.04	0.10	–0.02 ± 0.04	0.68	–0.07 ± 0.04	0.07	–0.06 ± 0.04	0.14
Metabolic syndrome	–0.02 ± 0.04	0.68	–0.07 ± 0.05	0.21	–0.01 ± 0.05	0.84	–0.03 ± 0.05	0.53
Brachial SBP	0.02 ± 0.03	0.56	–	–	–	–	–	–
24-hour SBP	–	–	0.007 ± 0.037	0.85	–	–	–	–
Aortic SBP	–	–	–	–	0.005 ± 0.036	0.88	–	–
Aortic PWV	–	–	–	–	–	–	0.07 ± 0.04	0.06

CKD, chronic kidney disease; SBP, systolic blood pressure; PWV, pulse-wave velocity; β-coeff, standardised β-coefficient; HOMA-IR, homeostasis model of insulin resistance. Also included in the regression models were gender, regular tobacco use and regular alcohol consumption. CKD was identified as eGFR values < 90 ml/min/1.73 m^2 from eGFR determined using the Chronic Kidney Disease Epidemiology equation.

to explain these relations. Furthermore, insulin resistance may be a consequence of CKD rather than a cause,[30] and reductions in eGFR may result in a reduced clearance of circulating substances and increases in circulating resistin concentrations. However, being a community-based study, few participants had late stages of CKD, which is more likely to result in insulin resistance or a reduced clearance of circulating resistin. Indeed, the relationships between insulin resistance or resistin concentrations and eGFR were largely in the early CKD stage range.

Second, the appropriate formula for calculating eGFR from serum creatinine concentrations in groups of black African ancestry is uncertain and ethnic-specific formulae have not been identified in Africa. As the relationships noted were largely in those with an eGFR > 60 ml/min/1.73 m^2, a range where creatinine-based formulae for estimating GFR are particularly inaccurate, either validation of the formula against inulin clearance in black African populations or validation of the results of the present study in other populations where obesity or the associated lipid or glucose abnormalities contribute little to reductions in eGFR, but where insulin resistance and adipocytokines may play a role, is required.

Third, we assessed the impact of resistin and CRP alone on eGFR and failed to evaluate the several additional adipocytokine changes that may influence eGFR. This was necessary to avoid the statistical imitations of multiple comparisons that would have been required if multiple adipocytokines (comparisons with multiple biomarkers) were evaluated. Consequently, we are likely to have underestimated the contribution of obesity-associated inflammatory changes to reductions in eGFR. Notwithstanding this possibility, we focused on resistin, as of all the adipokines, resistin demonstrates the most consistent and robust relationships with renal dysfunction.[16,18-26,28,29] Despite assessing the role of only one specific adipocytokine, we were able to show a combined effect of resistin and insulin resistance on eGFR, which was substantially greater than that of the combined impact of conventional risk factors, including adiposity indices *per se*. Nevertheless, the potential role of the multitude of additional adipocytokines requires further evaluation.

Fourth, we failed to identify CKD from urinary albumin-to-creatinine ratios in addition to eGFR, thus avoiding the impact of obesity-associated hyperfiltration on eGFR. However, the impact of HOMA-IR or resistin concentrations on eGFR was similar in obese versus non-obese participants.

Last, as the impact of HOMA-IR and resistin on eGFR were beyond obesity and associated metabolic changes, the question remains as to the factors responsible for HOMA-IR and increases in resistin in the community studied. Whether human immunodeficiency virus infection, the treatment thereof, or alternative factors contribute therefore requires further study.

Conclusions

We show in a large community-based sample with a high prevalence of obesity that the combined impact of insulin resistance, as indexed by HOMA-IR and circulating resistin concentrations, on eGFR or CKD was greater than that of all modifiable conventional cardiovascular risk factors together (including metabolic syndrome features). Importantly these effects were beyond even ambulatory or aortic BP and aortic stiffness. These data suggest that targeting conventional risk factors alone or the metabolic syndrome *per se* may result in a marked residual impact on the development of CKD in communities with a high prevalence of obesity. To adequately address the public health burden of CKD, approaches may therefore be required that influence insulin resistance and the adverse effects of resistin beyond obesity and associated metabolic syndrome features.

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Cameroon Country Report

PASCAR and WHF Cardiovascular Diseases Scorecard project

Anastase Dzudie, Jean M Fourie, Wihan Scholtz, Oana Scarlatescu, George Nel, Samuel Kingue

Abstract

Data collected by PASCAR for the World Heart Federation's Cardiovascular Diseases Scorecard project in Africa are presented. We summarise the strengths, threats, weaknesses and priorities identified from the collected data, which need to be considered in conjunction with the associated sections in the accompanying infographic. Data sets that were used include open-source data from the World Bank, World Health Organization and government publications.

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On behalf of the World Heart Federation (WHF), the Pan-African Society of Cardiology (PASCAR) co-ordinated data collection and reporting for the country-level Cardiovascular Diseases (CVD) Scorecard to be used in Africa.[1] The objective of the scorecard is to create a clear picture of the current state of CVD prevention, control and management, along with related non-communicable diseases (NCD) in 12 African countries. The Cameroon Cardiac Society, a member of PASCAR and the WHF, along with Professors Dzudie (scientific secretary) and Kingue (president), assisted in collating and verifying these data.

Part A: Demographics

According to the World Bank (2018), Cameroon is a lower-middle-income country with 44% of its people living in rural areas. In 2014, 23.8% of the population were living below the US$1.9-a-day ratio.[2] Life expectancy at birth in 2018 was 58 and 60 years for men and women, respectively. The general government health expenditure was 0.6% of the gross domestic product (GDP) in 2017, while the country GDP per capita was US$1 533.7 in 2018.[2]

Department of Internal Medicine, Yaoundé Faculty of Medicine and Biomedical Sciences, Yaoundé, Cameroon
Anastase Dzudie, MD
Samuel Kingue, MD

Pan-African Society of Cardiology, Cape Town, South Africa
Jean M Fourie
Wihan Scholtz, wihan@medsoc.co.za
George Nel

World Heart Federation, Geneva, Switzerland
Oana Scarlatescu

Part B: National cardiovascular disease epidemic

National response to CVD and NCD

In 2012, Cameroon's premature death rate attributable to CVD (age 30–70 years) was similar to its neighbouring country, Nigeria, at 12%.[3] In 2017, the age-standardised total CVD death rate was high at 11.85%, although much lower than the 31.8% for the global burden of disease (GBD) data.[4] The percentage of disability-adjusted life years (DALYs) resulting from CVD for men and women was 5.0 and 5.03%, respectively, which is lower than the GBD at 14.66% for both genders. The prevalence of atrial fibrillation (AF) and atrial flutter was 0.13%, while that of rheumatic heart disease (RHD) was 0.78%, which is higher when compared to the GBD RHD prevalence of 0.53%. The total RHD mortality rate was 0.02% of all deaths, which is lower than the GBD data (0.51%) (Table 1).[4]

Tobacco and alcohol

The prevalence of tobacco use in adult men and women (15+ years old) was 43.8 and 0.9%, respectively.[5] Comparative Global Health Observatory (GHO) data are 36.1% for men and 6.8% for women.[5] No data are available for adolescent tobacco use (13–15 years old) and the estimated annual direct cost of tobacco use is also not known. The premature CVD mortality rate attributable to tobacco is 2% of the total mortality rate, which is much lower than that of the global 10%.[6] The three-year (2015–17) average recorded alcohol consumption per capita (15+ years) was 6.5 litres (Table 1).[5]

Raised blood pressure and cholesterol

In 2015, 31.3% of men and 30.8% of women had raised blood pressure (BP) levels (systolic BP ≥ 140 mmHg or diastolic BP ≥ 90 mmHg), which is higher than the GHO level of 24.1 and 20.1% for men and women, respectively, and Africa's 27.4% for both.[5] In a screening study, only 31.7% of participants were found to be aware of their hypertension status, 59.9% of them were on treatment and of these, 24.6% had controlled BP levels.[7] In another study, Kingue *et al.* found a prevalence of 29.7%, with 14.1% awareness.[8] The percentage of individuals with raised total cholesterol levels (≥ 5.0 mmol/l or currently being on medication for raised cholesterol) was 26% compared to GHO data (38.9%).[5]

In 2017, the percentage of DALYs lost because of hypertension was 3.14%, whereas the mortality rate caused by hypertensive heart disease (0.64%) was lower than the 1.65% for global data (Table 1).[4]

Physical activity

No data were available for 11–17-year-old adolescents who were insufficiently active (< 60 minutes of moderate- to vigorous-intensity physical activity daily). However, the age-standardised estimate for adults who were insufficiently active (< 150 minutes of moderate-intensity physical activity per week, or < 75 minutes of vigorous-intensity physical activity per week) was 28.5%, which is higher than GHO data at 27.5% (Table 1).[5]

Overweight and obesity

In 2017, the prevalence of overweight [body mass index (BMI) ≥ 25 to < 30 kg/m^2] in adult men 25 years and older was 15.3% and in women 19.4% (May measurement month 2017 unpublished, permission granted). For obesity (BMI ≥ 30 kg/m^2), the prevalence was 7.7 and 18%, for men and women, respectively (May measurement month 2017 unpublished, permission granted). Cameroon's obesity prevalence for adults is lower (12.9%) compared to the global prevalence of 13.1%, as is that for the prevalence of overweight at 17.3 versus 38.9% globally (Table 1).[5]

Diabetes

The percentage of the population (adults 18 years and older) defined with fasting glucose ≥ 7.0 mmol/l or on medication for raised blood glucose levels (age-standardised) in 2014 was 6.5% for men and 6.9% for women.[5] In 2019, the prevalence of age-adjusted (20–79 years) diabetes was 6.0%, which is higher than that of Africa (3.9%) but lower than the global level of 9.3% (Table 1).[9]

Part C: Clinical practice and guidelines

Health system capacity

The country had 0.9 physicians and 0.058 nurses per 10 000 of the population in 2011 and 2013, respectively, while there were 13 hospital beds for every 10 000 people in 2010.[10]

No data for locally relevant clinical tools to assess CVD risk or national guidelines for the treatment of tobacco dependence were available by 2018.[11] However, locally relevant clinical guidelines for the management of acute rheumatic fever (ARF) and RHD are available.[12,13] Cameroon is involved in the INVICTUS (Investigation of Rheumatic Atrial Fibrillation Treatment using Vitamin K Antagonist, Rivaroxaban or Aspirin Studies) clinical trial, a comprehensive evaluation of RHD, including a multi-centre hospital-based registry.[14] Cameroon was one of 12 sub-Saharan countries that participated in the VALVAFRIC study, a multi-centre international hospital-based

Table 1. Cardiovascular disease indicators for Cameroon

Indicators	*Male*	*Female*	*Total*	*Year*
Status of the national CVD epidemic				
Premature CVD mortality (age 30–70 years) (% of deaths)	–	–	12	2012
Total CVD mortality (% of deaths)	10.79	13.08	11.85 (31.8)*	2017
DALYs attributable to CVD (%)	5.0	5.03	5.02 (14.66)*	2017
Total RHD mortality (% of deaths)	0.2	0.21	0.2 (0.51)*	2017
AF and atrial flutter (%)	0.14	0.11	0.13 (0.5)*	2017
Prevalence of RHD (%)	0.69	0.86	0.78 (0.53)*	2017
Tobacco and alcohol				
Prevalence of adult tobacco use (15+ years old) (%)	43.8 (36.1)*	0.9 (6.8)*	–	2015
Prevalence of youth (13–15-year-olds) tobacco use (%)	–	–		–
Estimated direct (healthcare-related) cost of tobacco use in your population (in current US$)			–	–
Proportion of premature CVD mortality attributable to tobacco (%)	–	–	2 (10)*	2004
Recorded alcohol consumption per capita (15+ years) (in litres of pure alcohol) (3-year average)			6.5	2015–17
Raised blood pressure and cholesterol				
Population with raised blood pressure (SBP ≥ 140 mmHg or DBP ≥ 90 mmHg) (%)	31.3 (24.1)*	30.8 (20.1)*	-	2018
Population with raised total cholesterol (≥ 5.0 mmol/l) (%)	29.5	22.3	26.0 (38.9)*	2018
DALYs attributable to hypertension (%)	3.23	3.02	3.14 (8.7)*	2017
Mortality caused by hypertensive heart disease (% of deaths)	0.42	0.91	0.64 (1.65)*	2017
Physical activity				
Adolescents (ages 11–17) who are insufficiently active (< 60 minutes of moderate- to vigorous-intensity physical activity daily) (%)	–	–	–	–
Adults (age-standardised estimate) who are insufficiently active (< 150 minutes of moderate-intensity physical activity per week, or < 75 minutes of vigorous-intensity physical activity per week) (%)	21.8	35.2	28.5 (27.5)*	2016
Overweight and obesity				
Adults who are overweight (BMI ≥ 25–< 30 kg/m^2) (%)	15.3	19.4	17.3 (38.9)*	2017
Prevalence of obesity (BMI ≥ 30 kg/m^2) (%)	7.7	18.0	12.9 (13.1)*	2017
Diabetes				
Defined population with fasting glucose ≥ 126 mg/dl (7.0 mmol/l) or on medication for raised blood glucose (age-standardised) (%)	6.5 (9)*	6.9 (8)*	–	2014
Prevalence of diabetes (ages 20–79) (%)	–	–	6.0 (9.3)**	2019

CVD, cardiovascular disease; DALYs, disability adjusted life years; RHD, rheumatic heart disease; AF, atrial fibrillation; SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, body mass index.
*WHO global data.[5]
**IDF Diabetes Atlas.[9]

WORLD HEART FEDERATION

PAN-AFRICAN SOCIETY OF CARDIOLOGY – PASCAR –

SCC Cameroon Cardiac Society

Cardiovascular Disease Scorecards – Africa

CAMEROON – APRIL 2020

Status of Cardiovascular Disease (CVD) and Non-communicable diseases (NCD)

Country Demographics

World Bank Classification

Lower-middle income

44%

of population living in rural areas

60% (Sub-Sahara Africa)

CAMEROON

0.2%

of total mortality caused by **RHD**

Global data: **0.51%**

0.78%

Prevalence of rheumatic heart disease **(RHD)**

Global data: **0.53%**

2%

of premature CVD mortality attributable to **tobacco**

Global data: **10%**

MALE **43.8%** **0.9** FEMALE

Prevalence of **tobacco** use **age ≥15**

Global data: **36.1%** (male) **6.8%** (female)

0.64%

of deaths caused by **hypertensive heart disease**

Global data: **1.65%**

31.3% **30.8%**

MALE FEMALE

of population with raised **blood pressure** (SBP ≥140 or DBP ≥90)

Global data: **24.1%** (male) **20.1%** (female)

12.9%

Prevalence of obese adults (BMI of ≥30 kg/m2)

Global data: **13.1%**

11.85%

of **deaths** caused by CVD

Global data: **31.8%**

26%

of population with raised total **cholesterol** (≥5.0 mmol/L)

Global data: **38.9%**

6%

Prevalence of diabetes **(ages 20-79)**

3.9% (Africa)

WORLD HEART FEDERATION

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CAMEROON

Health System Capacity

0.9
Number of physicians (per 10,000 population)

0.058
Number of nurses (per 10,000 population)

13
Number of hospital beds (per 10,000 population)

KEY: No data | Not in place | In process/ partially implemented | In place

Clinical Practice and Guidelines

Locally-relevant (national or subnational level):

- Clinical tool to assess CVD risk
- Guidelines for treatment of tobacco dependence

Clinical Guidelines for:

- The detection and management of atrial fibrillation
- The detection and management of acute rheumatic fever
- The detection and management of rheumatic heart disease
- The detection and management of diabetes
- CVD prevention (within the last 5 years)
- A system to measure the quality of care provided to people who have suffered acute cardiac events

CAMEROON

Cardiovascular Disease Governance

A national strategy or plan that addresses:

- CVDs and their specific risk factors
- NCD and their risk factors
- Rheumatic heart disease prevention and control as a priority
- A national surveillance system that includes CVDs and their risk factors

Stakeholder action

- Non-governmental organizations' advocacy for CVD policies and programmes
- Civil society involved in developing and implementing of national CVD prevention and control plan

For more information, please email info@worldheart.org info@pascar.org cameroon_cardiac_society@yahoo.fr

Source References: Global Health Data Exchange; WHO Global Health Observatory data repository; WHO NCD Document repository; Country specific publications.

retrospective registry of hospitalised RHD patients with valvular lesions.[15] International guidelines are followed regarding the detection and management of AF and pharyngitis.[12] Cameroon does have national guidelines on diabetes mellitus management or treatment.[16]

Essential medicines and interventions

The availability and affordability of essential CVD medicines were investigated in a study by Jingi *et al*.[18] Availability was higher in the urban informal sector, with 63.6% of these medicines available. Aspirin was the most affordable medicine and available at 70% of the study sites.[17] Metformin and insulin are not generally available in the public health sector.[18] Warfarin, clopidogrel, ACE inhibitors, beta-blockers and statins, which are mostly unaffordable, were not available. No data were available for CVD risk stratification in primary healthcare facilities, total cholesterol measurement at the primary healthcare level, and secondary prevention of ARF and RHD in public sector health facilities.[5]

Secondary prevention and management

Of the hypertensive persons, 11.5% is receiving medical treatment,[19] while oral anticoagulants are prescribed in 34.2% of high-risk patients with AF.[12] The percentage of people with a history of CVD taking aspirin, statins and at least one antihypertensive agent is unknown.

Part D: Cardiovascular disease governance

The National Integrated and Multi-sector Strategic Plan for the Control of Chronic NCD (NIMSPC-CNCD) of 2011–2015 included CVD and risk factors, such as hypertension, diabetes, tobacco use, unhealthy diets, physical inactivity and the harmful use of alcohol.[20] Although a unit for NCD is in place in the Ministry of Health,[21] no dedicated budget is available to ensure implementation. Preventing and controlling RHD as a priority in Cameroon was also included in the NIMSPC-CNCD, but this plan was never published or distributed.[21,22]

Ten-year NCD/CVD surveillance programmes have been reported, based on the STEPS approach and others.[20,23,24] However, a more comprehensive surveillance system for NCD was suggested.[23] Cameroon follows the World Health Organization (WHO) best-buy policies regarding tobacco use and has formulated a national tobacco control plan and multi-sectoral co-ordination mechanism for tobacco control.[20]

Developing the National Health Development Plan (NHDP) 2016–2020 was a collaborative project between the Ministry of Health and non-health ministries, which included NCD, of which CVD are prominent.[25] The health system is severely underfunded with NCD not prioritised and therefore affecting dedicated CVD funding, which has to rely on privately funded donors and out-of-pocket payments.[20] Cameroon was part of the WHO-CHOICE project, which incorporated a cost-effectiveness modelling tool that gathers national data to be used for developing the most effective interventions for leading causes of disease burden. The model can be adjusted according to the specific needs of the country and assist policymakers in planning and prioritising services at a national level.[26]

Assessment of policy response

Legislation that mandates health financing for CVD/NCD is lacking, as is that of essential CVD medicines at affordable prices.[20] Jingi *et al*.[17] noted aspirin was the most affordable CVD medicine with 70% availability and suggested improving access to affordable medicines through policy options, which include cost containment and promoting generics. No judicial orders protecting patients' rights and mandating improved CVD interventions, facilities, health-system procedures or resources have been implemented, although a few policies address individual interventions, such as tobacco and alcohol use, and physical activity.[20]

According to Cameroon's Framework Convention on Tobacco Control (FCTC) report, tobacco policy addressed the creation of smoke-free zones, warnings on tobacco products, a ban on advertising, and tax increases.[11,20] There were no measures to protect tobacco control policies from tobacco industry interference.[11]

The country does not have policies that ensure equitable nationwide access to healthcare professionals and facilities or screening of high-risk CVD individuals. However, the public sector provides most of the healthcare, which is burdened by a lack of funding.[20] Sustainable funding is also not available for CVD from taxation of tobacco and/or other 'sin' products. There are no taxes on unhealthy foods or sugar-sweetened beverages.[27] The percentage of the excise tax of the final consumer price of tobacco products in Cameroon was 19%, while that of the final consumer price of alcohol products was rated 25% in 2015.[11,20]

No legislation exists on banning the marketing of unhealthy foods to minors or mandating clear and visible warnings on foods that are high in calories/sugar/saturated fats. Cameroon developed a food and nutrition policy to improve food and nutrition, as well as one that addressed physical inactivity through mass media awareness.[20]

Stakeholder action

In Cameroon, non-governmental organisation (NGO) advocacy for CVD policies and programmes as such has not been demonstrated. However, NGO involvement in NCD policies has been reported, for example the multi-sectoral expert group on tobacco.[28] Clinical Research Education, Networking and Consultancy (CRENC) is the most active cardiovascular research organisation in the country.[29] Its primary goal is to educate young researchers, linking them and translating research findings into practice to improve healthcare programmes and improve the well-being of people.[29] The Cameroon Heart Foundation and the Fondation Coeur et Vie also play an active role in Cameroon.[19]

No involvement of patients' organisations in the advocacy for CVD/NCD prevention and management has been reported, and no evidence was found regarding advocacy champions and/or patient engagement for RHD groups.

Involvement of civil society organisations (CSO), such as the National Multi-sectoral Committee for Tobacco Control,[28] in the development and implementation of a national tobacco control plan was mentioned in the FCTC report.[11] Cameroon contributed to the Mapping of NCD Civil Society Organisations in Francophone sub-Saharan Africa, initiated by the NCD alliance with a focus on NCD, more specifically diabetes and CVD.[31]

CSO involvement in the national multi-sectoral co-ordination mechanism for NCD/CVD was documented by Juma *et al*.[30,32] An example is the Cameroon Civil Society NCD Alliance that

empowers CSO through capacity building, unified action and stakeholder consultations, along with promoting evidence-based advocacy in preventing and controlling NCD.[33] No specific activities by cardiology professional associations were reported that aim at a 25% reduction in premature CVD mortality rate by 2025, although Cameroon was represented at the 65th World Health Assembly in 2019.[34] BP screening by businesses has proved to be an effective strategy in early detection and monitoring of hypertension.[19] However, in Cameroon BP screening has not yet been addressed.

Based on the data collected for Cameroon, the following strengths, threats, weaknesses and priorities are summarised.

Strengths

Cameroon ratified the WHO FCTC in 2006, which motivated the development of policies to curb tobacco use and control NCD.[20] Taxation of cigarettes also emerged from the FCTC. Policies that address WHO best-buy interventions include those on the prevention of tobacco and alcohol use as well as physical inactivity and inadequate nutrition.[20] Promoting physical activity through mass media and public education and awareness has been reported.[28]

The CAMBoD (Cameroon Burden of Diabetes) survey provided data for implementing a programme on diabetes and hypertension as these risk factors had emerged as public health problems.[35]

May measurement month (MMM), an initiative started by the International Society of Hypertension, is a cross-sectional BP survey of volunteer adults age ≥ 18 years. Screening at public locations, including sponsorship from business entities, requesting their corporate responsibility, is promoted.[30] Organisations co-ordinating the MMM in Cameroon are the Cameroon Cardiac Society (CCS), CRENC, a non-profit research organisation, and the Fondation Coeur et Vie.[30]

Upgrading of the Shisong Cardiac Centre at St Elizabeth Catholic General Hospital ensured improved treatment of patients with heart diseases.[35] Total CVD death rates were lower than other West African countries, Mauritania (16.6%) and Senegal (16.9%), but higher than that of bordering Nigeria (7.7%) (Table 1).

Threats

Cameroon with its high mortality rate attributable to infectious diseases, inadequate health system characterised by absence of health insurance, and lack of healthcare professionals, is also burdened by an increase in NCD and specifically CVD.[35] Increased risk factors are obesity, hypertension and hyperglycaemia, with heart failure (HF) being the most significant form of CVD impacting on young, economically active individuals.[35]

In urban Cameroon, the hypertension prevalence is high, with a very low awareness, which is attributed to the rapid urbanisation along with high obesity, physical inactivity and diabetes rates, increased salt consumption and tobacco use.[8] In hypertensive patients, HF is common and often associated with co-morbidities.[36] Hypertension accounted for 43.9 and 54.49% of HF in sub-Saharan Africa (SSA) and Cameroon, respectively.[37] In a hospital study, HF was the reason for 5.77% of all admissions at the turn of the century, with a prevalence of 30% and overall mortality rate of 9.03%.[38] RHD also remains a significant cause of HF in SSA and is the third most common cause of HF after hypertension and cardiomyopathies.[37] Recent data from another hospital-based study confirmed hypertension (54.79%) to be the foremost risk factor associated with HF, along with diabetes (17.12%) and smoking (15.75%), as the most common co-morbidities.[39] As elsewhere in Africa, HF carries a poor prognosis with one out of five patients with HF in Cameroon dying, and one out of four hospitalised within one year.[40,41]

The rising burden of hypertension among people living with HIV/AIDS is another threat, with at least 20% of HIV-infected individuals found to be hypertensive. As in the general population, awareness, detection, treatment and control of hypertension are inadequate in these people.[42]

Weaknesses

Although the NIMSPC-CNCD was developed as a reference document for preventing and controlling NCD, it was never implemented as a comprehensive, preventative intervention strategy.[20] In the early 2000s, national programmes were introduced with a focus on controlling hypertension, CVD, diabetes and other NCD.[20] Discrepancies among the various tobacco control policies regarding implementation also exist, possibly because of the absence of a comprehensive tobacco-prevention control programme.[43]

As in most sub-Saharan countries, funding for health is insufficient, and no national strategy is available to secure funds.[44] Only in the 2016–2027 health sector strategy paper were funds allocated for NCD prevention and control, and optimal management of these resources could be sacrificed as there is no national multi-sectoral committee on NCD.[20] The NCD prevention policy is hampered by the absence of effective monitoring and evaluation plans, causing a risk of neglect.[20] No autonomous system exists for regulation of the pharmaceutical sector, allowing quality medical products at affordable prices.[44]

Although policies have been developed and the government showed interest in establishing an NCD unit in the Ministry of Public Health, no implementation is seen regarding regulations intended for alcohol, physical activity and diet.[20]

According to recent data, no community screening of RHD has been done in Cameroon.[45] Therefore, the true prevalence of RHD is possibly higher than recorded because patients are more likely to seek medical attention only when symptoms present.[45]

Priorities

Comprehensive interventions or programmes are needed to address nutrition, physical inactivity and obesity among adults and children, as has been done for tobacco control.[20] The high prevalence of hypertension highlights an urgent need to implement an up-to-date national programme targeting risk factors and community awareness of NCD, specifically CVD.[35] An adaptation and implementation of the PASCAR roadmap,[46] which identified practical and effective solutions to improve detection, treatment and control of hypertension on the continent, are among the best ways forward. Cost-effective strategies to prevent hypertension and improve awareness, treatment and control were also suggested.[47]

Guidelines to improve AF and HF treatment and prognosis include, among other things, improving access to cardiologists, training of staff, developing primary-prevention programmes, and providing cheaper medication and information to the general public.[12]

Shortages in human and financial resources need to be addressed mainly because of other competing health priorities. The fifth strategic priority of the WHO country co-operation strategy asks for the 'improvement of measures for the monitoring of the management of programmes, logistics, equipment and different materials, ICT (information and communication technologies) and finances'. As such, Cameroon is committed to focus on these and the other priority areas. These priority areas include, among other things, supporting the fight against NCD, improving health indicators and health security, promoting safe behaviour, and strengthening the health system.[44]

This publication was reviewed by the PASCAR governing council and approved by the president of the Cameroon Cardiac Society.

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Case Report

Large left ventricular non-infectious vegetation in patient with eosinophilic granulomatosis with polyangiitis

Yun-Seok Song, Sang-Hoon Seol, Jino Park, Dong-Kie Kim, Yeo-Jeong Song, Seunghwan Kim, Ki-Hun Kim, Doo-Il Kim, Chan-Seon Park, Yeon-Mi Kim

Abstract

Eosinophilic granulomatosis with polyangiitis (EGPA) is a rare form of systemic vasculitis in which cardiac involvement is relatively common and accounts for half of EGPA-related deaths. Cardiac involvement is more frequent in patients with an absence of anti-neutrophil cytoplasmic antibody and those with higher eosinophil counts. Clinical manifestations are various, including myocarditis, pericarditis, pericardial effusion, heart failure, arrhythmias, valvular insufficiencies and intra-cardiac thrombus formation. The pathology of cardiac involvement in EGPA is usually endomyocardial and pericardial eosinophilic infiltration. Considering the potentially adverse outcomes associated with cardiac involvement in EGPA, early detection is important. We experienced a rare case of EGPA with cardiac involvement presenting with non-infectious vegetations.

Keywords: eosinophilic granulomatosis with polyangiitis (EGPA), left ventricular non-infectious vegetation

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Department of Internal Medicine, Haeundae Paik Hospital, Inje University College of Medicine, Busan, Korea
Yun-Seok Song, MD
Sang-Hoon Seol, MD, shseol@paik.ac.kr
Jino Park, MD
Dong-Kie Kim, MD
Yeo-Jeong Song, MD
Seunghwan Kim, MD
Ki-Hun Kim, MD
Doo-Il Kim, MD
Chan-Seon Park, MD

Department of Pathology, Haeundae Paik Hospital, Inje University College of Medicine, Busan, Korea
Yeon-Mi Kim, MD

Case report

A 27-year-old man was transferred to our hospital after 10 days of persistent fever, skin rash, and pain and numbness in both ankles. At another hospital he had had antibiotic treatment with ceftriaxone and doxycycline on the presupposition that it was Tsutsugamushi disease, but it had no effect. The patient was regularly followed up at the Department of Allergy and Clinical Immunology for two years because of bronchial asthma and chronic rhinitis.

On admission, his body temperature was 38.1°C, blood pressure was 120/80 mmHg, heart rate was 80 beats/min, and respiratory rate was 20 breaths/min. Breath sounds were slightly decreased on the left lower lung field, and no heart murmur was audible. Petechial rash was found on his whole body. Electrocardiography was in normal sinus rhythm. A chest radiograph showed blunted left costopleural angle and the cardiac contour seemed to be slightly widened.

Initial laboratory tests showed mild leukocytosis (9.14×10^9 cells/l) with marked eosinophilia (39.0%). C-reactive protein was 11.5mg/dl and the cardiac markers, pro-BNP (3548.0 pg/ml), CK-MB (20.9 ng/ml) and troponin-I (2.92 ng/ml) were elevated. On additional laboratory examination, perinuclear anti-neutrophil cytoplasmic antibody (P-ANCA) and cytoplasmic anti-neutrophil cytoplasmic antibody (C-ANCA) were negative. Parasite-specific antibodies were all negative and total Ig E level was high at 2154.0 IU/ml. Blood cultures were all negative. The absence of platelet-derived growth factor receptor-α and -β (PDGRFA, PDGFRB) gene fusion made a diagnosis of idiopathic hyper-eosinophilic syndrome unlikely. A pleural effusion study revealed neutrophil-predominant exudate, the pH was 7.0, adenosine deaminase was 25 IU/l, glucose was 51 mg/dl (2.83 mmol/l), and Gram and AFB staining were negative. Bacterial and fungal cultures showed no growth.

Cardiac evaluation was performed because of the elevated cardiac markers. Transthoracic echocardiography showed oscillating mass-like lesions at the mid anteroseptal wall of the left ventricle. The heart chamber size and left ventricular (LV) wall thickness were in the normal range, and LV systolic and diastolic function were normal (Fig. 1). There was no evidence of pericardial effusion or inferior vena cava plethora. Coronary angiographic computed tomography revealed a normal coronary artery. Cardiac magnetic resonance imaging (MRI) was refused for the reason that the patient had panic disorder.

For lower extremity numbness, a nerve conduction study was performed, which showed decreased sensory nerve action potential amplitude in both sural nerves. Intravenous methyl-

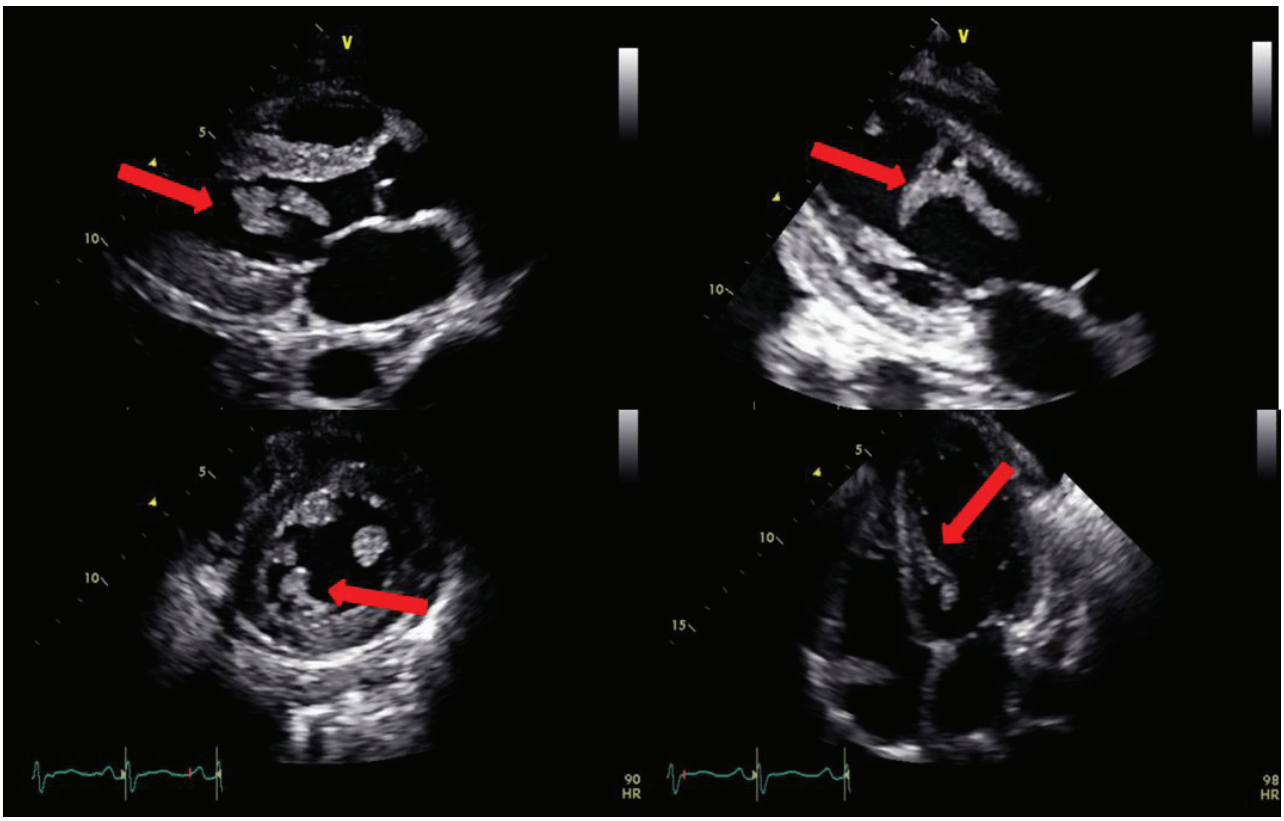

Fig. 1. Transthoracic echocardiography showing oscillating mass-like lesions at the mid anteroseptal wall of the left ventricle (arrows). The heart chamber size and systolic function were normal.

prednisolone (1 mg/kg/day) treatment was started from the second day in hospital, with a possible diagnosis of eosinophilic granulomatosis with polyangiitis (EGPA) or hyper-eosinophilic syndrome. After intravenous steroid treatment, the patient's clinical conditions, including body temperature, skin rash and numbness of the feet, improved rapidly.

On the fourth day in hospital, the patient had an operation for resection of the mass-like lesions in the left ventricle. Pathological gross findings showed fragments of pinkish-gray soft tissue measuring 3.0 × 1.0 and 2.5 × 1.0 cm (Fig. 2). Microscopic findings revealed non-infective vegetations that comprised a thrombus, granulation tissue, eosinophils, lymphoplasmic cells, neutrophils and histiocyte infiltrations (Fig. 3).

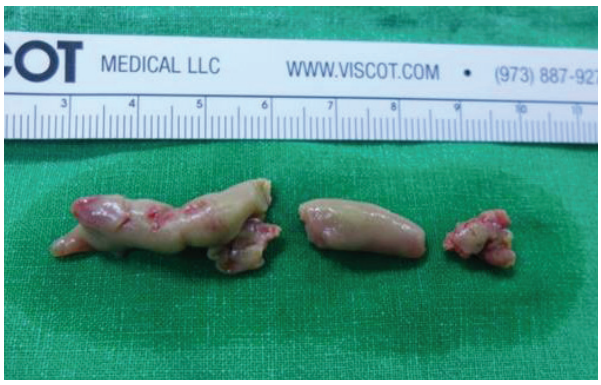

Fig. 2. Pathological gross findings showing fragments of pinkish-gray soft tissue measuring 3.0 × 1.0 and 2.5 × 1.0 cm.

Based on these pathological findings, namely hyper-eosinophilia, history of asthma, chronic sinusitis and polyneuropathy, a diagnosis of EGPA was made. Seven days after starting intravenous steroid treatment, all the laboratory results, including eosinophil count, C-reactive protein and cardiac markers, were normalised. The patient was discharged and is on oral methyl-prednisolone treatment at the out-patient clinic.

Discussion

Eosinophilic granulomatosis with polyangiitis or EGPA, previously named Churg-Strauss syndrome, which was first described in 1951, is a rare form of systemic, necrotising small-vessel vasculitis with accompanying bronchial asthma, eosinophilia and eosinophilic tissue infiltration of various tissues with granuloma formation.[1,2] The pathogenesis is not well known, however it is considered a T-helper type 2 (Th2)-mediated disease.[2-4] The immune response may be triggered by genetic or environmental factors such as allergens, infections, drugs or nutrition.[2-5] Eosinophils, T-lymphocytes, B-lymphocytes and various cytokines may also play a role in the process.[2-4]

The most commonly involved organ is the lung, followed by the skin and nervous system; however it can affect any organ

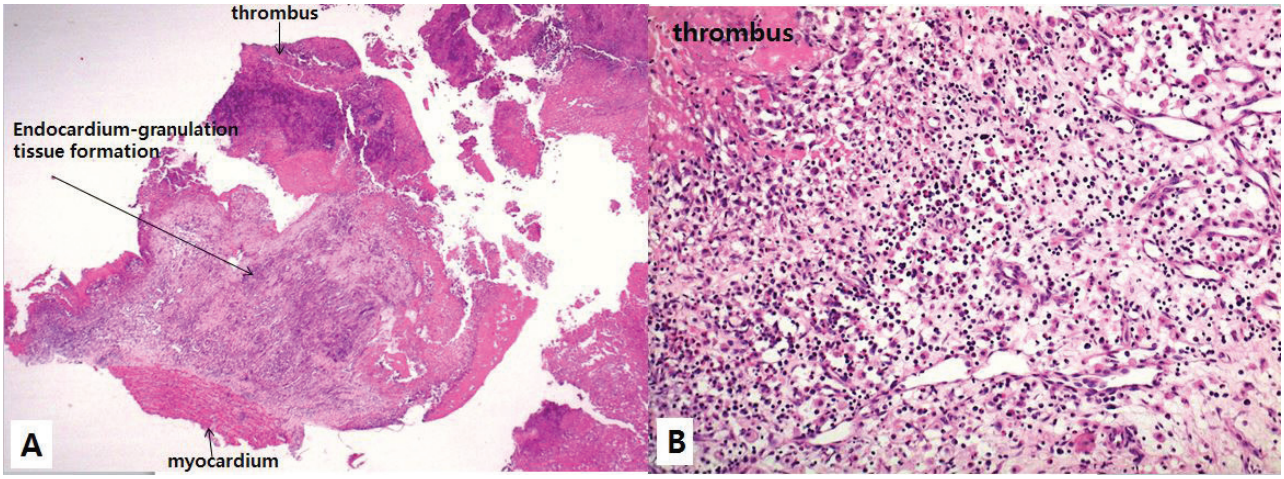

Fig. 3. A. Microscopic findings revealed marked inflammatory infiltration composed of granulation tissue, eosinophils, lymphoplasma cells, neutrophils and histiocytes, and thrombus formation (H&E, × 10.25). B. There was eosinophil-rich inflammation in the endocardium (H&E, × 200).

system.[4,6] Cardiac involvement is relatively frequent and one of the most serious manifestations of EGPA, accounting for approximately half of the deaths attributable to EGPA. It is more common in patients with an absence of ANCA and those with higher eosinophil counts.[2,7-9] Clinical manifestations are various, including myocarditis, pericarditis, pericardial effusion, heart failure, arrhythmias, valvular insufficiencies, intra-cardiac thrombus formation, and others.[7,9]

The histological features of EGPA are tissue eosinophilia, necrotising vasculitis and extravascular eosinophilic granulomas. However, histological findings may vary according to the organs involved. Cardiac pathology usually shows endomyocardial and pericardial eosinophilic infiltration and only rarely, coronary vasculitis.[1,3,9] Because cardiac involvement in EGPA is relatively frequent and could be fatal, early detection is important.[2,3,7]

Transthoracic echocardiography can show a wide spectrum of cardiac abnormalities, including systolic dysfunction, valvular insufficiencies, pericardial effusion and intra-cardiac thrombus.[7,9] Cardiac MRI is the most sensitive technique to evaluate cardiac involvement in EGPA. It can detect clinically silent and undisclosed myocardial involvement.[4,7,9] Late gadolinium enhancement in cardiac MRI suggests active endomyocarditis or endomyocardial fibrosis. Most enhancing lesions were apical and mid-cavity segments of the left ventricle.[7,10] Therefore late gadolinium enhancement of endocardial layers could be associated with eosinophilic Loeffler-like endocarditis.[7,11]

The general consensus for treatment is based on the usage of systemic glucocorticoids, adding other immunosuppressants if the prognosis is poor.[3,4] The most commonly used prognostic tool is the Five Factor Score (FFS) scale. According to this scale, one point is given for each of the following: cardiac involvement, severe gastrointestinal manifestation, central nervous system involvement and renal impairment. Patients with a good prognosis have a FFS of 0 points and are treated solely with corticosteroids, while for patients with a poor prognosis (FFS ≥ 1), consider the addition of immunosuppressants, usually cyclophosphamide.[4,12]

In this presented case, diagnosis was based on the clinical history and laboratory and pathology results. Hyper-eosinophilic syndrome (HES) is probably the most challenging differential diagnosis of EGPA. We could rule out reactive HES from parasitic infection, allergy and drug reaction by clinical history. The absence of PDGRFA and PDGFRB gene fusion suggested it was less likely to be myeloid or lymphoid HES, and idiopathic HES is rarely accompanied by asthma.[2,3] After ruling out HES, the diagnosis was made by American College of Rheumatology (ACR) criteria.[13]

Cardiac MRI is known as a sensitive modality to evaluate cardiac involvement,[4,7,9] however, we could not use it owing to the patient's refusal. Echocardiographic findings revealed intra-cardiac vegetative formations, which have not been reported before; hence it is quite a rare form of cardiac involvement of EGPA. We considered adding cyclophosphamide however the patient's clinical aspects rapidly improved after intravenous methyl-prednisolone and surgical treatment.

Conclusion

We experienced a patient with EGPA with cardiac involvement presenting with non-infectious vegetations. There have been reports of intra-cardiac thrombus formation in EGPA patients[7] but there are no reports of EGPA-related vegetative formation. The patient was successfully treated by surgical removal and a systemic corticosteroid.

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Case Report

A 31-year-old pericardial textiloma

Dong Kyu Kim, Su Kyung Hwang, Sang Cjeol Lee, Yong Jik Lee, Jong Pil Jung, Chang Ryul Park, Gwan Sic Kim

Abstract

Gossypibomas are uncommon but important complications of surgery. This case report is of a gossypiboma found accidentally 31 years after heart surgery. A 41-year-old man had lost 5 kg in the previous three months and suffered from intermittent epigastric discomfort. A computed tomography scan incidentally revealed a well-defined mass in the right lower anterior mediastinum. Given his history of previous cardiac surgery to repair a ventricular septal defect, the possibility of gossypiboma could not be excluded. Elective excision of the mass was performed through a median sternotomy, and a 5-cm ovoid mass consisting of a thrombus and gauze was removed. The postoperative course was uneventful. The patient's clinical findings were normal, with no abnormal findings on transthoracic echocardiogram performed one year later.

Keywords: textiloma, gossypiboma, gauzoma

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Gossypibomas are rare but can cause serious medicolegal issues after surgeries of the thorax, abdomen or pelvis. This complication occurs due a counting error with regard to the materials used during surgery and tends to be under-reported. If an abnormal mass is found in a patient who has undergone surgery, the possibility of a gossypiboma should be considered. If diagnosed, surgical removal is the treatment. We report a case of a 41-year-old man who presented with epigastric pain and weight loss and was diagnosed with a gossypiboma caused by heart surgery carried out 31 years before.

Department of Thoracic and Cardiovascular Surgery, Ulsan University Hospital, Ulsan University Medical School, Ulsan, Republic of Korea
Dong Kyu Kim, MD
Su Kyung Hwang, MD
Sang Cjeol Lee, MD
Yong Jik Lee, MD
Jong Pil Jung, MD
Chang Ryul Park, MD
Gwan Sic Kim, MD, lovingmylife@naver.com

Case report

A 41-year-old man had lost 5 kg in the previous three months and had suffered from intermittent epigastric discomfort. He presented to the emergency room with pain in the upper abdomen that began eight hours before his visit. He had undergone repair of a ventricular septal defect 31 years earlier.

The initial vital signs, simple radiography, 12-lead electrocardiogram and laboratory findings were unremarkable. An abdominal computed tomography (CT) scan incidentally revealed a well-defined mass in the right lower anterior mediastinum; no other abdominal findings were found. Subsequently, a chest CT scan showed a round, heterogeneous mass measuring 4.5 × 4 × 5 cm situated adjacent to the right atrium. A transthoracic echocardiogram revealed a heterogeneous and echogenic mass with a slight mass effect on the right atrium, and increased right ventricular outflow tract velocity (peak velocity = 2.46 m/s). The size of the left ventricle and systolic function were normal.

An elective operation to remove the mass was performed, to eliminate compression of the right atrium and obtain accurate histological findings. A median sternotomy was performed and a 5-cm ovoid mass was observed, strongly adhered to the right atrium and pericardium (Fig. 1A). The mass was not easy to peel off and a right atrial injury occurred during the dissection (Fig. 1B). It was necessary to resect and reconstruct part of the right atrium to completely remove the mass. We started cardiopulmonary bypass through the aorta, superior vena cava and right femoral vein. The mass was completely removed under cardiopulmonary bypass support. After removing the mass, a 2 × 3-cm defect formed in the right atrium, which was reconstructed using bovine pericardium (Fig. 2).

The cross-section of the mass indicated an old thrombus and blood clots. We found that the thrombus and gauze were adherent (Fig. 3). Specimens were sent to the Pathology Department for diagnosis and formal histopathological examination confirmed a textiloma. The patient's postoperative course was uneventful and the clinical findings were normal, with no abnormal findings on transthoracic echocardiogram performed one year after the operation.

Discussion

Gossypiboma, also known as a textiloma or gauzoma, refers to a foreign body left in the body after a surgical procedure, which becomes a mass. It usually consists of surgical gauze and inflammatory tissue caused by the foreign body reaction.[1]

This disease poses medicolegal issues, so it tends to be under-reported. The prevalence of this disease is about one

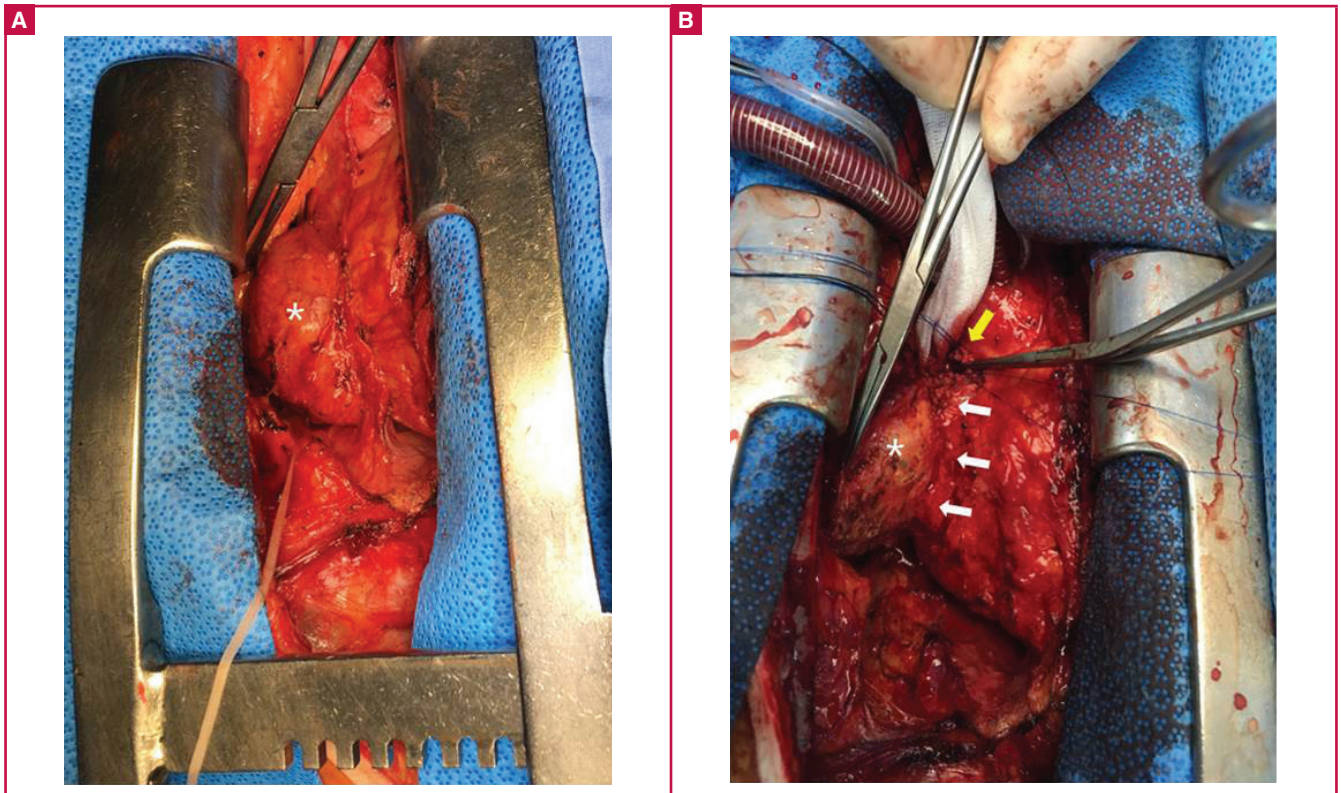

Fig. 1. (A) A 5-cm ovoid mass (asterisk) was strongly adhered to the right atrium after a median sternotomy. (B) Right atrial injury (yellow arrow) occurred during dissection of the mass (asterisk). Right atrium is indicated by white arrows.

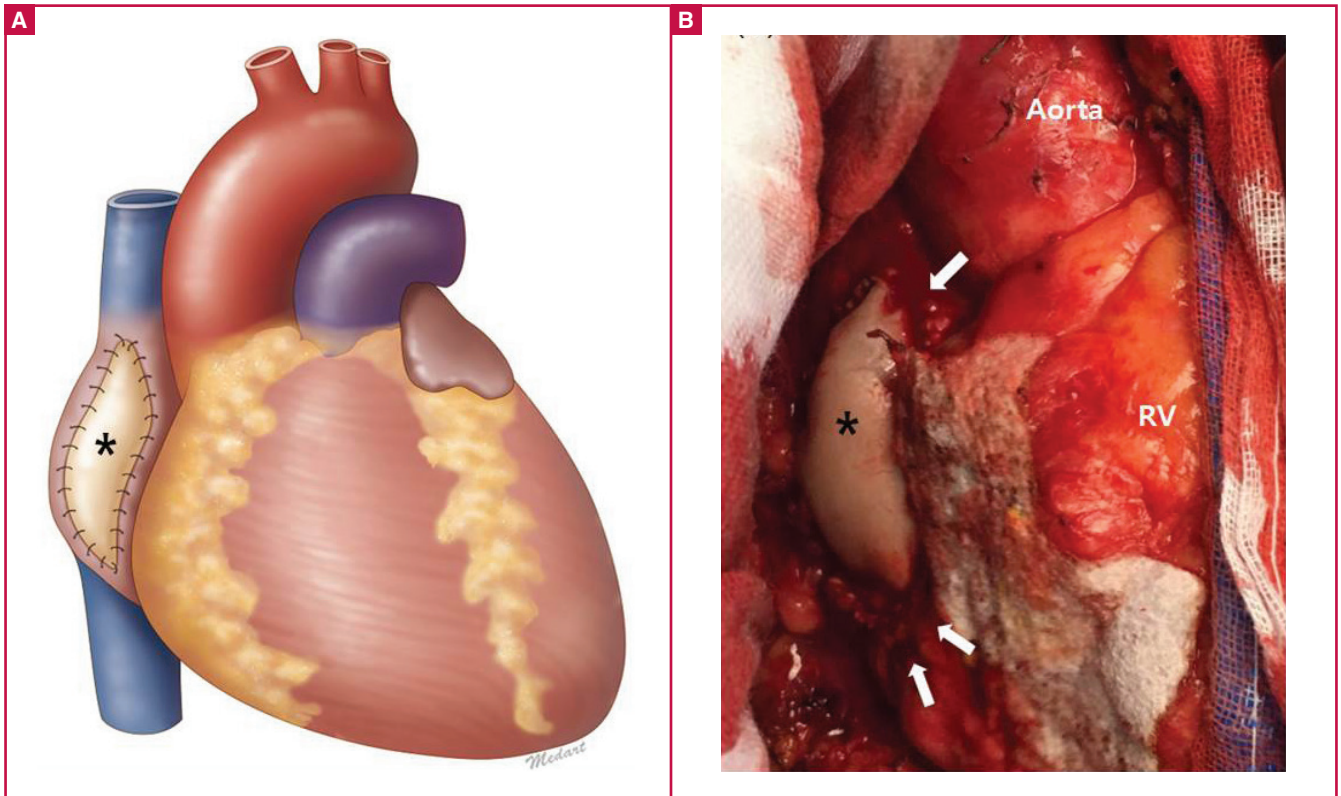

Fig. 2. (A) Schematic illustration of the right atrial reconstruction. (B) Intra-operative photograph. The right atrium (white arrows) was reconstructed using bovine pericardium (asterisk) after removing the mass. RV, right ventricle.

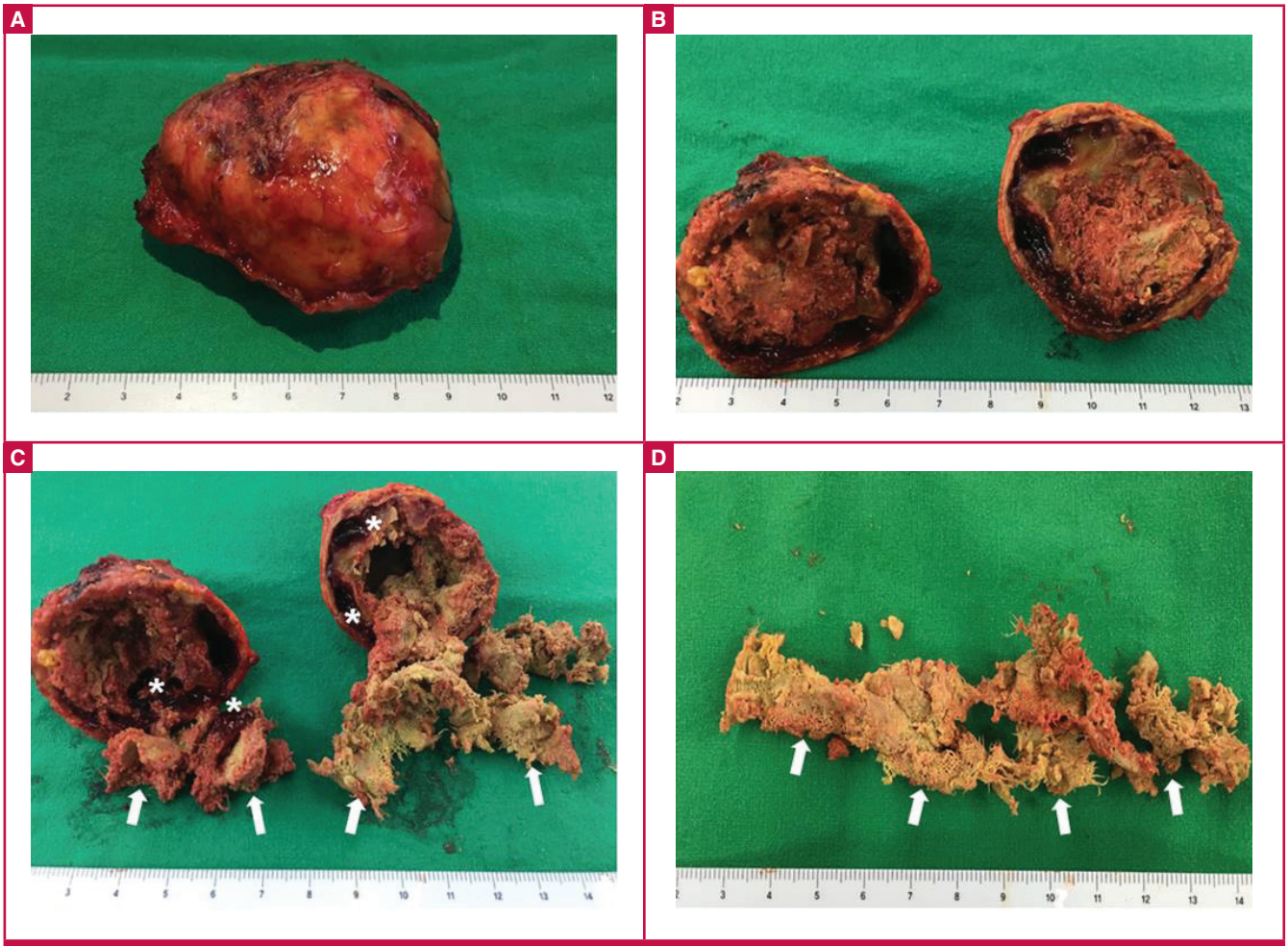

Fig. 3. (A) Gross image of the mass. (B) Cross-section of the mass. (C, D) Contents of the mass: thrombus (asterisks) along with gauze (arrows).

case per 3 000 surgeries.[2] Emergent surgery has a greater risk of gossypiboma than elective surgery.[3,4]

Gossypiboma can occur in any part of the body with an inner space but the most common location is the abdomen, followed by the pelvis and thorax.[5] Thoracic gossypibomas tend to be found in the lower third of the lung, with no difference in incidence between the left and right lungs.[6] Pericardial gossypibomas are much rarer.

Symptoms differ according to the size and site of the mass, as well as the degree of inflammation. Abdominal gossypibomas can cause abdominal pain and a palpable mass, whereas thoracic gossypibomas often result in chest pain, non-productive cough and fever.[7] Complications include adhesions, abscesses, fistulae and perforations, which require a surgical approach to remove the mass and control the inflammation. Despite the thoracic gossypiboma, the patient in this case showed non-specific symptoms such as abdominal pain and weight loss, probably due to prolonged inflammation.

Most cases of gossypiboma are detected on a CT scan. CT findings include well-encapsulated heterogeneous masses, often with calcification, gas and spongy or textured fragments.[8] The majority of masses does not exceed 10 cm in diameter and usually involves the adjacent peritoneum, pleura or pericardium. Magnetic resonance imaging findings are less accurate than CT, and biopsy results are inconclusive as the mass consists of foreign material and inflammatory tissue.[9] If a patient with a history of previous surgery shows non-specific symptoms and a CT scan reveals a mass at the operated site, a gossypiboma must be suspected.

Conclusion

The rate of gossypiboma cases is declining,[10] however, since it can occur during any surgery, a strict protocol for keeping track of the materials used during surgery is needed. The use of radiopaque gauze during surgery is one option to diagnose gossypiboma quickly if prevention fails. After the disease is diagnosed, surgical exploration and resection is the treatment, and extreme care is needed to prevent a second gossypiboma caused by the exploration itself. This case emphasises that pericardial gossypiboma can cause non-specific symptoms such as abdominal pain and weight loss, and can emerge after a long time, over 30 years.

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