Chronic Kidney Disease Prevalence and Ambulatory Blood Pressure Profile In Healthy HIV Positive Subjects Pre and Post Anti- Retroviral Therapy

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PLAGIARISM DECLARATION

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2. I have used the Vancouver convention for citation and referencing. Each contribution to, and quotation in this report from the work(s) of other people has been attributed, and has been cited and referenced.

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Dissertation abstract

Introduction: Few studies have been done in South Africa to establish the extent of chronic kidney disease (CKD) in stable outpatients infected with the human immunodeficiency virus (HIV). Both HIV and the anteretroviral therapy (ART) used to treat HIV have been associated with abnormal metabolic profile, increased cardiovascular risk and renal disease[1,2,3]. Hypertension has been found to be common in HIV infected individuals, in European and American cohorts, with a prevalence ranging from 13- 34%[2]. Nocturnal blood pressure (BP) is superior to daytime or office BP as a predictor of cardiovascular disease[4]. However, the relationship between circadian BP patterns, measured via ambulatory blood pressure (ABP) monitoring, and HIV has never been documented in the South African HIV infected population. Individuals with an abnormal diurnal rhythm and a blunted nocturnal decline in systolic BP (SBP), i.e. ≤ 10%, are referred to as 'non- dippers' and have the highest risk of cardiovascular complications[4]. HIV itself has been associated with a non- dipping status and may play a role in the HIV related increase in cardiovascular risk[5].

Our aims were to:

a) Document the prevalence of CKD at baseline in ART naive subjects, and document changes at 6 months on ART.
b) Document the prevalence of hypertension at baseline in ART naive subjects and document changes in BP at 6 months on ART.
c) Observe characteristics of ABP in a subset of patients at baseline and after 6 months of ART.

Subjects and Methods: We conducted a prospective cohort study of ART naive HIV positive patients at Crossroads Community Health Centre in Cape Town. Office BP and renal function parameters including: urine microalbumin: creatinine ratio, creatinine, urine dipsticks and estimated glomerular filatration rate calculation (eGFR) were measured at baseline and at 6 months, after the initiation of ART. A subset of patients underwent ABP monitoring. A control group of HIV negative patients, from Nolungile Clinic in Khayelitsha, were also recruited for ABP monitoring. Ethics approval was obtained from the University of Cape Town Research Ethics Committee (ref: 27/2006).

Findings: No subject had an eGFR below 60ml/min, 3 (4.7%) patients had microalbuminuria and only 1 (1.6%) had overt albuminuria. No patient was hypertensive but there was a significant rise in office SBP after 6 months of ART (p- value= 0.05, 95% CI: -0.007- 0.933), this increase was not confirmed on ABP. 80% of HIV positive patients and 52.9% of HIV negative controls were non- dippers (p- value= 0.05, odds ratio = 3.56, 95% CI: 0.96- 13.13). The high prevalence of non- dipping on ABP monitoring was not improved by ART.

Interpretation: The prevalence of CKD in ART naive patients in a typical HIV outpatient clinic is considerably lower than previously reported. This suggests that early introduction of ART may have a major impact on the prevalence of HIV associated nephropathy (HIVAN).

No subject was hypertensive, but non- dipping status is 3.6 times more likely among black HIV positive subjects than controls. The non- dipping status was not improved by ART. The phenomenon is unexplained and suggests an underlying dyregulation of the cardiovascular system and may be associated with future cardiovascular risk.

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Contents

PART A : PROTOCOL

Introduction

There have been 3 prospective studies performed in Africa assessing the prevalence of chronic kidney disease (CKD) in stable outpatients infected with the human immunodeficiency virus (HIV)[1,2,3]. No epidemiology study of this nature has taken place in South Africa (SA), the country housing the largest population of people living with HIV worldwide. Evidence of CKD at the time of initiation of antiretroviral therapy (ART) is an independent predictor of mortality in this population[4].

The first study was undertaken in western Kenya, which evaluated 373 patients, renal insufficiency (i.e. Creatinine Clearance (CrCl) <60ml/min) was identified in 11.5% with 4.8% having a CrCl <50ml/min[1]. Another study was performed in Uganda, of 508 patients studied 20% had reduced renal function evidenced by a CrCl between 25 and 50ml/min. However patients with a CrCl of less than 25ml/min were excluded from the study so the true prevalence is likely to be greater than this[2]. A large study, of well, ART naive non-pregnant adults from 7 sub-Saharan African nations, investigated the prevalence of CKD in a cohort of 2495 patients. The prevalence of CrCl <50ml/min was low at 3.4%. Median CD4 count was 295 cells/ml[3].

CKD in HIV includes HIV associated nephropathy (HIVAN), immune complex diseases, thrombotic microangiopathies and vasculitidies[5,6,7]. In these patients there is also a heavy burden of acute kidney injury caused by: diarrhoea,

sepsis, tuberculosis and the nephrotoxic effects of ART (e.g. tenofovir) and other drugs (e.g. cotrimoxazole, rifampicin)[7,8,9]. HIV infection has become a chronic and manageable disease especially where the roll- out of ART has been implemented for longer. With the rise of cardiovascular and metabolic disease in SA, hypertension and diabetes with ensuing nephropathy could become a greater problem in our ageing HIV infected population[10].

Hypertension, a major risk factor for cardiovascular disease in the general population, was found to be common in international studies of HIV infected individuals with a prevalence of up to 34%[11]. Nocturnal blood pressure (BP) is superior to daytime or office BP as a predictor of cardiovascular events and it is normally at least 10% lower than daytime BP (in normotensive and hypertensive patients)[12]. Individuals with an abnormal diurnal rhythm and a blunted nocturnal decline in systolic BP (SBP) are referred to as 'non-dippers'[12]. These patients have the highest risk of cardiovascular complications and target organ disease (e.g. left ventricular hypertrophy, microalbuminuria)[12]. However, the relationship between circadian BP patterns and HIV has never been documented in our HIV infected population, despite this being the most accurate measure of risk.

In studies conducted in Italy and Norway, of predominantly white HIV infected individuals, a high prevalence of non- dipping was found compared to equally matched HIV negative controls[13,14]. Our HIV infected population however is predominantly black and the incidence of non- dipping in black populations in general has been shown to be greater than in whites[15,16].

The overall aim of the study will be to determine the prevalence of CKD and hypertension in ART naive subjects, and assess the effect of ART on CKD and BP. A substudy will be conducted to determine the ambulatory BP (ABP) characteristics of ART naive subjects compared to controls, and the effect of ART on ABP trends.

Methods

A prospective cohort study will be conducted over a period of 6 months. The study population will be an ambulatory HIV- infected community clinic- based cohort attending a community health centre in Crossroads, Cape Town. Crossroads has a stable population size of 41,000 with an estimated 5000 HIV-infected individuals living in the community. It provides services for 60-80 HIV infected clients daily with approximately 1500 on ART, 1200 of whom are adults. The yet unpublished McHAART study is an established clinical trial at Crossroads investigating the effects of ART on metabolic syndrome parameters in ART naive subjects. This study will form an important sub-study.

The following measurements will be done: urine dipstick (AccuBioTech Co. Ltd, Beijing, China), serum creatinine (umol/L), spot urine microalbumin/creatinine ratio (mg/mmol), and estimated glomerular filtration rate (eGFR) (ml/min/1.73m^2). Measurements will be performed prior to initiation of ART and after 6 months of treatment.

Office BP will be similarly measured pre- and post 6 months of ART. A sub-group of patients will undergo ABP monitoring using a Spacelabs ambulatory BP monitor provided by the Hypertension Clinic at Groote Schuur Hospital (GSH). An group of confirmed serological HIV negative patients, of similar demographics, will be recruited from Nolungile Clinic in Khayelitsha and serve as a control group.

Any of the subjects found to have overt nephropathy or hypertension will be referred for further evaluation.

Ethics

After signing informed consent (appendix 1, 2) subjects will undergo study related procedures. The implications of participating in the study will be fully explained to all participants in IsiXhosa using fluent Xhosa speaking fieldworkers.

Consenting patients for ABP monitoring will receive R200 to account for any inconvenience caused and for transport fees to and from GSH. Ethics approval has been obtained from the University of Cape Town Ethics Committee (27/2006).

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PART B : LITERATURE REVIEW

The objectives of the literature review were to appraise published evidence addressing the:

- Prevalence of HIV related renal disease in South Africa (SA)
- Clinical application of ambulatory blood pressure (ABP) monitoring
- Relevance of circadian blood pressure (BP) patterns
- Relationship between circadian BP patterns and HIV
- Effect of HIV infection and antiretroviral therapy (ART) on cardiovascular risk

The following databases were accessed for literature; PubMed, Google Scholar, EbscoHost, the University of Cape Town Health Sciences Library and individual websites for the World Health Organisation (WHO) and South African Department of Health using keywords: HIV, kidney disease/ dysfunction, prevalence, antiretroviral therapy (ART)/ highly active antiretroviral therapy (HAART), ambulatory blood pressure monitoring, blood pressure, hypertension, diurnal variation and South Africa. The years 1985-2012 were searched and 39 articles were identified. 36 articles contained relevant information, namely: 11 on the prevalence and impact of renal disease in HIV positive individuals[1-11], 7 on measuring ambulatory BP and its clinical relevance[12-18] and 18 on the effect of HIV on circadian BP patterns and the metabolic consequences of HIV and ART[19-36]. Due to the paucity of data available all pertinent clinical research publication types (i.e. systemic reviews, letters to the editor) were evaluated.

There have been very few prospective studies on the prevalence of chronic kidney disease (CKD) in HIV positive patients in sub-Saharan Africa. The prevalence of CKD was estimated at: 2.4% in Rwanda[1], 11.5% in Kenya[2], 20% in Uganda[3], 25% in Tanzania[4], 33.5% in Zambia[5] and 3.4% in a large cohort from 7 sub-Saharan nations[6]. These studies (as described below) had different methods for defining CKD and the patients enrolled had various WHO clinical stages of HIV, which may account for the large discrepancies in prevalence.

In a cross- sectional study in western Kenya of the 373 patients enrolled 11.5% had CKD (creatinine clearance (CrCl) <60ml/min) and 4.8% of these patients had CrCl <50ml/min. Thus, in this medically stable cohort, CKD was not uncommon. Proteinuria (defined as ≥1+ protein on dipstick) was present in 6.2 % of patients[2]. This prevalence of proteinuria is similar to other African studies (Rwanda[1], SA[7], Uganda[3]) ranging between 6% and 9% of patients studied.

In a descriptive single centre study in Johannesburg, SA the prevalence of proteinuria (including microalbuminuria) was high in 253 (43.7%) of the 576 HIV positive patients that were screened. This study cohort was predominantly black (n=560, 97%). The aim of this study was to detect early kidney disease by screening for proteinuria but additional abnormalities, including leucocyturia 30.3% (n= 175) and microscopic haematuria 33.1% (n= 191), were found. Although this study suggests a high prevalence of proteinuria, inconsistent with the other studies in sub-Saharan Africa as

mentioned above, 482 of the 576 patients (84%) were classified as having AIDS (CD4 count <200 cells/mm^3). Also, in the group with microalbuminuria (n=107, 18.5%) the prevalence of co-morbid disease (tuberculosis, cardiovascular disease, infection, diabetes, malignancy) was high. This study concluded that urinary abnormalities in HIV infected, ART naive patients are common thus recommends routine urinary screening in HIV clinics. No follow up was done in this study to determine whether resolution or reversal of urinary screening abnormalities occurred with appropriate treatment[8].

A randomised controlled trial in Uganda followed a HIV positive cohort (n= 508) after initiation of ART. Most of the patients had advanced HIV disease (median CD4 cell count 122 cells per mm^3, median HIV viral load of 244500 copies per ml). The baseline prevalence of CKD was 20% with CrCl between 25 and 50ml/min. Patients with CrCl less than 25ml/ min were excluded meaning that the true prevalence of CKD was probably greater than this. Following 2 years of ART they also found that renal function improved with the serum creatinine decreasing by 16% ($p< 0.0001$) and CrCl significantly improving by 21% ($p< 0.0001$)[3].

A study using data from a mother- to- child transmission network investigated the prevalence of CKD in well (median CD4 count 295 cells/ml), HIV- infected, ART naïve non- pregnant adults from 7 sub- Saharan nations. Of the predominantly female cohort of 2495 the overall prevalence of CrCl< 50 ml/min, as calculated by the Cockroft and Gault equation, was low at 3.4%[6].

In a 3 year multicentre cohort study conducted in Lusaka, Zambia 36239 treatment naive HIV positive adults were recruited. They found 8456 (33.5%, 95% CI: 32.9-34.1%) of the 25779 eligible for statistical analysis had CKD. The researchers used published clinical guidelines from the U.S. National Kidney Foundation's Kidney Disease Outcome Quality Initiative (K/DOQI) to categorize renal insufficiency. Of the 8456, 73.5% had stage 2 CKD, 23.4% had stage 3 CKD and 3.1% had stage 4 CKD. The predictors of renal dysfunction in this study included: female sex, BMI<18.5 kg/m^2, haemoglobin <8g/dL and WHO HIV stage 3 or 4[5]. Microalbuminuria was found in 70% of patients which is also associated with mortality in HIV infected women[1]. Advanced HIV stage was also associated with decreased renal function in the studies conducted in Western Kenya[2] and Nigeria[9] but this was not the case in the Rwanda study[1].

An observational prospective study, comparing HIV positive and HIV negative Rwandan women, enrolled 936 participants. Of this group 710 were HIV positive and 226 HIV negative. The median CD4 count in these women was 256 and 41.5% were classified as having WHO stage 4 HIV. The prevalence of CKD was low with 2.4% (n= 21) having an estimated glomerular filtration rate (eGFR) <60ml/min/1.73m2 calculated by the 4- variable Modification of Diet in Renal Disease (MDRD) equation. Proteinuria was present in 9% (n= 64) of the patients which was not associated with HIV status (adjusted odds ratio (OR) 1.7, 95% CI: 0.7- 4.1). However, HIV infection was independently associated with decreased eGFR (adjusted OR 8.9, 95% CI: 1.6- 50.5) after adjusting for age, BP, body composition and albumin[1].

Some studies in sub-Saharan Africa assessed the prevalence of kidney disease by renal biopsies. These were done in SA (Cape Town[10], Johannesburg[11], Durban[7]) and in Nigeria[9]. These studies show a wide spectrum of histopathological lesions in HIV infected individuals but HIV-associated nephropathy (HIVAN) was the most common biopsy finding[7,9,10,11]. Additional lesions found were: immune complex disease[10,11], other glomerulonephritidies[7,10,11] and thrombotic microangiopathies[7,10].

Hypertension is a common in the adult population of SA and is a major cause of morbidity and mortality. Office BP is an important tool to diagnose hypertension, but has limited value for both diagnosis and prediction of cardiovascular outcome. ABP monitoring, on the other hand, enhances the ability to identify white coat hypertension and the masked effect as well as eliminating bias introduced by inaccuracy and human error[12]. ABP is also more closely associated with cardiovascular complications[12]. This has been confirmed in the SA population even in patients with normal or mildly raised BP[13]. ABP is better than office BP in assessing circadian rhythm and BP variability[12]. The normal circadian pattern is where the night-time systolic BP (SBP) falls by ≥10% and increases on waking[14,15]. Individuals with a nocturnal SBP fall less than 10%, referred to as ‘non- dippers’, have an increased risk of cardiovascular complications[13-17]. A non- dipping BP pattern is also associated with CKD, but it is not established whether this is cause or effect[18].

Two studies have evaluated 24-hour ABP in HIV positive patients[19,20]. The prevalence of non-dipping BP pattern, in a Norwegian study of 77 hypertensive HIV positive individuals, was high and significantly more than in 76 hypertensive controls (59.7% vs. 32.9%, p=0.001). Non- dipping was independently associated with HIV status (OR 0.33, 95% CI: 0.17-0.66, p=0.002), but not microalbuminuria (OR 1.001, 95% CI: 0.99-1.04, p=0.37) or office SBP (OR 1.56; 95% CI: 0.57-4.28, p=0.39). There were no statistically significant difference in office or ABP between ART naïve (n= 13) and ART treated subjects (n=64)[19].

An Italian study assessed ABP in ART naive HIV infected individuals (n= 52) and in healthy HIV negative control subjects (n= 156). The controls were accurately matched by: age, BP, sex and Framingham risk score. The prevalence of non- dipping was found to be 35% in the HIV positive group and 15% in the control group (p=0.003). The night-time SBP and diastolic BP (DBP) was significantly greater in the HIV positive individuals than the controls (113/69 ± 11/9mmHg vs. 109/67 ± 8/6mmHg, p=0.008 vs. 0.005). The mean nocturnal SBP fall was 8.8% in the HIV positive cohort and 11.7% in the control group. The independent contribution of HIV infection and other variables (sex, office SBP, age, sex, smoking status) to nocturnal BP reduction was tested in a stepwise multiple linear regression analysis. HIV infection was the only independent determinant of nocturnal SBP fall. These findings suggest that HIV infection is associated with circadian BP patterns[20].

As noted above the only published data on the effect of HIV on circadian BP patterns are from Norway and Italy. These studies involve white patients from developed countries. Our HIV infected population is predominantly black and younger. In addition to this HIV negative African Americans, both normotensive and hypertensive subjects, exhibit more non- dipping during ABP monitoring than do whites[21]. A SA study, comparing ABP in black and Indian medical students, revealed less nocturnal dipping and a higher left ventricular mass (identified by echocardiography criteria) in young black participants than young Indians[13]. A nocturnal non- dipping pattern has been linked to: high dietary sodium intake, lower dietary potassium intake, salt sensitivity, sleep apnoea, obesity, lower socio-economic status and CKD in blacks[21,22]. The contribution of HIV to nocturnal non- dipping status has not to date been assessed in the SA population.

In addition, HIV infection and the use of ART have been associated with an increase in: insulin resistance, dyslipidaemia and lipodystrophy[23,24]. In HIV infected individuals chronic infection promotes chronic arterial inflammation and injury and in turn causes endothelial dysfunction, hypertension accelerated atherosclerosis and a higher risk of acute myocardial infarction[25,26].

The effect of ART on BP has shown contrasting results in the literature. ART was shown to increase BP in 6 studies[24,27-32]. Further, the duration of therapy was associated with a greater risk of hypertension[27,32]. 3 studies found that ART did not significantly affect BP[33-35]. However the latter study was

conducted for only 6 months and a longer period of observation would be required to determine causation[35].

As illustrated the available literature suggests that CKD and proteinuria in medically well, HIV positive outpatients is highly varied. More research is needed to determine the risk of CKD in well HIV positive patients and the cost effectiveness of regularly screening these patients.

Our literature review revealed important implications for the design of the MMed research proposal. For comparison purposes with the available literature, on kidney disease in HIV, we decided to measure similar renal function parameters as used in these publications. It included: urine dispticks, creatinine, microalbuminuria and eGFR as calculated by the 4- variable MDRD formula[36]. Although the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation has shown improved performance, compared to the MDRD, at higher levels of eGFR it has not been validated amoung Africans[37]. GFR categories in CKD were assigned according to the latest clinical guidelines from the Kidney Disease: Improving Global Outcomes (KDIGO) group[38]. Although albuminuria categories in CKD have also been re-classified, in these guidelines, the terms ‘microalbuminuria’ and ‘macroalbuminuria’ are still predominantly used in the literature. A urinary albumin/creatinine ratio between 3-30 mg/mmol would be identified as microalbuminuria and a level greater than this as macroalbuminuria[39]. Due to the apparent beneficial effects of ART on renal function we planned to measure renal function parameters before and after 6 months on ART.

As most of our patients would be black and of a low socio-economic status, the need for a HIV negative control group of a similar racial and demographic profile for the ABP substudy was deemed necessary. This would eliminate the possible overestimation of non- dipping status in our black study population.

The literature on the development and clinical application of ABP informed our decision on how to measure and classify dipping status. It was decided to measure BP on a typical weekday when daytime activity and duration of night-time bed rest could be more accurately reproduced[14]. The process of BP dipping is not likely to occur when a person does not sleep at night, as exemplified by night shift workers[18,40]. To define night time we adopted the commonly used period from 22h00- 06h00[18,40].

Known conditions associated with non- dipping include: endocrine conditions such a hyperthyroidism, renal dysfunction, and disturbances of the autonomic system (i.e. diabetic neuropathy, obstructive sleep apnoea) and other influences (i.e. malignant hypertension, pre- eclamptic toxaemia)[18]. In order to determine the intrinsic effect of HIV on circadian BP trends we decided to include only well, ambulant outpatients in our study.

On review of the evidence it is clear that the exact mechanism explaining the apparent association between HIV infection and an impairment of nocturnal BP fall is yet to be fully determined. Also, guidelines and protocols regarding the role of ABP in our clinical practice have yet to be established.

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PART C : JOURNAL READY MANUSCRIPT

CHRONIC KIDNEY DISEASE PREVALENCE AND AMBULATORY BLOOD PRESSURE PROFILE IN HIV POSITIVE OUTPATIENTS PRE AND POST ANTIRETROVIRAL THERAPY.

Short title: CKD AND AMBULATORY BP IN HIV- POSITIVE PATIENTS

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Abstract

Objectives: HIV and antiretroviral therapy are associated with renal disease and increased cardiovascular risk. The relationship between HIV and ambulatory blood pressure (ABP) dipping status, a risk factor for cardiovascular events and target organ damage, has never been assessed in South Africa. Study objectives were to: establish the prevalence of chronic kidney disease and assess the ABP profile in well HIV positive outpatients.

Methods: This was a prospective cohort study of healthy HIV positive clinic outpatients. Office blood pressure (BP), urinary microalbumin/creatinine ratio, urine dipsticks, serum creatinine and estimated glomerular filtration rate (eGFR) were measured at baseline and 6 months after antiretroviral therapy initiation. A subset of HIV positive subjects, and HIV negative control group, underwent 24 hour ABP monitoring.

Results: No patient had an eGFR<60ml/min, 3 patients (4.7%) had microalbuminuria and 1 (1.6%) had macroalbuminuria. Mean office systolic BP was 111 ± 14 mmHg at baseline and increased by 5 mmHg to 116 ± 14 mmHg (p= 0.05) at 6 months. This increase was not confirmed by ABP monitoring. In the HIV positive and negative patients the prevalence of non-dipping was 80% and 52.9% respectively (p- value= 0.05, odds ratio = 3.56, 95%, CI: 0.96 - 13.13). No relationship between dipping status and antiretroviral usage was found.

Conclusion: The prevalence of chronic kidney disease (CKD) was lower than anticipated. HIV infection was associated with an ambulatory non-dipping status, which suggests an underlying dysregulation of the cardiovascular

system. Antiretroviral therapy does not seem to improve circadian rhythm loss.

Key words: Human immunodeficiency virus, antiretroviral therapy, microalbuminuria, chronic kidney disease, ambulatory blood pressure, non-dipping.

Condensed abstract

This is a prospective study of well HIV positive outpatients, and HIV negative controls. Office blood pressure (BP), chronic kidney disease (CKD) markers and ambulatory BP (in a subset of patients) were measured at baseline and 6 months after the initiation of antiretroviral therapy (ART). The CKD prevalence was lower than anticipated. HIV infection was associated with a high prevalence of ambulatory non-dipping status, which suggests an underlying dysregulation of the cardiovascular system. The pathophysiology of this phenomenon, which contributes to cardiovascular risk in HIV positive patients, remains unexplained. ART does not seem to improve the loss of circadian rhythm.

Introduction

South Africa has 5.6 million people living with HIV/AIDS and has the largest antiretroviral therapy (ART) program globally with more than 2 million people accessing ART [1]. Although ART has significantly decreased the mortality from HIV infection, these individuals are now living longer and are at risk of developing metabolic (dyslipidaemia, lipodystrophy, dysglycemia), cardiovascular and renal complications from ART and chronic exposure to HIV infection [2-7]. Chronic HIV and ART are associated with increased risk of developing hypertension [8]. In studies of HIV positive patients in high income countries, hypertension prevalence ranges from 13- 34% [9,10]. However, data from low and middle income countries remains sparse.

Nocturnal blood pressure (BP) is superior to daytime or office BP as a predictor of cardiovascular disease [11]. Non- dipping is defined as an abnormal diurnal rhythm manifested by a blunted nocturnal decline in systolic BP (SBP) [11]. It is associated with more severe hypertensive target organ damage (left ventricular hypertrophy, microalbuminuria and cerebrovascular disease) and is also a predictor of increased cardiovascular risk, both in hypertensive and normotensive populations [11]. Studies from high-income countries have shown an increased prevalence of non- dipping with HIV infection [9,12]. However, the participants in these studies were largely white, middle-aged, males. Since the majority of subjects with HIV infection in sub-Saharan Africa are young black females it is not known whether the same relationship between dipping status and HIV infection would be found. In

addition, there is data showing that black HIV negative individuals have less nocturnal dipping compared to their white counterparts [5,13,14].

Therefore, the aims of the present study were to document the prevalence of chronic kidney disease (CKD) and hypertension at baseline (ART-naive), in a healthy HIV positive cohort, and to assess changes in these parameters after 6 months on ART. The characteristics of ambulatory blood pressure (ABP) in a subset of patients were to be recorded and compared to a control group of HIV negative patients.

Methods

A longitudinal, prospective cohort study was conducted. The study was approved by the Research Ethics Committee of the Faculty of Health Sciences of the University of Cape Town. Before participating in the study, procedures and risks were explained to the patients, who gave written informed consent to participate in the study. This study formed part of a yet unpublished larger longitudinal study, investigating the metabolic complications of ART in a HIV- positive population, at a HIV clinic in a community health center in Cape Town, South Africa.

All patients recruited for the parent study, over a 6 month period, were enrolled into the present study. The following measurements were done at baseline and repeated at 6 months: urine dipstick (AccuBioTech Co. Ltd, Beijing, China), office BP, serum creatinine (umol/L), spot urine

microalbumin/creatinine ratio (mg/mmol), and estimated glomerular filtration rate (eGFR) (ml/min/1.73m^2). Three office BP readings were performed on the right arm with the patient in a seated position using a mercury barometer in accordance with the South African hypertension guidelines [15]. A urinary albumin/creatinine ratio between 3-30 mg/mmol was identified as microalbuminuria and a level greater than this as macroalbuminuria [16]. eGFR was estimated using the 4-variable Modification of Diet in Renal Disease (MDRD) equation which accounts for the sex, age, creatinine and race of a patient [17]. Clinical guidelines from the Kidney Disease: Improving Global Outcomes (KDIGO) work group were used to categorize CKD [18].

After the baseline measurements all patients were commenced on ART (Table 1). The treatment regimen used depended on the date of enrolment into the study. Initially patients were prescribed stavudine (D4T), lamivudine (3TC) and efavirenz (EFV) according to the previous National Guidelines, but later tenofovir (TDF) replaced D4T [19,20].

All enrolled patients were invited to participate in the ABP sub-study. Consenting individuals underwent ABP monitoring prior to and after the initiation of ART. A control group of confirmed serological HIV negative patients formed a control group of another study from our institution investigating HIV associated dementia [21]. They were originally recruited by trained fieldworkers from a community primary healthcare clinic in Cape Town. Seventeen individuals, from of a list of 32 contacted telephonically, were available to participate. They were equally matched for age, body mass

index (BMI) and socioeconomic background. Patients were excluded if they had underlying hypertension, diabetes mellitus, ischaemic heart disease, concurrent illness or any condition affecting BP (i.e. pregnancy or renal disease).

ABP monitoring was set up by a trained nurse, on a weekday with an oscillometric device (SpaceLabs Medical Inc, WA, USA). BP and heart rate were recorded every 20 minutes during the day (06h00- 22h00) and every 30 minutes at night (22h00- 06h00). Hypertension was defined as a SBP ≥140 mmHg or diastolic BP (DBP) ≥90 mmHg, in accordance with the South African Hypertension Guidelines 2011 [15]. Non- dipping was defined as a nocturnal reduction of SBP≤ 10% [22].

Statistical analyses were performed using STATA statistical software, version 11.0 (STATA Corporation, College Station, Texas, USA). Mean ± standard deviation was used for normally distributed data and median plus interquartile ranges for skewed data. Continuous and categorical variables were compared using chi^2, student t-test or Pearson's X^2 as appropriate. All P-values considered significant at $p \leq 0.05$.

Results

Sixty four patients were entered into the study with baseline characteristics as shown in table 1.

Table 1: Patient characteristics and demographics

	Baseline (n= 64)	**6 months (n= 53)**	**ABP group at baseline (n= 30)**	**ABP group at 6 months (n= 28)**	**Controls (n= 17)**
Age (years) mean ±SD	33 ± 7	33 ± 7	32 ± 8	32 ± 8	31 ± 9
Men (%)	23	23	37	36	40
Women (%)	77	77	63	64	60
BMI (kg/m^2) mean ±SD	24.8 ± 5.4	25.7 ± 5.2	24.6 ± 5.2	24.8 ± 5.4	24.0 ± 4.8
• Men	22.5 ± 4.6	23.1 ± 4.8	22.9 ± 5.0	22.7 ± 5.3	22.8 ± 5.1
• Women	25.5 ± 5.4	26.9 ± 5.6	25.4 ± 4.9	25.8 ± 5.4	25.2 ± 4.8
CD4 (cells/mm^3) median	239	359	242	361	N/A
(IQR)	(169- 322)	(231- 411)	(165- 330)	(240- 406)	
ART regimen (%)					-
• Current	-	67	-	72	-
• Earlier	-	29	-	26	-
• Other	-	4	-	2	

All were black South Africans, mean age 33 years ± 7, and 77% were female. Eleven patients were excluded on follow up [defaulted treatment (n=8), pregnancy (n=3)]. Mean BMI was 24.8 kg/m^2 ± 5.4 and increased to 25.7 kg/m^2 ± 5.2 after 6 months on ART (p= 0.39). At baseline median CD4 count was 239 (169- 322) cells/mm^3, and after 6 months of ART the CD4 count increased to 359 (231- 411) cells/mm^3. All patients had suppressed viral loads. Thirty two patients agreed to participate in the ABP substudy (2 were excluded due to pregnancy). There were no significant differences between

those who underwent ABP monitoring and those who did not according to age, gender, ethnicity, BMI, CD4 count, ART status or office BP. Baseline demographics were similar in the HIV negative control group except there were more males in this group compared with the HIV positive cohort. However, there were still a greater percentage of females than males in the control group (Table 1).

At baseline, 13 patients (20%) had an eGFR 60- 89 ml/min/1.73m^2 (GFR category G2). No patient had an eGFR <60ml/min/1.73m^2 (Table 2). Microalbuminuria was present in 3 of the 64 patients (4.7%) and only 1 patient (1.6%) had macroalbuminuria. At the end of 6 months, microalbuminuria persisted in the 3 patients and developed in 2 new cases. The patient who initially had macroalbuminuria resolved on follow-up sampling. No patient had a change in eGFR over the study period.

TABLE 2: BP and Renal parameters at baseline and 6 months

	Baseline (n= 64)	**6 Months (n=53)**	**p- value**
MDRD eGFR (ml/min/1.73m^2)	109 +/- 23	107 +/- 22	0.66
≥ 90, *n* (%)	51 (80)	51 (80)	-
60-89, *n* (%)	13 (20)	13 (20)	-
< 60, *n* (%)	0 (0)	0 (0)	-
Microalbuminuria (mg)	0.9 +/- 5.0	0.8 +/- 2.8	0.67
Office Systolic BP [SBP] (mmHg)	111+/- 14	116 +/- 14	0.05
Office Diastolic BP [DBP] (mmHg)	72 +/- 9	75 +/- 10	0.69

Mean office SBP increased significantly from 111 ± 14 mmHg at baseline to 116 ± 14 mmHg (p= 0.05) at 6 months, but this was not confirmed by the ABP substudy (Table 2). The mean day and night ABP values for each group are shown in table 3. The mean nocturnal SBP was higher at 110 ± 6 mmHg in the HIV positive group at baseline compared to 99 ± 6 mmHg in the control group (p< 0.0001). There were no significant differences in age, gender, ethnicity, BMI, CD4 count, ART status or office BP between patients who did or not undego ABP monitoring. The prevalence of non-dipping in HIV positive patients (Table 3), did not differ at baseline or after 6 months on ART. Twenty four of 30 subjects (80%) were non-dippers at baseline and 23 of 28 subjects (82%) (odds ratio= 1.15, p= 0.84, 95% CI: 0.31- 4.29) were non-dippers at 6 months. In the HIV negative control group 9 of 17 (52.9%) were non- dippers, thus non- dipping was 3.6 times more likely in HIV positive patients at baseline than controls (p= 0.05, 95% CI: 0.96 - 13.13).

TABLE 3: Mean day and night BP and dipping status in 30 patients with HIV and in 17 control subjects.

	HIV positive		HIV negative	Baseline BP vs. controls (p- value)
	Baseline	6 months		
Mean BP (mmHg)				
Daytime SBP	114 +/- 10	116 +/- 12	114 +/- 14	1.00
Daytime DBP	75 +/- 12	72 +/- 11	73 +/- 16	0.63
Nightime SBP	110 +/- 6	111 +/- 4	99 +/- 6	<0.0001
Nightime DBP	65 +/- 8	67 +/- 11	60 +/- 9	0.05
Non-dipper	24 (80%)	23 (82%)	9 (52.9%)	-
Dipper	6 (20%)	5 (18%)	8 (47.1%)	0.05
Total	30	28	17	-

Discussion

This is the first study from Africa, to our knowledge, to have used ABP monitoring to characterize differences in nocturnal blood pressure dipping status between HIV-positive and HIV-negative patients. The study found that: (1) there is a low prevalence of CKD and microalbuminuria in healthy HIV-positive patients; (2) there is a greater prevalence of non-dipping of nocturnal blood pressure in HIV-positive patients than HIV-negative controls.

Studies from a high income country found the estimated prevalence of CKD in HIV infected subjects to be 11 to 15.5% [23, 24]. Our study found a lower prevalence of microalbuminuria in HIV-positive patients. In contrast, a study from Johannesburg reported a prevalence of microalbuminuria of 18.5% in their cohort of HIV-positive patients [25]. A possible explanation for this difference is that their patients were significantly more immunosuppressed (CD4 < 200 cells/mm^3) with a mean CD4 count of 130 cells/mm^3. They also had a high prevalence of co-morbid disease whereas the patients in our study were all healthy, with a mean CD4 count of 239- 339 cells/mm^3.

Microalbuminuria is an important finding in HIV as it may reflect early kidney disease. In a study from Kwazulu-Natal, 6 of 25 (24%) patients with an eGFR >60 ml/min/1.73m^2 had persistent microalbuminuria and HIV-associated nephropathy (HIVAN) detected on renal biopsy [26]. This is an isolated study. It is important to note that renal biopsies are not routinely performed in patients with microalbuminuria and normal renal function. In a large biopsy

series from Cape Town, HIVAN presented with nephrotic range proteinuria and impaired renal function [7]. Patients not receiving ART had a poor prognosis [27,28]. Microalbuminuria is non specific and is a marker of inflammation and cardiovascular risk independent of renal function [29].

In this small study, no patient had a clinically relevant reduction in eGFR (<60 ml/min/1.73m^2), and only 1 patient had overt macroalbuminuria (which resolved on treatment with ART). This suggests that the approximate prevalence of CKD in an otherwise healthy HIV population is about 1.6%, considerably lower than previously reported [23, 30]. Importantly a high CD4 count and normal creatinine does not exclude renal disease in HIV [6, 7, 26]. Patients demonstrating proteinuria, who would not normally be eligible for ART due to an elevated CD4 count, benefit from timely initiation of ART which can greatly improve survival with stabilisation of eGFR [7,30]. Therefore screening of patients enrolling into an ART program, with urine dipsticks or spot urine sampling for proteinuria, should be standard practice and could have an impact on the prevalence of HIVAN. This is particularly important in SA where, due to the problem of limited access to renal replacement, there is a need for early identification and management of renal disease.

In this study no cases of hypertension were identified, and there was a small but significant increase in office SBP after 6 months on ART. However in a subset of patients ABP monitoring did not confirm these findings. ABP monitoring is the most reliable method of assessing BP and suggests that effect of ART on BP maybe minimal.

In the ABP substudy there was no difference in mean day time SBP and DBP between patients and controls. However the mean night time SBP was significantly higher in the HIV group as was the proportion of non- dippers compared to the control group with similar demographics (BMI, age, sex, socioeconomic status). A non- dipping pattern is an established entity with important clinical implications, and is associated with a higher cardiovascular morbidity and mortality [31]. The high prevalence of non- dippers in the HIV infected group, in this study, supports the data from Italy and Norway [9,12].

The potential mechanisms underlying the non- dipping phenomenon in HIV positive patients are uncertain. It does suggest an underlying dysregulation of the cardiovascular system. Chronic infection and arterial inflammation contribute to endothelial dysfunction which may be further exacerbated by ART [2,3]. In addition, HIV related endocrinopathies (i.e. hyperaldosteronism and hypercortisolism) and autonomic dysfunction may play a role [32,33]. However the lack of improvement in dipping status after 6 months of ART with suppressed viral loads suggests that other mechanisms may also be involved.

Our study has several limitations. Firstly, the sample size for the ABP substudy is small and the nocturnal dipping status between HIV positive and negative controls is marginal. Secondly, the short length of follow up (6 months) may be a limitation as the effects of ART on BP and nocturnal dipping may take longer to manifest. Thirdly, a single spot sample was used for establishing microalbuminuria. Lastly, ABP measurements were conducted during a presumed typical weekday and we could not objectively observe

daytime activity and duration of night time bed rest which has been shown to affect diurnal BP patterns.

The focus of HIV care, in our country, remains virological suppression and managing opportunistic infections. Our findings correlate with established evidence linking HIV to increased cardiovascular risk. Young black females are traditionally at low risk for cardiovascular disease. However a non- dipping status, in the context of HIV infection, could confer a greater risk in this group. If there are approximately 5.6 million HIV positive people in South Africa potentially 4.48 million (80%) are non- dippers. Therefore, as HIV infected patients are living longer, investigating and addressing the cardiac and metabolic complications related to HIV is becoming more important.

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Conflict of interest. None.

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PART D : APPENDICES

Appendix 1

Patient information sheet and consent form: Ambulatory blood pressure study

You are invited to participate in a research project conducted by the Nephrology Department at Groote Schuur Hospital.

Patients will be referred to the **high blood pressure clinic at Groote Schuur Hospital (E17)** to undergo ambulatory blood pressure monitoring. This is a **24 hour blood pressure monitor** that is applied to your upper arm. It must stay on for the whole day and night. There are no risks to your health by wearing the monitor and it is painless. It may inconvenience you, especially at night, as the machine needs to blow up and down approximately every 20 minutes. However, it will give us useful information about your blood pressure throughout the day and night. If you are found to have high blood pressure we can arrange further treatment for you.

We will pay you R200 for participating in the study and **to cover transport costs** to and from Groote Schuur hospital. Two trips to Groote Schuur hospital will be required to fit the machine and then to return it.

You do not have to participate in this study. Your participation is voluntary and if you agree to participate you will be required to sign this form. You can withdraw from this study at any time (provided blood pressure monitors are returned to Groote Schuur hospital) and this will not affect your future treatment.

Your details will not be viewed by anybody not involved in this study and **we will strive t**o **keep your records confidential**.

Should you have any **questions** please contact Dr Borkum on **0722465633**.

If you wish to participate please sign below.

_____ _____

Patient print name Patient signature

Date: Place:

_____ _____

Witness print name Witness signature

Appendix 2

Patient information sheet and consent form: Prevalence of kidney disease study

You are invited to participate in a research project conducted by the Nephrology Department at Groote Schuur Hospital.

Patients will be required to have blood taken and to give a urine sample at Crossroads clinic. We will also test your blood pressure. There are no risks to your health by participating. However, these tests will give us useful information about your kidney function and blood pressure. If you are found to have high blood pressure or kidney problems we will inform you and can arrange further treatment for you.

In 6 months, after starting antiretroviral treatment, we will repeat the blood and urine tests if you agree.

You do not have to participate in this study. Your participation is voluntary and if you agree to participate you will be required to sign this form. You can withdraw from this study at any time (provided blood pressure monitors are returned to Groote Schuur hospital) and this will not affect your future treatment.

Your details will not be viewed by anybody not involved in this study and **we will strive t**o **keep your records confidential**.

Should you have any **questions** please contact Dr Borkum on **0722465633**.

If you wish to participate please sign below.

_____ _____

Patient print name Patient signature

Date: Place:

_____ _____

Witness print name Witness signature

Appendix 3

Instructions to authors:
Journal of Hypertension

Journal of Hypertension
Online Submission and Review System

Guidance for Authors on the Preparation and Submission of Manuscripts to Journal of Hypertension

These instructions comply with those formulated by the International Committee of Medical Journal Editors. For further details, authors should consult the following article: International Committee of Medical Journal Editors, "Uniform Requirements for Manuscripts Submitted to Biomedical Journals". The complete document appears at www.icmje.org.

The Journal is a member of the Committee on Publication Ethics (COPE) which aims to define best practice in the ethics of scientific publishing. COPE has established a number of guidelines including a Code of Conduct that can be found at www.publicationethics.org.

Scope
The Journal of Hypertension publishes papers reporting original clinical and experimental research which are of a high standard and which contribute to the advancement of knowledge in the field of hypertension. The Journal publishes full papers and reviews or editorials (normally by invitation). Authors who submit papers to the Journal must document that all persons acknowledged have seen and approved the mention of their name in the paper.

Points to consider before submission
Redundant or duplicate publication
Submissions are accepted on the understanding that they have not been published in their current form or a substantially similar form (in print or electronically, including on a web site), that they have not been accepted for publication elsewhere, and they are not under consideration by another publication.

Conflicts of interest
Authors must state all possible conflicts of interest in the manuscript, including financial, consultant, institutional and other relationships that might lead to bias or a conflict of interest. If there is no conflict of interest, this should also be explicitly stated as none declared. All sources of funding should be acknowledged in the manuscript. All relevant conflicts of interest and sources of funding should be included on the title page of the manuscript with the heading "Conflicts of Interest and Source of Funding:". For example:

Conflicts of Interest and Source of Funding: A has received honoraria from Company Z. B is currently receiving a grant (#12345) from Organization Y, and is on the speaker's bureau for Organization X – the CME organizers for Company A. For the remaining authors none were declared.

In addition, each author must complete and submit the journal's copyright transfer agreement, which includes a section on the disclosure of potential conflicts of interest based on the recommendations of the International Committee of Medical Journal Editors, "Uniform Requirements for Manuscripts Submitted to Biomedical Journals" (www.icmje.org/update.html). The form is readily available on the manuscript submission pagewww.editorialmanager.com/jh and can be completed and submitted electronically. Please note that authors may sign the copyright transfer agreement form electronically. For additional information about electronically signing this form, go to http://links.lww.com/ZUAT/A106.

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Authors should include with their submission copies of written permission to reproduce material published elsewhere (such as illustrations) from the copyright holder. Authors are responsible for paying any fees to reproduce material.

Patient consent forms

Patients have a right to privacy that should not be infringed without informed consent. Identifying details (written or photographic) should be omitted if they are not essential, but patient data should never be altered or falsified in an attempt to attain anonymity. Complete anonymity is difficult to achieve, and a consent form should be obtained if there is any doubt. For example, masking the eye region in photographs of patients is inadequate protection of anonymity. When informed consent has been obtained it should be indicated in the published article.

Ethics committee approval

All authors must sign a declaration that the research was conducted within the guidelines below and under the terms of all relevant local legislation. (Such a statement is included in the model submission letter on the journal's web site.) The Editors reserve the right to judge the appropriateness of the use and treatment of humans or animals in experiments for publication in the journal.

Human experiments: All work must be conducted in accordance with the Declaration of Helsinki. Papers describing experimental work on human participants which carries a risk of harm must include (1) a statement that the experiments were conducted with the understanding and the consent of each participant, and (2) a statement that the responsible ethical committee has approved the experiments.

Animal experiments: In papers describing experiments on living animals, include (1) a full description of any anaesthetic and surgical procedure used, and (2) evidence that all possible steps were taken to avoid animals' suffering at each stage of the experiment.

Experiments on isolated tissues: Indicate precisely how you obtained the donor tissue.

Systematic Reviews and Meta-analysis

Authors should follow the PRISMA guidelines (www.prisma-statement.org) on reporting items for systematic reviews and meta-analyses. Such reviews often serve as a basis for many health policy decisions and direction for further research, and following these guidelines will assist in improving the quality of reports available.

Clinical Trials and Behavioural and Public Health Evaluations

Authors reporting results of randomised controlled trials should include with their

submission a complete checklist from the CONSORT statement (www.consort-statement.org). For behavioural and public health evaluations involving non-randomised designs, authors should include with their submission a complete checklist from the TREND statement (www.cdc.gov/trendstatement/).

Registration of clinical trials: As a condition for publication of a clinical trial in the Journal, registration of the trial in a public registry is required. The editor does not advocate one particular registry but require that the registry utilised meet the criteria set out in the statement of policy of the ICMJE (www.icmje.org).

Authorship

All authors must sign the letter accompanying their submission to confirm that they have read and approved the paper, that they have met the criteria for authorship as established by the International Committee of Medical Journal Editors, that they believe that the paper represents honest work, and that they are able to verify the validity of the results reported In addition to those from the ICJME the International Society for Medical Publication Professionals, ISMPP (www.ismpp.org) have produced some useful guidelines on authorship of studies sponsored by companies: Good Publication Practice (GPP2) (www.ismpp.org/initiatives/gpp2.html).

Compliance with NIH and Other Research Funding Agency Accessibility Requirements

A number of research funding agencies now require or request authors to submit the post-print (the article after peer review and acceptance but not the final published article) to a repository that is accessible online by all without charge. As a service to our authors, LWW will identify to the National Library of Medicine (NLM) articles that require deposit and will transmit the post-print of an article based on research funded in whole or in part by the National Institutes of Health, Wellcome Trust, Howard Hughes Medical Institute, or other funding agencies to PubMed Central. The revised Copyright Transfer Agreement provides the mechanism.

Copyright assignment

Papers are accepted for publication on the understanding that exclusive copyright in the paper is assigned to the Publisher. Authors are asked to submit a signed copyright assignment form with their submission. They may use material from their paper in other works published by them after seeking formal permission.

Submissions

Authors are strongly encouraged to submit their manuscripts through the web-based tracking system at http://www.editorialmanager.com/jh. Signed author forms may be included in the submission as a 'supporting document' or mailed to the journal office. Authors should submit the text of the paper as a word-processed document, and not as a PDF. The site contains instructions and advice on how to use the system. Authors should NOT in addition then post a hard copy submission to the editorial office, unless you are supplying artwork, letters or files that cannot be submitted electronically, or have been instructed to do so by the editorial office. Include the following where appropriate: subject consent forms; transfer of copyright form; permission to reproduce previously published material; checklist. Editor address : Alberto Zanchetti, The Editor, Journal of Hypertension, Centro di Fisiologia Clinica e Ipertensione, University of Milan, Ospedale Maggiore, Via F. Sforza 35, 20122, Milan; tel: 39 02 5518 4606, fax: 39 02 503 20480, email:j.hypertension@centroipertensione.191.it

Margins should be not less than 3 cm. Double spacing should be used throughout the manuscript, which should include the following sections, each starting on a separate page: title page, abstract and keywords, text, acknowledgements, references, individual tables and captions. Pages should be numbered consecutively, beginning with the title page, and the page number should be placed in the top right hand corner of each page. Abbreviations should be defined on their first appearance in the text; those not accepted by international bodies should be avoided.

Please note that as a new feature of the Journal of Hypertension, published articles will be followed by a short summary of strengths and weaknesses prepared by each of the reviewers.

Presentation of Papers

Title Page

The title page should carry the

- full title of the paper, consisting of no more than 20 words (only common abbreviations should be used if absolutely necessary); titles should be clear and brief, conveying the message of the paper
- a brief short title, which will be used as running head (consisting of not more than 40 characters, including spaces)
- all authors' names: the full first name, middle initial(s) and last (family name) name of each author should appear; if the work is to be attributed to a department or institution, its full name and location should be included. The last (family name) must appear in CAPITAL letters. Persons listed as authors should be those who substantially contributed to the study's conception, design, and performance
- the affiliations of all the authors; when authors are affiliated to more than one institution, their names should be connected using a,b,c, etc. These letters should follow the surname but precede the address; they should be used for all addresses
- information about previous presentations of the whole or part of the work presented in the article
- the sources of any support, for all authors, for the work in the form of grants, equipment, drugs, or any combination of these
- Disclose funding received for this work from any of the following organizations: National Institutes of Health (NIH); Wellcome Trust; Howard Hughes Medical Institute (HHMI); and other(s).
- a statement on potential conflicts of interest: if authors have financial interests relevant to the research or constituting a conflict of interest, these must be stated. If not applicable, state NONE disclaimers, if any
- the name and address of the author responsible for correspondence concerning the manuscript, and the name and address of the author to whom requests for reprints should be made. If reprints are not to be made available, a statement to this effect should be included. The peer-review process as well as publication will be delayed if you do not provide up to date telephone and fax numbers, and E-mail address, if available

- word count: please list full word count (including references, but not tables and legends)
- number of tables
- number of figures
- number of supplementary digital content files

Authors are encouraged to submit colour and non-colour versions of illustrative figures, should the editor choose to publish gratis the colour version online only. Colour images should be prepared to the standards indicated in the section below on illustrations, and take into account that colour and non-colour versions need to be interpretable by the reader. Please ensure that the different versions of the illustrations are labeled for easy identification.

Authors are also encouraged to submit supplementary digital content that may include figures, tables, a PowerPoint slide deck, audio or videos. Material submitted should not duplicate what is in the paper but contain extra material that a reader would find useful to access, but not critical for interpretation of the study. Audio or video should be no longer than 5 minutes in length. Please consult the Supplementary Digital Content section below for further advice.

Abstracts

The second page should carry a structured abstract of no more than 250 words. The abstract should state the Objective(s) of the study or investigation, basic Methods (selection of study subjects or laboratory animals; observational and analytical methods), main Results (giving specific data and their statistical significance, if possible), and the principal Conclusions. It should emphasise new and important aspects of the study or observations.

Review articles and case reports should include an unstructured summary of no more than 150 words.

Condensed Abstracts

A condensed abstract will be published in the 'forthcoming contents' section of the issue preceding the published article. This should be supplied with the submission, and should consist of no more that 100 words, this abstract should briefly summarise the main findings of your study.

Key Words

The abstract should be followed by a list of 3–10 keywords or short phrases which will assist the cross-indexing of the article and which may be published. When possible, the terms used should be from the Medical Subject Headings list of the Index Medicus (http://www.nlm.nih.gov/mesh/ meshhome.html).

Abbreviations and symbols

Use only standard abbreviations. Avoid abbreviations in the title and abstract. A short list of non-standard abbreviation definitions that may not be familiar to readers should be included in a separate mandatory document submitted with your paper.

Text

Full papers of an experimental or observational nature may be divided into sections headed Introduction, Methods (including ethical and statistical information), Results and Discussion (including a conclusion), although reviews may require a different format.

Acknowledgements

Acknowledgements should be made only to those who have made a substantial contribution to the study. Authors are responsible for obtaining written permission from people acknowledged by name in case readers infer their endorsement of data and conclusions.

References

References should be numbered consecutively in the order in which they first appear in the text. They should be assigned Arabic numerals, which should be given in brackets, e.g. [17]. References should include the names of all authors when seven or fewer; when eight or more, list only the first six names and add et al. References should also include full title and source information. Journal names should be abbreviated as MEDLINE (www.nlm.nih.gov/tsd/serials/lji.html).

Articles in journals

Zhou M-S, Schulman IH, Raij L. Vascular inflammation, insulin resistance, and endothelial dysfunction in salt-sensitive hypertension: role of nuclear factor kappa B activation. J Hypertension 2010; 28:527–535

More than seven authors:

Grassi G, Vailati S, Bertinieri G, Seravalle G, Stella ML, Dell'Oro R, et al. Heart rate as a marker of sympathetic activity. J Hypertens 1998; 16:1635–1639.

Supplements:

Dean RT, Wilcox I. Possible atherogenic effects of hypoxia during sleep apnea. Sleep 1993; 16 (suppl 8):S15–S21.

Letter/Abstract:

Perk G, Bursztyn M. Changes in body position effect measurements during 24 hr ambulatory blood pressure monitoring [Letter]. J Hypertens 2001; 19:1513.

Hostetter TH, Kren S, Ibrahim HN. Mineralocorticoid receptor blockade in the remnant kidney model [Abstract]. J Am Soc Nephrol 1999; 10:85A.

Books

Book:

Katz AM, Konstam MA. Heart Failure. Pathophysiology, Molecular Biology, and Clinical Management. Philadelphia: Lippincott Williams & Wilkins; 2008

Chapter in a book:

Wakhloo AK. Carotid artery revascularization. In: Kandarpa K (editor). Peripheral Vascular Interventions. Philadelphia: Lippincott Williams & Wilkins; 2008. pp. 137–153.

Personal communications and unpublished work should not feature in the reference list but should appear in parentheses in the text. Unpublished work accepted for publication but not yet released should be included in the reference list with the words 'in press' in

parentheses beside the name of the journal concerned. References must be verified by the author(s) against the original documents.

Tables

Each table should be typed on a separate page in double spacing. Tables should not be submitted as photographs. Each table should be assigned an Arabic numeral, e.g. (Table 3) and a brief title. Vertical rules should not be used. Place explanatory matter in footnotes, not in the heading. Explain in footnotes all non-standard abbreviations that are used in each table. Identify statistical measures of variations, such as standard deviation and standard error of the mean.

Be sure that each table is cited in the text. If you use data from another published or unpublished source, obtain permission and acknowledge the source fully.

Illustrations

A) Creating Digital Artwork

1. Learn about the publication requirements for Digital Artwork: http://links.lww.com/ES/A42
2. Create, Scan and Save your artwork and compare your final figure to the Digital Artwork Guideline Checklist (below).
3. Upload each figure to Editorial Manager in conjunction with your manuscript text and tables.

B) Digital Artwork Guideline Checklist

Here are the basics to have in place before submitting your digital artwork:

- Artwork should be saved as TIFF, EPS, or MS Office (DOC, PPT, XLS) files. High resolution PDF files are also acceptable.
- Crop out any white or black space surrounding the image.
- Diagrams, drawings, graphs, and other line art must be vector or saved at a resolution of at least 1200 dpi. If created in an MS Office program, send the native (DOC, PPT, XLS) file.
- Photographs, radiographs and other halftone images must be saved at a resolution of at least 300 dpi.
- Photographs and radiographs with text must be saved as postscript or at a resolution of at least 600 dpi.
- Each figure must be saved and submitted as a separate file. Figures should not be embedded in the manuscript text file.

Remember:

- Cite figures consecutively in your manuscript.
- Number figures in the figure legend in the order in which they are discussed.
- Upload figures consecutively to the Editorial Manager web site and enter figure numbers consecutively in the Description field when uploading the files.
- Photomicrographs must have internal scale markers.

- If photographs of people are used, their identities must be obscured or the picture must be accompanied by written consent to use the photograph.
- If a figure has been published before, the original source must be acknowledged and written permission from the copyright holder for both print and electronic formats should be submitted with the material. Permission is required regardless of authorship or publisher, except for documents in the public domain.
- Figures may be reduced, cropped or deleted at the discretion of the editor.
- Colour illustrations for reproduction in print are acceptable but authors will be expected to cover the extra reproduction costs (for current charges, contact the publisher).

Legends for illustrations

Captions should be typed in double spacing, beginning on a separate page. Each one should have an Arabic numeral corresponding to the illustration to which it refers. Internal scales should be explained and staining methods for photomicrographs should be identified.

Supplemental Digital Content

Supplemental Digital Content (SDC): Authors may submit SDC via Editorial Manager to LWW journals that enhance their article's text to be considered for online posting. SDC may include standard media such as text documents, graphs, audio, video, etc. On the Attach Files page of the submission process, please select Supplemental Audio, Video, or Data for your uploaded file as the Submission Item. If an article with SDC is accepted, our production staff will create a URL with the SDC file. The URL will be placed in the call-out within the article. SDC files are not copy-edited by LWW staff, they will be presented digitally as submitted. For a list of all available file types and detailed instructions, please visit http://links.lww.com/A142.

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Supplemental Digital Content must be cited consecutively in the text of the submitted manuscript. Citations should include the type of material submitted (Audio, Figure, Table, etc.), be clearly labeled as "Supplemental Digital Content," include the sequential list number, and provide a description of the supplemental content. All descriptive text should be included in the call-out as it will not appear elsewhere in the article.
Example:
We performed many tests on the degrees of flexibility in the elbow (see Video, Supplemental Digital Content 1, which demonstrates elbow flexibility) and found our results inconclusive.

List of Supplemental Digital Content

A listing of Supplemental Digital Content must be submitted at the end of the manuscript file. Include the SDC number and file type of the Supplemental Digital Content. This text will be removed by our production staff and not be published.
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Supplemental Digital Content 1. wmv

SDC File Requirements

All acceptable file types are permissible up to 10 MBs. For audio or video files greater than

10 MBs, authors should first query the journal office for approval. For a list of all available file types and detailed instructions, please visit http://links.lww.com/A142.

Units of measurement

Measurements of length, height, weight, and volume should be reported in metric units (metre, kilogram, or litre) or their decimal multiples. Temperatures should be given in degrees Celsius. Blood pressures should be given in millimetres of mercury.

All haematologic and clinical chemistry measurements should be reported in the metric system in terms of the International System of Units (SI). Editors may request that alternative or non-SI units be added by the authors before publication.