Longitudinal study to assess the prevalence of hypogonadism in HIV-infected South African men and its association with bone density, body composition, metabolic abnormalities (dysglycaemia, dyslipidaemia) and quality of life.

by

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DECLARATION

I, *Le Roux van der Merwe*, hereby declare that the work on which this dissertation/thesis is based is my original work (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other university.
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SUPERVISOR

This study was conducted from March 2019 to March 2021 under the supervision of Associate Prof JA Dave, Division of Endocrinology, Department of Medicine, University of Cape Town.

As the candidates Supervisor, I have approved this dissertation for submission.

Name: Joel Dave

Signed:

Date: 25 April 2021

Abstract

Background: Hypogonadism has been described in association with HIV infection and anti-retroviral therapy (ART). Furthermore, hypogonadism is associated with co-morbidity and a reduced quality of life. The prevalence of hypogonadism in HIV-infected South African men is unknown. We examined the prevalence of hypogonadism in HIV-infected men before and 12 months after the initiation of ART.

Methods: This is a sub-study of the McHAART Study designed to investigate the metabolic complications of ART. HIV-infected men attending the Crossroad Community Healthcare Clinic were conveniently sampled prior to commencing ART and then followed for 6-months, 12-months and 24-months. Here we report on total testosterone, LH, FSH, SHBG and free testosterone measured on blood samples taken at 08h00 prior to initiating ART (baseline) and then again at 12 months on ART.

Results: There were 44 patients at baseline and 30 patients at the 12-month visit. There were no participants with hypogonadism at baseline or after 12 months on ART. Testosterone levels or change in testosterone levels were not associated with alcohol intake, bone miner density, body mass index, waist circumference, fasting glucose, HOMA IR, HOMA β, fasting cholesterol or blood pressure at baseline or 12 months.

Conclusions: There were no cases of hypogonadism in HIV-infected ART-naïve men and there were no cases of hypogonadism in HIV-infected men on ART for 12 months in this study population. Testosterone levels or change in testosterone levels were not associated with any measures of body composition, glucose metabolism, lipids or bone mineral density.

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List of Tables and Figures

Abbreviations

ART- Anti- retroviral therapy

BMD- Bone mineral density

BMC- Bone mineral content

ELISA- Enzyme-linked immunosorbent assay

HIV- Human immunodeficiency virus

NRTI- Nucleoside reverse transcriptase inhibitors

NNRTI- Non-nucleoside reverse transcriptase inhibitors

SHBG- Sex hormone binding globulin

TNF- Tumour necrosis factor

PI- Protease inhibitors

MLWH- Men living with HIV

PLWH- People living with HIV

HOMA IR – Homeostatic model assessment for insulin resistance

HOMA β – Homeostatic model assessment for Beta-cell function

SBP – Systolic blood pressure

DBP – Diastolic blood pressure

BMI – Body mass index

HDL – High density lipoprotein

LDL-Low density lipoprotein

FSH– follicle stimulating hormone

LH - Luteinising hormone

SHBG – sex hormone binding globulin

CVD- Cardiovascular disease

CHAPTER 1: LITERATURE REVIEW

INTRODUCTION

The Human Immunodeficiency Virus (HIV) is a species of Lentivirus that infects humans and was first identified in 1981 in the United States(1). It is a virulent virus that is transmitted amongst humans via bodily fluids, most commonly sexually and by blood transfusions, but can also be transmitted during childbirth and breastfeeding (2, 3). CD4 cells, especially T-Helper cells, are particularly vulnerable to being infected, resulting in low levels thereby causing impaired cell-mediated immunity(4). This predisposes the individual to a large number of opportunistic infections, such as bacterial, viral and fungal infections as well as certain types of cancer(5).

In communities, at risk individuals are screened with an HIV rapid test, which detects circulating antibodies to HIV 1 and HIV 2 antigens, and confirmed by an HIV ELISA, which is also an antibody test but performed in a laboratory(6).

HIV-infected patients are treated with anti-retroviral therapy (ART) following the South African Department of Health's national guidelines (7).

Current first line treatment of HIV is Tenofovir, Lamivudine/Emtricitabine and Dolutegravir. Second line regimens are usually tailored to the individual patient, but can include Zidovudine/Tenofovir plus Lamivudine/Emtricitabine plus Lopinavir/Ritonavir or Atazanavir (8).

Common side-effects of ART include (9):

- Tenofovir: Most notably renal failure and other common effects are headache, insomnia nausea and vomiting as well as low bone mineral density
- Lamivudine: Pure red cell aplasia is very rare, but the most notable.
- Dolutegravir: Weight gain, insomnia, dizziness and headache.
- Lopinavir/Ritonavir: Diarrhoea can be debilitating, abnormal lipid profile in certainindividuals, abnormal glucose metabolism
- Atazanavir: Jaundice is very common and transient, skin rash and dysrhythmias.
- Efavirenz: Dizziness, insomnia, lowers seizure threshold and worsening of existing mental health care issues, vitamin D deficiency and abnormal glucose metabolism
- Zidovudine: Headache, anaemia, macrocytosis.
- Emtricitabine: Headache, dizziness and hyperpigmentation.

There are 36.9 million people living with HIV (PLWH) infection worldwide. Sub-Saharan Africa bears the brunt of this epidemic with over 50% of those infected living in this region. South Africa harbours nearly 20% of the global HIV-infected population, being about 7.7 million individuals. Of these, only 38% are males over the age of 15 years(10)(9)(8). The roll-out of ART has reduced the mortality from HIV-infection rendering it a chronic disease with many people now living with HIV infection (8).

With patients living longer they are now more prone to developing complications from HIV itself, chronic diseases of aging (for example, hypertension, diabetes, dyslipidaemia) as well as long-term complications of ART(11).

HIV AND THE ENDOCRINE SYSTEM

HIV has an effect on multiple endocrine organs resulting in a wide spectrum of presentation (Table 1). These endocrinopathies can be due to the direct effect of HIV itself, opportunistic infections, ART and, rarely, neoplasms (Table 1). There is a very complex interaction between HIV infection and the endocrine system(12). This ranges from subtle abnormalities in hormone secretion, transport and metabolism on the one end of the spectrum to rare instances of hormonal resistance and organ failure on the other (12).

Endocrinopathies commonly found in PLWH

1. HIV lipodystrophy syndrome

   The HIV lipodystrophy syndrome is associated with metabolic derangements, changes in body composition and abnormal fat distribution. Although mostly occurring in patients on ART it may also occur in ART-naïve patients(13). It is characterized by changes in the distribution of adipose tissue (for example peripheral fat loss, truncal obesity, atrophy of facial fat and breast enlargement in females) and is often associated with insulin resistance, hyperglycaemia and an abnormal lipid profile. These changes predispose the individual to developing the metabolic syndrome with all its associated complications, for example ischaemic heart disease(14, 15)

2. Low bone mineral density (BMD)

People living with HIV are also at increased risk for low BMD mainly due to calcium and vitamin D imbalance(16). Osteopenia and osteoporosis are common among HIV-infected individuals. The cause for Vitamin D

deficiency is multifactorial, including malabsorption (HIV enteropathy) and a side-effect of ART such as Tenofovir-based regimens, as well as Efavirenz and the protease inhibitors (PI). Tenofovir-associated bone loss is postulated to be due to proximal tubular dysfunction in the kidney causing phosphorous wasting with resultant increased bone turnover(17). Both Efavirenz and PI-based regimens are thought to cause low BMD by altering vitamin D metabolism. In South Africa males living with HIV are usually of lower socioeconomic circumstances and are also more prone to smoking and alcohol abuse which contributes to low BMD(18).

Table 1: Common endocrinopathies associated with HIV:

Direct effect
HIV adrenalitis and impaired adrenal reserve
Hypercortisolism
Idiopathic adenohypophysis necrosis
Hyperprolactinaemia
Primary hypogonadism
Growth Failure
AIDS wasting syndrome
Structural damage of glands
Infection leading to haemorrhage or abscesses in glands
Secondary hypogonadism
Drug-related
Insulin resistance and diabetes mellitus
Dyslipidaemias
Bone mineral and electrolyte disturbances
Immune reconstitution syndromes
HIV lipodystrophy syndrome
Pancreatitis

3. Adrenal insufficiency

Adrenal insufficiency is frequently found in PLWH admitted to a medical ward and is more severe with more advanced HIV infection(19). At autopsy the adrenal gland shows features of both inflammation and necrosis(20). Adrenal insufficiency is a common finding in HIV-infected people admitted to the ward. These patients usually present with fatigue and may have hyponatraemia and hypotension(21).

4. Diabetes mellitus

The association of HIV and diabetes has become of great importance as both are an economic burden to our healthcare system, and often co-exist. The development of the metabolic syndrome in PLWH is multi-factorial, including advancing age, male gender, longer duration of HIV infection, low CD4 count, high viral burden, high body mass index, greater waist circumference or waist- to- hip ratio, lower socio economic class, and certain ethnic backgrounds (15, 22).

In PLWH diabetes is usually due to insulin resistance as there is no evidence for destruction of beta-cells or islet cell autoimmunity (23, 24). PLWH often have an altered body composition with increased visceral fat and peripheral fat wasting. The increase in visceral fat is thought to be associated with a pro-inflammatory state resulting in high levels of inflammatory cytokines, especially TNF-alpha(25). The high levels of TNF-alpha are thought to be responsible for increasing insulin resistance, thereby causing glucose intolerance, or diabetes(25).

The major contributor to hyperglycaemia in PLWH however is iatrogenic. There are multiple benefits of ART, these include, viral load suppression, improvement in CD4 count, less opportunistic infections, decreased length of hospital stay and an overall decrease in all-cause mortality, but ART also has other deleterious effects including, insulin resistance, diabetes, dyslipidaemia and lipodystrophy(26).

Protease inhibitors have been associated with the development of insulin resistance and diabetes by acutely and reversibly inhibiting GLUT4, an insulin-responsive glucose transporter(27). NRTI's have also been implicated in causing diabetes(28). The mechanism of NRTI-induced diabetes is thought to be with its complex interaction with DNA polymerase, the enzyme responsible for mitochondrial replication. As mitochondria are dysregulated throughout the body, this leads to its various clinical manifestations, including insulin resistance and diabetes(29).

HYPOGONADISM

Hypogonadism is a clinical syndrome resulting from low levels of sex steroids. In men a low testosterone level could be due to a testicular problem or as a consequence of disruption of the hypothalamic-pituitary-testicular axis(30). To confirm a diagnosis of hypogonadism an early morning (08h00) testosterone level is required to be below the lower level of normal for the specific assay being used. For adults (over 18 years of age) there is no validation of age-specific reference ranges for testosterone and these are not used clinically. Primaryhypogonadism is due to testicular disease and secondary (hypogonadotropic)hypothalamus or pituitary gland (Table 2). To distinguishing between primary and secondary hypogonadism measurement of the serum gonadotrophins (LH and FSH) is needed. In primary hypogonadism, LH is elevated due to loss of negative feedbackinhibition due to low testosterone levels, whilst in secondary hypogonadism, LH is inappropriately normal or low(30). An acute illness may cause transient secondary hypogonadism, the sick gonadotroph syndrome, so men should not be investigated for hypogonadism until an inter-current illness has resolved. Testosterone circulates in the blood bound to SHBG or as biologically free active testosterone(31). It is not clinically practical to measure free testosterone as the methods are labour intensive andexpensive. Measuring the bound testosterone has its own limitations as it is affected by many variables for example: HIV infection, obesity, insulin resistance and diabetes as well as certain medications(32).

Sex-hormone binding globulin (SHBG), is produced in the liver and tightly binds oestrogen, dihydrotestosterone and testosterone.

Several chronic and systemic diseases, for example, chronic kidney disease, cirrhosis, chronic lung disease and HIV infection may cause hypogonadism by a combination of primary and secondary effects. This is referred to as the “sick gonadotroph syndrome”(33).

In men living with HIV (MLWH), early studies suggested that primary hypogonadism was more prevalent due to the direct effect of HIV on the germinal epithelium, while others suggested that the aetiology lies more in the higher centres causing secondary hypogonadism(34). The cause of the abnormality in the hypothalamic-pituitary-gonadal axis might be explained by chronic HIV infection itself, poor health status of the patient, increased visceral adiposity, opportunistic infections or the use of

antiretroviral medication(30, 35).

Table 2. Causes of primary and secondary hypogonadism:

Primary (Hypergonadotropic)	**Secondary (Hypogonadotropic)**
Klinefelter's Syndrome	Panhypopituitarism
XX males	Hyperprolactinaemia
XY/XO mixed gonadal dysgenesis	Isolated gonadotrophin deficiency
XYY Syndrome	Kalman syndrome and variants
Myotonic dystrophy	Congenital hypogonadotropic hypogonadism
Enzymatic defects in testosterone biosynthesis	Isolated LH or FSH deficiency
Disorders of sexual development	Prader-Willi Syndrome
Viral Orchitis	Laurence-Moon-Biedl syndrome
Cryptorchidism	Severe systemic illness
Polyglandular autoimmune disease	Haemochromatosis
Testicular trauma	Morbid obesity
Testicular tumour, chemotherapy or irradiation	Nutritional deficiency or starvation
Congenital or acquired anorchia	Constitutional delay of puberty

MLWH generally have a lower testosterone level than men without HIV infection, however, the levels of serum LH and FSH may be variable(30). Inflammatory cytokines are thought to cause endocrine dysfunction which can contribute to the symptoms of loss of weight, fatigue and poor quality of life(36). This is applicable to PLWH as they are often chronically ill and with a decline in their CD4 counts they are susceptible to a multitude of opportunistic infections. Hypogonadism has multiple symptoms, including erectile dysfunction, infertility, decreased body and facial hair, decrease in muscle mass, loss of bone mass, fatigue, difficulty in concentrating and overall decrease in quality of life. However, these symptoms may be easily missed as they are non-specific and may mimic the symptoms of advanced HIV infection such as fatigue, psychosocial problems and loss of weight. In addition, MLWH are likely to be on a variety of medications with several potential side effects. The initiation of ART may restore testosterone levels into the normal range, but protease inhibitors may be associated with sexual dysfunction, independent of the testosterone level(35).

The clinical consequences of prolonged hypogonadism are diverse and include the following:

1. Emotional: Depression, low self-esteem and poor concentration.
2. General: Loss of libido, hot flushes, palpitations, abdominal obesity and anaemia.
3. Reproductive: Subfertility, erectile dysfunction and sexual dysfunction.

More severe complications include, osteoporosis (causing fractures and debility), insulin resistance, raised lipids and loss of muscle bulk (sarcopenia). These complications can increase the risk for CVD in PLWH. Since hypogonadism has detrimental effects on the long-term health, quality of life and longevity it is important that HIV-infected men are tested for the presence of hypogonadism.

Currently for men with hypogonadism there is a number of potential benefits with testosterone replacement therapy. This includes improvement in the following: muscle bulk, mood symptoms, libido, erectile dysfunction and body composition(37). The benefit of testosterone therapy must be weighed against potential side effects. Some adverse effects include, acne, erythrocytosis, elevation of PSA with worsening of prostate conditions and worsening of obstructive sleep apnoea(37). A large number of different formulations for testosterone replacement are currently available, including injectables, gels, transdermal patches and implants(38). Little is known about managing hypogonadism in MLWH, but some studies have shown that using intramuscular depot testosterone for short courses does improve mood, libido and overall well-being(39).

Literature

There is a lack of data indicating the prevalence of hypogonadism in MLWH. This is largely due to the lack of validated methods and standardization in approach to research and results. Studies from high-income countries demonstrate a variable prevalence (12,5%- 54%) of hypogonadism in MLWH, however, data from low- and middle-income countries is sparse, with little data from sub-Saharan Africa, the epicentre of the HIV epidemic. Aggarwal *et al* (2015) found gonadal dysfunction to be the most prevalent endocrine abnormality followed by thyroid and adrenal dysfunction (40). Dutta *et al* (2015) concluded that hypogonadism was a frequent occurrence in MLWH, affecting around 39% of their study population, with most cases found to have hypogonadotropic hypogonadism(41). Pongener *et al* (2019) reported a prevalence of hypogonadism of 23.3% (20/120 patients) with 85.7% shown

to have hypogonadotropic hypogonadism. They showed a significant association between hypogonadism and CD4 count but found no association with BMI and duration of ART (42). Lachatre *et al* (2017) reported in their study of 113 French MLWH on ART for 6 months that 1 in 6 men had hypogonadism (43). Similarly, Gomes *et al* (2016) showed a high prevalence (29.4%) of hypogonadism in Portuguese MLWH, the majorityof which were also shown to have hypogonadotrophic hypogonadism(30). Wunder *et al* (2007) reported that 70% of their cohort of 139 ART-naive Swiss MLWH had below- normal levels of free testosterone, which did not improve on 2 years of anti-retroviral therapy (44).

In stark contrast to these studies, Dube *et al* (2007) reported a low (6%) prevalence of hypogonadism in American MLWH and also showed that their testosterone levels improved on ART, especially in patients who received lamivudine and zidovudine in comparison with patients who received stavudine and didanosine (45). Dobs *et al* (1988) showed that 50% of their cohort of 70 MLWH who were classified as having AIDS were found to be hypogonadal, with 75% having hypogonadotropic hypogonadism(46).

Data from sub-Saharan Africa remains sparse, essentially represented by a single report from South Africa that was presented in 2000 at a HIV Congress held in Durban (47). Deystanik *et al* (2000) conducted a retrospective chart review on 88 MLWH who had been on ART for at least six months and reported a 20% prevalence of hypogonadism(47).

Chapter 2: Aims and Hypothesis

AIMS

1. To determine the prevalence of hypogonadism in HIV - infected men prior to and 12 months after initiating anti-retroviral therapy.

2. To investigate the association between testosterone levels and metabolic variables such as glucose metabolism and lipid changes in HIV-infected men prior to and 12-months after initiating anti-retroviral therapy

3. To investigate the association between testosterone levels and changes in body composition of HIV-infected men prior to and 12-months after initiating anti-retroviral therapy.

4. To investigate the association between testosterone levels and changes in bone density in HIV-infected men prior to and 12-months after initiating anti-retroviral therapy.

HYPOTHESIS

HIV-infected men will have a high prevalence of hypogonadism which will result in an increased risk for elevated body mass index, metabolic derangement, low bone mineral density and a decrease in quality of life.

CHAPTER 3: PUBLICATION-READY MANUSCRIPT

Low prevalence of hypogonadism in HIV-infected South African men - a longitudinal study to assess the association between testosterone and bone mineral density, metabolic abnormalities and quality of life.

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Background

HIV is a global pandemic with South Africa at the epicentre. HIV-infection and its associated co-morbidities remains a major contributor to morbidity and mortality of South Africans. Hypogonadism has been described amongst HIV-infected men, however, most of the current data is from developed countries with the prevalence of hypogonadism differing widely amongst these studies. Data from sub-Saharan Africa remains sparse with only one abstract describing the prevalence of hypogonadism in HIV-infected men to be around 20% (47). Since hypogonadism can contribute to morbidity and reduced quality of life in HIV-infected men the aim of this study was to assess the prevalence and predictors of hypogonadism in HIV-infected men and its association with body composition, metabolic derangement and bone mineral density (BMD). This data will help inform screening and management protocols.

Methods

This is a sub-study of a parent study, the McHAART Study, which was a two-year longitudinal study assessing the mechanisms and risks for the development of metabolic abnormalities in a cohort of HIV-infected patients receiving ART. In this study a longitudinal assessment of body composition [anthropometric measures and dual-energy X-ray absorptiometry (DEXA)] and BMD was also done.

a) Brief description of the study design and methods of the parent study:

A prospective longitudinal two-year cohort study of ambulatory HIV-infected subjects enrolled in a community HIV treatment program. HIV-infected patients commencing ART were conveniently sampled from clinic lists at Crossroads Community Health Care Centre in Cape Town.

Inclusion criteria: age > 18 years, antiretroviral therapy -naïve, able to provide informed consent, no past history of diabetes or dyslipidaemia, not taking corticosteroids or statins

Exclusion criteria: any acute opportunistic infection, < 1 month of anti-tuberculous therapy, renal failure or liver failure

Patients satisfying inclusion (and exclusion) criteria and providing written informed consent were assessed at baseline and then again at 3, 6, 12, 18 and 24 months after the commencement of ART.

The following assessments were done at each time-point:

1) a field worker-administered questionnaire (known diabetes risk factors, level of physical activity, history of prior illness, socioeconomic factors, quality of life, nutrition, smoking, drugs and toxins)
2) clinical records were reviewed to provide all prior information on weight, CD4 count, viral load, antiretroviral therapy regimens, and any associated medical data
3) oral glucose tolerance test (OGTT) with glucose and insulin at 0, 30, 90 and 120 mins. These values were used to provide a measure of insulin resistance (HOMA-IR) and β-cell function (IGI and OGIS).
4) measurement of total cholesterol, low density lipoprotein (LDL), high density lipoprotein (HDL), triglyceride (TG), LDL particle size, lactate, TSH, T4, T3.
5) anthropometry - weight, height, waist circumference, hip circumference, mid-upper arm circumference, mid-thigh circumference, calf circumference and sagittal height were measured using standardized procedures
6) body composition was measured using dual-energy x-ray absorptiometry.

b) Brief description of the study design and methods of the current study:

1. All men recruited into the parent study were included in this study
2. Blood samples taken at 08h00-09h00 after an overnight fast (10-12h) from these participants (stored at -80^{o}C) at baseline and 12-months after initiating ART were used to measure testosterone, follicle stimulating hormone (FSH) and luteinizing hormone (LH). Serum samples were run with an automated analyser through a Roche Cobas e601 machine and analysed via immunoassay.
3. Other variables included in the analyses were:
    a. CD4 count
    b. Body composition: Basic anthropometric measurements, including weight, height and waist (at the level of the umbilicus), hip (largest gluteal area) and calf (mid-calf) circumferences were taken. All measurements were taken twice and recorded to nearest 0.1 cm using a flexible, non-elastic tape, approximately 0.7 cm wide.
    c. Bone density: Dual-energy X-ray absorptiometry (DEXA) (Hologic Discovery-W, software version 12.7; scan region 195 x 65 cm^2 and weight

limit 160kg).

d. Fasting blood samples: In the morning (08h00- 09h00), after an overnight fast (10-12h), an indwelling cannula was inserted into the antecubital vein. Venous blood samples (10ml) were drawn at 0 min, 30 min and 120 min after the participants ingested 75 g glucose in 250 ml water. Specimens were centrifuged on the day of collection and plasma samples for glucose analysis were stored at -20^{0}C while serum samples were stored at -80^{0}C until analysed further. Glucose, insulin, total cholesterol (TC) and triglycerides were measured by enzymatic colorimetry on the Cobas 6000 autoanalyzer (Roche, Switzerland). HDL cholesterol (HDCL) was measured using a direct method (Roche HDLC3 assay, Switzerland). LDL cholesterol (LDLC) was calculated using the Friedewald formula, provided triglycerides were less than 4.5mmo/L.
e. Blood pressure: Blood pressure was measured 3 times at 1-min intervals in each subject using an appropriately sized cuff and a calibrated sphygmomanometer after at least 20min of seated rest. An average of the last 2 readings will be used in the analyses.

Study Population

All men over the age of 18 years, who were HIV-infected and had given written informed consent for the parent study were included in this study.

Statistical analysis

Descriptive statistics were means and standard deviations for normally distributed data and median and interquartile range (IQR) for non-normally distributed data. Pearson correlation assessed the relationship between baseline testosterone levels and all other variables(at 0, 3, 6 and 12 months), and between change in testosterone (from baseline to 12-months) and all variables at 12 months (Bonferroni corrected *p*-value =0.010). Independent sample t-tests compared baseline testosterone levels and change in testosterone levels for the variable's: smoker and ART.

Results

The study population consisted of 44 participants. Of these 44 participants, 30 were available for review at 12 months. The reasons for 14 participants not being available

for follow-up included lost to follow up, relocation to another area and death.

There was no significant difference in baseline characteristics in participants who had blood samples at baseline and 12-months when compared to those with samples only at baseline.

Patient characteristics

The patient characteristics at baseline and at 12- months are described in Table 3.

The average age of our participants was 36.1 ± 9.2 years with most participants being smokers (n=35, 79.5%). Baseline CD4 counts were 149 ± 66.5 cells/mm^3 with an average viral load of 4522.2 copies/ml. None were on ART at baseline and at 12 months all were established on ART.

At baseline body composition measurements were waist circumference 78.8 ± 7.1 cm, waist: hip ratio 1.0 ± 0.1, calf skinfold thickness 6.5 cm ± 3 cm and BMI were 21.4 ± 2.8 kg/m^2. At 12 months body composition measurements were waist circumference 81.5 ± 7.7 cm, waist: hip ratio 0.90 ± 0.07, calf skinfold thickness 7.2 ± 3.5 cm and BMI were 22.6 ± 2.5 kg/m^2.

Systolic blood pressure was 121 ± 14.5 mmHg at baseline and 123.1 ± 11.0 mmHg at 12 months. Diastolic blood pressure on the other hand was 76.5 ± 11.1 mmHg and 74.6 ± 7.6 mmHg at baseline and 12 months, respectively.

The fasting glucose at baseline was 4.7 ± 0.50 mmol/L whilst the 2-hour glucose value after an OGTT was 5.3 ± 1.2 mmol/L. At 12 months the mean fasting glucose was 4.9 ± 0.5 mmol/L and the 2-hour glucose after an OGTT was 5.5 ± 1.6 mmol/L. HOMA-IR was 0.88 ± 0.68 at baseline and 1.03 ± 0.8 at 12 months.

At baseline the total cholesterol was 3.57 ± 0.70 mmol/L, triglycerides were 0.94 ± 0.35 mmol/L, HDL was 0.92 ± 0.31 mmol/L and LDL cholesterol was 2.21 ± 0.61 mmol/L. At 12 months the total cholesterol was 4.40 ± 0.87 mmol/L, triglycerides were 1.18 ± 1.17 mmol/L, HDL was 1.46 ± 0.50 mmol/L and the LDL cholesterol was 2.39 ± 0.75 mmol/L.

Testosterone levels

Testosterone and gonadotroph levels of the participants at baseline and at 12-months are described in Table 4.

At baseline the mean level of testosterone was 23.65 ± 7.7 nmol/L (normal: 10.4 – 32.6) and the mean levels of LH and FSH were 7.64 ± 3.7 IU/L (normal: 1.93 – 9.7)

and 7.95 ± 6.2 IU/L (normal: 2.04 – 12.4), respectively. After 12 months on ART there was no significant change with the mean levels of testosterone 23.84 ± 5.60 nmol/L, LH 6.71 ± 2.49 IU/L and FSH 5.74 ± 3.2 IU/L. The levels of free testosterone were also normal at baseline [0.34 ± 0.12 pmol/L (normal: 0.06 – 1.08)] and at 12 months [0.36 ± 0.08 pmol/L].

No participants were found to have hypogonadism at baseline or at 12 months.

All participants had normal LH and FSH values.

Association between testosterone levels and body composition, glucose metabolism, lipids and blood pressure

The association between levels of testosterone and change in levels of testosterone with body composition, glucose metabolism, lipids and blood pressure are described in Table 5 and Table 6

There was no significant association between levels of testosterone or change in levels of testosterone with any measure of body composition, glucose metabolism, lipids or blood pressure.

Bone mineral density

Results of the bone mineral density measurements by DEXA are described in Table7.

At baseline the BMC and BMD at L1-L4 was 65.2 ± 12.6 g and 0.96 ± 0.14 g/cm^2, at left hip it was 41.69 ± 8.0 g and 1.01 ± 0.14 g/cm^2 and at the right hip it was 41.26 ± 7.8 g and 1.00 ± 0.15 g/cm^2.

At 12 months the BMC and BMD at L1-L4 was 66.5 ± 12.0 g and 0.98 ± 0.13 g/cm^2, at the left hip it was 41.47 ± 8.41g and 1.00 ± 0.15 g/cm^2 and at the right hip it was 41.50 ± 7.85 g and 1.00 ± 0.15 g/cm^2.

Association between testosterone levels and bone mineral density

The association between levels of testosterone and change in levels of testosterone with measures of bone mineral density are described Table 8 and Table 9.

There was no association between levels of testosterone and change in levels of testosterone with bone mineral content or bone mineral density.

Discussion

This is the first longitudinal study in South Africa and sub-Saharan Africa to assess

hypogonadism in HIV-infected men. In this cohort of HIV-infected men there were no participants with hypogonadism at baseline or after 12 months on ART. In addition, there was no association between levels of testosterone or change in levels of testosterone with body composition, glucose metabolism, lipids and measures of bone mineral density.

Since all of the participants commenced ART at a CD4 count < 200 cells/mm^3 and had lost weight it was expected that a significant proportion may have a low testosterone level due to the sick gonadotroph syndrome. However, surprisingly, in this cohort and despite being ill all participants had a normal total testosterone level, a normal free testosterone level (calculated), normal gonadotrophs and there were no participants identified with hypogonadism at baseline. This is in contrast to data from the developed world where studies report an increased risk of developing hypogonadism in MLWH.(12, 48). As an example, a study of 70 HIV-infected men from The Johns Hopkins Hospital outpatient research centre, reports a 50% prevalence of hypogonadism(49). Depending on the numbers of participants in a study and the geographic area the prevalence of hypogonadism in MLWH does vary quite widely from 6% to > 50% (41) (43). A single unpublished report from South Africa describes a prevalence of hypogonadism of 20% (47).

After initiating ART all participants experienced an increase in their BMI and waist circumference signifying improved health and a return towards their baseline. This was also associated with an improvement in their CD4 count and their quality of life scores. All this points to them being ill prior to initiating ART yet no participant had hypogonadism. At the 12-month assessment all participants had a normal total testosterone level, a normal free testosterone level (calculated), normal gonadotrophs and there were no participants identified with hypogonadism at after 12 months of ART.

It is possible that a percentage of the 14 participants not available for the 12-month follow-up may have had hypogonadism. However, this group of 14 participants at baseline were not significantly different from the group of 30 participants that were available for the 12-month follow-up.

Since no participant had hypogonadism we sought to assess the association between levels of testosterone or change in levels of testosterone with various outcomes shown

to be associated with hypogonadism, namely body composition, metabolic variables such as glucose and cholesterol, and bone mineral density. Again, no significant association was shown between levels of testosterone or change in levels of testosterone with body mass index, waist circumference, glucose metabolism, lipids and measures of bone mineral content and bone mineral density.

Limitations and strengths

This study has several limitations. Firstly, the sample size is small compared to other studies. The HIV pandemic in sub-Saharan Africa predominantly affects women and it is often difficult to gain consent from men to enrol in studies. Although 32% of participants were not available for follow-up this seems to be consistent with many longitudinal studies, especially those from South Africa, where participants are quite mobile in search of employment opportunities. However, despite this dropout rate it remains significant that none of our patients had hypogonadism at baseline even though they had advanced HIV infection with a mean CD4 count of 149 cells/mm^3 and a low BMI of 21,4kg/m^2.

The strength of this study is its longitudinal design and that it is the first report on hypogonadism in MLWH in South Africa. This study can serves as a pilot study to design a larger longitudinal study to assess many of the potential endocrine abnormalities that may occur in MLWH.

Conclusion

In this longitudinal study of hypogonadism in MLWH, the first in South Africa, no participants were found to have hypogonadism. In addition, levels of testosterone or change in levels of testosterone were not significantly associated with body composition, glucose metabolism, lipids or any measure of bone mineral density. This study serves as a pilot study showing that future studies assessing hypogonadism in MLWH need to be adequately powered in order to definitively show the true prevalence of hypogonadism in MLWH or the association between levels of testosterone with body composition, metabolic variables and bone mineral density.

Table 1: Patient Characteristics at baseline and at 12-months

	Baseline	12 months
	(n=44)	(n=30)
Age (in years)	35.5 (28.25, 42.50)	
Smoker	35 (79.5)	
BMI (kg/m^2)	21.4 ± 2.8	22.6 ± 2.5
CD 4 count (cells/mm^3)	149.5 ± 66.5	248.8 ± 99.8
ART (number of patients on treatment)		
Zidovudine (AZT)	0	1
Stavudine (D4T)	0	29
Lamivudine (3TC)	0	41
Efavirenz	0	24
Nevirapine	0	17
Lopinavir/Ritonavir	0	0
Tenofovir	0	11
Viral load (copies/ml)	4522.1931	-
Waist circumference (cm)	78.8 ± 7.1	80.0 (75.0, 86.0)
Waist: hip ratio	1 ± 0.1	0.88 (0.85, 0.94)
Calf circumference (cm)	33 ± 2.3	-
Calf skinfold thickness (mm)	5.20 (4.60, 7.80)	7.2 ± 3.5
SBP (mmHg)	119 (111, 133)	123.1 ± 11.0
DBP (mmHg)	76.5 ± 11.1	74.6 ± 7.6
Fasting glucose (mmol/L)	4.7 ± 0.50	4.9 ± 0.5
2-hour glucose (mmol/L)	5.40 (4.60, 6.40)	5.5 ± 1.6
Fasting insulin (pmol/L)	4.0 ± 2.9	-
HOMA-IR**	0.85 (0.36, 1.25)	1.03 ± 0.8
Total cholesterol (mmol/L)	3.63 (2.96, 4.11)	4.4 ± 0.87
Triglycerides (mmol/L)	0.94 ± 0.35	1.18 ± 1.17
HDL (mmol/L)	0.92 ± 0.31	1.29 (1.04, 1.79)
LDL (mmol/L)	2.21 ± 0.61	2.39 ± 0.75

**(Glucose x insulin)/22.5

BMI-Body mass index; HOMA IR-Homeostatic Model Assessment for Insulin Resistance; TC-Total cholesterol; TG-Triglycerides; HDL-High density lipoproteins; LDL- Low density lipoproteins; SBP-Systolic blood pressure; DBP- Diastolic blood pressure
All values are mean ± standard deviation or median (IQR)

Table 2: Testosterone and gonadotroph levels at baseline and at 12 months

	Baseline	12-months	Normal range
	n=44	n=30	
FSH (IU/L)	7.95 ± 6.20	5.74 ± 3.20	2.04 -12.4
LH (IU/L)	7.64 ± 3.70	6.71 ± 2.49	1.93 - 9.7
Total testosterone (nmol/L)	23.65 ± 7.70	22.30 (19.60, 26.60)	10.4 - 32.6
SHBG (nmol/L)	51.62 ± 30.82	50.06 ± 25.01	13.5 - 57.4
Free testosterone (pmol/L)	0.34 ± 0.12	0.36 ± 0.08	0.06- 1.08

FSH- Follicle stimulating hormone; LH- Luteinizing hormone; SHBG- Sex hormone binding globulin All values are mean ± standard deviation or median (IQR)

Table 3: Pearson correlation between baseline testosterone levels and body composition, glucose metabolism, lipids and blood pressure at baseline and at 12 months

Characteristic	Baseline		12 months	
	p	r	p	r
BMI	0.114	-0.244	0.412	-0.161
Waist	0.192	-0.203	0.698	-0.077
Waist: hip	0.434	-0.122	0.985	-0.004
Calf skinfold	0.910	-0.018	0.948	0.13
Fasting glucose	0.007	0.409	0.289	0.193
2-hour glucose	0.874	0.025	0.714	-0.067
Fasting insulin	0.112	0.249	N/A	N/A
HOMA IR	0.068	0.284	0.683	0.075
TC	0.931	0.014	0.293	-0.192
TG	0.128	-0.239	0.096	-0.299
HDL	0.979	-0.004	0.919	0.019
LDL	0.614	0.080	0.903	-0.022
SBP	0.716	-0.057	0.324	0.190
DBP	0.310	-0.159	0.552	-0.115

BMI- Body mass index; HOMA IR- Homeostatic model assessment for insulin resistance; TC- Total cholesterol; TG- Triglycerides; HDL- High density lipoproteins; LDL- Low density lipoproteins; SBP- Systolic blood pressure; DBP- Diastolic blood pressure

Table 4: Pearson correlation between change in levels of testosterone and body composition, glucose metabolism, lipids and blood pressure at 12 months

Characteristic	Change at 12 months	
	p	r
BMI	0.978	-0.005
Waist	0.698	-0.077
Waist: hip	0.661	0.087
Calf skinfold	0.359	-0.180
Fasting glucose	0.151	-0.269
2-hour glucose	0.968	0.008
Fasting insulin	N/A	N/A
HOMA IR	0.152	-0.268
TC	0.526	0.121
TG	0.473	0.136
HDL	0.687	-0.077
LDL	0.635	0.090
SBP	0.992	-0.002
DBP	0.403	0.161

BMI-Body mass index; HOMA IR-Homeostatic model assessment for insulin resistance; TC- Total cholesterol; TG- Triglycerides; HDL- High density lipoproteins; LDL- Low density lipoproteins; SBP- Systolic blood pressure; DBP- Diastolic blood pressure

Table 5: DEXA measures of bone mineral density at baseline and at 12 months

	0 months	12 months
n	40	28
L1-L4 BMC	65.2 ± 12.6	66.5 ± 12.0
L1-L4 BMD	0.96 ± 0.14	0.98 ± 0.13
L neck BMC	4.62 (4.19, 5.27)	4.71 (4.26, 5.29)
L neck BMD	0.85 (0.78, 0.97)	0.85 (0.79, 0.94)
L HTOT BMC	41.69 ± 8.0	41.47 ± 8.41
L HTOT BMD	1.01 ± 0.14	1.00 ± 0.15
R HTOT BMC	41.51 (34.91, 48.68)	41.50 ± 7.85
R HTOT BMD	1.00 ± 0.15	1.00 ± 0.16
R neck BMC	4.66 ± 0.80	4.71 ± 0.79
R neck BMD	0.86 ± 0.14	0.85 ± 0.14

L-left; R-Right; L HTOT-Left hip total; R HTOT- Right hip total; BMC-Bone mineral content in grams; BMD- Bone mineral density in g/cm2
All values are mean ± standard deviation or median (IQR)

Table 6: Pearson correlation between baseline levels of testosterone and measures of bone mineral density at baseline and at 12 months

	Baseline		12 months	
	p	r	p	r
L spine				
ToT_Area	0.883	-0.024	0.915	-0.021
ToT_BMC	0.262	-0.182	0.558	-0.116
ToT_BMD	0.133	-0.242	0.425	-0.157
Left Hip				
Neck_Area	0.780	0.044	0.679	0.080
Neck_BMC	0.913	0.014	0.580	0.107
Neck_BMD	0.957	-0.009	0.727	0.068
HToT_Area	0.512	-0.104	0.288	-0.204
HToT_BMC	0.660	-0.070	0.734	-0.066
HToT_BMD	0.826	-0.035	0.839	0.039
Right hip				
Neck_Area	0.482	-0.114	0.716	-0.070
Neck_BMC	0.544	-0.099	0.934	0.016
Neck_BMD	0.644	-0.075	0.864	0.033
HToT_Area	0.108	-0.258	0.097	-0.314
HToT_BMC	0.138	-0.239	0.323	-0.190
HToT_BMD	0.425	-0.130	0.838	-0.040

ToT-Total; HTOT-Hip total; BMC-Bone mineral content; BMD-Bone mineral density

Table 7: Pearson correlation between change in levels of testosterone and measures of bone mineral density at 12 months

Bone density	12 months	
	p	r
L spine		
ToT_Area	0.917	-0.021
ToT_BMC	0.748	0.065
ToT_BMD	0.590	0.108
Left Hip		
Neck_Area	0.662	-0.086
Neck_BMC	0.218	-0.240
Neck_BMD	0.249	-0.225
HToT_Area	0.882	0.029
HToT_BMC	0.396	-0.167
HToT_BMD	0.039	-0.222
Right hip		
Neck_Area	0.882	-0.029
Neck_BMC	0.364	-0.178
Neck_BMD	0.396	-0.167
HToT_Area	0.511	0.130
HToT_BMC	0.775	-0.057
HToT_BMD	0.461	-0.145

ToT-Total; HTOT-Hip total; BMC-Bone mineral content; BMD-Bone mineral density

JEMDSA

Journal of Endocrinology, Metabolism and Diabetes of South Africa

JEMDSA representation

The Journal of Endocrinology, Metabolism and Diabetes of South Africa (JEMDSA) is the official journal of **SEMDSA** (Society for Endocrinology, Metabolism and Diabetes of SA), **DESSA** (Diabetes Education Society of SA), **NOFSA** (National Osteoporosis Foundation of SA), **SASOM** (SA Society of Obesity and Metabolism), **LASSA** (Lipid and Atherosclerosis Society of SA) and **PAEDS-SA** (The Paediatric Endocrinology Society of SA).

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Books

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Foley KM, Gelband H, editors. Improving palliative care for cancer [monograph on the Internet]. Washington: National Academy Press; 2001 [cited 2002 Jul 9]. Available from: http://www.nap.edu/books/0309074029/html/. Accessed 6 January 2007

Homepage/Web site

Cancer-Pain.org [homepage on the Internet]. New York: Association of Cancer Online Resources, Inc.; c2000-01 [updated 2002 May 16; cited 2002 Jul 9]. Available from: http://www.cancer-pain.org/. Accessed 3 May 2008

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Ethical Considerations

This study was approved by the University of Cape Town's Human Research Ethics Committee and the Provincial Administration of the Western Cape, HREC 728/2018. An unrestricted educational grant was obtained from Merck Pharmaceuticals for the measurement of testosterone, LH and FSH. There are no conflicts of interest.

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A/Prof J **Dav•**
Division of Endoa1nology
J-Floor, 0MB

Dear A/Prof Dave

PROJI!CT nn.E1LONGITUDINAL STUDY TO ASSI!SS THI! PR!VALI!NCE 01' HYPOGONADISM IN HIV-INFECTED SOUTH **APRICAN MEN AND** rrs ASSOCIAnON WITH BONE Dl!NSrTY, **BODY COMPOSffION, MI!TABOUC ABNORMALITII!S AND QUALITY *OF* LIPI!. (SUB-STUDY LINKED TO 221/2001)**
(MMacl C.ndldata - Dr Le Roux van der Merw•)

Thank you for submtttrng your study to the Faculty of Health Sciences Human Research Ethics Committee (HREC) for **review.**

It Is a pleasure to Infonn you that the HREC has **formally approved** the above-mentioned study.

Approv■l granted for on• yur untll th• JO Nonmber 2019.

Please submit a progress fonn, using the standardised Annual Report Form If the study continues beyond the approval period. Please submit• Standard Closure fonn If the study Is completed within the approval period.
(Fonns am be round on our website: www,beattb,uct,1c.zalflts/research/humaoetblcslfprms)

We *Klcnowledge* tut the *lltrldent: Dr Le Roux v•n tier Henn, will •• be Involved In th,. dully.*

PINN quote the HRIC Rl!P In ■II your correspondence.

Please note that the ongoing ethlcal conduct of the study remains the responsibility of the principal Investigator.

Please note that for ell studies **approved** by t REC, the principal Investigator 11111d obtain appropriate Institutional approval, where necessary ore he research may occur.

Yours sincerely

PROFESSOR M BLOCKMAN
CHAIRPERSON, FHS HUMAN RESEARCH ETHICS COMMITTEE

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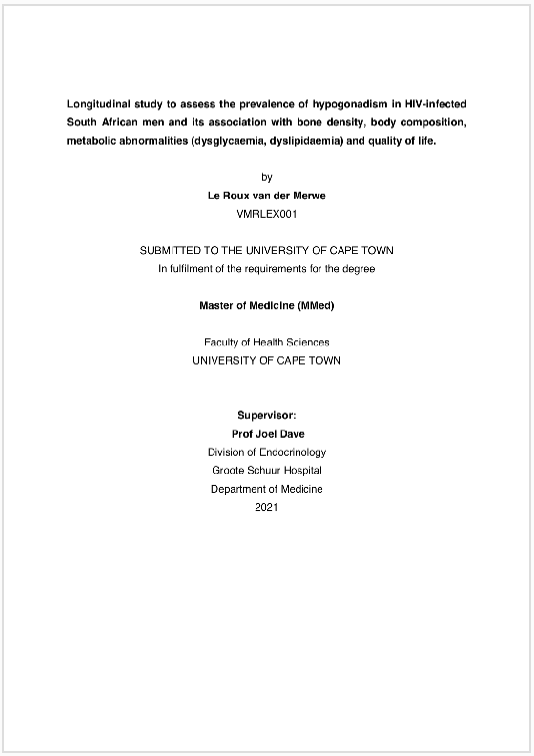

Longitudinal study to assess the prevalence of hypogonadism in HIV-infected South African men and its association with bone density, body composition, metabolic abnormalities (dysglycaemia, dyslipidaemia) and quality of life.

by

Le Roux van der Merwe

VMRLEX001

SUBMITTED TO THE UNIVERSITY OF CAPE TOWN

In fulfilment of the requirements for the degree

Master of Medicine (MMed)

Faculty of Health Sciences

UNIVERSITY OF CAPE TOWN

Supervisor:

Prof Joel Dave

Division of Endocrinology

Groote Schuur Hospital

Department of Medicine

2021

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