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The prevalence and associations of low testosterone levels and erectile dysfunction in a male diabetic urban population

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I, **Tanja Kemp**, hereby declare that the work which I hereby submit as partial fulfilment for the degree **MSc (Clinical Epidemiology),** on which this thesis is based, is original (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of it has been submitted, for another degree at this or any other university.

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ABSTRACT

Introduction: According to the literature, low serum testosterone levels are associated with diabetes mellitus. Minimal data exist for its prevalence or predictors in South Africa. Erectile dysfunction is a common condition in diabetic patients. The prevalence and predictors in our patient population is unknown.

Methods: An observational, cross-sectional study was performed in 150 consecutive male diabetic patients over the age of 50 years in the Diabetic clinic of Steve Biko Academic Hospital. These patients were evaluated for diabetes control and complications, the presence of erectile dysfunction and for hypogonadism symptoms. Morning serum testosterone levels were done. Subjects with low testosterone levels were compared to those with normal levels.

Results: The mean age of the patients was 62 years (standard deviation (SD) 7.87), 91.3% had type 2 diabetes, and 84.7% were on insulin. The mean duration of diabetes was 15 years (SD 8.65). The mean body mass index was 30.7 (SD 5.37), the mean waist circumference was 112.4cm (SD 16.42), the median creatinine was 96µmol/L (interquartile range (IQR) 79-133) and the median HbA1C was 7.85% (IQR 6.80-9.30). Ischaemic heart disease was previously diagnosed in 40.7% of patients.

Some degree of erectile dysfunction was reported in 95.3% of the patients with 51.3% reporting serious dysfunction. The prevalence of androgen deficiency symptoms was 94.7%. Fifty percent of the men had low total testosterone levels; 40.7% had low modified calculated bioavailable testosterone levels, and in 27.3% both were low.

With multivariate logistic regression the significant factors associated with low total testosterone were waist circumference and known cardiovascular disease. For a low modified calculated bioavailable testosterone level significant variables were age, diabetes duration and body mass index and for an outcome defined as both the above the significant factors were diabetes duration, body mass index, and known cardiovascular disease. With multivariate logistic regression the significant factors associated with erectile dysfunction were age, body mass index, peripheral neuropathy score, and diuretic therapy.

The prevalence of symptoms of androgen deficiency was very high with 94.7% of all patients reporting a significant amount of symptoms on the Androgen Deficiency in Adult

Males (ADAM) questionnaire. If only the total serum testosterone level was evaluated instead of the modified calculated bioavailable testosterone, the sensitivity was 69%, the specificity was 63%, with a poor positive predictive value of only 56%. The negative predictive value was better at 75%. Differences in quality of life scores were only seen for some erectile dysfunction subgroups but not for low testosterone levels.

Conclusion: This study confirms the high prevalence of low testosterone levels and of erectile dysfunction in diabetic male patients in a tertiary setting, and argues in favour of universal screening of this population group. Multiple predictors of low testosterone levels and of erectile dysfunction were identified. The ADAM questionnaire was not useful in identifying subjects with a low testosterone level. Total testosterone testing alone performed poorly in comparison with modified calculated bioavailable testosterone and is not the recommended test of choice. Erectile dysfunction negatively affected the quality of life.

Key words:

- Testosterone
- Hypogonadism
- Androgen deficiency
- Diabetes mellitus
- Erectile dysfunction
- Quality of life

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ABBREVIATIONS

ADAM	Androgen Deficiency in the Aging Male
BP	Blood pressure
DM	Diabetes mellitus
ED	Erectile dysfunction
EuroQol Group	European Quality of Life group
EQ-5D	European Quality of Life-5 Dimensions
HbA1c	Glycosylated haemoglobin / haemoglobin A1c
HH	Hypogonadotrophic hypogonadism
HT	Hypertension
IHD	Ischaemic heart disease
IQR	Interquartile range
LDL	Low density lipoprotein
LH	Luteinizing hormone
LOH	Late-onset hypogonadism
Modified NSS	Modified neuropathy symptom score
n	Numbers
NPV	Negative predictive value
PN	Peripheral neuropathy
PPV	Positive predictive value
PDR	Proliferative diabetic retinopathy
ROC	Receiver operating characteristic
SD	Standard deviation
SHBG	Sex hormone binding globulin
SHIM	Sexual health inventory for men
T1DM	Type 1 diabetes mellitus
T2DM	Type 2 diabetes mellitus
TDS	Testosterone deficiency syndrome

CHAPTER 1: LITERATURE REVIEW

1.1. BACKGROUND

The prevalence of male hypogonadism varies among different populations and age groups studied. Its prevalence together with that of erectile dysfunction is increased in male patients with diabetes mellitus. Minimal data is available for South Africa men with diabetes mellitus. Erectile dysfunction is often not asked about, and testing for low testosterone levels in every diabetic male is probably not cost-effective. This study aimed to evaluate the scope of the problem, to evaluate the influence on quality of life, and to identify predictors by evaluating different clinical and demographic factors and routine blood tests. This was done to try and identify male patients with diabetes at high risk for hypogonadism who needs further evaluation in the form of serum testosterone levels.

1.2. LITERATURE OVERVIEW AND MOTIVATION

(1) Testosterone deficiency syndrome (TDS)

a) Introduction

Hypogonadism is a clinical condition consisting both of symptoms and biochemical evidence of low testosterone levels. A condition called testosterone or androgen deficiency syndrome was described with advancing age, although this entity remains controversial.[1,2] It is recommended that this diagnosis only be made in men with clear clinical symptoms and signs of androgen deficiency, and with unequivocally low serum testosterone levels.[2]

b) Associations and prevalence of hypogonadism

The list of causes of testosterone deficiency is long and varied, as is the spectrum of clinical manifestations. Complex alterations in the hypothalamic-pituitary-testicular axis function exist in aging men against a background of age-related testicular impairment.[3] These changes are linked to specific risk factors such as age, obesity, comorbidities and smoking. A 4- 5-kg/m2 increase in body mass index or loss of a spouse can be associated with declines in total serum testosterone comparable to that associated with approximately

10 years of aging, thus both chronological aging and changes in health and lifestyle factors influence serum testosterone levels.[4]

It is well-known that age has significant effects on both total and free testosterone levels, with the levels decreasing with advancing age.[4,5] In the Baltimore Longitudinal Study on Aging, total testosterone levels dropped to hypogonadal levels in about 20% of men older than 60 years, 30% over the age of 70 years, and 50% in men above 80 years of age.[5]

In a large population-based study approximately 24% of subjects had low total testosterone levels and 11% low free testosterone levels.[6] Crude prevalence of symptomatic androgen deficiency was 5.6%. Prevalence was only 3.1-7.0% in men less than 70 years, but increased to 18.4% in 70 year old men.

c) Consequences of hypogonadism

Common symptoms and signs include poor morning erection, low sexual desire, erectile dysfunction, inability to perform vigorous activity, depression and fatigue.[7,8] Symptoms of hypogonadism in a large population-based study mentioned above included low libido (12%), ED (16%), osteoporosis or fracture (1%), and two or more of the nonspecific symptoms such as sleep disturbance, depressed mood, lethargy, or diminished physical performance (20%).[6]

Testosterone deficiency can lead to a decrease in lean body mass, increased fat mass, impaired mood, decreased bone mineral density and erythropoiesis, and a decrease in body and facial hair.[7] In older men, lower testosterone levels are associated with greater dependency, impaired balance, falls, increased bone resorption, poor mobility, poorer general health, and low muscle strength.[1,9,10]

Several studies demonstrated a relationship between low serum testosterone levels and obesity and that it predisposed these men to develop the metabolic syndrome, insulin resistance and type 2 diabetes.[1,11,12,13,14] In the Massachusetts Male Aging Study, low serum SHBG, low total testosterone, and clinical androgen deficiency were associated with increased risk of developing metabolic syndrome over time.[14] There was also an

association with an increased mortality due to cardiovascular disease.[15,16] Testosterone deficiency in older men is associated with increased risk of death over the following 20 years, independent of other risk factors and of preexisting health conditions.[17,18]

d) Treatment of hypogonadism

Therapy with testosterone in relatively frail elderly men with borderline or low testosterone levels can improve body composition, bone mineral density, quality of life, frailty, and physical function.[2,19,20,21] Treatment of older men with low testosterone levels remain controversial, especially since there is uncertainty on whether this is a physiological condition associated with aging or whether this is a pathological condition that warrants therapy. [8]

Testosterone therapy is contraindicated in men with prostate or breast cancer, a haematocrit of more than 50%, untreated severe obstructive sleep apnoea, uncontrolled heart failure, severe lower urinary tract symptoms, and men with prostate nodules or high prostate-specific antigen without prior urological evaluation.[2]

e) Screening for hypogonadism

It remains unclear which men from the general population to screen for the presence of hypogonadism. In one large study in men with erectile dysfunction (including non-diabetics) the incidence of hypogonadism was too low to recommend screening all the patients by measuring their serum testosterone levels.[22] Screening was however recommended for those over the age of 50 years, in whom the prevalence of hypogonadism was 9%. Unfortunately determining testosterone only in patients with low sexual desire or abnormal physical findings can miss up to 40% of men with hypogonadism.

(2) Hypogonadism in men with diabetes mellitus.

a) Introduction

There has been debate as to whether low testosterone levels contribute to the development of diabetes and its complications, or whether low testosterone is only a biomarker which coexists with diabetes because of mutual risk factors, or whether it is a consequence of the diabetes.[12]

Men with obesity, the metabolic syndrome, and type 2 diabetes often have low total and free testosterone and low sex hormone binding globulin (SHBG).[12] Hypogonadism seems to be a common finding in diabetic men, and the majority of these men have symptoms of hypogonadism.[23] Studies over the last few years found that at least 25% of men with type 2 diabetes had subnormal testosterone levels with inappropriately low LH and FSH concentrations, indicative of hypogonadotrophic hypogonadism.[24] Another 4% had subnormal testosterone levels with elevated LH and FSH concentrations implying primary hypogonadism. One study found a prevalence of 33% of low free testosterone in type 2 diabetic men.[25]

b) Pathophysiological mechanisms underlying hypogonadism in type 2 diabetes

The nature of the relationship between diabetes and hypogonadism is unclear, since even young men with type 2 diabetes and newly diagnosed type 2 diabetic patients have a high prevalence of hypogonadotropic hypogonadism (HH). [24] One systemic review and meta-analysis demonstrated that men with diabetes had a significantly lower serum free testosterone than non-diabetic men.[12,26] Longitudinal studies in this review also demonstrated that men with higher testosterone levels had a 42% lower risk of developing type 2 diabetes. A higher SHBG also appeared to be protective against developing type 2 DM. In the Rancho Bernardo study, low testosterone levels in men predicted insulin resistance and incident type 2 diabetes in older adults.[27] Obesity is also associated with HH, thus it is possible that HH precedes diabetes.[23,24]

Obesity is a proinflammatory state.[12] Visceral fat produces inflammatory cytokines,

adipokines, and other proinflammatory factors such as interleukin(IL)-6, IL-1β, tumor necrosis factor(TNF)-α, plasminogen activator inhibitor-1, angiotensinogen, vascular endothelial growth factor, and serum amyloid A. Obesity also leads to an increased release of oestrogen and free fatty acids. These factors can all potentially contribute to suppression of the hypothalamus-pituitary-gonadal axis, and can lead to androgen deficiency. A past study demonstrated that in severely obese men (BMI > 40) total testosterone, free testosterone, LH levels as well as LH pulse amplitude were decreased, indicating a functional impairment of the gonadostat.[28]

There might be also a direct effect of testosterone on insulin sensitivity, with testosterone deficiency leading to insulin resistance.[1,13,29] Interactions between visceral adiposity and low testosterone levels through proinflammatory factors can result in insulin resistance and vascular endothelial dysfunction.[12] These can be potential causal factors for increased ED and cardiovascular disease. Leptin resistance at the hypothalamic-pituitary and testicular levels can also contribute to lowered testosterone levels.[30]

c) Consequences of hypogonadism in type 2 diabetes

Type 2 diabetic men with low testosterone levels had been found to have a high prevalence of symptoms of hypogonadism such as erectile dysfunction, low libido, and fatigability.[23,31] Men with ED who do not respond to sildenafil frequently have low levels of testosterone; testosterone replacement can often in these men convert them to responders.[32] One group also demonstrated that severity of erectile dysfunction and hypogonadism was independently associated with a poorer health related quality of life.[33]

A recent prospective study demonstrated that low testosterone levels put diabetic men at a significantly increased risk of death.[34] There is increasing evidence from multiple studies that low testosterone levels are associated with an increase in all-cause mortality that is independent of the metabolic syndrome and diabetes even after adjusting for confounders, as summarized in a review article recently.[12] Cardiovascular events and death can be two to three times elevated.[24,35] A high endogenous serum testosterone level predicted a reduced 5-year risk of cardiovascular events in elderly males in the MrOS study.[36]

d) Screening of male type 2 diabetics for hypogonadism

The Endocrine Society now recommends the routine measurement of testosterone in patients with type 2 diabetes due to the high prevalence.[23] A total testosterone level may not be a satisfactory test for hypogonadism in diabetic men, especially obese patients. Determination of free testosterone or of bioavailable testosterone are better tests to perform, since they are independent of sex hormone binding globulin (SHBG).[16] SHBG is the major carrier protein of testosterone in the circulation, and can be low as a consequence of insulin resistance, thus leading to a low total testosterone when the free testosterone can still be within the normal limits.[37] Even so, free testosterone levels, which are independent of SHBG, are low in one-third of diabetic men.[25]

e) Consequences of treating type 2 diabetics who have low testosterone levels with testosterone replacement therapy

In the recent TIMES2 Study in hypogonadal men with type 2 diabetes or the metabolic syndrome, it was demonstrated that transdermal testosterone replacement had beneficial effects on insulin resistance, total and LDL-cholesterol, lipoprotein a (Lpa), body composition, libido and sexual function.[38] There was not an increased risk of side-effects. A clinical review from 2010 concluded that the adverse effects of testosterone therapy included an increase in haemoglobin and haematocrit, and a small decrease in HDL.[39] This is of unknown clinical significance. Evidence about the safety of testosterone therapy is of poor quality and does not include long follow-up.

Wang C et al recently summarized the results of several trials evaluating the metabolic effect of testosterone replacement. [12] Testosterone therapy improved body composition, and decreased leptin levels. Total cholesterol and in some studies also LDL cholesterol, showed a small but significant fall; HDL can increase, decrease, or remain unchanged. Triglycerides do not change; but lipoprotein a (Lpa) fall significantly after therapy. The bone mineral density at several sites can increase on testosterone therapy, although it is uncertain whether it is a direct effect of testosterone or an indirect effect of aromatization to oestradiol which has an anabolic effect on bone.[40]

Some trial data over the last few years showed that patients with low testosterone levels who received testosterone replacement had a significantly better survival than those who were not treated.[34,41,42] This will need to be confirmed in larger trials; if confirmed, testosterone therapy when indicated could prevent many deaths per year. It will also be necessary to evaluate whether testosterone therapy will improve the quality of life of these diabetic men. The risk-benefit ratio of such therapy can only be determined by adequately designed and powered randomized clinical trials.[13]

The decision to treat older men with low testosterone levels or men with chronic diseases associated with low testosterone levels remains controversial.[13,41,43] Few clinical trials have demonstrated clear benefit, and there is a lack of large trials evaluating potential risks such as prostate cancer and cardiovascular events.[8,41,42] Since it is controversial whether low testosterone levels are a normal or adaptive response to an abnormal environment or pathological condition, therapy may be inappropriate and may cause adverse effects.[41] During therapy, monitoring should include assessment of symptom improvement, glycaemic control, lipid levels, the haematocrit, and for adverse effects such as CVD and prostate disease.[12]

(3) Erectile dysfunction in diabetes[44]

a) Introduction

Many middle-aged and older adults with diabetes are sexually active; one survey showed that more than 60% of partnered diabetic patients engage in sexual activity.[45] Erectile dysfunction (ED) is a common problem in diabetic men. ED occurs at a younger age and with greater frequency in men with diabetes compared with non-diabetic men.

ED is often missed by treating physicians.[46] In one large study in men with type 2 diabetes, 63% reported that their physicians had never investigated their sexual problems. Moreover, in one large epidemiological study where the prevalence of ED in diabetic patients was 71%, the majority of patients was never even asked about ED and thus remained untreated.[47]

b) Aetiology of ED in diabetic men

ED is often related to organic causes such as vasculogenic and neurological abnormalities. [47] Organic ED results mainly from vascular problems due to atherosclerosis.[48] Vasculogenic ED is now thought to be caused by endothelial dysfunction, which is the first step of atherosclerosis.[49,50] (see below under ED and cardiovascular disease). The medical therapy these patients are placed on can also contribute to the ED.[51]

In a large study in medical outpatients (including non-diabetic patients) in which the prevalence of ED was 34%, an underlying cause was found in 93% of the patients after extensive evaluation.[52] Twenty nine percent of patients had hormonal disorders including hypogonadism in 19%. In a large cohort of patients presenting with ED, abnormal results were found including of testosterone in 5.7%, prolactin in 0.5%, thyroid function tests in 0.13%, liver function tests in 12.8%, glucose in 9.3%, cholesterol in 15%, and ferritin in 1.6%.[53] The frequency of sexual dysfunction in a cohort of men with benign prostatic hyperplasia increased with testosterone concentrations below 225 ng/dl (7.8 nmol/l).[54]

It is controversial whether routine determination of a serum testosterone may be indicated, since testosterone modulates erectile function, hypogonadism is prevalent among men with ED and elderly men, and symptoms alone seldom detect hypogonadism.[55,56] The American College of Physicians though does not recommend for or against routine use of hormonal blood tests or hormonal therapy in patients with ED, due to insufficient evidence to determine net benefits and harm.[57]

Another study in 1,022 men with erectile dysfunction also concluded that the low prevalence of low testosterone and high prolactin in ED cannot justify their routine determination.[56] However, determining testosterone only in cases of low sexual desire or abnormal physical examination would have missed 40% of the cases in their study with low testosterone, including 37% of those subsequently improved by androgen therapy.

c) Prevalence and associations of ED

Analysis of data from the National Health and Social Life Survey, a probability sample study of sexual behavior in a demographically representative cohort of US adults, which included 1410 men aged 18 to 59 years, demonstrated a prevalence of 31% of sexual dysfunction in the men.[58] Experience of sexual dysfunction was more likely among people with poor physical and emotional health. When data from the National Health and Nutrition Examination Survey was analyzed, it was found that ED affected almost 1 in 5 respondents.[59] In men above the age of 75 years, 77.5% were affected. Several modifiable risk factors were independently associated with ED, including diabetes mellitus, obesity, current smoking, and hypertension.

In a Canadian study in men between the ages of 40 and 88 years, the prevalence of ED was 49.4%.[60] The presence of diabetes or cardiovascular disease increased the probability of ED. Among patients without these two conditions, ED was independently associated with the 10-year Framingham coronary risk, fasting blood glucose, undiagnosed hyperglycaemia, impaired fasting glucose and the metabolic syndrome.

In diabetic patients, a study as early as 1980 already found that 35% of their study population was affected.[44] The prevalence of ED increased progressively with age; the other most important associations were treatment with insulin or oral hypoglycaemic agents, retinopathy, symptomatic autonomic neuropathy, and symptomatic peripheral neuropathy. The duration of diabetes, the presence of ischaemic heart disease, nephropathy and poor glycaemic control may also be associated with ED.

These findings were confirmed in a more recent, large study in Israel.[51] In their diabetic male patients, severe ED was found in 30.1%. ED severity increased with age, diabetes duration, poor glycaemic control, presence of microvascular complications, diuretic therapy, and cardiovascular disease. Consumption of small amounts of alcohol and physical activity might be protective. One other study demonstrated a prevalence of ED in their diabetic patients of as high as 71%.[47]

d) Prevalence of ED in South Africa

In South Africa, minimal data exists. De Klerk et al found a prevalence of 77.1% of ED among users of primary care in a black and mixed race urban population in the Western Cape.[61] Diabetes mellitus was one of the significant associated diseases, with a crude odds ratio (OR) of 3.35 (p=0.001). Webb et al found some degree of ED in 88% of diabetic patients screened for complications at primary health care clinics in Tshwane. [62] Thirty six percent of the patients had severe ED.

e) ED and cardiovascular disease

Erectile dysfunction and atherosclerosis share similar risk factors such as smoking, diabetes, dyslipidaemia, hypertension and obesity, and there seems to be a strong link between ED and atherosclerotic vascular disease.[63,64] In the Rancho-Bernardo Study cardiovascular risk factors including mean age, body mass index, cholesterol and triglycerides were significantly associated with an increased risk of ED 25 years later.[65] Men with ED are also more likely to be hypertensive and to have undiagnosed hyperglycaemia.[64]

ED is related to cardiovascular disease through an impairment of endothelial function.[50,66] Endothelial dysfunction seems to be an important aetiologic factor in the development of atherosclerosis and systemic vascular diseases such as diabetes, hypertension, dyslipidaemia, ischaemic heart disease, stroke or claudication.[50]

Therefore, symptoms of ED probably precede cardiovascular events.[66] It can be an early marker for atherosclerosis, cardiovascular risk, and subclinical vascular pathology.[49,50,63,67] ED can predict the presence and extend of subclinical atherosclerosis independent of traditional risk factors.[66] In one study onset of sexual dysfunction occurred before coronary artery disease onset with a mean time interval of 24 months.[68]

In a study in India the overall ED prevalence in men with coronary artery disease was 47%.[68] ED prevalence in acute coronary syndrome was found to differ according to the extent of coronary artery disease, with a higher prevalence in patients with worse

atherosclerosis. In men referred for nuclear stress testing, ED was present in 54.8%.[63] Patients with ED had more severe coronary heart disease and left ventricular dysfunction than those without. Multivariate analysis demonstrated that ED was an independent predictor of severe coronary artery disease.[63] Coronary atherosclerosis is more severe in patients with vascular ED.[67] Another study identified left ventricular dysfunction as an independent risk factor for ED, linked through the common mechanism of endothelial dysfunction.[66]

In diabetic patients specifically, several prospective studies have also demonstrated that ED can predict coronary heart disease, cardiovascular events and death.[51,61] Among patients with coronary artery disease and ED, statin therapy and possibly 5-phosphodiesterase inhibitors were found to be protective.[69]

Patients should probably be systematically screened for ED as part of periodic examination programs, which can lead to early detection of modifiable vascular risk factors or already existing vascular disease.[68] Symptoms of ED should be independently sought to identify patients at high risk for cardiovascular events.[70] Since ED is suggested to be an early symptom of generalized cardiovascular disease; cardiovascular evaluation is recommended in all these patients to optimize preventional therapy. [67,70,71] ED should be included as an outcome in clinical trials of lifestyle modifications and of lipid-lowering drugs.[65]

f) Quality of life in ED

Diabetic men who develop ED experience a significant decline in quality of life, and an increase in depressive symptoms.[46,72] Depression is also an important factor in the development of ED in diabetic patients. In one study with a prevalence of mild or moderate ED of 24%, and that of severe ED of 34%, only severe ED was associated with diabetic severity.[73] Mild or moderate ED was only associated with the severity of depressive symptoms. This implies that there is an important interplay of clinical and psychological factors in the development of ED in diabetic men.

ED in diabetic patients has a poor prognosis; in one prospective study it was found that

only 9% of these men regained erectile function over a 5 year period.[74] The development of ED was also significantly associated with poor glycaemic control and the appearance of neuropathic symptoms in the intervening 5 years.

g) Therapy of ED in DM

Several therapies for ED are available, none of which is specific for diabetic patients. Phosphodiesterase inhibitors are the first-line therapy for ED. Several trials evaluated the efficacy and safety in diabetic men.[75,76] Both sildenafil and tadalafil appeared to be effective and well-tolerated in diabetic men with ED. A potential protective role against cardiovascular events was seen for 5-phosphodiesterase inhibitors in one study.[69]

Testosterone therapy may be useful for improving vasculogenic ED in men with low or low-normal testosterone levels, especially with serum testosterone levels below 12nmol/L.[77] It can moderately improves the number of nocturnal erections, sexual thoughts, number of successful intercourses and erectile function, while it has no effect on erectile function in eugonadal men. This effect tends to decline over time, and the risks and benefits as well as long-term safety data are not available.[55,77]

Prevention of ED is also important. In the Diabetes Control and Complications Trial, a period of intensive glycaemic therapy significantly reduced the prevalence of ED 10 years later in men with type 1 DM who had some target organ damage at baseline.[78] The risk of ED was directly associated with mean HbA1C during the trial duration.

1.3. RATIONALE FOR THIS STUDY GIVEN THE LITERATURE REVIEW

It appears from the literature that hypogonadism is a common finding in men with diabetes, but it is often missed. Erectile dysfunction doesn't appear to be a good predictor of hypogonadism in those with diabetes. Low testosterone levels appear to predict many adverse events, which might be potentially reversible with testosterone therapy. There are very little documented data in South Africa on the prevalence of erectile dysfunction or of hypogonadism. Due to limited resources it isn't cost-effective to screen all diabetic men for hypogonadism. This study attempted to measure the scope of the problem, and then to identify predictors of hypogonadism, so that screening can be limited to a group with a high pre-test probability. The serum calculated bioavailable testosterone is the more accurate test to determine hypogonadism but is expensive, so the serum total testosterone which is cheaper was evaluated for sensitivity and specificity in this population group.

CHAPTER 2: METHODS

2.1. SETTING

This study was conducted in a tertiary care diabetes clinic at Steve Biko Academic Hospital. The majority of patients came from urban referral areas. Diabetic patients referred to Steve Biko Academic Hospital are usually poorly controlled, complicated to manage or have severe target organ damage. Due to the much higher prevalence of type 2 than type 1 diabetes in the population, the majority of the patients had type 2 diabetes. Most patients were on insulin therapy to control their disease.

2.2. STUDY DESIGN

An observational, cross-sectional study was performed in 150 consecutive male diabetic patients older than 50 years, who met the inclusion criteria. Subjects with low testosterone levels were compared to those with normal testosterone levels. The study started in January 2012, and continued until 150 patients were selected.

2.3. BIAS AND CONFOUNDING

By selecting 150 consecutive patients selection bias was minimised. Selection bias might have occurred if a large percentage of patients refused to participate with potentially only well controlled patients or those without major complications volunteering and thus systematically differing from the general diabetic population at the clinic. However, none of the patients approached to take part refused consent.

One potential problem causing selection bias that could not be eliminated was that blood samples had to be taken before 10h00; patients from rural areas often arrived much later which eliminated them from the study.

2.4. AIM AND OBJECTIVES

Aim: to identify predictors of low testosterone levels in diabetic men by using different demographic, clinical, and other variables.

Objectives:

1) to determine the prevalence of low testosterone levels and of erectile dysfunction in a diabetic population
2) to determine which demographic, clinical or biochemical variables can help identify diabetic men at risk of having low testosterone levels
3) to evaluate the test characteristics of the ADAM questionnaire in identifying patients with a low serum testosterone level
4) to evaluate the sensitivity and specificity of using only the total testosterone value instead of a modified calculated bioavailable testosterone for screening in this population group
5) to measure how the presence of low serum testosterone or ED effects the health related quality of life

2.5. PATIENT/RESEARCH OBJECT SELECTION AND CLASSIFICATION

Inclusion criteria:
- All male diabetic patients older than 50 years were invited to participate in the study.
- The first 150 patients who gave consent and who went for blood tests were included.

Exclusion criteria:
- Patients not willing or able to give consent.
- Men on hormonal therapy.
- Men who had a surgical or chemical castration (for prostate cancer).

Patients were classified based on whether their serum testosterone levels were normal or abnormal.

Even though there is data that a serum testosterone level below 12 nmol/L is probably abnormal, for the diagnosis of hypogonadism or testosterone deficiency syndrome the guidelines still advice that the level should be unequivocally low and not just borderline low, accompanied by symptoms suggestive of a low serum testosterone level .[2]

Patients with low serum testosterone values:

50 - 60 years:

1) Total testosterone: < 9.9 nmol/L, and/or:
2) Calculated bioavailable testosterone: < 1.7 nmol/L

≥ 60 years:

1) Total testosterone: < 9.9 nmol/L, and/or:
2) Calculated bioavailable testosterone: < 1.57 nmol/L

Patients with normal testosterone values:

50 - 60 years:

1) Total testosterone: 9.9 – 27.8 nmol/L
2) Calculated bioavailable testosterone: 1.7 – 6.31 nmol/L

≥ 60 years:

1) Total testosterone: 9.9 – 27.8 nmol/L
2) Calculated bioavailable testosterone: 1.57 – 6.38 nmol/L

Patients were classified as having ischaemic heart disease if they had:

- A previous myocardial infarction
- ECG changes indicative of a previous myocardial infarction
- A previous coronary artery bypass graft
- A previous angioplasty or stent insertion
- A previous abnormal angiogram
- An abnormal stress ECG or Nuclear medicine stress test
- Angina with objective evidence of ischaemia such as an abnormal ECG

2.6. ETHICAL CONSIDERATIONS

All patients were asked to provide informed consent as a pre-requisite for inclusion. Data was captured under a randomly selected number, and patient confidentiality was strictly observed. The protocol was approved by the University of Pretoria Ethics Committee.

2.7. MEASUREMENT OF COVARIATES

The following information was obtained from the patients themselves, their hospital and clinic files, the hospital laboratory system, and from questionnaires that the patients completed.

a) Demographic variables.
b) Clinical variables
c) Androgen Deficiency in the Aging Male (ADAM) questionnaire[79,80]
d) Sexual health inventory for men (SHIM) questionnaire[81]
e) Health related quality of life questionnaire (EuroQoL group / EQ-5D questionnaire)[82]
f) Peripheral neuropathy questionnaire (modified neuropathy symptom score / NSS) [83]
g) Rose questionnaire[84]
h) Biochemical variables: Total testosterone, SHBG, calculated bioavailable testosterone, serum creatinine, urine albumin/creatinine ratio, serum LDL values, HbA1c

a) Demographic variables

The age, race, marital status / relationship status, smoking history, alcohol consumption and employment status of all patients were recorded.

Table 1. Demographic variables

Age
Race
Marital status
Smoking history
Alcohol consumption
Employment status

b)Clinical variables

The type of Diabetes mellitus and the time since diagnosis as well as the presence of hypertension and the time since diagnosis were recorded. A previous history of stroke, myocardial infarction, amputation, foot ulceration, cataracts, revascularization, nephropathy or laser therapy of the eyes were obtained. Patients were also asked about the presence of intermittent claudication. The blood pressure was taken, the BMI was calculated and the waist measured. All the current medications patients were using were recorded.

Table 2. Clinical variables

History	**Examination**
Type of Diabetes mellitus Time since diagnosis	Weight
Hypertension Time since diagnosis	Height
Previous stroke	Body mass index
Previous myocardial infarction	Waist circumference
Previous amputation	Foot examination
Previous foot ulceration	Eye examination
Previous cataracts	
Previous revascularization	
Previous nephropathy	
Previous laser therapy	
Current medication	

c) Androgen Deficiency in the Aging Male (ADAM) questionnaire about symptoms of low testosterone [79,80]

The ADAM questionnaire consists of 10 questions that evaluate the kind and severity of low testosterone symptoms. A diagnosis of ADAM was suspected if there are “yes” answers to questions 1 and 7, or to any other 3 questions. Patients who couldn’t read or write in English or were illiterate were assisted in his/her own language by a as far as possible a male medical doctor or professional nurse who completed the questionnaires

on their behalf. Patients were categorised as being ADAM positive or ADAM negative. This questionnaire has not been validated in a South African population.

d) Sexual health inventory for men (SHIM) questionnaire [81]

The SHIM questionnaire is a basic 5 point questionnaire on erectile dysfunction. Each answer is graded from 0 (no sexual activity or attempts at intercourse) to 5 (very good sexual function). The maximum score patients could obtain was 25, the minimum was 1. Based on the SHIM questionnaire patients are divided into groups: 1-7 = Severe ED; 8-11 = Moderate ED; 12-16 = Mild to Moderate ED; 17-21 = Mild ED; $\geq$22 = No ED. This questionnaire was completed by all patients. If a patient couldn't read or write in English or were illiterate, the patient was assisted in his/her own language by as far as possible a male medical doctor or professional nurse who completed the questionnaire on their behalf. This questionnaire was not validated in a South African population.

e) Health related quality of life questionnaire (EQ-5D)[82]

A basic health related quality of life questionnaire the EuroQol Group EQ-5D health questionnaire was completed by all patients. Although this questionnaire was not validated in a South African population it was chosen for its brevity and simplicity. If patients couldn't read or write in English or were illiterate, they were assisted in their own language by a medical doctor or professional nurse who completed the questionnaire on their behalf.

f) Peripheral neuropathy (modified neuropathy symptom score / NSS)[83]

This questionnaire consists of 5 questions regarding symptoms of peripheral neuropathy. Patients could answer 'no', 'yes', and grade it by answering 'worse at night'. This questionnaire was also chosen for its simplicity and brevity, although it wasn't validated in a South African population.

g)The Rose questionnaire[84]

This questionnaire can be used to diagnose intermittent claudication. It consists of 9 questions and based on the patients' choice (out of 2 possible answers) intermittent claudication was diagnosed or ruled out. This questionnaire was also not validated in a South African population.

h) Biochemical variables

1) Blood collected in the fasting state following venepuncture.

Routine tests

- **serum creatinine,**
- **lipogram (including Low density lipoprotein LDL),**
- **HbA1C.**

2) Urine collected as a random spot urine specimen

- **albumin creatinine ratio after exclusion of possible UTI (nitrates and / or leucocytes on urine dipstick; if positive it was sent for culture, and the urine was repeated if clear)**

Non routine tests

- Blood for serum total testosterone and sex hormone binding globulin (SHBG) was collected in clotting tubes between 7h00 and 10h00 in the diabetic clinic, and was immediately refrigerated. It was transported to dr WJH Vermaak Inc. laboratory for analysis on the same day. The laboratory also provided a modified calculated bioavailable testosterone by using the SHBG value with testosterone in a computerised formula.

Testosterone test principle (from cobas® Testosterone II package insert)

- 1^{st} incubation: 20µL of sample are incubated with a biotinylated monoclonal testosterone-specific antibody
- The binding sites of the labeled antibody becomes occupied by the sample analyte, depending on its concentration
- 2^{nd} incubation: after addition of streptavidin-coated microparticles and a testosterone derivate labeled with a ruthenium complex, the complex becomes bound to the solid phase via interaction of biotin and streptavidin
- The reaction mixture is aspirated into the measuring cell where the microparticles are magneticallly captured onto the surface of the electrode.
- Unbound substances are then removed with ProCell/Procell.

- Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier
  Results are determined via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve provided via the reagent barcode

The background on the validation of this method can be found:
"J Clin Endocrinol Metab 84:3666-3672, 1999 – A critical evaluation of simple methods for the estimation of free testosterone in serum" [85]

Concentration Testosterone = FT(free) + Alb-bound-T + [SHBG]-bound-T

Testosterone = [S] + [S_A] + [SP]

- ***Albumin***

$$\frac{[S_A]}{[S]} = \text{constant} = K_A \times C_{conc.}Alb = 3.6\times10^4 \times \frac{43 g/l}{69000} = \mathbf{22.4}$$

69000 =(molecular weight alb.)
$K_{A\,=} = 3.6\times10^4$
for an average albumin conc. of 4.3 g/dL

or **[S_A] = 22.43 [S]**

[S] + [S_A] = (1 + 22.43)[S] = **23.43 [S]**

- ***SHBG***

[P] = free SHBG

[SP] = steroid bound SHBG

$K = 10^9$ M

[S] +[P] $\leftrightarrow$ *[SP]* or $[S] = \frac{[SP]}{[P]\,[K]}$

[P] + [SP] = [SHBG] **or [P] = [SHBG] –[SP]**

- ***Bioavailable***

[Bio T] = [S] + [SA]

- ***Bioavailable***

  - Bio T = [S] + [SA] = [S] + 22.43 [S] (for the default albumin concentration of 4.3 g/dL)

Normal Reference ranges:

50 - 60 years:

1) Total testosterone: 9.9 – 27.8 nmol/L
2) Calculated bioavailable testosterone: 1.7 – 6.31 nmol/L

≥ 60 years:

1) Total testosterone: 9.9 – 27.8 nmol/L
2) Calculated bioavailable testosterone: 1.57 – 6.38 nmol/L

i) Modified calculated bioavailable testosterone

As demonstrated above, to correctly calculate the bioavailable testosterone the value of SHBG and of albumin should also be determined.[85,86] These values can then be added into a computerized formula to obtain the calculated bioavailable testosterone. In practice the albumin is often not determined; instead a default albumin concentration of 4.3 g/dL (= 43 g/L) is used. Dr WJH Vermaak Inc. does not use the true albumin value in the formula unless explicitly requested to do so. This can potentially underestimate the calculated bioavailable testosterone if the patients have low serum albumin levels.

In an outpatient setting, with relatively stable ambulatory patients, most patients can be assumed to have normal albumin levels. An unknown percentage of our patients might have had chronic diseases with associated hypoalbuminaemic states such as nephrotic syndrome, liver disease, or malnutrition. Due to financial constraints we could not determine the albumin values as well in all the patients, so we had to use the formula with albumin set at a default level of 43 g/L. We will therefore call it a modified calculated bioavailable testosterone level since the correct calculated bioavailable testosterone takes the albumin value also into account.

2.8. SAMPLE SIZE

Exact sample size calculations are not presented. A sample of 150 patients was deemed sufficient for statistical calculations.

2.9. DATA ANALYSIS

Patient data was captured on a data spreadsheet on Microsoft Excel. This data was transferred to STATA 12 (StataCorp. 2011. *Stata Statistical Software: Release 12*. College Station, TX: StataCorp LP) for analysis.

Exposures between cases and non-cases were compared using appropriate tests for continuous and categorical data (Unpaired Students-T-test, Mann Whitney or Kruskal Wallisand Pearson Chi square test or Fisher Exact test).

Logistic regression was utilized to determine predictors of outcome with tests of calibration and validation as required. To determine which variables to use in the multivariate model univariate logistic regression was done to evaluate the relationship between demographic variables (age, race, smoking history, alcohol consumption), clinical variables (such as type of DM, duration of disease, HT and duration of disease, history of stroke, myocardial infarction, amputation, revascularization, diabetic complications, medications etc), biochemical variables (HbA1C, LDL, creatinine, urine microalbumin), different health related questionnaires, and the outcome (low or normal testosterone levels).

Variables with a p-value < 0.25 were entered into a multivariate model with manual backward elimination based on the p-values in the model (using Likelihood ratio tests). Variables were dropped if the p-value was non-significant (>0.05). Sensitivity, specificity, positive and negative predictive values were calculated. To determine the calibration of the final model, ROC analysis was done with calculation of the C-statistic. For validation 10-fold cross validation of the area under the ROC curve was used.

The outcome variable “testosterone” (both total and calculated bioavailable) was modeled as a dichotomous variable (low or normal); this was done for simplicity due to different reference ranges for different age groups, and because there isn’t convincing evidence that a high normal or low normal testosterone level is necessarily pathologic.

CHAPTER 3: RESULTS OF THE STUDY

3.1. PATIENT DEMOGRAPHICS

Table 3 shows patients' clinical and biochemical demographic characteristics at baseline. The mean was reported for data that was normally distributed, with the standard deviation. For data with a skew distribution a median was reported with an interquartile range from the 25th to the 75th percentiles.

Table 3. Baseline characteristics of patients: clinical and biochemical findings

Variable	n (%)	Mean (SD)	Median (IQR)
Type of DM			
Type 1	13 (8.7)		
Type 2	137 (91.3)		
Race			
White	79 (52.7)		
Black	45 (30.0)		
Coloured	15 (10.0)		
Asian	11 (7.3)		
Other			
Age (years)		62 (7.9)	
Diabetes duration (years)		15 (8.7)	
Hypertension	142 (94.7)		
Hypertension duration (years)			12 (7 -22)
Systolic blood pressure (mmHg)		134 (15.5)	
Diastolic blood pressure (mmHg)		77(9.3)	
Body mass index		30.7 (5.37)	
Waist circumference (cm)		112 (16.4)	
Current smoker	24 (16.0)		
Past smoker	42 (28.0)		
Serum Creatinine (µmol/L)			96 (79-133)
HbA1C (%)			7.9 (6.8-9.3)
Total cholesterol (mmol/L)		4.09 (0.97)	
TG (mmol/L)			1.90 (1.20-2.50)
HDL (mmol/L)		0.99 (0.32)	
LDL (mmol/L)		2.33 (0.70)	
Serum Total Testosterone (nmol/L)			9.88 (7.04-14.13)
Serum Sex Hormone Binding Globulin (SHBG) (nmol/L)			35.85 (23.50-49.90)
Serum calculated Bioavailable Testosterone (nmol/L)			1.82 (1.35-2.30)

Ninety-one percent of the patients were type 2 diabetics, and the mean age was 62 years (SD=7.9). Just over half of the patients were white (53%) and 30% were black. The mean diabetes duration was 15 years (SD=8.7). Ninety-five percent of the patients were previously diagnosed with hypertension, but this was relatively well-controlled with a mean systolic blood pressure of 134mmHg (SD=15.5) and mean diastolic blood pressure of 77mmHg (SD=9.3). The patients were relatively obese with a mean BMI of 31 (SD=5.37) and a mean waist circumference of 112 cm (SD=16.4). Sixty-six percent of the patients were current (24%) or past (42%) smokers.

The median serum creatinine was 96 µmol/L (IQR=79-133). The patients' diabetes was better controlled than expected with a median HbA1c of 7.9 % (IQR=6.8-9.3). The mean LDL was above target at 2.33 mmol/L (SD=0.7) and the median TG was 1.90 mmol/L (IQR=1.20-2.50). The median serum total testosterone was 9.88 nmol/L (IQR=7.04-14.13) and the median serum calculated bioavailable testosterone was 1.82 nmol/L(IQR=1.35-2.30).

Table 4. Baseline characteristics of patients: therapy and complications

Variable	**n (%)**
Past or present cardiovascular disease	61 (40.7)
Known with proliferative diabetic retinopathy	38 (25.3)
Significant Peripheral neuropathy present	64 (43.2)
Intermittent claudication (Rose questionnaire)	10 (6.7)
Present or past cataracts	86 (58.5)
Microalbuminuria present	72 (48.0)
On insulin	127 (84.7)
On Metformin	96 (64.0)
On statin	140 (93.3)
On diuretics	123 (82.0)
On fibrates	22 (14.7)
On beta-blockers	68 (45.3)

Table 4 shows the baseline characteristics of the patients regarding their therapy and diabetic complications. Forty-one percent of patients were known to have cardiovascular disease, and less than 7% had intermittent claudication. Microvascular complications were common with symptoms of significant peripheral neuropathy present in 43% of the study

population, microalbuminuria in 48% of patients, and proliferative diabetic retinopathy in 25%. Present or past cataracts affected almost 59% of the patients.

Eighty-five percent of the patients had to be managed on insulin therapy. Metformin was prescribed to 64% of the patients. Statin usage was high at 93% and diuretics were prescribed to 82% of the study population. The percentage of patients on beta-blockers was 45.3%. Fibrate use was low at 14.7%.

3.2. PREVALENCE OF LOW TESTOSTERONE LEVELS

Table 5. The prevalence of low serum Testosterone levels

Variable	n	Percentage
Low Total testosterone	75	50.0
Low calculated Bioavailable Testosterone	61	40.7
Low Total Testosterone and low calculated Bioavailable Testosterone	41	27.3

Table 5 demonstrates the prevalence of low serum testosterone levels. Low serum total testosterone levels were found in 50% of the patients, a higher than expected prevalence. Evaluating just low modified calculated bioavailable testosterone the prevalence dropped to 41%. Twenty-seven percent of patients had both low serum total and modified calculated bioavailable testosterone levels.

3.3. PREVALENCE OF ERECTILE DYSFUNCTION (ED)

Table 6. The prevalence of Erectile Dysfunction (ED)

ED (grade)	n	Percentage
0 (no ED)	7	4.7
1	17	11.3
2	28	18.7
3	21	14.0
4 (severe ED)	77	51.3
Total	150	100.0

Table 6 shows the very high prevalence of erectile dysfunction. Less than 5% had no ED, and 51% of patients had severe ED. Forty-four percent had milder degrees of ED.

3.4. TESTOSTERONE LEVELS IN PATIENTS WITH ED

In Tables 7, 8 and 9 Fisher's exact test was used. This is a statistical significance test used in the analysis of contingency tables to determine if there are non-random associations between two categorical variables. Although in practice it is employed when sample sizes are small such as in some of the ED categories, it is valid for all sample sizes.

Table 7. Total testosterone in patients with ED

ED	Normal total testosterone	Low total testosterone	Total (%)
None (%)	3 (42.7)	4 (57.1)	7 (100)
Mild (%)	11 (64.7)	6 (35.3)	17 (100)
Mild to moderate (%)	10 (35.7)	18 (64.3)	28 (100)
Moderate (%)	12 (57.1)	9 (42.9)	21 (100)
Severe (%)	39 (50.7)	38 (49.4)	77 (100)
Total (%)	75 (50.0)	75 (50.0)	150 (100)

Fisher's exact p= 0.369

Table 7 shows the distribution of low and normal total testosterone in the different ED categories. The Fisher's exact result of 0.369 was non-significant (>0.05) which implied that there was no association between the two variables namely total testosterone and ED.

Table 8. Modified calculated bioavailable testosterone in patients with ED

ED	Normal calculated bioavailable testosterone	Low calculated bioavailable testosterone	Total (%)
None (%)	5 (71.4)	2 (28.6)	7 (100)
Mild (%)	11 (64.7)	6 (35.3)	17 (100)
Mild to moderate (%)	19 (67.9)	9 (32.1)	28 (100)
Moderate (%)	15 (71.4)	6 (28.6)	21 (100)
Severe (%)	39 (50.7)	38 (49.4)	77 (100)
Total (%)	89 (59.3)	61 (40.7)	150 (100)

Fisher's exact p= 0.291

Table 8 shows the distribution of low and normal calculated bioavailable testosterone in

the different ED categories. The Fisher's exact result of 0.291 was non-significant (>0.05) which meant that there was no association between the two variables namely modified calculated bioavailable testosterone and ED.

Table 9. Total and modified calculated bioavailable testosterone in patients with ED

ED	Normal total calculated bioavailable testosterone	Low total and calculated bioavailable testosterone	Total (%)
None (%)	6 (85.7)	1 (14.3)	7 (100)
Mild (%)	13 (76.5)	4 (23.5)	17 (100)
Mild to moderate (%)	19 (67.9)	9 (32.1)	28 (100)
Moderate (%)	18 (85.7)	3 (14.3)	21 (100)
Severe (%)	53 (68.8)	24 (31.2)	77 (100)
Total (%)	109 (72.7)	41 (27.3)	150 (100)

Fisher's exact = 0.547

Table 9 demonstrates the distribution of low and normal total and calculated bioavailable testosterone in the different ED categories. The Fisher's exact value of 0.547 was non-significant (>0.05) which meant that there was no association between the two variables namely total and modified calculated bioavailable testosterone, and ED.

3.5. UNIVARIATE ANALYSIS OF FACTORS ASSOCIATED WITH LOW TESTOSTERONE LEVELS

In Tables 10, 11 and 12 different tests for statistical significance were used. A t-test was used for continuous data where the distribution was normal and wasn't skewed. It can be used to determine if two sets of data are significantly different from each other, and is most commonly applied when the data follows a normal distribution.

Table 10. Univariate analysis of multiple variables with total testosterone

Variable	Normal total testosterone	Low total testosterone	p-value
Age [Mean (SD)]	61 (7.8)	63 (8.0)	0.32
Type 1 diabetes [n (%)]	9 (12)	4 (5.3)	0.25
Type 2 diabetes [n (%)]	66 (88)	71 (94.7)	
Race - White [n (%)]	38 (50.7)	41 (54.7)	0.30
- **Black [n (%)]**	27 (36.0)	18 (24.0)	
- **Coloured [n (%)]**	5 (6.7)	10 (13.3)	
- **Asian [n (%)]**	5 (6.7)	6 (8.0)	
Diabetes duration [Mean (SD)]	14.7 (8.3)	15.3 (9.1)	0.67
Hypertensive [n (%)]	68 (90.7)	74 (98.7)	0.063
Hypertension duration [Median (IQR)]	12 (5 to 21)	12 (8 to 22)	0.28
Systolic blood pressure [Mean (SD)]	134 (15.2)	134 (15.8)	0.84
Body mass index [Mean (SD)]	29.4 (4.9)	32(5.5)	0.004
Waist circumference [Mean (SD)]	108 (14.1)	117 (17.3)	<0.001
PN score [Mean (SD)]	2.5 (2.5)	3.1 (3.1)	0.192
PDR [n (%)]	22 (29.3)	16 (21.3)	0.26
Smoker			0.057
- **Never [n (%)]**	41 (54.7)	43 (57.3)	
- **Current [n (%)]**	17 (22.7)	7 (9.3)	
- **Past [n (%)]**	17 (22.7)	25 (33.3)	
Known cardiovascular disease [n (%)]	22 (29.3)	39 (52)	0.005
On insulin [n (%)]	63 (84)	64 (85.3)	0.82
Units of insulin [Mean (SD)]	50 (42.7)	69 (55.8)	0.022
Serum creatinine [Median (IQR)]	95 (76 to 111)	99 (81 to 137)	0.199
HBA1C [Median (IQR)]	8 (6.7 to 9.3)	7.5 (6.8 to 9.3)	0.75
LDL [Mean (SD)]	2.31 (0.71)	2.35 (0.69)	0.71
Urine microalbumin present [n (%)]	39 (52)	33 (44)	0.33
Erectile dysfunction			0.36
- **Grade 0 [n (%)]**	3 (4.0)	4 (5.3)	
- **Grade 1 [n (%)]**	11 (14.7)	6 (8.0)	
- **Grade 2 [n (%)]**	10 (13.3)	18 (24.0)	
- **Grade 3 [n (%)]**	12 (16.0)	9 (12.0)	
- **Grade 4 [n (%)]**	39 (52.0)	38 (50.7)	

As demonstrated in table 10 and table 11, univariate associations of a low total testosterone that were statistically significant ($p<0.05$) were BMI, waist circumference, known cardiovascular disease, and units of insulin used per day.

Table 11. Summary of univariate analysis showing statistically significant variables with total testosterone

Variable	Normal total testosterone	Low total testosterone	p-value
Body mass index [Mean (SD)]	29.4 (4.9)	32 (5.5)	0.004
Waist circumference[Mean (SD)]	108 (14.1)	117 (17.3)	<0.001
Known cardiovascular disease [n (%)]	22 (29.3)	39 (52)	0.005
Units of insulin per day [Mean (SD)]	50 (42.7)	69 (55.8)	0.022

Table 12. Univariate analysis of multiple variables with modified calculated bioavailable testosterone

Variable	Normal calculated bioavailable testosterone	Low calculated bioavailable testosterone	p-value
Age [Mean (SD)]	60 (7.4)	64 (8.0)	0.002
Type 1 diabetes [n (%)]	8 (9.0)	5 (8.2)	1.00
Type 2 diabetes [n (%)]	81 (91.0)	56 (91.8)	
Race - White [n (%)]	45 (50.6)	34 (55.7)	0.71
- **Black [n (%)]**	28 (31.5)	17 (27.9)	
- **Coloured [n (%)]**	8 (9.0)	7 (11.5)	
- **Asian [n (%)]**	8 (9.0)	3 (4.9)	
Diabetes duration [Mean (SD)]	13.3 (7.8)	17.4 (9.3)	0.004
Hypertensive [n (%)]	84 (94.4)	58 (95.1)	1.00
Systolic blood pressure [Mean (SD)]	134 (14.2)	133 (17.2)	0.77
Hypertension duration [Median (IQR)]	12 (6 to 20)	13 (8 to 22)	0.144
Body mass index [Mean (SD)]	29.7(4.6)	32.2 (6.0)	0.006
Waist circumference [Mean (SD)]	110 (15.6)	116 (16.8)	0.011
PN score [Mean (SD)]	2.7 (2.7)	3 (3.0)	0.47
PDR [n (%)]	21 (23.6)	17 (27.9)	0.55
Smoker			0.34
- **Never [n (%)]**	50 (56.2)	34 (55.8)	
- **Current [n (%)]**	17 (19.1)	7 (11.5)	
- **Past [n (%)]**	22 (24.7)	20 (32.8)	
Known cardiovascular disease [n (%)]	22 (29.3)	39 (52)	0.005
On insulin [n (%)]	73 (82.0)	54 (88.5)	0.28
Units of insulin [Mean (SD)]	51 (43.6)	71 (57.4)	0.018
Serum creatinine [Median (IQR)]	96 (81 to 119)	95 (78 to 146)	0.89
HBA1C [Median (IQR)]	7.9 (6.8 to 9.3)	7.8 (6.8 to 9.1)	0.80
LDL [Mean (SD)]	2.32 (0.74)	2.34 (0.65)	0.86
Urine microalbumin present [n (%)]	40 (44.9)	32 (52.5)	0.37
Erectile dysfunction			0.27
- **Grade 0 [n (%)]**	5 (5.6)	2 (3.3)	
- **Grade 1 [n (%)]**	11 (12.4)	6 (9.8)	
- **Grade 2 [n (%)]**	19 (21.4)	9 (14.8)	
- **Grade 3 [n (%)]**	15 (16.9)	6 (9.8)	
- **Grade 4 [n (%)]**	39 (43.8)	38 (62.3)	

As demonstrated in table 12 and in table 13, univariate associations of a low calculated bioavailable testosterone that were statistically significant ($p<0.05$) were age, diabetes duration, units of insulin used per day, BMI, waist circumference and known cardiovascular disease.

Table 13. Summary of univariate analysis showing statistically significant variables with modified calculated bioavailable testosterone

Variable	Normal calculated bioavailable testosterone	Low calculated bioavailable testosterone	p-value
Age [Mean (SD)]	60.3 (7.4)	64.3 (8)	0.002
Diabetes duration [Mean (SD)]	13.3 (7.8)	17.4 (9.3)	0.004
Units of insulin per day [Mean (SD)]	51 (43.6)	71 (57.4)	0.018
Body mass index [Mean (SD)]	29.7 (4.7)	32.2 (6.0)	0.006
Waist circumference [Mean (SD)]	110 (15.6)	116 (16.8)	0.011
Known cardiovascular disease [n (%)]	22 (29.3)	39 (52)	0.005

Table 14. Univariate analysis of multiple variables with both low total testosterone and low modified calculated bioavailable testosterone

Variable	Normal calculated bioavailable and/or total testosterone	Low calculated bioavailable and low total testosterone	p-value
Age [Mean (SD)]	61 (7.5)	64 (8.3)	0.017
Type 1 diabetes [n (%)]	11 (10.1)	2 (4.9)	0.52
Type 2 diabetes [n(%)]	98 (89.9)	39 (95.1)	
Race - White [n (%)]	57 (52.3)	22 (53.7)	0.93
- **Black [n (%)]**	34 (31.2)	11 (26.8)	
- **Coloured [n (%)]**	10 (9.2)	5 (12.2)	
- **Asian [n (%)]**	8 (7.3)	3 (7.3)	
Diabetes duration [Mean (SD)]	13.8 (8.0)	18.1 (9.5)	0.005
Hypertensive [n (%)]	102 (93.6)	40 (97.6)	0.45
Systolic blood pressure [Mean (SD)]	134 (15.3)	133 (16.1)	0.56
Hypertension duration [Median (IQR)]	12 (6 to 21)	15 (10 to 22)	0.043
Body mass index [Mean (SD)]	29.6 (5.0)	33.8(5.2)	<0.001
Waist circumference [Mean (SD)]	109 (16.1)	120 (14.6)	<0.001
PN score [Mean (SD)]	2.6 (2.7)	3.3 (3.1)	0.21
PDR [n (%)]	28 (25.69)	10 (24.39)	0.87
Smoker			0.132
- **Never [n (%)]**	63 (57.8)	21 (51.2)	
- **Current [n (%)]**	20 (18.4)	4 (9.8)	
- **Past [n (%)]**	26 (23.9)	16 (39.0)	
Known cardiovascular disease [n (%)]	22 (29.3)	39 (52)	0.005
On insulin [n (%)]	89 (81.7)	38 (92.7)	0.095
Units of insulin [Mean (SD)]	53 (46.1)	77 (57.6)	0.009
Serum creatinine [Median (IQR)]	95 (78 to 119)	103 (82 to 146)	0.23
HBA1C [Median (IQR)]	7.9 (6.8 to 9.3)	7.5 (6.8 to 8.9)	0.69
LDL [Mean (SD)]	2.35 (0.72)	2.27 (0.67)	0.57
Urine microalbumin present [n (%)]	52 (47.7)	20 (48.8)	0.91
Erectile dysfunction			0.49
- **Grade 0 [n (%)]**	6 (5.5)	1 (2.4)	
- **Grade 1 [n (%)]**	13 (11.9)	4 (9.8)	
- **Grade 2 [n (%)]**	19 (17.4)	9 (22)	
- **Grade 3 [n (%)]**	18 (16.5)	3 (7.3)	
- **Grade 4 [n (%)]**	53 (48.6)	24 (58.5)	

In table 14 and table 15 the univariate associations of both a low total and modified calculated bioavailable testosterone that were statistically significant ($p<0.05$) are shown. These were age, diabetes duration, hypertension duration, BMI, waist circumference, known cardiovascular disease, and units of insulin used per day.

Table 15. Summary of univariate analysis showing statistically significant variables with both low total and modified calculated bioavailable testosterone

Variable	Normal total and calculated bioavailable testosterone	Low total and calculated bioavailable testosterone	p-value
Age [Mean (SD)]	61 (7.5)	64 (8.3)	0.017
Diabetes duration [Mean (SD)]	13.3 (7.8)	17.4 (9.3)	0.004
Hypertension duration [Mean (SD)]	12 (6 to 21)	15 (10 to 22)	0.043
Body mass index [Mean (SD)]	29.6 (5.0)	33.8 (5.2)	<0.001
Waist circumference [Mean (SD)]	109 (16.1)	120 (14.6)	<0.001
Known cardiovascular disease [n (%)]	22 (29.3)	39 (52)	0.005
Units of insulin per day [Mean (SD)]	53 (46)	77 (57.6)	0.009

Table 16. Summary of univariate analysis of multiple statistically significant variables with ED (no - mild-moderate ED versus moderate - severe ED)

Variable	No-moderate ED	Moderate-severe ED	p-value
Age [Mean (SD)]	59 (6.6)	64 (8.0)	<0.001
Race - White [n (%)]	21 (40.4) (50.7)	58 (59.2)	0.033
- **Black [n (%)]**	16 (30.8)	29 (29.6)	
- **Coloured [n (%)]**	8 (15.4)	7 (7.1)	
- **Asian [n (%)]**	7 (13.5)	4 (4.1)	
Smoker			0.026
- **Never [n (%)]**	30 (57.7)	54 (55.1)	
- **Current [n (%)]**	13 (25)	11 (11.2)	
- **Past [n (%)]**	9 (17.3)	33(33.7)	
Systolic blood pressure [Mean (SD)]	130 (14.7)	136 (15.6)	0.034
Body mass index [Mean (SD)]	29 (5.0)	31.6 (5.4)	0.006
Waist circumference [Mean (SD)]	108 (15.7)	115 (16.4)	0.021
Serum creatinine [Median (IQR)]	85 (76 to 106)	99 (83 to 146)	0.008
PN score [Mean (SD)]	1.9 (2.2)	3.3 (3.0)	0.005
On a diuretic [n (%)]	33 (63.5)	90 (91.8)	<0.001

Table 16 summarises the statistically significant univariate associations of ED. These variables were age, race, smoking status, systolic blood pressure, BMI, waist circumference, serum creatinine, peripheral neuropathy score and diuretic usage. To simplify statistical analysis, and because of the low numbers in some of the ED categories, ED were regrouped into 2 groups: no to mild-moderate ED and moderate-severe ED.

Table 17. Summary of univariate associations

	Low total testosterone	Low calculated bioavailable testosterone	Low total and calculated bioavailable testosterone	Erectile dysfunction
Body mass index	**X**	**X**	**X**	**X**
Waist circumference	**X**	**X**	**X**	**X**
Ischaemic heart disease	**X**	**X**	**X**	
Units of insulin	**X**	**X**	**X**	
Age		**X**	**X**	**X**
Diabetes duration		**X**	**X**	
Hypertension duration			**X**	
Race				**X**
Smoking history				**X**
Diuretic therapy				**X**
Systolic blood pressure				**X**
Peripheral neuropathy				**X**
Creatinine				**X**

"**X**" denotes the statistically significant associations

Table 17 summarises the univariate associations of the different groups: low total testosterone, low modified calculated bioavailable testosterone, low both total and modified calculated bioavailable testosterone, as well as that of erectile dysfunction. Common factors in all four groups were the BMI and waist circumference. Common factors in three of the four groups were age, ischaemic heart disease, and units of insulin used.

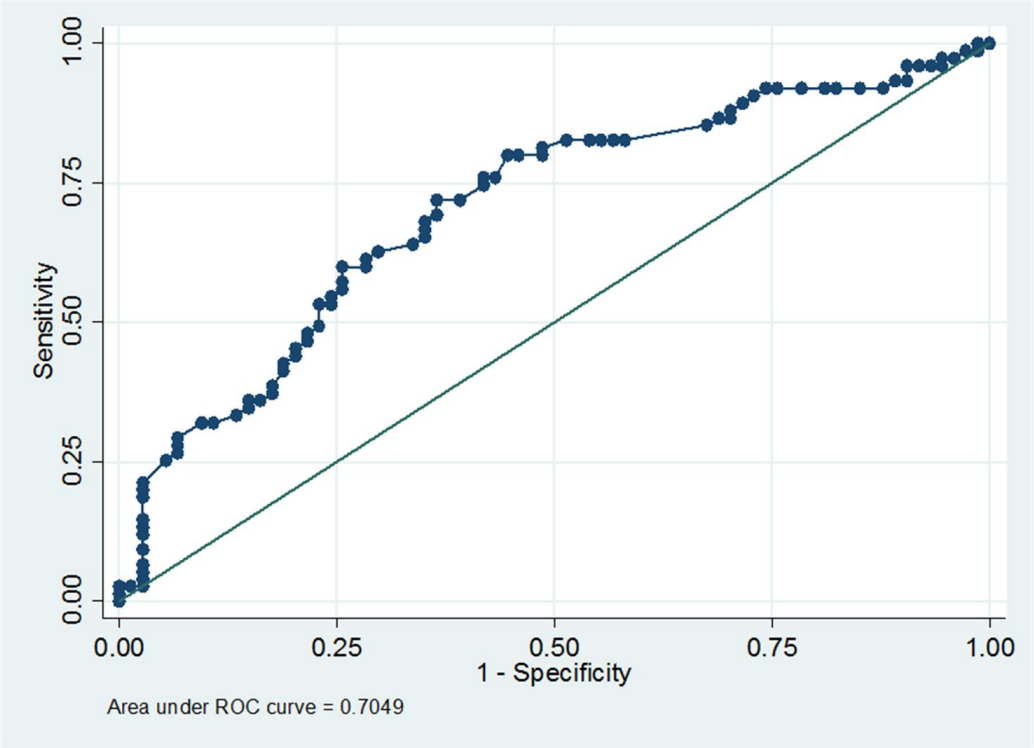
3.6. MULTIVARIATE ANALYSIS OF FACTORS ASSOCIATED WITH LOW TESTOSTERONE LEVELS

Table 18. Multivariate associations: Low total testosterone

	OR	SE	95% CI (lower limit)	95% CI (upper limit)	p-value
Waist circumference	1.04	0.01	1.01	1.06	0.003
Ischaemic heart disease	2.18	0.78	1.09	4.39	0.029

Statistically significant multivariate associations of a low total testosterone were waist circumference and known ischaemic heart disease.

Figure 1. ROC curve: model for low total testosterone



Using this model to predict low total testosterone, the area under the ROC curve was 0.70, which just reached an acceptable discrimination level.

- Sensitivity = 67%
- Specificity = 65%
- Positive predictive value = 66%
- Negative predictive value = 66%
- Correctly classified = 66%

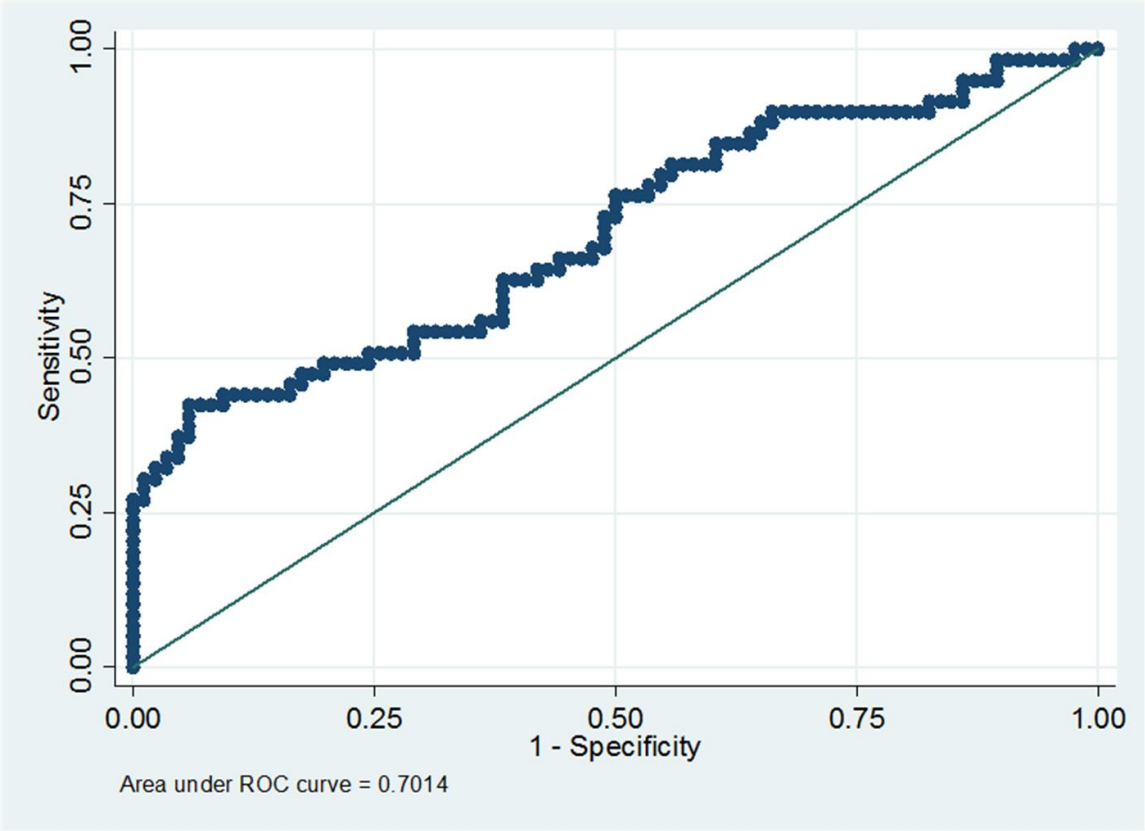
This model did not perform very well looking at the statistical measures such as sensitivity, specificity, PPV and NPV with percentages all in the sixties. Only 66% were correctly classified.

Table 19. Multivariate associations: Low calculated bioavailable testosterone

	OR	**SE**	**95% CI (lower limit)**	**95% CI (upper limit)**	**p-value**
Age	1.07	0.03	1.02	1.12	0.010
Diabetes duration	1.05	0.02	1.00	1.10	0.049
Body mass index	1.11	0.04	1.03	1.19	0.005

Statistically significant multivariate associations of a low modified calculated bioavailable testosterone were age, diabetes duration, and BMI.

Figure 2. ROC curve: model for low modified calculated bioavailable testosterone



The area under the ROC curve was 0.70, which reached an acceptable but not excellent discrimination level.

- Sensitivity = 47%
- Specificity = 83%
- Positive predictive value = 65%
- Negative predictive value = 70%
- Correctly classified = 68%

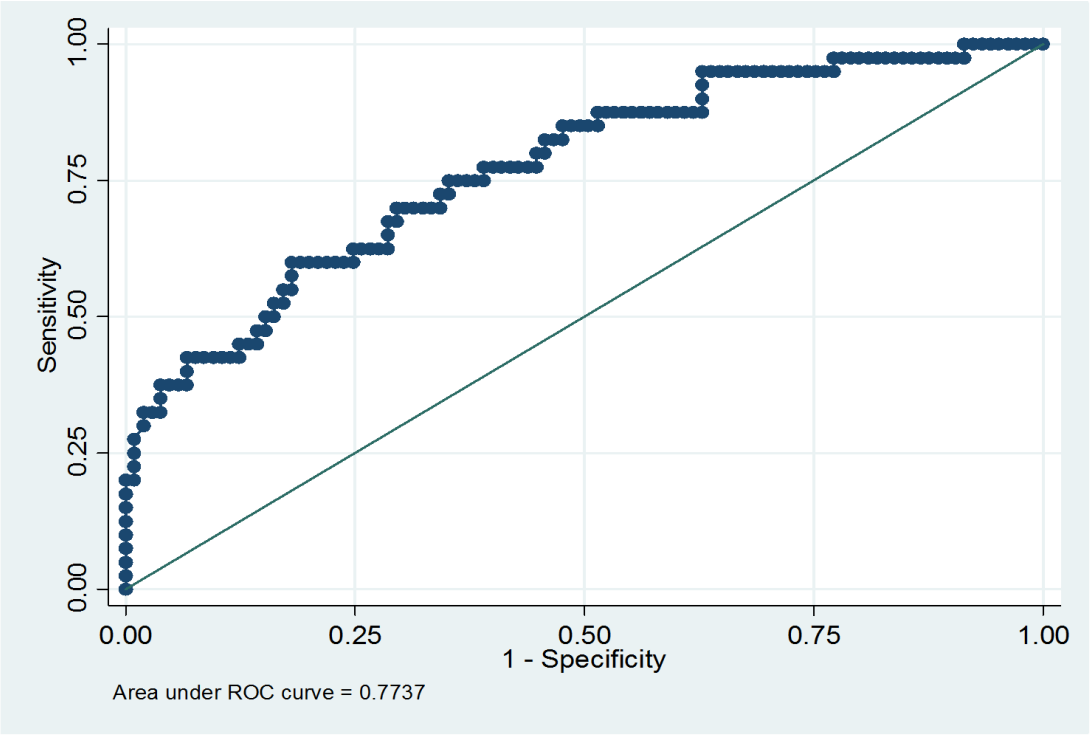
This model did not perform very well looking at the statistical measures. Although the specificity was very good at 83% the sensitivity was only 47% implying more than half of cases would be missed using this model. Only 68% were correctly classified.

Table 20. Multivariate associations: Low total and modified calculated bioavailable testosterone

	OR	SE	95% CI (lower limit)	95% CI (upper limit)	p-value
Diabetes duration	1.07	0.03	1.02	1.13	0.007
Body mass index	1.19	0.05	1.09	1.30	<0.001
Ischaemic heart disease	2.36	0.99	1.04	5.38	0.041

Statistically significant multivariate associations of a low total and modified calculated bioavailable testosterone were diabetes duration, BMI, and a history of ischaemic heart disease.

Figure 3. ROC curve: model for low total and modified calculated bioavailable testosterone



The area under the ROC curve was 0.77 for this model to predict low testosterone, which showed fair discrimination.

- Sensitivity = 40%
- Specificity = 93%
- Positive predictive value = 70%
- Negative predictive value = 80%
- Correctly classified = 79%

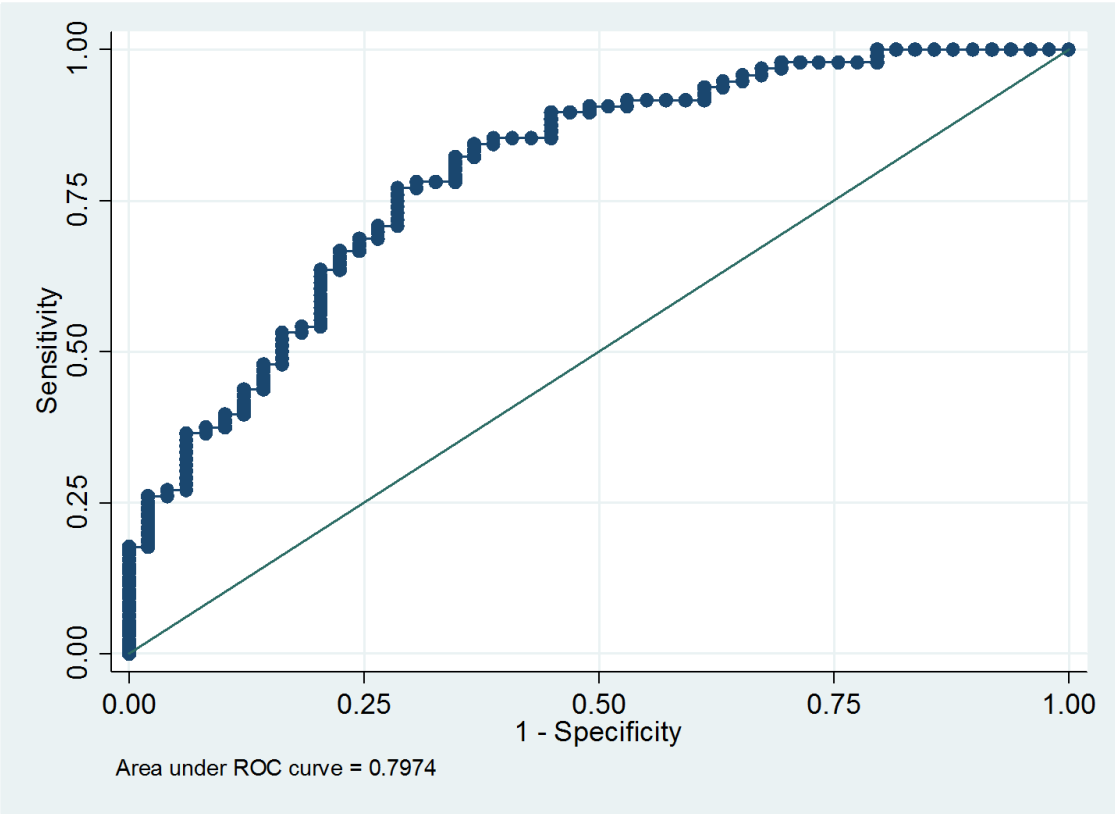
This model did not perform very well looking at the statistical measures. Although the specificity was very good at 93% the sensitivity was only 40% implying more than half of cases would be missed using this model. Seventy-nine percent of cases were correctly classified.

Table 21. Multivariate associations: No to Mild-Moderate ED versus Moderate-Severe ED

	OR	**SE**	**95% CI (lower limit)**	**95% CI (upper limit)**	**p-value**
Age	1.11	0.03	1.05	1.17	<0.001
Body mass index	1.09	0.05	1.00	1.18	0.050
Peripheral neuropathy score	1.22	0.10	1.04	1.45	0.018
On diuretics	5.26	2.75	1.89	14.68	0.002

Significant multivariate associations of ED (no to mild-moderate versus moderate to severe) were age, BMI, peripheral neuropathy score and diuretic therapy.

Figure 4. ROC curve: model for No to Mild-Moderate ED versus Moderate to Severe ED



The area under the ROC curve was 0.797 for this model to predict ED, which implied good discrimination (almost excellent).

- Sensitivity = 90%
- Specificity = 55%
- Positive predictive value = 80%
- Negative predictive value = 73%
- Correctly classified 78%

This model performed well on most of the statistical measures. Although the specificity was poor at 55%, the sensitivity was 90% which meant this model can be used for screening for this condition. Seventy-eight percent of cases were correctly classified.

Table 22. Summary of Multivariate associations

	Low total testosterone	Low bioavailable testosterone	Low total and bioavailable testosterone	Erectile dysfunction
Body mass index		X	X	X
Age		X		X
Diabetes duration		X	X	
Ischaemic heart disease	X		X	
Waist circumference	X			
On diuretics				X
Peripheral neuropathy				X

"X" denotes the statistically significant associations

Table 23. Summary of the 10-fold cross validation of the area under the ROC curve

Model	10-fold cross validation	ROC area	Standard error	95% confidence interval	
				Lower limit	Upper limit
Low Total testosterone	Before	0.705	0.043	0.621	0.789
	After	0.678	0.044	0.591	0.764
Low Calculated bioavailable testosterone	Before	0.701	0.045	0.612	0.790
	After	0.678	0.047	0.585	0.771
Low Total & Calculated bioavailable testosterone	Before	0.774	0.044	0.687	0.860
	After	0.746	0.047	0.654	0.837
Erectile dysfunction	Before	0.770	0.041	0.690	0.850
	After	0.720	0.045	0.632	0.807

Prediction error measures how well the model will be able to predict the outcome for new observations or factors that were not used in the development of the prediction model, for example in a new set of patients. Cross-validation uses distinct and independent sets of observations to estimate the model and to evaluate prediction error.

The estimates of the areas under the ROC curves shown in figures 1 to 4 were larger than the cross-validated estimates. The differences though were relatively small, suggesting that the logistic models for the different categories of low testosterone and for ED were not very over-fitted. In a new group of patients one would expect that the areas under the ROC curves would probably not be much higher than 0.70 for all four models evaluated.

3.7. THE TEST CHARACTERISTICS OF THE ADAM QUESTIONNAIRE

Table 24. The prevalence of Androgen Deficiency in Adult Males (ADAM) symptoms

ADAM	n	Percentage
Negative	8	5.3
Positive	142	94.7
Total	150	100.0

The prevalence of symptoms of androgen deficiency was very high with 95% of patients reporting a significant amount of suggestive symptoms on the ADAM questionnaire. Only 5% of patients had no symptoms.

Table 25. The statistical measures of the Androgen Deficiency in Adult Males (ADAM) questionnaire in patients with a low total testosterone

Statistical measures	Results	95% Confidence Interval
Sensitivity	94.7%	86.9 - 98.5%
Specificity	5.3%	1.5 – 13.1%
Positive predictive value	50%	41.5 – 58.5%
Negative predictive value	50%	15.7 – 84.3%

Unfortunately the ADAM questionnaire was not helpful in identifying patients with low testosterone levels, due to the very high prevalence of suggestive symptoms even in patients with normal testosterone levels. The sensitivity to identify those with low total

testosterone was very high at 95%, but the trade-off was a low specificity of 5%. The PPV and NPV's were both 50%, similar to flipping a coin and thus not useful.

Table 26. The statistical measures of the Androgen Deficiency in Adult Males (ADAM) questionnaire in patients with a low modified calculated bioavailable testosterone

Statistical measures	Results	95% Confidence Interval
Sensitivity	96.7%	88.7 – 99.6%
Specificity	6.7%	2.5 – 14.1%
Positive predictive value	41.5%	33.3– 50.1%
Negative predictive value	75%	34.9 – 96.8%

The sensitivity of the ADAM questionnaire to identify those with low calculated testosterone was very high at 97% but the specificity was only 7%. This makes it a good screening tool for low calculated bioavailable testosterone. The NPV was good at 75% but the PPV was poor at 42%. This questionnaire can possibly be used to rule out disease.

Table 27. The statistical measures of the Androgen Deficiency in Adult Males (ADAM) questionnaire in patients with a low total and low modified calculated bioavailable testosterone

Statistical measures	Results	95% Confidence Interval
Sensitivity	100%	91.4 - 100%
Specificity	7.3%	3.2 - 14%
Positive predictive value	28.9%	21.6 – 37.1%
Negative predictive value	100%	63.1 - 100%

The sensitivity of the ADAM questionnaire to identify those with low calculated testosterone was excellent at 100% but the specificity was only 7%. This will make the ADAM questionnaire a possible tool to screen for patients with both a low total and low modified calculated bioavailable testosterone. The NPV was excellent at 100% but there was a poor PPV of only 29%. The questionnaire can be used to rule out disease.

3.8. THE STATISTICAL MEASURES OF TOTAL TESTOSTERONE VERSUS MODIFIED CALCULATED BIOAVAILABLE TESTOSTERONE FOR DETECTING HYPOGONADISM

Table 28. The statistical measures of the total testosterone level compared with the modified calculated bioavailable testosterone for screening of hypogonadism

Statistical measures	Results	95% Confidence Interval
Sensitivity	68.9%	55.7 – 80.1%
Specificity	62.9%	52 – 72.9%
Positive predictive value	56%	44.1 – 67.5%
Negative predictive value	74.7%	63.3 – 84%

Calculated bioavailable testosterone is the more reliable test to evaluate as a reflection of free or active testosterone, especially in the obese or elderly. A modified form of this calculation using a default value for serum albumin is probably less reliable. This test was used as the gold standard in calculations. If the total serum testosterone level was evaluated as a screening test the sensitivity was only 69%, which meant that more than 30% of patients with low modified calculated bioavailable testosterone would be missed.

The specificity was 63%, with a poor PPV of only 56%. The NPV was better at 75%. This implied that total testosterone is not an acceptable diagnostic test to perform in the place of a modified calculated bioavailable testosterone. Unfortunately the modified calculated bioavailable testosterone will be less accurate than the true calculated bioavailable testosterone, so two tests with specific limitations were compared to one another.

3.9. ED AND QUALITY OF LIFE

To simplify statistical analysis, and because of the low numbers in some of the five original ED categories, ED was firstly grouped into two: no to mild-moderate ED, and moderate-severe ED. When these two categories were evaluated against quality of life, no association could be found. When, however, ED was regrouped into 3 categories: no-mild, moderate, or severe, an association could be demonstrated with quality of life, especially between the normal to mild, and the severe ED groups.

Table 29. ED and Quality of life score

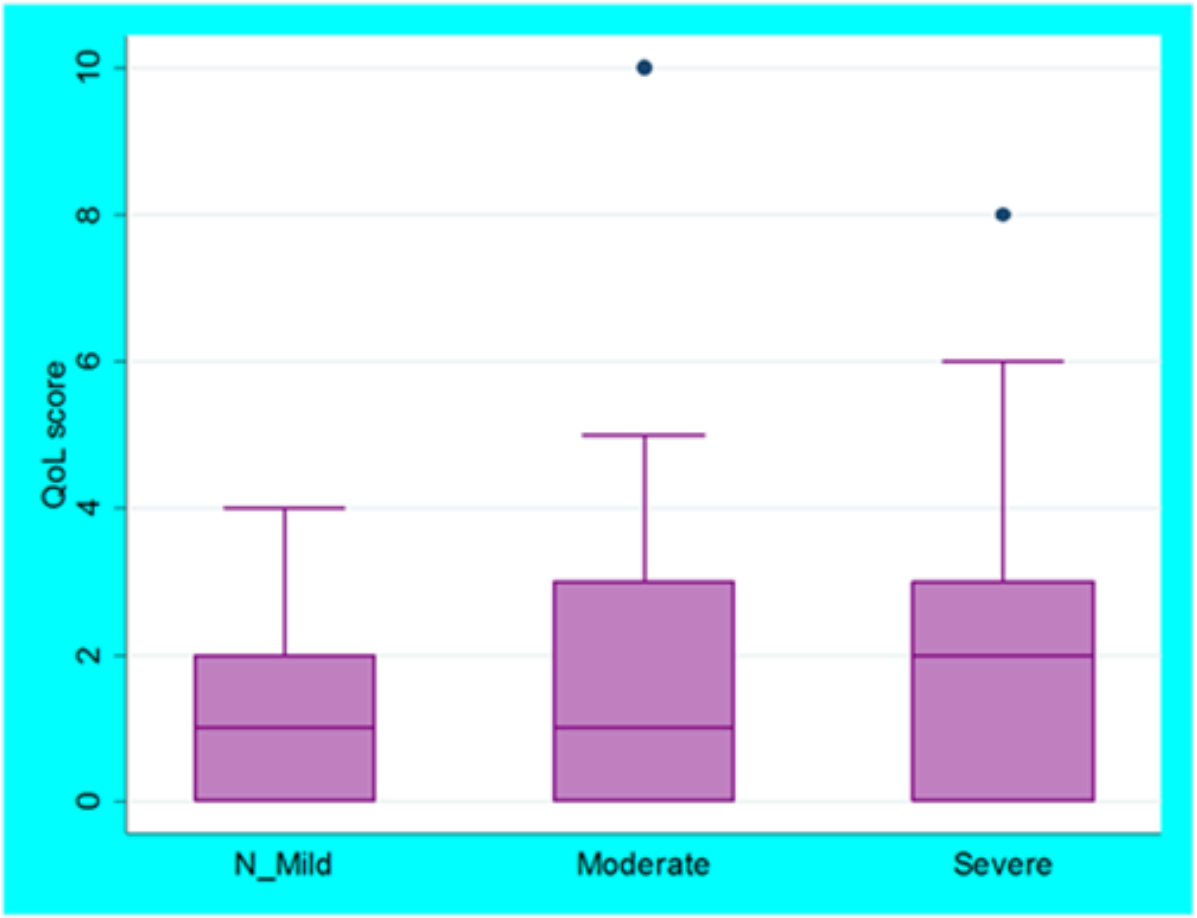
ED category	n	Quality of life score		
		Median	IQR	
			p25	p75
Normal to mild	24	1	0	2
Moderate	49	1	0	3
Severe	77	2	0	3

Kruskal-Wallis p=0.0513

This test approached statistical significance. This result implies that the different ED subgroups differed regarding quality of life and this came close to reaching statistical significance.

Although the EQ-5D was not validated in a South African population, the Chronbach Alpha - Internal consistency was 0.7610, which meant it was acceptable to use in our setting.

Figure 5. Box plot: ED and Quality of life



This box-plot graphically demonstrates the difference in quality of life between the three different subgroups of erectile dysfunction: no-mild, moderate or severe. An association could be demonstrated with quality of life, especially between the normal to mild, and the severe ED groups.

3.10. TESTOSTERONE LEVELS AND QUALITY OF LIFE

Table 30. Comparison between low Testosterone groups and Quality of life score

	Normal total testosterone	**Low total testosterone**	**p-value**
Quality of life score [Median (IQR)]	1 (0 to 3)	2 (0 to 3)	0.52

	Normal modified calculated bioavailable testosterone	**Low modified calculated bioavailable testosterone**	**p-value**
Quality of life score [Median (IQR)]	1 (0 to 3)	2 (0 to 3)	0.131

	Normal total and modified calculated bioavailable testosterone	**Low total and modified calculated bioavailable testosterone**	**p-value**
Quality of life score [Median (IQR)]	1 (0 to 3)	2 (1 to 3)	0.081

None of the p-values reached statistical significance, which meant that no clear correlation between testosterone groups and quality of life could be detected.

CHAPTER 4: DISCUSSION

4.1. DISCUSSION OF RESULTS

The prevalence of low testosterone levels was higher than expected. Fifty percent of the men had low total testosterone levels; 40.7% had low calculated bioavailable testosterone levels, and 27.3% had both low total and low calculated bioavailable testosterone levels. Contributors to this high prevalence was probably the high prevalence of comorbid diseases, advanced diabetic complications, and the age of patients (mean age of 62 years). These results should not be expected in the general diabetic population, but only in similar tertiary care facilities.

Some degree of erectile dysfunction was reported in 95.3% of the patients, and 51.3% reported serious erectile dysfunction. ED can have neurogenic or vascular components. Again, this very high prevalence probably reflected the high prevalence of micro- and macrovascular diabetic complications in this population group and other comorbid conditions which all contributed to this condition.

Both autonomic neuropathy and peripheral vascular disease ED which are highly prevalent in diabetic patients are well-known to be associated with ED. Most patients were also on multiple drugs that could cause or contribute to ED. The high prevalence of ED and the well-known association of this condition with ischaemic heart disease, and this in the presence of other known cardiovascular risk factors, strongly support universal cardiovascular screening for all the men in the study not already known with ischaemic heart disease.

No novel predictors of low testosterone levels could be identified in this study. Variables univariately associated with low total testosterone were found to be the body mass index, the waist circumference and the presence of known cardiovascular disease. With multivariate logistic regression confounders were excluded; the only significant factors remaining were waist circumference and known cardiovascular disease.

Both these factors are well-known to be associated with low testosterone levels although causality is unclear; a low testosterone value can be a contributing cause or a complication of obesity and of cardiovascular disease. The effect of obesity on serum testosterone levels is multifactorial (see above: “*b) Pathophysiological mechanisms underlying*

hypogonadism in type 2 diabetes"). Using these factors in a model to predict low total testosterone, the area under the ROC curve was 0.70, which just reached an acceptable discrimination level. This model did not perform very well; only 66% were correctly classified.

The calculated bioavailable testosterone may be the better test to determine testosterone deficiency especially in elderly or more obese subjects. A modified form of calculated bioavailable testosterone that was used may unfortunately overestimate the amount of patients with low values especially in the presence of hypoalbuminaemic states which can underestimate the bioavailable testosterone level.

For a low modified calculated bioavailable testosterone level univariate associations were age, diabetes duration, body mass index, waist circumference and known cardiovascular disease. After multivariate logistic regression the remaining significant variables were age, diabetes duration and body mass index. Again, these factors are well-known to be associated with serum testosterone levels. Age and diabetes duration are closely related since older patients tend to have had diabetes for a longer duration.

Using these factors to predict low modified calculated bioavailable testosterone, the area under the ROC curve was 0.70, which reached an acceptable but not excellent discrimination level. This model further did not perform very well with a good specificity but poor sensitivity; only 68% were correctly classified.

Univariate associations for both low total and low modified calculated bioavailable testosterone levels were age, diabetes duration, hypertension duration, body mass index, waist circumference and the presence of known cardiovascular disease. After multivariate logistic regression the significant factors were diabetes duration, body mass index, and known cardiovascular disease.

These are not novel risk factors for low serum testosterone levels but have been described in the literature before. Using these associations in a model to predict low testosterone, the area under the ROC curve was 0.77 which showed fair discrimination. This model did not perform very well looking at the statistical measures, with an excellent specificity but a very poor sensitivity. Seventy-nine percent of cases were correctly classified.

For erectile dysfunction univariate associations were age, race, smoking status, systolic blood pressure, body mass index, waist circumference, serum creatinine, peripheral neuropathy score, and diuretic therapy. After multivariate logistic regression the remaining significant factors were age, body mass index, peripheral neuropathy score, and diuretic therapy. Other drug classes such as beta-blockers and fibrates did not show statistically significant associations with ED.

Using these variables in a model to predict ED, the area under the ROC curve was 0.797, which implied good discrimination. This model performed well on most of the statistical measures. Although the specificity was poor at 55%, the sensitivity was 90% which meant this model can be used for screening for this condition. Seventy-eight percent of cases were correctly classified.

Cross-validation uses distinct and independent sets of observations to estimate the model and to evaluate prediction error. The estimates of the areas under the ROC curves for above four models were larger than the cross-validated estimates. The differences though were relatively small, suggesting that the logistic models for the different categories of low testosterone and for ED were not very over-fitted. In a new group of patients one would expect that the areas under the ROC curves would probably not be much higher than 0.70.

Testosterone levels in ED did not differ significantly between different ED groups.
Total testosterone for screening of hypogonadism performed poorly when compared with using a modified calculated bioavailable testosterone; 31% of patients with a low modified calculated bioavailable testosterone would be missed if only the total testosterone was evaluated. Unfortunately the accuracy of using a modified calculated bioavailable testosterone level is questionable in the presence of a low serum albumin, which can falsely lower the testosterone level. We do not have data on the trial patients' albumin levels to know whether a significant proportion of them had hypoalbuminaemia.

On the ADAM questionnaire the prevalence of androgen deficiency symptoms was 94.7%. The almost universal presence of these symptoms even in patients with normal testosterone levels made this questionnaire less useful for screening purposes; it has a very high sensitivity but a very poor specificity for detecting low testosterone levels.

No clear correlation between testosterone deficiency and quality of life could be

determined in this population group. There was some negative effect on quality of life in the ED patients with the main difference being between the none-mild and the severe groups, the latter group being worse affected. This is in keeping with numerously literature supporting the negative effect that ED has on quality of life. The low testosterone groups may have been too small to demonstrate a clear association with quality of life.

4.2. POSSIBLE LIMITATIONS OF THE STUDY

This study was conducted in a tertiary outpatient diabetic clinic where most of the patients had complications of their disease and numerous comorbid conditions. The results can therefore not be generalised to the majority of diabetic patients who follow up at primary health care facilities.

By enrolling 150 consecutive patients selection bias was to some degree minimized but not totally eliminated, for example most rural patients arrived regularly after 10h00 at the clinic which was too late to be included in the study, since blood tests had to be performed in the morning. A limited number of Asian and Mixed ancestry patients were included which would make the results more difficult to interpret in these population groups.

Some of the information was subjectively obtained from patients without outside collaboration such as a history of ischaemic heart disease. Due to multiple examiners with different levels of expertise, only proliferative diabetic retinopathy (either objectively observed, documented in the Ophthalmology notes, or a history of laser therapy or haemorrhage by the patients) was reported on.

Due to a lack of funds and time restrictions, a repeat confirmatory testosterone level or a serum LH to distinguish primary from secondary hypogonadism could not be done. Due to time constraints the questionnaires were also picked for their simplicity and brevity, not necessary because of superior accuracy.

An unknown percentage of our patients might have had chronic diseases with associated hypoalbuminaemic states such as nephrotic syndrome. Due to financial constraints we could not determine the albumin values as well in all the patients, so we had to use the

formula to calculate bioavailable testosterone with albumin set at a default level of 43 g/L. Patients with low albumin levels can have false low calculated bioavailable testosterone levels. Even so, in an outpatient setting with relatively stable ambulatory patients, most patients can be assumed to have normal albumin levels. The high percentage of patients who had low total testosterone levels (50% versus 40.7% with low modified calculated bioavailable testosterone levels) argues against significant overdiagnosis of low calculated bioavailable testosterone in patients.

Even though there is data that a serum testosterone level below 12 nmol/L is probably abnormal, for the diagnosis of hypogonadism or testosterone deficiency syndrome the guidelines still advice that the level should be unequivocally low and not just borderline low, accompanied by symptoms suggestive of a low serum testosterone level.[2] We chose to use the laboratory cut-off value of 9.9 nmol/L to increase specificity at the expense of a lower sensitivity.

This was a descriptive, cross-sectional observational study. A prospective randomised controlled trial would have been superior especially to answer questions regarding causality between diabetes and hypogonadism.

4.3. STUDY RESULTS IN RELATION TO OTHER STUDIES

The prevalence of low testosterone levels were higher than that reported in the literature.[15,16,17] This can be because our diabetic population had multiple comorbid diseases and advanced diabetic complications which can all influence the testosterone levels , and were older than patients in some other studies. Some degree of ED was almost universally present; this higher than reported prevalence can also be due to the patients' advanced complications, comorbid diseases and numerous drug therapy.[32,34,35] Predictors for both testosterone deficiency and ED were very similar to those reported in the literature.[7,8,32]

4.4. QUESTIONS ARISING FROM THIS STUDY FOR FUTURE STUDY

Questions regarding causality could not be evaluated with this study design. The main question that has arisen from this study is whether low testosterone levels cause or contribute to the development of diabetes mellitus, or whether diabetes mellitus is the main aetiologic factor of the low testosterone levels.

The second question is whether the patients with a low testosterone level have a poorer long-term prognosis, especially regarding cardiovascular mortality than the patients with normal testosterone levels. For this a larger prospective study will be necessary.
A third question is whether testosterone therapy for hypogonadal men will ameliorate their diabetes mellitus.

A fourth question arising is that of the association of ED with cardiovascular disease, well-described in the literature. It is uncertain how many of the patients with ED have underlying ischaemic heart disease. These questions can better be answered with prospective randomised controlled trials. It will also be interesting to create prediction models with the current information and to use and evaluate its performance regarding screening in the clinical setting.

4.5. CONCLUSIONS

This study confirmed the high prevalence of low testosterone levels in diabetic male patients in a tertiary setting. Multiple associations and predictors of low testosterone levels were identified by evaluating different demographic, clinical and other variables. However, poor prediction argued in favour of universal testing of this population group for low testosterone values.

It also confirmed the high prevalence of ED in a tertiary diabetic clinic setting, and that it negatively affected the quality of life. Total testosterone testing alone performed poorly in comparison with calculated bioavailable testosterone and is not the recommended test of choice. The ADAM questionnaire was not useful in identifying subjects with a low testosterone level since almost all the test subjects had symptoms suggestive of hypogonadism irrespective of their testosterone status.

The presence of low serum testosterone levels could not be demonstrated to have a detrimental effect on the health related quality of life. The high prevalence of both low testosterone levels and of ED should prompt the screening of all diabetic male patients older than 50 years at tertiary clinics for these conditions. Moreover, the clear association well described in the literature between ED and cardiovascular disease should prompt the screening of patients with ED for ischaemic heart disease.

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ADDENDUM: QUESTIONNAIRES

1) ED-5D QUESTIONNAIRE

By placing a tick in one box in each group below, please indicate which statements best describe your own health state today.

Mobility

I have no problems in walking about ❑

I have some problems in walking about ❑

I am confined to bed ❑

Self-Care

I have no problems with self-care ❑

I have some problems washing or dressing myself ❑

I am unable to wash or dress myself ❑

Usual Activities (*e.g. work, study, housework, family or leisure activities)*

I have no problems with performing my usual activities ❑

I have some problems with performing my usual activities ❑

I am unable to perform my usual activities ❑

Pain/Discomfort

I have no pain or discomfort ❑

I have moderate pain or discomfort ❑

I have extreme pain or discomfort ❑

Anxiety/Depression

I am not anxious or depressed ❑

I am moderately anxious or depressed ❑

I am extremely anxious or depressed ❑

2) ADAM QUESTIONNAIRE

Androgen Deficiency in Aging Males (ADAM) Questionnaire	Yes	No
1. Do you have a decrease in sex drive? 2. Do you have a lack of energy? 3. Do you have a decrease in strength and/or endurance? 4. Have you lost height? 5. Have you noticed a decreased enjoyment of life? 6. Are you sad and/or grumpy? 7. Are your erections less strong? 8. Has it been more difficult to maintain your erection throughout sexual intercourse? 9. Are you falling asleep after dinner? 10. Has your work performance deteriorated recently?		

Note: A diagnosis of ADAM is suspected if there are "yes" answers to 1 and 7, or any other 3 questions.

www.landerurology.com/pdf/adam_questionnaire.pdf
Dr John Morley

3) SHIM QUESTIONNAIRE

MALE PATIENT: SEXUAL HEALTH INVENTORY:

Each question has several possible responses. Circle the number of the response that **best describes** your own situation. Please be sure that only one response for **each question** is selected.

Over the past 6 months:

1. How do you rate your confidence that you could get and keep an erection?		VERY LOW	LOW	MODERATE	HIGH	VERY HIGH
		1	2	3	4	5
2. When you had erections with sexual stimulation, how often were your erections hard enough for penetration (entering your partner)?	NO SEXUAL ACTIVITY	ALMOST NEVER OR NEVER	A FEW TIMES (MUCH LESS THAN HALF THE TIME)	SOMETIMES (ABOUT HALF THE TIME)	MOST TIMES (MUCH MORE THAN, HALF THE TIME)	ALMOST ALWAYS OR ALWAYS
	0	1	2	3	4	5
3. During sexual intercourse, how often were you able to maintain your erection after you had penetrated (entered) your partner?	DID NOT ATTEMPT INTERCOURSE	ALMOST NEVER OR NEVER	A FEW TIMES (MUCH LESS THAN HALF THE TIME)	SOMETIMES (ABOUT HALF THE TIME)	MOST TIMES (MUCH MORE THAN, HALF THE TIME)	ALMOST ALWAYS OR ALWAYS
	0	1	2	3	4	5
4. During sexual intercourse, how difficult was it to maintain your erection to completion of intercourse?	DID NOT ATTEMPT INTERCOURSE	EXTREMELY DIFFICULT	VERY DIFFICULT	DIFFICULT	SLIGHTLY DIFFICULT	NOT DIFFICULT
	0	1	2	3	4	5
5. When you attempted sexual intercourse, how often was it satisfactory for you?	DID NOT ATTEMPT INTERCOURSE	ALMOST NEVER OR NEVER	A FEW TIMES (MUCH LESS THAN HALF THE TIME)	SOMETIMES (ABOUT HALF THE TIME)	MOST TIMES (MUCH MORE THAN, HALF THE TIME)	ALMOST ALWAYS OR ALWAYS
	0	1	2	3	4	5

Source: http://www.murdockurology.com/PDF/shim.pdf *(Accessed 14 April 2010*

4) MODIFIED NEUROPATHY SYMPTOM SCORE (NSS)

FEET

Do you experience numbness in your lower legs and feet?	**Yes**	**Worse @ night**	**No**
Do you experience abnormal hot or cold sensations in your lower legs and feet?	**Yes**	**Worse @ night**	**No**
Do you experience any tingling sensations in your lower legs and feet?	**Yes**	**Worse @ night**	**No**
Do you experience any burning pain in your lower legs and feet?	**Yes**	**Worse @ night**	**No**
Do you experience any aching pain in your lower legs and feet?	**Yes**	**Worse @ night**	**No**

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5) ROSE QUESTIONNAIRE

Intermittent claudication

If an answer is recorded in a box marked * no further questions need to be asked

1) Do you you get pain in either leg on walking ?
.....□ **yes** □ no *

2) Does the pain ever begin when you are standing or sitting?.....□ yes * □ **no**

3) In what part of your leg do you feel it ?
Pain includes calf/calves.....□
Pain does not include calves.....□ *
if calves not mentioned ask "Anywhere else?"

4) Do you get it when you walk uphill or hurry ?
.....□ yes □ no *
Never hurries or walk uphill .....□

5) Do you get it when you walk at an ordinary pace on the level ?.....□ yes □ no

6) Does the pain ever disappear while you are walking ?
.....□ yes-* □ **no**

7) What do you do if you get it while walking ?
Stop or slow down.....….□
Carry on.....□ *

8) What happens to it if you stand still ?
Relieved.....□
Not relieved.....□ *

9) How soon ?
10 min or less.....□
more than 10 min .....□

Intermittent claudication if subject answers as follows:

Q1 yes
Q2 no
Q3 includes calf
Q4 or 5 yes
Q6 no
Q7 stop or slow down
Q8 relieved
Q9 10 minutes or less

Q5 No Grade 1
Q5 Yes Grade 2

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