Bone health in Graves' disease: A comparison of black and white South African women.

By

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Dedication:

I would like to dedicate this thesis to my wife and best friend, Lidelle, who has been my constant inspiration, motivation, and source of wisdom. Without her support and sacrifice this work would never have been possible.

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Abbreviations:

1,25-dihydroxyvitamin D	$1,25(OH)_2D$
25-hydroxyvitamin D	25(OH)D
Aspartate transaminase	AST
Basic multi-cellular unit	BMU
Black control	BC
Black patient	BP
Body mass index	BMI
Bone mineral density	BMD
Bone remodelling compartment	BRC
Bone surface	BS
Bone volume	BV
Bone-specific alkaline phosphatase	BAP
Computerized tomography	CT
Coefficient variant	CV
C-reactive protein	CRP
Cross-linked C- telopeptide of type 1 collagen	CTX
c-terminal propeptide of type I procollagen	PICP
Direct, two-site, sandwich type chemiluminescence immunoassay	CLIA
Dual-energy x-ray absorptiometry	DXA
Electrocardiogram	ECG
Endosteal	ES
Erythrocyte sedimentation rate	ESR
Ethics Committee of the Faculty of Health Sciences, University of the Free State	ECUFS
Fat mass index	FMI
Fibroblast growth factor reptor-1	FGFR1
Fracture risk assessment tool	FRAX
Free deoxypyridinoline	D-PYR
Free pyridinoline	PYD
Fisher's exact tests	(F)
Graves' disease	GD
Human immunodeficiency virus	HIV

Hydroxyproline	HYP
Insulin-like growth factor 1	IGF-1
Insulin-like growth factor–binding proteins	IGFBPs
Interleukin	IL
International Osteoporosis Federation	IOF
Interquartile range	IQR
Lipoprotein receptor-related protein 5	LRP-5
Lumbar spine	AP-spine
Magnetic resonance imaging	MRI
Mineralization Lag Time	MLT
Monocarboxylate transporter	MCT
National Health and Nutrition Examination Survey	NHANES
National Health Laboratory Services	NHLS
National Osteoporosis Foundation of South Africa	NOFSA
Not available	NA
n-terminal propeptide of type I procollagen	PINP
Nuclear factor kappa-B	NFκB
Organic anion-transporting polypeptide 1C1	OATP1c1
Osteoclast surface	Oc. S.
Osteoclastic resorptive surfaces	Oc. S/ BS%
Osteoid surface	OS/ BS
Osteoid thickness	O. Th.
Osteoid volume	OV/ BV
Osteomalacia	OM
Osteoporosis and Ultrasound Study	OPUS
Parathyroid hormone	PTH
Phosphate	PO_4
Relative osteoid volume	OV/ TV%
Serum isoform 5b of tartrate resistant acid phosphatase	TRACP5b
Standard deviation	SD
Subcutaneous adipose tissue	SAT
Surface density osteoid seams	SDOS

The International Society of Clinical Densitometry	ISCD
Thyroglobulin	TGB
Thyroid peroxidase	TPO
Thyroid peroxidase antibodies	TPOAb
Thyroid receptor	TR
Thyroid-stimulating hormone	TSH
Thyroid-stimulating hormone receptor	TSH-R
Thyroid-stimulating hormone receptor antibodies	TSH-R Abs
Thyroxine	T_4
Tissue volume	TV
Total bone volume	BV/ TV%
Total osteoid surface	OS/ BS
Total osteoid volume	OV/ BV%
Total resorptive surfaces	ES/ BS%
Triiodothyronine	T_3
Tumour necrosis factor-α	TNFα
United Kingdom	UK
United States	US
United States of America	USA
Universitas Academic Hospital	UAH
University of the Free State	UFS
Urinary deoxypyridinoline (Expressed as a ratio to urinary creatinine)	Urine DPD
Vertebral fracture assessment	VFA
Visceral adipose tissue	VAT
White cell count	WCC
White control	WC
White patient	WP
World Health Organization	WHO
γ-Glutamyl Transferase	GGT

Reference values:

Investigation	Normal	Unit
Thyroid-stimulating hormone	0.27-4.20	mIU/L
Thyroxine	12.0-22.0	pmol/L
Triiodothyronine	3.1-6.8	pmol/L
Thyroid peroxidase antibodies	< 10	IU/ml
Thyroglobulin antibodies	< 116	IU/ml
Thyroid- stimulating hormone antibodies	< 1.75	IU/L
Parathyroid hormone	15-65	pg/ml
Calcium	2.15-2.50	mmol/L
Phosphate	0.78-1.42	mmol/L
25-hydroxyvitamin D	32-80	ng/ml
1,25-dihydroxyvitamin D	19.9-67	ng/L
Serum bone specific alkaline phosphatase Premenopausal	3-19	µg/L
Serum bone specific alkaline phosphatase Postmenopausal	6-26	µg/L
Osteocalcin Premenopausal	6.5-42.3	ng/ml
Osteocalcin Postmenopausal	5.4-59.1	ng/ml
Urinary deoxypyridinoline (Expressed as a ratio to urinary creatinine)	3.0-7.4	nm/mm
White cell count	4-10	cells x 10^9
Interleukin 6	0.0-6.4	pg/ml
Tumour necrosis factor alpha	1.47-5.93	pg/ml
C-reactive protein	0.0-5.0	mg/L
Erythrocyte sedimentation rate	0-31	mm/hr
Insulin-like growth factor 1	107.8-246.7ng	ng/ml

Investigation	Normal	Unit
Leptin	*According to BMI*	ng/ml
BMI < 25	0.2-45.8	
BMI 25.00-29.99	3-65.7	
BMI 30.00-34.99	8.1-79.1	
BMI ≥ 35.00	11.9-137.4	
Bone Histomorphometry	*In text*	

Abstract:

Objectives

The negative effects of thyrotoxicosis on bone health in white populations have been widely studied. In non-South African black populations, it has been showed that the skeleton is protected against the negative effects of other endocrinopathies, for e.g.: hyperparathyroidism. The aim of the study was to determine whether black South African women are also protected against the detrimental effects of Graves' disease (GD)?

Background and motivation

The detrimental effects of thyrotoxicosis on bone health has been known for more than a century. In white females, thyrotoxicosis has been shown to increase the rate of bone resorption along with bone formation. However, the rate of bone formation is inadequate to compensate for bone resorbed, leading to a net loss of bone volume. This negatively impacts on the structural integrity of bone with an increased fracture risk. Little is known about the effect of thyrotoxicosis on bone health in black African-, and especially black South African women.

Ethnic differences in bone metabolism and fat distribution do exist between black and white South African women.

Dual-energy X-ray absorptiometry (DXA) studies have shown that the bone mineral density at the lumbar spine of healthy premenopausal black South African women, is equal or lower than that of their white counterparts. The bone mineral density of postmenopausal black- and white women at the lumbar spine is comparable. Vertebral fracture risk of the lumbar spine is equal for black- and white South African women.

Significant differences in femoral bone density do exist when black and white South African women are compared. Black South African women have a greater bone mineral density at the femur neck site. Histomorphometric and radiometric differences between these two ethnic groups may explain the lower incidence of femoral fragility fractures in the black

population. Black South African women, when compared to their white counterparts, have thicker and less porous cortices as well as thicker trabeculae. These micro architectural differences could explain the decreased number of fractures seen in black females when compared to white females.

There are also ethnic differences in the abdominal adipose tissue depot distribution. White men and women have increased abdominal visceral adipose tissue and decreased subcutaneous adipose tissue when compared to black men and women.

Thyrotoxicosis has detrimental effects on bone metabolism and bone structure in white women. These effects can lead to an increased risk of fracture that persists even after normalization of the bone mineral density.

Study design

This was a prospective exploratory and comparative study.

Methods

A convenience sample of 40 consecutive and consenting black female patients (age ≥ 25 and ≤ 65 years) and 20 consecutive and consenting white female patients (age ≥ 25 and ≤ 65 years) with confirmed GD referred to the Endocrine service at Universitas Hospital were recruited for the study. Patients were matched with 40 black and 20 white healthy control females according to age (± 5 years), body mass index (BMI) and seasonality.

Black- and white South African women suffering from GD were compared with each other at baseline, 6- and 12 months according to pre-determined objectives. Women suffering from GD were also compared with healthy controls from the same ethnic group. This comparison was performed to rule out effects of ethnicity versus effects of GD on bone health. The main objectives included differences in biochemical markers, bone mineral density and body composition.

Histomorphometric data on the effect of GD on bone in white ethnic groups has been published before. Therefore, bone histomorphometry was only performed in black women suffering from GD to ascertain whether ethnic differences do exist.

Results

In this prospective study, 39 black women and 20 white women with newly diagnosed GD were included.

The median parathyroid hormone (PTH) level of black women suffering from GD was suppressed and significantly lower when compared to white patients ($p = 0.04$). The suppressed PTH in black patients were accompanied by increased serum calcium levels. The markers of bone formation as well as bone resorption were increased in both patient groups. The median urine deoxypyridinoline (DPD) to creatinine ratio (Urine DPD), a marker of bone resorption, was significantly higher in black women suffering from GD compared to white patients ($p = 0.026$). Although the median 25-hydroxyvitamin D (25(OH)D) level of black patients with GD was lower compared to their white counterparts and suppressed below the lower limit of the laboratory threshold, it did not differ significantly. The median 1,25-dihydroxyvitamin D ($1,25(OH)_2D$) levels of black- and white patients were normal and not different. A marker of inflammation, tumour necrosis factor alpha (TNFα), was significantly higher in black patients compared to white patients ($p = 0.022$) while the other markers included, interleukin 6 (IL-6) and C-reactive protein (CRP), did not differ. The median insulin-like growth factor 1 (IGF-1) levels of both patient groups were lower compared to healthy controls. The median IGF-1 of black patients was significantly lower compared to that of healthy black controls ($p = 0.001$). The same was observed in white patients and –controls with white patients having a significantly lower median IGF-1 level ($p = 0.05$). There was no difference between the two patient groups.

The actual bone mineral density (BMD) of white patients at the left femoral neck was significantly lower compared to black patients at baseline ($p = 0.033$). This difference was not observed between white patients and –controls. The BMD at the left forearm distal 3rd was lower in black patients compared to white patients ($p = 0.049$). Although the same pattern was observed when comparing the median Z-scores at baseline, it did not reach significance. The median Z-score at the left total hip of white patients were significantly

lower when compared to white controls (p = 0.039). A greater proportion of black patients had a median Z-score of the lumbar spine ≤ -2.0 compared to white patients (p = 0.028). The difference observed of actual BMD at the left femoral neck between black- and white patients at baseline was maintained at 6- and 12 months after therapy. The difference at the left forearm distal 3rd disappeared at months 6 and 12. The actual BMD of white patients at the right femoral neck was significantly lower at 12 months compared to black patients (p = 0.030).

The body composition of both black- and white patients were comparable at baseline. However, the percentage change in body mass index (BMI) did differ significantly from 0-6 and 0-12 months between the two patient groups. Black patients had a significantly higher percentage increase in BMI at 0-6 months (p = 0.042) and 0-12 months (p = 0.01) compared to white patients. The body composition of white patients and –controls did not differ significantly at baseline, 6- and 12 months. Although the body composition of black patients and –controls were comparable at baseline, black patients had a significant increase of especially fat tissue after treatment. This is confirmed by a significantly higher fat mass index (FMI) found in black patients at 12 months compared to controls (p = 0.011).

The bone histomorphometry revealed a state of accelerated bone turnover, predominant stimulation of bone resorption and histological evidence of demineralization as evidenced by abundant osteoid on histology.

Conclusions

Ethnic differences between black- and white South African women suffering with GD were shown. Black South African women are not protected against detrimental skeletal effects of GD.

It is hoped that this study will contribute to a better understanding of bone health in the South African population in general, but especially those patients with GD. It is also envisioned that this may lead to improved management of patients once thought to be protected against the skeletal complications of GD. Further South African research is warranted.

Key words:

South Africa; thyrotoxicosis; Graves' disease; bone mineral density; histomorphometry; fracture risk; body composition.

Chapter 1

Introduction

1.1 **Background & Motivation**

1.1.1 ***Thyrotoxicosis and hyperthyroidism***

The term "thyrotoxicosis" refers to the physiologic manifestations that arise from inappropriately excessive thyroid hormone action in tissues, usually resulting from elevated tissue thyroid hormone levels, mainly tri-iodothyronine (T_3) and its precursor, thyroxine (T_4) (1, 2). The term "hyperthyroidism" is reserved for disorders resulting from excess thyroid hormone secretion (1). Hyperthyroidism can be overt or subclinical (3). Overt hyperthyroidism is characterised by low serum thyroid-stimulating hormone (TSH) concentrations and raised serum concentrations of thyroid hormones: T_4, T_3, or both. Subclinical hyperthyroidism is characterised by low serum TSH, but normal serum T_4 and T_3 concentrations (1, 3).

The prevalence of hyperthyroidism worldwide varies greatly depending on methodology used for diagnosis, region, age, sex, and iodine intake (Mandel *et al.*, 2011; Ross *et al.*, 2016). In the United States of America (USA) the prevalence of hyperthyroidism in subjects 12 years and older was 1.3% (4) compared to 0.75% of subjects of varying ages in Europe (5). Subjects, who unknowingly had laboratory evidence of hyperthyroidism, were included in these data. The prevalence of hyperthyroidism in the USA varied according to ethnicity with 1.1% of black non-Hispanic subjects in the National Health and Nutrition Examination Survey III (NHANES III) (4), compared to 1.4% of white subjects. Good quality data on the prevalence of hyperthyroidism in sub-Sahara Africa is lacking. Kalk (6) commented that hyperthyroidism is rare in black South Africans. The frequency of admission of black patients with proven hyperthyroidism to UAH in recent years indicates that this condition is no longer rare in this racial group [Personal observation].

Graves' disease (GD) is the most common cause of thyrotoxicosis in iodine sufficient areas of the world and accounts for up to 88% of cases in both black and white patients (3, 6-8). The aetiology of GD is multifactorial with a strong autoimmune component. It is

characterised by the development of autoantibodies that stimulate thyroid follicular cells by binding to the TSH receptor in genetically predisposed individuals (3). This form of thyrotoxicosis is more common in women and is characterised by a diffuse goitre. It may also be accompanied by infiltrative orbitopathy and ophthalmopathy and occasionally by infiltrative dermopathy (1). A prolonged state of excessive and unabated thyroid hormone action in tissues may lead to a myriad of other clinical manifestations that generally also affects the musculoskeletal system (1). Bone disease is more common in subjects with thyrotoxicosis than controls. The risk of hip fracture in women with hyperthyroidism is increased threefold and vertebral fractures fourfold (9). Compared to subjects with euthyroidism, subjects with subclinical hyperthyroidism also have a significantly increased risk of hip and other fractures (10).

1.1.2 ***Metabolic Bone Disease***

Bone health is maintained through the sophisticated process of bone remodelling (11, 12). Metabolic bone disease arises due to conditions affecting the matrix, minerals and/or cells in bone tissue. There are multiple forms of metabolic bone disease (13, 14), which can be associated with low or high bone mineral density (BMD) (14, 15). Conditions associated with an increased BMD are rare, but may include acromegaly, osteopetrosis and renal osteodystrophy. Conditions associated with low BMD are much more common and may be due to osteoporosis, osteomalacia or a combination of both.

Osteomalacia is a pathological bone condition in which there is defective mineralization of bone, leading to the accumulation of unmineralized bone matrix and an increase in osteoid thickness (16, 17). The most common cause of osteomalacia is vitamin D deficiency, but other causes include renal tubular acidosis and X-linked hypophosphataemic rickets (14). Osteomalacia can be due to primary- or secondary vitamin D deficiency. Primary (nutritional) vitamin D deficiency occurs mainly due to a decreased exposure to sunlight and to a lesser extent to decreased dietary intake (16-18). Causes of secondary vitamin D deficiency include partial gastrectomy, the prolonged use of anticonvulsants, for e.g. phenobarbitone, etc.

Osteoporosis is the most common form of metabolic bone disease (19). In adults, osteoporosis is characterized by low bone mass, and a deterioration of bone tissue and

architecture (20). This leads to reduced bone strength and an increased risk for fragility fractures. The most common fracture sites are the vertebral bodies, distal radius and femur, but fractures can also occur at other sites (21). One in 3 women and 1 in 5 men over the age of 50 will suffer an osteoporotic fracture (22-24).

Hip fractures are associated with the highest morbidity and mortality (25). In white women the lifetime risk for the development of a hip fracture is 1 in 6 (26). This figure can be compared to the risk for the development of breast cancer, which is 1 in 8 in women (27). Up to one third of hip fractures occur in men (28). The mortality rate of a hip fracture is 25% in the first year for women and as high as 35% for men. Early studies showed that an increased risk of dying may persist for more than a year after the initial hip fracture (29). A more recent study found an increased mortality rate for up to 10 years after a hip fracture (30).

The causes of osteoporosis can be divided into primary and secondary (21). Primary osteoporosis denotes reduced bone mass and fractures in postmenopausal women or in older men due to age-related factors. Secondary osteoporosis occurs when an underlying disease, deficiency, or drug causes osteoporosis, for example: hyperparathyroidism, myeloma, and hyperprolactinaemia to name a few. Thyrotoxicosis is considered a secondary cause of osteoporosis (31).

It was estimated that in 2000 there were 9 million new osteoporotic fractures world-wide (19). Europe was hardest hit with 36.6% of new fractures, followed by the Western Pacific region and the Americas. A report based on data from the year 2010 estimated that there were 22 million women and 5.5 million men suffering from osteoporosis in the European Union (32). In the USA during the same period, 10 million people 50 years and older suffered from osteoporosis (33). World Health Organization (WHO) data showed that about 1% of osteoporotic fractures occurred in Africa (19). These rates were less than reported for non-Hispanic black persons in the USA (33). Unfortunately, South African data on the prevalence of osteoporosis is limited – this statement is supported by a 2011 IOF audit (34). Until recently it has been thought that black South African women suffer less vertebral fractures than their white counterparts, but a recent study in the Western Cape showed the prevalence to be the same (35). This raises the question whether there are true racial differences in fracture prevalence between white and black South African women.

1.1.3 *Thyrotoxicosis & Bone*

The association between thyroid and bone had been suggested by Von Recklinghausen more than a century ago (36). The thyroid gland, through mainly T_3, plays an integral role in bone health (37). Thyroid hormones influence skeletal development and linear growth and are pivotal for the maintenance of adult skeletal health. The effect of T_3 on bone is through its binding with the receptor isoforms, TR-α1, TR-α2 and TR-β1, which are expressed in osteoblasts, osteoclasts, osteocytes and chondrocytes (38-42). Mice with mutations of TR-α1 receptor suffer growth retardation, delayed endochondral bone formation, reduced ossification, and reduced growth hormone and insulin-like growth factor-1 (IGF-1) production (43). The phenotypical picture fits with hypothyroidism. Mutations of TR-β1 are associated with increased fibroblast growth factor receptor-1 (FGFR1), increased trabecular bone mineralization, advanced endochondral and intra-membranous ossification, and short stature. The phenotypical picture fits with a mixture of hypo- and hyperthyroidism in different tissue types (36). There is similarity between the clinical picture found in mice and humans suffering from TR-α1 mutations (44).

Thyrotoxicosis is associated with abnormal bone metabolism (45). Kinetic studies have shown that there is a shortening of the bone remodelling cycle (46) with the amount of bone resorbed staying constant, although the resorption rate being accelerated. An increased rate of bone formation does not adequately compensate for the shortened remodeling cycle, leading to a net loss of bone per cycle. This abnormal metabolism leads to a decrease in bone mineral density (BMD) and an increased risk of fracture (47-49).

1.2 **The Problem & Hypothesis**

1.2.1 *The Problem*

The detrimental effect of thyrotoxicosis on bone health in white women has been confirmed and was studied extensively (49-51). In certain black populations it is suggested that the skeleton is relatively protected against the negative effects of endocrinopathies like hyperparathyroidism (52, 53). This raises the question of whether the skeleton of black women is also protected against the detrimental effects of GD?

1.2.2 ***The Hypothesis***

The skeleton of black women is protected against the detrimental effects of GD.

1.3 **Objectives**

The main objective of the study was to assess indices of bone health in black and white South African women suffering from GD at the time of diagnosis, and at 6 and 12 months after commencement of treatment for GD.

The specific aims of this study were formulated as follows:

1.3.1 To determine if biomarkers of bone turnover in black women suffering from GD at baseline (pre-treatment) are like or different from that of white women with GD.

At baseline (at the time of diagnosis of GD):

- To determine and compare biomarkers of bone turnover of black women suffering from GD with that of healthy black control subjects.
- To determine and compare biomarkers of bone turnover of white women suffering from GD with that of healthy white control subjects.
- To compare biomarkers of bone turnover of black women suffering from GD with that of white women suffering from GD.

1.3.2 To determine if inflammatory markers in black women suffering from GD at baseline (pre-treatment) are like or different from that of white women with GD.

At baseline (at the time of diagnosis of GD):

- To determine and compare inflammatory markers of black women suffering from GD with that of healthy black control subjects.
- To determine and compare inflammatory markers of white women suffering from GD with that of healthy white control subjects.
- To compare inflammatory markers of black women suffering from GD with that of white women suffering from GD.

1.3.3 To determine effect of thyrotoxicosis on the baseline (pre-treatment) BMD of black women suffering from GD in comparison to white women with GD.

At baseline (at the time of diagnosis of GD):

- Determine and compare the BMD of black women suffering from GD to that of healthy black control subjects (BMD measured at vertebral-, distal radius- and femur sites. Include Vertebral Fracture Assesment (VFA)).
- Determine and compare the BMD of white women suffering from GD to that of healthy white control subjects.
- Compare the BMD of black women suffering from GD to that of white women suffering from GD.

1.3.4 To determine if BMD increases after commencement of treatment for GD, and if so, to determine whether the rate of recovery is similar in black and white women.

At 6 & 12 months after commencement of treatment for GD:

- Determine and compare the BMD of black women suffering from GD with BMD at baseline as well as with BMD of healthy black control subjects.
- Determine and compare the BMD of white women suffering from GD with BMD at baseline as well as with BMD of healthy white control subjects.
- Compare the rate of recovery of BMD between black- and white women suffering from GD at 6 and 12 months following commencement of treatment.

1.3.5 To determine the effect of thyrotoxicosis on the baseline (pre-treatment) body composition of black women suffering from GD in comparison with white women suffering from GD.

At baseline (at the time of diagnosis of GD):

- Determine and compare the body composition of black women suffering from GD with that of healthy black control subjects.
- Determine and compare the body composition of white women suffering from GD to that of healthy white control subjects.

- Compare the body composition of black women suffering from GD to that of white women suffering from GD.

1.3.6 To determine if body composition changes after commencement of treatment for GD, and if so, to determine if these changes are different between black and white women.

- Determine and compare the body composition of black women suffering from GD at 6 and 12 months after commencement of therapy, with baseline body composition of healthy black control subjects.
- Determine and compare the body composition of white women suffering from GD at 6 and 12 months after commencement of therapy, with baseline body composition of healthy white control subjects.
- Compare the changes in body composition between black- and white women suffering from GD at 6 and 12 months following commencement of treatment.

1.3.7 To describe and compare features of iliac crest bone histomorphometry of black and white women diagnosed with GD.

To realise these objectives:

- Forty consecutive black and 20 consecutive white women, between 25 and 65 years of age with confirmed GD referred to the endocrine service at Universitas Hospital were recruited.
- An euthyroid healthy matched control was selected for each of the study women (40 black and 20 white). These controls were matched according to age, BMI, and seasonality with the study cases.
- BMD, body composition and biomarkers of bone turnover were determined at baseline and after 6 and 12 months (subjects with GD) and
- Bone histomorphometry were performed on a subset of black women at presentation with GD.

1.4 **Structure of the Thesis**

The study will be reported on as follows:

In Chapter 1, **Introduction**, the background to the study is provided, the problem stated, and the overall goals and objectives provided.

In Chapter 2, **Literature Review**, the current knowledge on the effect of thyroid function on bone is reviewed, including the effect of thyrotoxicosis on body composition. Currently known ethnic differences are also described in this chapter.

In Chapter 3, **Methodology**, the methods of the study are described.

In Chapter 4, **Results and Discussion: Biochemical Measurements**, the baseline clinical characteristics, thyroid function test results, and thyroid autoantibody levels of subjects and controls are presented and compared. Biochemical measurements related to bone metabolism at baseline are also presented and compared. Results are presented for each ethnic group and compared with their respective controls. Finally, results for black and white women are also compared.

In Chapter 5, **Results and Discussion: Dual-energy X-ray Absorptiometry – Bone Mineral Density**, the BMD results obtained at baseline, 6- and 12 months are reported and compared. Results are presented for each ethnic group and compared with their respective controls at the different time-points. Finally, results for black and white women are also compared.

In Chapter 6, **Results and Discussion: Dual-energy X-ray Absorptiometry - Body Composition**, the Dual-energy X-ray absorptiometry data on body composition is analysed. Measurements of body composition obtained at baseline, 6- and 12 months are reported and compared. Results are presented for each ethnic group and compared with their respective controls at the different time-points. Finally, results for black and white women are also compared.

In Chapter 7, **Bone Histomorphometry**, the bone histology performed on consenting black female patients are analysed and discussed. Bone biopsies were not performed on white female patients as there were deemed to be sufficient information available in the literature.

In Chapter 8, **Combined Discussion, Conclusions and Recommendations,** the most important conclusions, limitations, and recommendations to have emanated from the study, are provided.

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Chapter 2

Literature Review

2.1 **Introduction**

2.1.1 ***Thyrotoxicosis and GD***

Thyrotoxicosis has been associated with metabolic bone disease for more than a century (1). Of the various causes of thyrotoxicosis, GD makes up 60-80% of patients suffering from thyrotoxicosis (2, 3) and is 10 times more common in women than men.

GD is an auto-immune disorder that develops due to the production of thyroid-stimulating hormone receptor antibodies (TSH-R Abs) (4). These TSH-R Abs were identified 50 years ago as a cause of thyrotoxicosis (5). The stimulating antibodies are usually mono- or oligoclonal and belong to the IgG1 class (6, 7). The thyroid-stimulating hormone (TSH) receptor (TSH-R) on the thyroid consists of three domains including an extracellular site, a transmembrane-spanning receptor region and an intracellular effector (8). The TSH-R Abs interact with the TSH-R through the extracellular domain (8). A Danish twin study has demonstrated that genetic factors contribute up to 80% to the risk of development of GD (9). Exposure to environmental factors including stress, smoking, and infections, trigger the auto-immune process against the thyroid (10). If the auto-immune process is triggered in an at-risk individual, TSH-R Abs are produced. TSH-R Abs bind to the TSH-R on the thyroid, and stimulate the production of thyroid hormones (11). This leads to thyrotoxicosis.

Thyrotoxicosis tends to decrease bone mineral density (BMD) and increase fracture risk by up to 2.5-fold, a phenomenon that may get worse with ageing (12-14). Thyrotoxicosis may directly affect skeletal integrity, but the disease is also accompanied by weight loss and changes in body composition which may also impact negatively on bone health (15, 16). It has also been shown that there is a negative relationship between BMD and the levels of TSH-R Abs in patients suffering from GD (17). The exact nature of the metabolic bone disease(s) that occurs during and after resolution of thyrotoxicosis (18) remain poorly defined, and little is known of the severity and prevalence of metabolic bone disease associated with thyrotoxicosis in sub-Saharan Africa.

2.1.2 *Bone health among women from different ethnic groups*

Differences in the risk of development of osteoporosis and subsequent fragility fractures do exist between different populations and ethnic groups (19). Osteoporotic fractures can occur at any site, but the three most common sites include the distal radius, spine and hip (20, 21). Although vertebral fractures are the most common osteoporotic fractures, representing up to a third of all osteoporotic fractures, hip fractures are associated with the highest morbidity and mortality (21, 22). Women above the age of 50 years from the United States of America (USA) have double the risk of sustaining a hip fracture when compared to Hispanic women of the same age. Asian women also have a much lower risk of sustaining a hip fracture when compared to white women from the USA (23). The highest lifetime risk for the development of hip fractures are in women from northern Europe (19). There is much less variability when comparing vertebral fracture risk between different populations (23-25). This variability is even less with advancing age (23). It has been shown that the prevalence of vertebral fractures whether looking at the USA-, Latin American- or Chinese populations are the same (25). Ethnic differences within a population also do exist (21). A recent study from the USA showed that up to 90% of osteoporotic fractures occur in white women, with less than 10% occurring in black- and Hispanic women combined (21). Although there are abundant data available on world-wide osteoporosis and fracture risk, Sub-Saharan- and specifically South African data are currently lacking (26).

Studies have shown that differences in bone metabolism and fat distribution exist between black and white South African women (27-35). It is thought that these differences may explain the differences in fracture risk between black and white women.

An epidemiological study investigating the incidence of hip fractures was performed in Johannesburg, South Africa (27). The study compared the incidence of hip fractures in black South Africans with fracture data from two western European countries. It showed the hip fracture risk of black South Africans to be less than 10% of that of Europeans. The study estimated the incidence of hip fractures in black women to be 4.3 per 100 000 per year with no significant increase associated with aging. A recent publication from KwaZulu-Natal demonstrated that black women do suffer more hip fractures than previously thought (36). The age corrected incidence of hip fractures for this population of black women was 69 per 100 000 per year with a significant increase from the age of 75.

The prevalence of vertebral osteoporosis was investigated in Durban, South Africa (28). The study compared three groups with each other namely, rural- and urbanized black women, and urban white women. The rate of vertebral osteoporosis was equal between the two black groups and 5 times less than the rate found in white women. Using dual-energy x-ray absorptiometry (DXA), BMD differences between black- and white nurses from Johannesburg were investigated (30). The distal radius, lumbar spine and femur were evaluated. The researchers found that peak distal radius- and vertebral BMD were the same in black- and white women, but femoral BMD was significantly higher in black women. It was indicated that weight plays an important role in the attainment of peak femoral BMD in black women. The same study also showed that black women have a decreased rate of bone loss during the peri-menopausal transition when compared to white women. A more recent study, looking at premenopausal black- and white South African women, evaluated BMD differences at the hip and lumbar spine (35). Hip BMD was once again higher, but lumbar spine BMD was lower in black women compared to whites. This study also showed that the higher hip BMD in black women may be attributable to higher body mass. Other factors that were shown to have a positive impact on BMD was level of education and having children. The use of injectable contraception was detrimental to BMD. A group from Cape Town confirmed that the lumbar spine BMD of premenopausal black women is similar or lower than that of white women (33). The lumbar spine BMD of black- and white postmenopausal women was comparable. The hip BMD of black women, irrespective of menopausal status, was once again significantly higher than whites. The same group also showed that vertebral fracture risk is the same for black- and white women (34).

Body weight plays an important role in the higher hip BMD found in black South African women, but histomorphometric differences also do exist between black- and white individuals (30, 31, 35, 37). Evaluating ethnic differences in trabecular microstructure, it was found that trabecular thickness and turnover were increased in blacks (37). This may lead to improved bone quality and a decreased fracture risk in blacks. The hip mainly consists of cortical bone (38). A cortical bone histomorphometry study showed more efficient cortical bone metabolism along with increased cortical thickness in blacks (31). The more effective osteoblast function was associated with a greater mineral apposition rate and bone formation. These histomorphometric differences may play a role in the lower hip fracture prevalence found in black South African women (South African FRAX study: Unpublished), as the hip is mainly composed of cortical bone.

Finally, in certain ethnic groups it has been suggested that the skeleton is relatively protected against the negative effects of endocrinopathies like hyperparathyroidism (39, 40). In an African-American patient cohort suffering from hyperparathyroidism, no skeletal involvement could be detected on x-ray (39). Synthetic parathyroid hormone (h(1-34)PTH) was administered to black and white women from the USA, and their skeletal response monitored (40). There was no difference in the markers of bone formation, but the African American women had significantly lower markers of bone resorption, indicating resistance to the detrimental effects of excess PTH. This raises the question of whether the skeleton of black women is also protected against the detrimental effects of GD.

2.2 **The Thyroid and Bone**

The effect of thyroid hormones on bone health can be divided into two main categories, namely: direct and indirect effects. Direct effects of the thyroid on bone are largely mediated by triiodothyronine (T_3) and thyroid-stimulating hormone (TSH). The indirect effects include the influence of the thyroid on cytokine metabolism, body composition, bone-mineral metabolism and sex hormones (15, 16, 18, 41-49).

2.2.1 ***The Direct Effects of Thyroid Hormones on Bone:***

2.2.1.1 **Triiodothyronine (T_3) and Bone Growth / Development**

The thyroid hormone, triiodothyronine (T_3), plays an important role in the development and maintenance of healthy bone. The thyroid hormones, T_3 and T_4, enter target cells via active transport mechanisms (50). The thyroid hormones transport proteins include monocarboxylate transporter 8 (MCT8), MCT10, and organic anion-transporting polypeptide 1C1 (OATP1c1) (50, 51). The thyroid mainly produces thyroxine (T_4) (52). T_4 is converted intracellularly to T_3 by deiodinases (52). The thyroid hormone receptor forms part of the nuclear receptor family (53), and two different genes encode for the two receptor types, TR-α and TR-β (54, 55). There are multiple mRNA thyroid receptor isoforms and TR-α1, TR-α2 and TR- β1 are expressed in osteoblasts, osteoclasts, osteocytes and chondrocytes (56) (Fig. 2.1).

It is through these receptors that T_3 has its effects on bone metabolism (57-60). Adult mice, devoid of the thyroid receptors, TR-α1 and TR- β (TR-$\alpha 1^{-/-}$ TR- $\beta^{-/-}$), have growth retardation. Bone histology of these animals displays delayed endochondral bone formation and reduced ossification (61, 62). Mutations of TR-α1 in humans are associated with growth retardation, delayed endochondral bone, formation, reduced ossification, reduced growth hormone and IGF-1 (63). This manifests clinically with features of severe hypothyroidism, including delayed bone age (64). Mutations in TR-β can be associated skeletal phenotypes ranging from severely abnormal to normal (52).

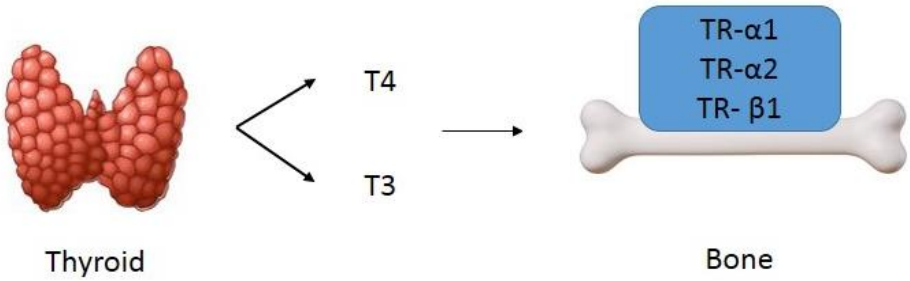

Figure 2.1: Thyroid hormone receptors in bone. T3: Triiodothyronine (T_3). T4: Thyroxine. TR: Thyroid receptors. (56)

Endochondral bone formation is the process whereby long bones and vertebrae grow (65). The process is initiated when mesenchymal stem cells at the epiphyseal growth plate are transformed into chondrocytes. These chondrocytes then undergo a regulated process of proliferation, maturation, and hypertrophy. New bone is formed when the hypertrophied chondrocytes start producing matrix components and undergo apoptosis. The surrounding cartilage matrix then mineralizes (57, 58, 65-67). T_3 plays an important role in regulating endochondral bone formation via direct and indirect pathways (57, 65, 68-70). T_3 promotes terminal differentiation of the chondrocytes and stimulates mineralization (71-73).

Childhood thyrotoxicosis, which is attended by increased T_3 levels, is associated with accelerated endochondral bone formation, skeletal maturation and linear growth (57, 74). This leads to accelerated growth and advanced bone age, which may be complicated by early closure of skull sutures or craniosynostosis, premature growth plate closure, and impaired adult height (58).

2.2.1.2 **Triiodothyronine (T_3) and Bone Remodelling**

Bone health is maintained by the sophisticated process of bone remodelling (75). Remodelling occurs on the surface of the trabeculae in cancellous bone (76), while in cortical bone remodelling takes place on the cortical surfaces as well as intra-cortically (77). A Basic Multi-cellular Unit (BMU) is formed during remodelling (76). The BMU consists of osteoclasts, osteoblasts, and osteocytes. Bone remodelling starts when osteoclasts are activated, and these activated osteoclasts remove bone down to a predetermined depth. New bone is then formed by the osteoblasts. This whole process takes place within the Bone Remodelling Compartment (BRC) and the mean duration of the process is 200 days (76, 78).

Increased bone resorption by osteoclasts is associated with an increase in urinary cross-linked N-telopeptide of type 1 collagen (NTX), cross-linked C- telopeptide of type 1 collagen (CTX), free deoxypyridinoline (D-PYR), free pyridinoline (PYD), hydroxyproline (HYP) and serum isoform 5b of tartrate resistant acid phosphatase (TRACP5b). Increased osteoblast activity is associated with an increase in serum bone-specific alkaline phosphatase (BAP), osteocalcin, c-terminal propeptide of type I procollagen (PICP) and n-terminal propeptide of type I procollagen (PINP) (78, 79).

Thyrotoxicosis is associated with an increase in bone turnover (18). Hyperthyroid rats and humans alike show an increase in osteoblast activity as illustrated by an increase in BAP and osteocalcin (18, 80, 81). Elevated BAP levels can persist up to a year after normalization of thyroid function (82). Hydroxyproline levels (reflecting bone resorption) are also increased during thyrotoxicosis (46, 83). Kinetic studies of bone metabolism in hyperthyroid patients have shown that there is a shortening of the bone remodeling cycle (46). The amount of bone resorbed stays constant, although the resorption rate is accelerated. An increased rate of bone formation does not adequately compensate for the shortened remodeling cycle and leads to a net loss of bone per cycle.

A bone histomorphometric study, performed on white females from the northern hemisphere, confirmed the development of metabolic bone disease during thyrotoxicosis (18). A significant increase in osteoclast function during thyrotoxicosis was found. This leads to a decrease in trabecular and especially cortical bone volume and an increase in urinary excretion of calcium and phosphorus. Bone mineralization is also increased. The

investigators could not define a specific type of metabolic bone disease but concluded that the bone disease found during thyrotoxicosis is specific to the endocrinopathy.

Drug-induced thyrotoxicosis in a rat model resulted in a reduction of BMD (84). This was especially true of the femoral BMD. Data in humans are conflicting (85-87). T_4 therapy at high dosages reduces BMD at the radial, lumbar and hip sites (88). Other authors have not confirmed this and have either shown that thyroid replacement therapy has no effect on BMD or that it's effect is very site specific, e.g.: the distal radius (85-87, 89). It seems that the effect of thyroid replacement therapy may be dose, gender and age specific, with postmenopausal women being most at risk of deteriorating BMD (87, 90-92). Kim *et al.* (91) demonstrated that the effect of levothyroxine therapy on the female skeleton may be related to menopausal status and time elapsed after surgery. In their study, BMD determined by DXA was measured at baseline and at one year in an early postoperative group and a late postoperative group (long-term thyroid replacement therapy). Postmenopausal women, within 1 year after undergoing thyroidectomy for thyroid carcinoma, and initiated on TSH-suppressive therapy, had increased loss of BMD when compared to baseline. No decrease in BMD was observed in the late postoperative group. The risk of developing osteoporosis after thyroidectomy for thyroid carcinoma and being on suppressive doses of levothyroxine with a median TSH of 0.4mIU/L was also evaluated in the USA (92). The risk of developing osteoporosis in women on suppressive doses of levothyroxine was 3.5 times greater compared to those who were not suppressed. It was shown that the risk was even greater with advancing age (hazard ratio of 4.3). This increased risk of fracture in patients with suppressed levels of TSH has been confirmed by others (93). Replacement therapy, which is insufficient to suppress TSH levels, does not seem to be associated with increased fracture risk (89, 93).

The deterioration in BMD associated with thyrotoxicosis is reversible (17, 94-96). Although the BMD returns to baseline after normalization of the thyroid function (14), the data on fracture risk is inconclusive. Some studies have shown a persisting fracture risk after normalization of the thyroid function, suggesting a long lasting effect of thyroid hormone on bone quality (12).

2.2.1.3 **Thyroid-stimulating Hormone (TSH) and Bone**

TSH receptors (TSH-R) are avidly expressed in extra-thyroidal tissue including lymphocytes, the pituitary, adipose tissue, fibroblasts as well as in osteoblast and osteoclast precursors (97-99).

TSH affects skeletal remodeling directly via binding to its receptor on osteoblasts and osteoclast precursors (97, 100). Studies in mice have shown that TSH inhibits osteoclastogenesis through binding to the TSH-R on osteoclast precursors (100). This leads to an inhibition of c-Jun N-terminal kinase (JNK) phosphorylation and nuclear factor kappa-B (NFκB) translocation, subsequently suppressing osteoclast formation. TSH also increases osteoclast apoptosis (100). Interestingly, TSH also inhibits osteoblast differentiation directly by down regulation of low-density lipoprotein receptor-related protein 5 (LRP-5) and the kinase insert domain receptor, Flk-1, which are important factors in osteoblast differentiation (97).

Studies in humans found a significant inverse correlation between levels of TSH and markers of bone turnover (99). It has also been shown that there is a strong association between low TSH values and BMD, with a lower TSH being associated with a lower BMD, even in euthyroid individuals, independent of the thyroid hormone (T_3 and T_4) levels (98, 101, 102). This low BMD also correlates with an increased fracture risk (89). The administration of recombinant human TSH alpha (rhTSH) to female subjects leads to an increase in the markers of osteoblast activity and an inhibition of bone resorption (103-105).

In conclusion, it seems that thyroid hormone stimulates bone remodeling and that TSH is an independent negative regulator of bone resorption.

2.2.1.4 **Skeletal Consequences of Thyroid Disease in Adults**

2.2.1.4.1 *Subclinical thyroid disease*

Subclinical thyroid disease or mild thyroid disease is defined as a TSH-level above or below the reference range, accompanied by a T_3- and T_4 level within the reference range (106).

Blum *et al.* (107), performed a meta-analysis evaluating the effect of sub-clinical thyroid disease on fracture risk. Sub-clinical hyperthyroidism was defined as a TSH level below 0.45 mIU/L. During 762 401 patient years of subclinical hyperthyroidism and after adjusting for age and gender, it was found that the hazard ratio for sustaining a hip fracture was 1.36, any fracture 1.28, non-spine fracture 1.16 and spinal fracture 1.51. The risk of fracture increased with decreasing TSH levels and a TSH of less than 0.1mIU/L was associated with a more than 2-fold increase in risk, especially for spinal fracture. A more recent meta-analysis of cohort studies, including patients that were followed for 3 months and up to 13 years, was recently published (108). The analysis showed that the lumbar spine BMD of both females and males were not influenced, but the femoral BMD of females were decreased. An increased femoral fracture risk was observed in both females and males.

Subclinical hypothyroidism is characterized by T_3- and T_4 levels within the reference range and a TSH level that is raised above the normal reference value (106). It is classified into two categories: a TSH value between 4.5-10 mIU/L or a TSH value above 10 mIU/L. A study of bone turnover markers in women with subclinical hypothyroidism showed an increase in markers of bone remodeling once thyroid hormone replacement was initiated (109). The active therapy group had a significant decrease in bone mineral density at the lumbar- and trochanteric sites when compared to the placebo group at the end of the trial period. Unfortunately, no fracture data are available for this study. Two recent meta-analyses looking at subclinical hypothyroidism and fracture risk could not confirm an increased risk associated with subclinical hypothyroidism (107, 110).

2.2.1.4.2 *Overt thyroid disease*

Overt thyroid disease can be divided into hypothyroidism and hyperthyroidism. Overt hyperthyroidism is characterised by low serum thyroid-stimulating hormone (TSH) concentrations and raised serum concentrations of thyroid hormones: T_4, T_3, or both (111). Overt hypothyroidism is characterised by increased serum thyroid-stimulating hormone (TSH) concentrations and decreased serum concentrations of thyroid hormones (112).

Hyperthyroidism is associated with a decrease in BMD and an increase in fracture risk (17, 113). In a recent publication it was shown that up to 6% of newly diagnosed fractures in patients above the age of 50 years were associated with hyperthyroidism (113). The decrease

in BMD is associated with a two- to four fold increase in risk of fracture risk when compared to healthy controls (13, 89). This increased risk is augmented by advancing age (14). Hyperthyroidism is not only associated with increased risk of fractures of the distal radius or spine, but also the hip (13, 14). The risk of hip fractures in hyperthyroid patients increases exponentially with ageing (14). It has been postulated that this phenomenon may be due to the inability of the ageing skeleton to respond adequately to the increased bone loss (14, 46, 114). The BMD loss is reversible with adequate treatment of the hyperthyroidism (14). The fracture risk may persist up to 5 years after the diagnosis of hyperthyroidism and the use of anti-thyroid drugs, compared to radio-active iodine, is associated with accelerated recovery (115). It does not seem that the cause of thyrotoxicosis has differing effects on BMD (96, 116, 117).

Hypothyroidism arises when the thyroid gland is unable to produce sufficient amounts of T_4 and/ or T_3 in order to fulfil the body's requirements (118). Hypothyroidism can be classified as either primary or secondary (119).

Primary- or overt hypothyroidism occurs when the thyroid gland fails to produce enough thyroid hormones (120). This decrease in thyroid hormones influences the hypothalamus-pituitary-thyroid feedback loop and an increase in TSH (121). Auto-immune thyroiditis is the most common cause of primary hypothyroidism in iodine sufficient countries (122). Other causes include surgery, radio-active iodine therapy and drugs. Secondary hypothyroidism occurs in conditions where the hypothalamus and/ or pituitary is affected and leads to insufficient TSH production (119). Patients with secondary hypothyroidism may have normal to high levels of TSH, but with low levels of T_4 or low levels of TSH accompanied by low T4 levels (119, 123). Secondary hypothyroidism is rare and may be due to pituitary mass lesions, trauma, and infarctions.

Most patients suffering from hypothyroidism has primary hypothyroidism. 4.6% (0.3% clinical and 4.3% subclinical) of the U.S. population was found to suffer from hypothyroidism during NHANES III (124). In the Colorado Thyroid Disease Prevalence Study, using a TSH level of > 5.1mIU/L as raised, 9.5% of the population had increased TSH levels (125). In patients older than 60 years of age and using a TSH of more than 10mIU/L, it was found that 5.9% of women had abnormal values in the Framingham study (126). This was almost three times higher than the prevalence observed in men. 9.3% of women and

1.2% of men had a TSH level above 10mIU/L during the Wickham study conducted in the United Kingdom (127, 128).

Histomorphometric studies comparing bone remodelling in hypothyroid versus euthyroid females have been performed (129, 130). It was found that hypothyroidism is a low bone turnover state associated with reduced osteoclastic bone resorption and osteoblastic bone formation. Hypothyroidism is associated with decreased levels of markers of bone resorption and bone formation (131). This correlates with the histomorphometric studies. Bone turnover markers in patients on long-term replacement therapy correlate with those of euthyroid individuals (132, 133). At least two studies have concluded that hypothyroidism has no impact on BMD (134, 135). Currently it seems that overt hypothyroidism is not associated with an increased fracture risk (52).

2.2.1.4.3 *Variations within the normal reference range*

The Osteoporosis and Ultrasound Study (OPUS) investigated the effect of variations of thyroid function within the normal reference range on BMD and non-vertebral fractures in postmenopausal women (136). It was found that higher T_3- and T_4 levels were associated with lower BMD at the hip and an increased risk of non-vertebral fractures. A higher TSH was protective against fractures. A low normal TSH has been shown to correlate with a decreased BMD and an increased risk of vertebral fracture in post-menopausal women with normal thyroid function (137, 138). In a Norwegian study it was found that a high normal TSH along with positive thyroid peroxidase antibodies (TPOAb) may be associated with a higher hip fracture risk (102). It seems that this increased fracture risk with thyroid function within the normal reference range is more prominent in women (139, 140). The association between TSH level and fracture risk has not been confirmed in all studies (101).

2.2.2 ***The Indirect Effects of Thyrotoxicosis on Bone:***

2.2.2.1 **The Cytokines**

Patients suffering from thyrotoxicosis have increased levels of the cytokines interleukin-6 (IL-6), interleukin-8 (IL-8), TNFα and its soluble receptor, sTNFR-I (141-145). T_3 directly stimulates IL-6 and IL-8 production from bone (143, 146), and the underlying autoimmune

disorder associated with GD may stimulate IL-6 production from adipose tissue (147). It has been suggested that TSH-R Abs directly stimulate TNFα production (148). In a mouse model it was shown that TNFα stimulates osteoclastogenesis (41).
TSH can affect bone metabolism indirectly via its effect on the production of the cytokine tumor necrosis factor-α (TNFα) (41). TSH is usually reduced during thyrotoxicosis. TSH dramatically reduces TNFα production by osteoclast precursors and thereby reduces its stimulatory effects on bone resorption (41). Resolution of the thyrotoxicosis is followed by a return of the TNFα- and its receptor levels to normal (141).

2.2.2.2 **Calcium, PTH and Vitamin D**

Thyrotoxicosis is associated with an increase in serum calcium- and a decrease in parathyroid hormone levels (PTH) (149). This leads to an increase in urinary calcium- and phosphorus excretion (18, 46, 47). The net effect is a negative calcium balance. The increase in serum calcium is independent of PTH and may be due to thyroid hormone induced bone resorption (150). Calcitriol (1, 25-dihydroxyvitamin D) ($1,25(OH)_2D$) levels are also reduced during thyrotoxicosis (45, 48, 49). These factors have a negative impact on bone mineral content.

2.2.2.3 **Body Mass and Leptin**

The relationship between body mass index (BMI) and bone mineral density (BMD) has been studied extensively. Early studies reported a positive association between total body weight and BMD (151, 152). This relationship, however, is dependent on the type of body fat and its distribution. BMD and abdominal visceral adipose tissue are inversely related (153-155) while subcutaneous fat has a positive effect on bone structure and strength (156). The risk of fracture in overweight and obese patients is site specific (157). Fractures of the humerus, ankle and upper forearm are much more common in the overweight group. Hip-, pelvis- and wrist fractures are decreased. Differences in the fall patterns of obese patients compared to non-obese subjects may influence the fracture sites.

Hyperthyroid patients usually lose large amounts of fat and lean mass. After treatment these patients have a sharp increase in body weight (15, 16, 42). It has been shown that during the early recovery phase preference is given to the recovery of muscle mass and the deposition of visceral adipose tissue while subcutaneous adipose tissue increases at a later stage (42).

Leptin is a cytokine that is derived from the Obese (*ob)* gene (158). Although it is mainly produced by adipose tissue, it has been shown that leptin is also produced by a series of other tissues, for example the placenta and stomach (159). Leptin is an indicator of fat mass and plays an important role in regulating appetite and subsequently energy expenditure. It has an anorexogenic effect via its receptors in the hypothalamus (160, 161). Patients suffering from obesity have increased levels of leptin (162). Leptin values differ between patients from different ethnicities (162, 163). African American and black South African women seem to have higher levels of leptin than their white counterparts of comparable BMI (35, 164, 165).

Leptin plays an important role in regulating skeletal health. A mouse study investigated the effect of leptin receptor-deficiency on bone health (166). The investigators found that reduced leptin-signalling led to reduced bone mass, confirming the anabolic effects of leptin. These findings have been confirmed by other studies (167-170). The positive effects of leptin on bone formation in mice also relates to a decrease in fractures (171).

Although the relationship between leptin and BMI in humans have been well-defined, the relationship between leptin and BMD seems less clear. There have been reports of a positive correlation between leptin and BMD (172-174), but others have showed either a neutral or a negative relationship (163, 175-180) . Data on the effect of leptin on fractures show that there is an inverse relationship, even after controlling for BMI (172, 179, 181, 182), although others could not confirm this (183).

Patients suffering from thyrotoxicosis experience severe weight loss. This weight loss includes losses of both fat and lean mass. Studies investigating the association between thyrotoxicosis and leptin have revealed conflicting results, with some showing an increase and others a decrease in serum leptin values (184, 185). Opinions are also divided on whether the changes in the leptin values found during thyrotoxicosis are due to the changes in BMI, or due to the direct effects of thyroid-stimulating hormone (TSH) and the thyroid hormones (T_3 and T_4) (147, 159, 186-196).

2.2.2.4 **Sex Hormones**

Sex hormone levels also change during thyrotoxicosis (43, 44). It has been shown that the free (active) fractions of the sex hormones, oestrogen, and testosterone, are reduced during thyrotoxicosis. These reductions may compound the negative effects of thyrotoxicosis on bone health.

2.3 **Racial Differences in Body Fat Distribution**

There are racial differences in the abdominal adipose tissue depot distribution. White men and women have increased abdominal visceral adipose tissue and decreased subcutaneous adipose tissue compared to black men and women (197, 198). A South African study evaluating racial differences in BMD, found that black women had significantly higher BMD at the hip (35). Fat free soft tissue mass was lower in black women but had the biggest effect on BMD. Body fat had a negative effect on BMD at all sites in black women, but fat mass was the biggest contributor to BMD in white women at the lumbar site.

The incidence of thyrotoxicosis in South Africa is unknown. During 2011, 116 patients suffering from thyrotoxicosis were admitted by the Division of Endocrinology, UAH [Endocrine Annual Report, 2011]. The majority of these were black women suffering from GD. These admission figures correlate well with a previous study performed in Johannesburg, South-Africa (3).

2.4 **Conclusion**

Thyrotoxicosis has detrimental effects on bone metabolism, bone mass and bone structure which are reasonably well characterized in white female patients. These effects may lead to an increased risk of fragility fractures that persists even after normalization of the BMD in some (e.g. postmenopausal women) but not all subjects.

In black populations from the USA, it has been documented that the skeleton is relatively protected against the negative effects of endocrinopathies like hyperparathyroidism (39, 40). This raises the question whether they are also protected against the detrimental effects of GD?

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Chapter 3

Methodology

3.1 **Study Design**

This was a prospective exploratory and comparative study.

3.2 **Patient Selection**

A convenience sample of 40 consecutive and consenting black female patients (age ≥ 25 and ≤ 65 years) and 20 consecutive and consenting white female patients (age ≥ 25 and ≤ 65 years) with confirmed GD referred to the Endocrine service at Universitas Hospital were recruited for the study. Patients were matched with 40 black and 20 white healthy control females according to age (± 5 years), body mass index (BMI) and seasonality (Table 3.2.1). Patients and controls were matched according to BMI ranges (Underweight, Normal, Overweight, Obesity class 1/2/3), e.g.: a patient with a BMI of 27 kg/m^2 (overweight) was matched with an overweight control (1). For seasonality, the year was divided into 2 seasons: Season 1 = Winter & Spring; Season 2: Summer & Autumn. A patient from season 1 was matched with a control from season 1. All three these criteria had to be met before enrolment into the study. Thyrotoxicosis was defined as a suppressed or an undetectable serum TSH level, and an increased serum T_4 and/or T_3 level(s), above the reference range. GD was defined as the biochemical confirmation of thyrotoxicosis along with positive thyroid-stimulating hormone receptor antibodies (TSH-R Abs) and/or a diffusely increased radionuclide uptake during a technetium- and an I^{123} thyroid scintigram. Menopause was defined according to the World Health Organisation criteria (2). The absence of menses for 12 consecutive months without any other obvious reason or iatrogenic menopause was used to diagnose menopause. The control group consisted of healthy volunteers that were recruited by means of an advertisement posted on the intranet of the University of the Free State as well as an advertisement placed on notice boards at UAH (Appendix 3A). The advertisement was approved by the Ethics Committee of the Faculty of Health Sciences, University of the Free State (ECUFS).

The sample size was because this was an exploratory study with an invasive component. The study design was reviewed by the University of the Free State (UFS) School of Medicine's PhD-evaluation committee and approved by the Ethics Committee of the Faculty of Health Sciences, University of the Free State (ECUFS 49/2012). The inclusion and exclusion criteria for subjects and controls are shown in Table 3.3.2 and Table 3.3.3 respectively.

Table 3.2.1. Number of study subjects planned

	Black Females	**White Females**
Patients	40	20
Controls	40	20

Table 3.2.2. Patients: Inclusion & exclusion criteria

Inclusion Criteria:	**Exclusion Criteria:**
Black/ white consenting female	Pregnancy
Age ≥ 25 and ≤ 65 years	Thyroid storm (3) (Appendix 3B)
GD	Patients suffering from chronic diseases other than thyrotoxicosis (e.g. HIV) that might affect bone metabolism
	Previous- (< 6 months) and current bone-affecting drugs (e.g.: glucocorticoids; diuretics, etc.)
	Patients who refuse to participate
	Patients not capable of giving consent (e.g. due to mental health problems).

Table 3.2.3. Healthy controls: Inclusion & exclusion Criteria

Inclusion Criteria:	**Exclusion Criteria:**
Black/ white consenting female	Pregnancy
Age ≥ 25 and ≤ 65 years	Previous thyroid disease
Normal thyroid function	Patients suffering from any chronic disease (e.g. HIV) that might affect bone metabolism
	Previous- (< 6 months) and current bone-affecting drugs (e.g.: glucocorticoids; diuretics, etc.)
	Patients who refuse to participate
	Patients not capable of giving consent (e.g. due to mental health problems).

3.2.1 ***Investigation Site***

Universitas Academic Hospital (UAH) is one of two tertiary hospitals situated in the Mangaung metropolitan area. The hospital is situated in the city of Bloemfontein, the judicial capital of the Republic of South Africa. The hospital mainly serves patients from the Free State province, but also delivers services to patients from the Northern Cape Province and the small neighbouring country of Lesotho. According to Statistics South Africa, the Free State province had a population of 2.7 million in 2011 (4). Women comprised 52% of the population. Black Africans made up 88% of the population and two thirds were Sotho speaking.

During 2011, 116 patients suffering from thyrotoxicosis were admitted by the Division of Endocrinology, UAH (Appendix 3C). Ninety percent (90%) of these patients suffered from GD. The majority were black women.

3.3 **Duration**

The planned recruitment period of both subjects and controls was 2 years.

3.4 **Study Method**

3.4.1 ***Consent***

Informed patient consent for participating in the study as well as for an iliac crest bone biopsy was obtained on the first day of admission. Patients received verbal information (English, Afrikaans, and Sotho) along with an information leaflet (English and Afrikaans) before entering the study (Appendix 3D). Patients participating in the study signed two separate consent forms: The first for participating in the study and the second for the bone biopsy (Appendix 3E & 3F). Both consent forms were approved by the ECUFS. After obtaining consent, the consent for participating in the study was kept in the patient's study folder along with the other study records and data, while the consent for the biopsy was stored in the patient's hospital records.

Informed consent for participating was obtained from the control females on the day of the interview (the same protocol was followed as above). The form was stored in the control subject's study file that was generated when the patient came for a bone mineral density test (Appendix 3G & H).

3.4.2 ***Routine investigations***

The following ***routine investigations*** as per Endocrine Unit protocol, UAH, for investigating and managing patients with thyrotoxicosis, were performed. The laboratory investigations were performed by the National Health Laboratory Services (NHLS).

Table 3.4.1. Baseline investigations

Patient questionnaire[1]		(Appendix 3I)
Full Blood Count	Haemoglobin Haematocrit White cell count Platelets	
Urea &electrolytes	Sodium Potassium Creatinine Calcium Magnesium Phosphate	
Liver tests	Total bilirubin Conjugated bilirubin Aspartate transaminase (AST) Alanine transaminase (ALT) γ-Glutamyl transferase (GGT) Alkaline phosphatase (ALP)	
C-reactive protein (CRP)		
Thyroid function tests	Thyroid stimulating hormone (TSH) Triiodothyronine (T_3) Thyroxine (T_4)	

Table 3.4.1. (Continued)

Thyroid antibodies	Thyroid stimulating hormone receptor Thyroglobulin Thyroxine peroxidase	
β-Human Chorionic Gonadotropin		
Lipid profile		
Fasting plasma glucose		
Pro-BNP		Performed if left ventricular dysfunction suspected on clinical grounds.
ECG		
Echocardiogram		Performed if cardiac involvement of heart suspected on clinical grounds.
X-Ray chest		If indicated
DXA Bone Scan	Distal radius Lumbar spine Femoral (Total Hip & Femoral Neck)	
CT / MRI Orbit		If indicated
Technetium-99m radionuclide uptake scintigram		
Thyroid ultrasound		If indicated

[1]All documents listed were part of original ethics application.

3.4.3 ***Additional laboratory investigations***

The following additional laboratory investigations (specific to study) were performed on day 3 of admission after the diagnosis of GD had been confirmed. Blood samples were taken by a phlebotomist at 8 am after the patient had fasted for 8 hours. All additional laboratory analyses were performed by Ampath Laboratories, Universitas Private Hospital, Logeman St, Universitas, Bloemfontein.

Table 3.4.2. Additional laboratory investigations

Category	**Laboratory test:**	**Specimen handling**	**Method of analysis:**
Bone formation	*Serum osteocalcin* (Supplier: Roche, Germany)	Blood collected into cold plain tubes. The serum was separated from the cells by centrifugation, aliquoted and frozen immediately into a plastic tube. Sent on ice.	Direct, two-site, sandwich type chemiluminescence immunoassay (CLIA).
	Serum bone specific alkaline phosphatase (Supplier: Beckman Coulter, USA)	Transported at 2-8°C to national reference laboratory.	Access Ostase-Immuno-enzymatic assay.
Bone Resorption	*Urinary deoxypyridinoline* (Expressed as a ratio to urinary creatinine) (Supplier: Siemens, USA)	Urine was wrapped in foil.	Solid phase, enzyme-labelled chemiluminescent competitive immunoassay.

Table 3.4.2. (Continued)

Category	**Laboratory test:**	**Specimen handling**	**Method of analysis:**
Vitamin D	*25-hydroxyvitamin D* (25(OH)D) (Supplier: Beckman Coulter, USA)	Sent ambient to national referencing laboratory.	Chemiluminescent immunoassay.
	1,25-dihydroxyvitamin D ($1,25(OH)_2D$) (Supplier: Beckman Coulter, USA)	Sent on ice to national referencing laboratory.	Vitamin D radio immunoassay.
Bone metabolism	*Parathyroid hormone* (PTH) (Supplier: Roche, Germany)	Centrifuge in an EDTA tube and then centrifuged to separate plasma from cells. Plasma was pipetted and frozen.	Electrochemiluminescence immunoassay.
Body composition	*Leptin*	Information not available.	
	Insulin growth factor 1 (IGF-1) (Supplier: Siemens, USA)	Sample centrifuged and serum separated from cells. Serum frozen immediately.	Chemiluminescent immunometric assay.
Inflammation	*Interleukin 6* (IL-6) (Supplier: Beckman Coulter, USA)	Sent at ambient temperature to national referencing laboratory.	Chemiluminescent immunoassay.

Table 3.4.2. (Continued)

Category	**Laboratory test:**	**Specimen handling**	**Method of analysis:**
Inflammation (Continued)	*Tumour necrosis factor alpha* (TNFα) (Supplier: Randox, UK)	Collected and stored at -20°C. Transferred to national reference laboratory.	Sandwich chemiluminescent immunoassay.

3.4.4 ***Additional non-laboratory investigations***

The following special investigations (non-laboratory) were performed once the diagnosis of GD had been confirmed.

3.4.4.1 **Standard posterior-anterior and lateral spinal X-Ray**

Images of the thoraco-lumbar spine were obtained if indicated. Indications included lower back pain, kyphosis, etc. The images obtained were reported by the department of Radiology, UAH, and reviewed by the investigator.

3.4.4.2 **Total Body DXA Scan**

A total body DXA scan was performed to obtain body composition. Lateral imaging of the thoracic- and lumbar vertebrae was performed to assess vertebral morphology. Vertebral Fracture Assessment (VFA) for vertebral fractures was done according to the Genant classification (5).

The DXA scan was performed on a Discovery W QDR Hologic Densitometer (model S/N 70494) Hologic USA.

A single operator, Me. P Pienaar, performed all the DXA scans. Before commencing with the study, Me. Pienaar attended the National Osteoporosis Foundation of South Africa

(NOFSA) DXA course to review current standards of operation. The densitometer was serviced annually.

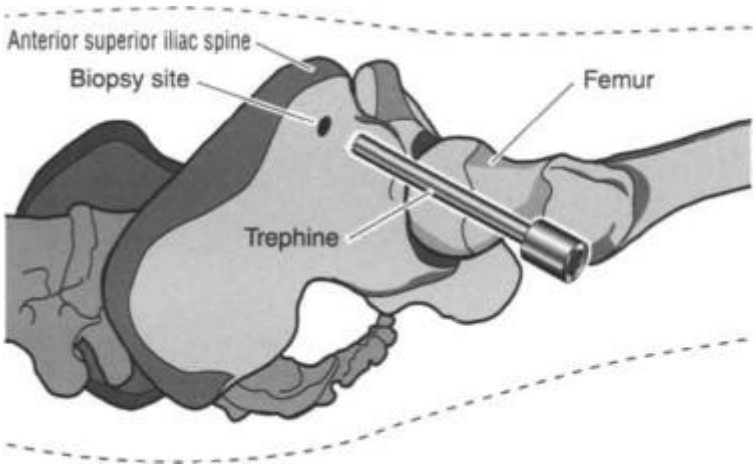
To maintain quality control daily phantom screening was performed and coefficient of variance (CV) was calculated. The CV for the lumbar spine was 1.5 and the other sites 2.3.

3.4.4.3 **Double Tetracycline-Labelled Bone Histomorphometry**

A Danish histomorphometry study on white female patients suffering from GD has been performed and published previously (6). Bone histomorphometry was therefore only limited to black female patients diagnosed with GD. Black GD patients with a DXA bone mineral density Z-score of ≤ -2 standard deviation (SD) (low bone mass) or a T-score of ≤ -2.5 SD (osteoporosis) at any of the measured skeletal sites and / or a prevalent fragility fracture at the time of diagnosis were considered. The diagnosis of fracture was based on history of a fragility fracture, or the presence of a fracture on x-ray or an important deformity (≥ 25% abnormality) on VFA. The white female database supplied by Hologic® was used as reference population. The purpose of performing bone histomorphometry on this subset of black women was to assess the type of metabolic bone disease and more specifically to detect evidence of osteomalacia or a mineralization defect induced by thyrotoxicosis.

Consent for bone biopsies was obtained on the standard Universitas hospital consent form. Prior to the biopsy, patients received a course of oral tetracycline at a dosage of 300mg q.i.d for the first three days. This course of tetracycline was repeated 14 days later. Two to four days later a bone biopsy was performed under local anaesthetic along with light sedation, by the Department of Orthopaedic Surgery, University of the Free State. The biopsy was obtained from a standard site, namely the anterior iliac crest, using a Meunier or Bordier trephine needle (Image 3.1). Care was taken to include both cortices in the biopsy.

Figure 3.1 Trans-iliac Bone Biopsy (7)



The biopsy material was placed in absolute alcohol, clearly marked, sent via overnight courier to the Metabolic Bone Disease Unit, Division of Endocrinology, University of Stellenbosch where the biopsy specimen was handled for interpretation of the specimen through standardized histomorphometry criteria.

Patients found to have an abnormal result on histology were requested (voluntarily) to have a repeat biopsy one year after commencement of treatment.

3.4.4.4 **Stored blood**

Consent for genetic testing was obtained during the initial informed consent process.

Blood samples were collected and frozen at minus 40 degree Celsius for future analyses.

Stored blood samples are meant for future genetic testing.

3.4.5 ***Procedure for control subjects***

The following investigations were performed on ***control subjects*** after fulfilling the study entry criteria:

- Control Questionnaire & Results Sheet (Appendix 3J)

- TSH (T3 & T4 if TSH-value abnormal)
- Serum osteocalcin
- Urinary deoxypyridinoline
- 25(OH)D
- $1,25(OH)_2D$
- Parathyroid hormone
- Leptin, IGF-1, TNFα & IL-6
- DXA Bone scan (distal radius; lumbar spine & femoral [total hip & femoral neck) & VFA
- DXA measurement of body composition

3.4.6 ***Follow-up investigations in black- and white female patients***

The following investigations were ***repeated*** at 6 weeks, at 6- and 12 months in all participating patients:

- 6 Weeks:
  a. TSH & T_4

- 6 Months:
  a. TSH & T_4
  b. DXA & VFA
  c. DXA for body composition

- 12 Months:
  a. TSH & T_4
  b. DXA & VFA
  c. DXA for body composition

- Bone histomorphometry (black female patients who underwent a bone biopsy at baseline and who gave consent for a repeat bone biopsy)

3.5. **Data handling and statistical analyses**

3.5.1 An Excel® database was created. The data capturing was done by Dr. G.M. Oosthuizen Department of Internal Medicine and me. The document was inspected for errors and outliers, and the data was cleaned before statistical analysis.

3.5.2 The Department of Biostatistics, under supervision of Prof. G Joubert, was responsible for the data analysis as agreed on during periodic face to face meetings with the biostatistician.

Results were summarised by frequencies and percentages for categorical variables and medians and interquartile ranges for numerical variables due to skew distributions.

Patient groups were compared using Wilcoxon tests and 95% confidence intervals for median differences (numerical variables) and Chi-square or Fisher's (F) exact tests depending on sparseness of cells (categorical variables).

Due to the skewness of numerical data distributions, and the paired nature of the data, values were log transformed for parametric generalised linear modelling considering the matched pairs. The comparison of cases and controls regarding categorical variables was done using Mantel-Haenszel tests, stratified by matched pair. ANOVA method could not be used. See above. See Appendix 3M for matching of patients with controls.

Correlation coefficient was calculated using the Pearson method.

3.6 **Ethical approval**

3.6.1 The Post-Graduate Evaluation Committee, Faculty of Health Sciences, University of the Free State reviewed and approved the study.

3.6.2 Ethical approval from the Ethics Committee of the Faculty of Health Sciences, University of the Free State (ECUFS) was obtained to conduct the study (Appendix 3K). **Ethics Number:** ECUFS 49/2012

3.6.3 Consent to conduct the study within UAH was obtained from the then chief executive officer, Dr. N.R.J. van Zyl (Appendix 3L).

3.6.4 Subject information (patients and controls) was always kept confidential and patient identity was concealed from non-clinical staff.

3.6.5 There was no conflict of interest.

3.6.6 Consent was obtained from participants upon entry into the study (Refer 3.5.1).

3.7 **Funding**

3.7.1 The researcher deposited R80 000 of his own savings into an UFS Research Entity to get started.

3.7.2 A grant of R150 000, was obtained from Research Development at the UFS via a competitive process.

3.8 **References**

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Chapter 4

Results and Discussion: Biochemical Measurements

4.1 **Introduction**

Data collection for black and white women with GD took place in parallel from successive consenting individuals. Enrolment to the patient arm commenced on 28 May 2012 and was completed on 6 September 2014. Enrolment of black and white control subjects commenced on 14 June 2012 and was completed on 22 November 2017. Euthyroid healthy control subjects were carefully matched for age, ethnicity, body mass index (BMI) and seasonality.

In this chapter baseline biochemical measurements are reported and discussed. Data comparing white and black female subjects with GD are presented first followed by a comparison of subjects with GD (white and black) with their respective control subjects.

The baseline clinical characteristics of black and white subjects diagnosed with GD are shown in Table 4.1.1.

Although 40 black patients were initially included at baseline, patient number 6 (BP6) was excluded from the analysis as the diagnosis of GD could not be confirmed by TSH-receptor antibodies (TSH-R antibodies) or Technetium-99m radionuclide uptake scintigram.

The median mean blood pressure of the black patients (93 [90to 100] mmHg) was significantly higher compared to that of white patients (90 [90 to 93]) mmHg ($p = 0.036$) but still within the normal range. Forty percent of white patients were smoking at entry into the study compared to none of the black patients ($p = 0.0003$). There were no other significant differences between the two groups at baseline.

Eleven black patients and four white patients suffered from Graves' orbitopathy at presentation ($p = 1.0$) [Not shown in table]. Also not shown is that there was no difference between the patient groups when matched according to seasonality ($p = 0.425$ [F: Fisher's exact method due to sparse cells]).

The baseline clinical characteristics of the control subjects were well-matched with their patient groups.

Table 4.1.1. Baseline clinical characteristics of patients[1]

	Black Females with GD n = 39	**White Females with GD** n = 20	**p-value (95% confidence limits for median differences)[2] GD: Black- vs. White patients**
Age (years)	39 (30 to 48)	33.5 (31 to 46)	*0.442* (-9 to 3)
BMI (kg/m^2)	25 (21 to 32)	25 (21 to 28.5)	*0.344* (-5.0 to 2.0)
Smoking (at entry) (%)	0	40	*0.0003* (F)
Mean Blood Pressure (mmHg)[3]	93 (90 to 100)	90 (90 to 93)	*0.036* (-10.0 to 0.0)
Pulse rate at presentation (without β-blockers) (beats/ minute)	89 (80 to 110)	96 (80 to 100)	*0.811* (-12.0 to 10.0)
Duration of symptoms before presentation (months)	6 (4 to 13.5)	3 (1 to 12)	*0.079* *(-5.0 to 0.0)*

[1] Values are medians (interquartile range).
[2] Analysed with a Wilcoxon two-sample test.
[3] Diastolic pressure + (pulse pressure ÷ 3) mmHg.
BMI, body mass index. TSH, thyroid-stimulating hormone. Graves' disease, GD.

Discussion: *Baseline Characteristics*

The median mean blood pressure of both patient groups in this was within normal limits. In a study by Kandala *et al.* it was shown that up to a third of the South African population above the age of 15 years may suffer from hypertension (1). In the same study the prevalence of hypertension in the Free State province was only second to the North West province when looking at the highest prevalence in South Africa. The study also showed being black and female carried the highest risk for the development of hypertension. Although ethnic differences in the prevalence of hypertension do exist, it must be noted that thyrotoxicosis itself can be associated with increased blood pressure (2).

According to the South Africa Demographic and Health Survey 2016 more than two thirds of South African women are overweight or obese (3). Hyperthyroidism is associated with excessive weight loss (4). The median BMI of both patient groups in this study fell into the overweight (BMI = 25.00-29.99 kg/m^2 (5)) category at presentation. It must be kept in mind that the patients presented while suffering from hyperthyroidism and this may have impacted on the BMI at presentation.

Approximately 25% of patients in both patient groups suffered from Graves' Orbitopathy. Although interesting, it is not completely surprising. In a study by Tanda *et al.* it was found that more than 70% of patients did have Graves' orbitopathy at presentation with GD (6). In a Swedish study it was also shown that only 20% of patients have Graves' orbitopathy at diagnosis of the GD (7).

4.2 **Measurements of thyroid function and thyroid related autoantibodies**

Thyroid function tests results (TSH, T_4 & T_3) and serum thyroid antibody levels (TPO, TGB & TSH-R) of patients are summarised in Table 4.2.1. No statistically significant differences were found between the two ethnic groups when the median values of thyroid function tests and thyroid autoantibody levels were compared. As expected, median TPO and TGB antibody levels were raised in both groups.

Table 4.2.1. Baseline thyroid function test results and serum thyroid antibody levels[1]

	Black Females n = 39	**White Females** n = 20	**p-value (95% confidence limits for median differences)[2] GD: Black- vs. White patients**
TSH (mIU/L)	0.01 (0.01 to 0.01) n = 39	0.01 (0.01 to 0.01) n = 18	*0.554* (0.0 to 0.0)
T_4 (pmol/L)	100.0 (43.3 to 100.0) n = 39	56 (37.8 to 100) n = 18	*0.139* (-38.0 to 0.0)
T_3 (pmol/L)	37.0 (14.2 to 47.0) n = 31	18.3 (9.0 to 37.0) n = 15	*0.152* (-22.80 to 1.20)
TPO antibodies (IU/ml)	116 (18.0 to 328.0) n = 35	97.9 (13.1 to 595.0) n = 11	*0.837* (-100.0 to 225.0)
TGB antibodies (IU/ml)	34.15 (19.1 to 136.1) n = 36	25.4 (16.1 to 185.4) n = 12	*0.552* (-42.08 to 9.80)
TSH-R antibodies (IU/L)	22 (6.0 to 38.0) n = 37	10.7 (4.2 to 37.0) n = 17	*0.279* (-15.81 to 2.14)

[1] Values are medians (interquartile range).
[2] Analysed with a Wilcoxon two-sample test.
Graves' disease, GD. TSH, thyroid-stimulating hormone (reference range = 0.27-4.20 mIU/L); T4, thyroxine (reference range = 12.0-22.0 pmol/L); T3, tri-iodothyronine (reference range = 3.1-6.8 pmol/L); TPO, thyroid peroxidase antibodies (reference range < 10 IU/ml); TGB, thyroglobulin antibodies (reference range < 116 IU/ml); TSH-R antibodies, thyroid- stimulating hormone receptor antibodies (reference range < 1.75 IU/L). White females: n = 18, 18 white female patients paired with 39 black female patients. (See Chapter 3: 3.5. Data handling and statistical analyses)

Baseline thyroid function status of the two patient groups according to laboratory cut points are compared in Table 4.2.2. No statistically significant differences between the two ethnic groups were found.

A single black patient (BP33) had a suppressed TSH, normal T_4 and T_3 at presentation. Her TSH-receptor antibody titre was 6.37 mIU/L at presentation confirming GD. A radionuclide uptake scintigram also showed increased- and uniform uptake, confirming active GD. A single white patient had a normal TSH at presentation, but a T4-level of 30.54 pmol/L ($N \leq 22.0$) confirming thyrotoxicosis. Although her TSH-R antibody titre was normal, Technetium-99m radionuclide uptake scintigram confirmed active GD.

Table 4.2.2. Frequency (%) of subjects at baseline according to thyroid function status

	Black Females n = 39	**White Females** n = 20	**p-value**[1]
TSH			
Low	39 100%	17 94.4%	
Normal	0	1 5.6%	*0.316* (F*)
T_4			
Low	0	0	
Normal	1 2.6%	0	
High	37 94.8%	18 100%	*1.000* (F)
T_3			
Low	0	0	
Normal	2 6.5%	1 6.7%	
High	29 93.5%	14 93.3%	*1.000* (F)

Footnote (Table 4.2.2.)
[1]Chi-square p-value, except where indicated by (F) for Fisher's exact method due to sparse cells.
TSH, thyroid-stimulating hormone (reference range = 0.27-4.20 mIU/L); T4, thyroxine (reference range = 12.0-22.0 pmol/L); T3, triiodothyronine (reference range = 3.1-6.8 pmol/L).

TSH-R- and TPO-antibody levels were increased in more than 90% of women from both ethnic groups (Table 4.2.3.).

Table 4.2.3. Frequency (%) of subjects at baseline according to thyroid antibody level status

	Black Females n = 39	**White Females** n = 20	**p-value[1]**
TPO antibodies			
Normal	1 2.9%	1 9.1%	
High	34 97.1%	10 90.9%	*0.425* (F)
TGB antibodies			
Normal	27 75%	9 75%	
High	9 25%	3 25%	*1.000* (F)
TSH-R antibodies			
Normal	3 8.1%	1 5.9%	
High	34 91.9%	16 94.1%	*1.000* (F)

[1]Chi-square p-value, except where indicated by (F) for Fisher's exact method due to sparse cells.
TPO, thyroid peroxidase antibodies (reference range < 10 IU/ml); TGB, thyroglobulin antibodies (reference range < 116 IU/ml); TSH-R, thyroid- stimulating hormone receptor (reference range < 1.75 IU/L).

Discussion: *Thyroid function and autoimmunity*

The diagnosis of GD can be based on clinical and/or laboratory investigations (8). Clinical manifestations include a diffuse goitre and Graves' ophthalmopathy.

Laboratory investigations include positive TSH-R antibodies and/or a thyroid radionuclide scan demonstrating a diffusely enlarged goitre. In this study, median TSH-R antibody levels were increased in almost all patients with GD in both groups as was expected. A recently published study from the United Kingdom (UK) evaluated the sensitivity and specificity of the clinical diagnosis of GD versus that of the TSH-R antibody assay (9). It was found that clinical diagnosis of GD had a sensitivity of 88% and specificity 66%, while the latest generation antibody assay had a sensitivity of 98% and specificity of 99%. The normal TSH-R antibody levels found in patients included in the study thus do not exclude the diagnosis of GD but might be related to the sensitivity of the assay used.

It must be noted that the TPO and TGB antibodies were raised in both groups as well. Up to 10% of the normal healthy population may have positive TPO and TGB antibodies (10). Almost all patients with Hashimoto's thyroiditis will have positive TPO and TGB antibodies (11). In GD, 50% to 90% of patients may also have positive TPO and TGB antibodies (11). The absence of TPO antibodies, but not TGB antibodies, in GD may predict an increased risk for the development of Graves' ophthalmopathy (12). Increased TPO antibodies may also have a detrimental impact on pregnancy outcomes (11).

4.3 **Biochemical measurements related to bone metabolism**

Black and white patients

The median serum parathyroid hormone (PTH) level was significantly lower among black females with GD (5.7 pg/ml [3.1 to 15.0]) compared to white females with this condition (21.9 pg/ml [4.8 to 29.0]) ($p = 0.04$) (Table 4.3.1). The median serum calcium level in black female subjects with GD was mildly elevated (2.56 mmol/L [2.48 to 2.65]) which is in keeping with the decreased PTH levels in this group. The median serum calcium level in white women with GD was within the normal reference range (2.46 mmol/L [2.44 to 2.56]). However, the difference in median serum calcium levels between the two groups was not significant ($p = 0.133$).

Median serum bone-specific alkaline phosphatase (BAP) levels for both premenopausal groups were elevated (black females: 28.95 µg/L [16.25 to 43.90]; white females: 24.3 µg/L [16.6 to 34.2] (normal ≤ 19 µg/L) and differed significantly from their respective controls. In the black postmenopausal group the median BAP level (34.15 µg/L [28.85 to 46.55]) was also raised (normal ≤ 26 µg/L) while the median BAP level in white women was within the reference range (21.4 µg/L [14.7 to 29.1]). In the postmenopausal category the numbers of subjects and controls for both ethnic groups were, however, small. The differences in BAP levels between the two groups race were not significant.

Median serum osteocalcin levels for both ethnic groups including the pre- and postmenopausal subgroups, were within the reference range and there was no significant difference between the two groups.

The median urine deoxypyridinoline (DPD) to creatinine ratio was increased in both groups (black females: 30.6 nmol/mmol [22.9 to 45.2]; white females = 19.9 nmol/mmol [12.6 to 36.2]) and was significantly greater in black compared to white females ($p = 0.026$).

No differences in serum phosphate (PO_4) values were observed.

White patients and controls

Although the median PTH levels of both race groups were within the normal reference range, the median PTH level of the white patients (21.9 pg/ml [4.8 to 29.0]) was significantly lower than that of their control subjects (30.0 pg/ml [27.0 to 38.0]; $p = 0.005$)

The median BAP of the white patients (23.4 µg/L [(15.9 to 33.9]) was significantly higher than that of white controls (9.5 µg/L [7.1 to 12.5]) ($p < 0.0001$). The median BAP of premenopausal white patients (23.4 µg/L [15.9 to 33.9]) was above the upper limit of the reference range (≤ 19 µg/L), while the median BAP level in the control group (9.2 µg/L [7.0 to 12.5]) was well within the reference range. This difference was statistically significant ($p < 0.0001$). Although the median BAP of postmenopausal patients was higher than that of the control group, this difference did not reach statistical significance. This may be due to the limited number of patients included in this age group or other postmenopausal factors that may influence bone metabolism.

The median osteocalcin levels of both groups were within the reference range, although the level of the GD group of patients (24.4 ng/ml [16.9 to 31.3]) was significantly higher than that of the controls (12.6 ng/ml [10.1 to 15.80]) ($p = 0.0009$). The premenopausal subgroup

of patients had a significantly higher median osteocalcin level (22.3 ng/ml [15.7 to 30.1]) (reference range: 6.5- 42.3 ng/ml) compared to the control group (10.65 ng/ml [9.6 to 15.1]) ($p = 0.004$). The postmenopausal subgroup of patients also had a higher median osteocalcin level compared to the controls, but this difference did not reach statistical significance. This may once again have been due to a limited number of participants in this subgroup.

The median urine DPD to creatinine ratio of the white patient group (19.9 nmol/mmol [12.6 to 36.2]) was raised almost 3 times above the upper limit of the reference range (3.0 – 7.0 nmol/mmol), while the median level of the controls (6.5 nmol/mmol [4.9 to 7.5]) was within reference range. This difference was statistically significant ($p = 0.0002$).

Black patients and controls

The median PTH level of the black patients (5.7 pg/ml [3.1 to 15.0]) (reference range: 15-65 pg/ml) was decreased. The median PTH level along with the 25^{th}- and 75^{th} centile values were below the lower limit of normal for the reference range. The median PTH level (36 pg/ml [27 to 57]) of the control group was within the reference range and the difference in median PTH levels between patients and controls was statistically significant ($p < 0.0001$).

The median BAP level of the black patients (31.4 µg/L [18.2 to 43.9]) (reference range: 3-19µg/L) was above the upper limit of normal and significantly higher compared to that of the control group (11.9 µg/L [9.6 to 20.4]) ($p < 0.0001$). The same pattern was evident in the premenopausal subgroup. Premenopausal black patients had a median BAP level above the upper limit of normal (28.95 µg/L [16.25 to 43.90]) and this was significantly higher compared to the median BAP level in black premenopausal controls (12.10 µg/L [10.10 to 18.10]) ($p = 0.0002$). The median BAP level in the postmenopausal subgroup of black patients was higher compared to that of the controls, but this difference was not statistically significant.

Median serum osteocalcin levels of both black patients and controls were within the reference range, but the level was significantly higher in the patient group (34.5 ng/ml [23.0 to 45.0]) compared to that of the control group (11.2 ng/ml [8.1 to 13.6]) ($p = 0.0001$). The median osteocalcin level of the premenopausal patient subgroup (33.15 ng/ml [22.0 to 45.15]) was significantly higher than that of the control group (9.4 ng/ml [6.9 to 13.5]) ($p = 0.0001$). The median osteocalcin level in the black postmenopausal subgroup of patients was also higher compared to controls, but this difference was not statistically significant. The median urine DPD to creatinine ratio of the black patients (30.6 nmol/mmol [22.9 to 45.2]) was

significantly greater compared to that of controls (6.9 nmol/mmol [4.6 to 8]) ($p < 0.0001$). The median urine DPD to creatinine ratio found in black patients was more than four times above the upper limit of the reference range (reference range: 3-7.4 nmol/mmol).

Table 4.3.1. Baseline biochemical measurements related to bone metabolism[1]

	Black Females with GD n = 39	**Black Controls** n = 39	**p-value (95% confidence limits for ratio)[2] Black patients vs. Black controls**	**White Females with GD** n = 20	**White Controls** n = 20	**p-value (95% confidence limits for ratio)[2] White patients vs. White controls**	**p-value (95% confidence limits)[3] GD: Black- vs. White patients**
PTH (pg/ml)	5.7 (3.1 to 15.0) n = 35	36 (27 to 57) n = 27	*< 0.0001* (0.113 to 0.308)	21.9 (4.8 to 29.0) n = 15	30.0 (27.0 to 38.0) n = 17	*0.005* (0.237 to 0.727)	*0.04* (0.20 to 18.30)
Calcium (corrected) (mmol/L)	2.56 (2.48 to 2.65) n = 38	N.D.		2.46 (2.44 to 2.56) n = 17	N.D.		*0.133* (-0.13 to 0.02)
Phosphate (mmol/L)	1.3 (1.03 to 1.43) n = 38	N.D.		1.36 (1.27 to 1.53) n= 13	N.D.		*0.443* (-0.13 to 0.28)
BAP (µg/L)	31.4 (18.2 to 43.9) n = 36	11.9 (9.6 to 20.4) n = 27	*<0.0001* (1.608 to 2.830)	23.4 (15.9 to 33.9) n = 20	9.5 (7.1 to 12.5) n = 17	*< 0.0001* (1.84 to 3.573)	*0.20* (-14.20 to 2.60)
BAP Premenopausal (µg/L)	28.95 (16.25 to 43.90) n = 28	12.10 (10.10 to 18.10) n = 22	*0.0002* (1.452 to 2.792)	24.3 (16.6 to 34.2) n = 17	9.2 (7.0 to 12.5) n = 14	*< 0.0001* (1.899 to 4.052)	*0.59* (-12.30 to 6.20)
BAP (µg/L) Postmenopausal	34.15 (28.85 to 46.5) n = 8	11.5 (9.6 to 22.1) n = 5	*0.105* (0.777 to 5.907)	21.4 (14.7 to 29.1) n = 3	9.9 (9.8 to 17.8) n = 3	*0.179* (0.538 to 5.646)	*0.066* (-66.40 to 3.40)
Osteocalcin (ng/ml)	34.5 (23.0 to 45.0) n = 36	11.2 (8.1 to 13.6) n = 25	*< 0.0001* (1.942 to 3.710)	24.4 (16.9 to 31.3) n = 20	12.6 (10.1 to 15.80) n = 17	*0.0009* (1.403 to 3.013)	*0.081* (-16.70 to 1.30)
Osteocalcin (ng/ml) Premenopausal	33.15 (22.0 to 45.15) n = 28	9.4 (6.9 to 13.5) n = 21	*< 0.0001* (1.749 to 3.777)	22.3 (15.7 to 30.1) n = 17	10.65 (9.6 to 15.1) n = 14	*0.004* (1.329 to 3.343)	*0.143* (-17.70 to 4.30)
Osteocalcin (ng/ml) Postmenopausal	36.75 (30.7 to 43.55) n = 8	13.65 (11.75 to 24.05) n = 4	*0.137* (0.565 to 12.651)	28.6 (25.8 to 35.4) n = 3	15.9 (14.5 to 18.8) n = 3	*0.069* (0.889 to 3.723)	*0.184* (-36.00 to 17.60)
Urine DPD/ Creatinine ratio (nmol/mmol)	30.6 (22.9 to 45.2) n = 36	6.9 (4.6 to 8) n = 27	*< 0.0001* (3.114 to 5.783)	19.9 (12.6 to 36.2) n = 18	6.5 (4.9 to 7.5) n = 17	*0.0002* (1.821 to 4.665)	*0.026* (-19.80 to -1.60)

Footnote (Table 4.3.1.)
[1] Values are medians (interquartile range).
[2]Generalized linear model of log transformed data.
[3] Analysed with a Wilcoxon two-sample test.
Graves' disease, GD. ND, not done; PTH, parathyroid hormone (reference range = 15-65pg/ml; Calcium, (reference range = 2.15-2.50 mmol/L); Phosphate, (reference range = 0.78-1.42mmol/L); BAP, serum bone-specific alkaline phosphatase (premenopausal reference range = 3-19 µg/L; postmenopausal reference range = 6-26 µg/L); Osteocalcin, (premenopausal reference range = 6.5-42.3 ng/ml; postmenopausal reference range = 5.4-59.1ng/ml); Urine DPD, urinary deoxypyridinoline (expressed as a ratio to urinary creatinine) (reference range 3.0-7.0 nmol/mmol).

Table 4.3.2. categorizes the subjects at baseline according to the respective biochemical measurements related to bone metabolism as being low, normal, or high.

Black- and white patients

When subjects were grouped according to s-PTH and corrected s-calcium reference ranges (low, normal or high) significantly more black female patients (74.3%) had decreased median PTH levels compared to white females (33.3%) ($p = 0.006$) (Table 4.3.2). Likewise, significantly more black females (63.2%) had elevated corrected serum calcium levels compared to their white counterparts (29.4% to $p = 0.021$). There were no differences comparing phosphate levels.

White patients and controls

Significantly more white patients (33.3%) had a decreased median serum PTH level compared to controls (0%) ($p = 0.025$), although the median s-calcium level was within the reference range in 70.6% of patients.

The median BAP level was raised in 60% of patients while all the controls had a normal BAP level ($p = 0.001$). The BAP level was raised in 64.7% of premenopausal patients compared to normal BAP levels in all the controls ($p = 0.002$). The numbers in the postmenopausal group were too small to reach a definitive conclusion.

The proportion of subjects and controls did not differ significantly between different osteocalcin cut points.

Most white patients (83.3%) had an increased urine DPD/creatinine ratio, compared to less than half of the controls (41.2%) ($p = 0.033$).

Black patients and -controls

Significantly more black patients (74.3%) had a decreased PTH level compared to the control group (0%) ($p < 0.0001$).

A greater proportion of black patients (69.4%) had increased levels of BAP compared to controls (18.5%) ($p = 0.0005$). Two thirds of black patients in the premenopausal subgroup had increased BAP levels compared to only 22.7% in the control group ($p = 0.007$). There was no significant difference in BAP levels between the postmenopausal subgroups.

More patients (25%) had a high osteocalcin level compared to the controls (0%) ($p = 0.01$). Most of these patients with high osteocalcin were in the premenopausal subgroup (28.6%) compared to none in the control group ($p = 0.014$). Osteocalcin levels in patients and controls in the postmenopausal subgroup did not differ significantly.

Almost all (97.2%) of the patients had a high urine DPD to creatinine ratio compared to only a third of controls ($p < 0.0001$).

Table 4.3.2. Frequency (%) of subjects at baseline according to biochemical measurements related to bone metabolism

	Black Females n = 39	**Black Controls** n = 39	**p-value[1] Black patients vs. Black controls**	**White Females** n = 20	**White Controls** n = 20	**p-value[1] White patients vs. White controls**	**p-value[2] GD: Black- vs. White patients**
PTH							
Low	26 74.3%	0		5 33.3%	0		
Normal	9 25.7%	24 88.9%		10 66.7%	17 100%		
High	0	3 11.1%	*< 0.0001*	0	0	*0.025*	*0.006*
Corrected s-calcium							
Low	0	Not done		0	Not done		
Normal	14 36.8%			12 70.6%			
High	24 63.2%			5 29.4%			*0.02*
BAP							
Low	0	0		0	0		
Normal	11 30.6%	22 81.5%		8 40%	17 100%		
High	25 69.4%	5 18.5%	*0.001*	12 60%	0	*0.001*	*0.474*

Table 4.3.2. (Continued)

	Black Females n = 39	**Black Controls** n = 39	**p-value[1] Black patients vs. Black controls**	**White Females** n = 20	**White Controls** n = 20	**p-value[1] White patients vs. White controls**	**p-value[2] GD: Black- vs. White patients**
BAP							
Premenopausal							
Low	0	0		0	0		
Normal	9 32.1%	17 77.3%		6 35.3%	14 100%		
High	19 67.9%	5 22.7%	*0.007*	11 64.7%	0	*0.002*	*0.828*
Postmenopausal							
Low	0	0		0	0		
Normal	2 25%	5 100%		2 66.7%	3 100%		
High	6 75%	0	*0.117*	1 33.3%	0		*0.491 (F)*

Table 4.3.2. (Continued)

	Black Females n = 39	**Black Controls** n = 39	**p-value[1] Black patients vs. Black controls**	**White Females** n = 20	**White Controls** n = 20	**p-value[1] White patients vs. White controls**	**p-value[2] GD: Black- vs. White patients**
Osteocalcin							
Low	1 2.8%	3 12.0%		0	1 5.88%		
Normal	26 72.2%	22 88.0%		17 85%	16 94.12%		
High	9 25%	0	*0.01*	3 15%	0	*0.063*	*0.68 (F)*
Premenopausal							
Low	1 3.5%	3 14.3%		0	1 7.14%		
Normal	19 67.9%	18 85.7%		14 82.3%	13 92.86%		
High	8 28.6%	0	*0.014*	3 17.7%	0	*0.063*	*0.68 (F)*
Postmenopausal							
Low	0	0		0	0		
Normal	7 87.5%	4 100%		3 100%	3 100%		
High	1 12.5%	0	*0.317*	0	0	No difference	*1.000 (F)*

Table 4.3.2. (Continued)

	Black Females n= 39	**Black Controls** n = 39	**p-value[1] Black patients vs. Black controls**	**White Females** n = 20	**White Controls** n = 20	**p-value[1] White patients vs. White controls**	**p-value[2] GD: Black- vs. White patients**
Urine DPD/ Creatinine ratio							
Low	0	1 3.7%		0	0		
Normal	1 2.8%	17 63%		3 16.7%	10 58.82%		
High	35 97.2%	9 33.3%	*< 0.0001*	18 83.3%	7 41.18%	*0.033*	*0.103 (F)*

[1]Cochran-Mantel-Haenszel p-value. [2]Chi-square p-value, except where indicated by (F) for Fisher's exact method due to sparse cells. Graves' disease, GD.

Discussion: *Biochemical measurements related to bone metabolism*

Several noteworthy differences emerged between the two groups of patients as well as between patients and their respective controls in the biochemical variables studied.

The median serum PTH level was decreased, and median serum calcium level was raised in more than two thirds of black female patients with GD. This is in line with what other researchers have found in mostly white ethnic groups (13). The increase in serum calcium is independent of PTH and may be due to thyroid hormone induced bone resorption (14). Although a third of white patients had a PTH level in the low range, PTH and calcium levels were mostly normal in this group

There is some controversy about the effect of hyperthyroidism on PTH- and calcium levels. In the study by Mosekilde *et al.*, evaluating white females, up to 50% of the subjects had hypercalcemia (13). Pantazi *et al.* also found the PTH levels to be decreased and calcium levels to be increased in pre- and post-menopausal females suffering from hyperthyroidism before initiation of therapy (15). Although the PTH was not decreased, a Polish study found that thyrotoxic patients had a lower PTH before treatment compared to after treatment (16). A recent sub-Saharan African study could not detect a difference in PTH levels between patients suffering from hyperthyroidism and normal controls (17).

Differences in the markers of bone formation and resorption were also observed. The BAP was raised in both black and white premenopausal subgroups and in the black postmenopausal subgroup, but not in the white postmenopausal subgroup. BAP plays an important role in the regulation of bone mineralization and is a marker of bone formation (18). Increased levels of BAP signal increased osteoblast activity. It must also be noted that it has been shown that BAP levels increase with age and the onset of menopause (19).

Osteocalcin is also a marker of osteoblast activity (20). The osteocalcin levels for both patient groups were within the reference range, and there was no difference between the two groups. It must be noted, however, that more black premenopausal patients had a raised osteocalcin level compared to black controls. Although the median osteocalcin levels of black- and white patients suffering from GD were not raised above the upper limit of normal, the levels were significantly higher in patients compared to controls and more patients had a raised osteocalcin level compared to controls. Osteocalcin has been shown to be increased during hyperthyroidism (21, 22). It has been shown by Garnero *et al.* that a discrepancy do

exist between osteocalcin- and BAP levels in hyperthyroid patients and this may explain the different levels of markers observed (23).

The urine DPD to creatinine ratio was significantly higher in black females compared to their white counterparts. Increased excretion of urinary deoxypyridinoline DPD (expressed as a ratio to urinary creatinine) is a marker of osteoclast activity (Robins *et al.*, 1994). The increased urine DPD to creatinine ratio in black females may signal increased osteoclast activity which may be more detrimental to bone health in this ethnic group compared to white females.

4.4 **Vitamin D measurements**

Median 25-hydroxyvitamin D (25(OH)D) levels of the two ethnic groups according to age and body mass index (BMI) are shown in Table 4.4.1. The patients were matched with controls according to seasonality.

Black- and white patients with GD

Median 25(OH)D levels were decreased in all subjects except for white females with GD. The median 25(OH)D level for black patients (27.0 ng/ml [20.5 to 40.0]) was below the lower limit of the reference range (32 ng/ml) and was also lower than the median level found in white patients (37.9 ng/ml [28.4 to 44.6]). Among black patients median 25(OH)D levels decreased progressively from 31.0 ng/ml ([15.5 to 45.2]) to 22.4 ng/ml ([19.2 to 30.8]) with increasing BMI while median 25(OH)D levels remained fairly stable in white patients as BMI increased. Comparing black to white females no significant inter-group differences were found within respective BMI categories.

White patients and controls

The median 25(OH)D level of the control group (26.0 ng/ml [22.0 to 29.2]) was significantly lower compared to the patient group (37.9 ng/ml [28.4 to 44.6]) ($p = 0.002$). The same pattern was evident for menopausal status and BMI category. The premenopausal patients had a significantly higher median 25(OH)D level (37.8 ng/ml [28.8 to 44.8]) compared to the control group (26.45 ng/ml [22.0 to 30.0]) ($p = 0.003$). The 25(OH)D level in the postmenopausal subgroup of patients was higher than in controls, but the difference did not reach statistical significance. Median 25(OH)D levels tended to be higher in the patient BMI

subgroups group compared to controls, but only reached statistical significance in the overweight sub-group. With increasing BMI 25(OH)D levels remained relatively stable in patients and controls.

Black patients and controls

Median 25(OH)D levels in patients and controls were below the lower limit of the reference range (32 ng/ml) – i.e. all black female subjects were thus 25(OH)D insufficient.

The median 25(OH)D level of black patients (27.0 ng/ml [20.5 to 40.0]) was significantly higher compared to black controls (20 ng/ml [16 to 27]) ($p = 0.021$). When evaluating the effect of menopausal status on 25(OH)D levels, it was found that the above difference persisted. Premenopausal black patients had a significantly higher 25(OH)D level (28.4 ng/ml [20.5 to 40.0]) compared to black controls (20.1 ng/ml [16 to 24]) ($p = 0.013$). The 25(OH)D levels were higher in black postmenopausal patients compared to controls, but this difference did not reach statistical significance. The 25(OH)D levels decreased with an increase in BMI, but there were no significant differences between the two groups (small numbers).

Table 4.4.1. Baseline 25-hydroxyvitamin D levels (ng/ml) according to menopausal status and BMI[1]

	Black Females n = 39	**Black Controls** n = 39	**p-value (95% confidence limits for ratio)[2] Black patients vs. Black controls**	**White Females** n = 20	**White Controls** n = 20	**p-value (95% confidence limits for ratio)[2] White patients vs. White controls**	**p-value (95% confidence limits for median differences)[3] GD: Black- vs. White patients**
25(OH)D (ng/ml)							
All subjects	27.0 (20.5 to 40.0) n = 37	20 (16 to 27) n = 27	*0.021* (1.044 to 1.636)	37.9 (28.4 to 44.6) n = 19	26.0 (22.0 to 29.2) n = 17	*0.002* (1.198 to 1.982)	*0.093* (-1.10 to 14.70)
Premenopausal	28.4 (20.5 to 40.0) n = 29	20.1 (16 to 24) n = 22	*0.013* (1.072 to 1.709)	37.8 (28.8 to 44.8) n = 16	26.45 (22.0 to 30.0) n = 14	*0.003* (1.177 to 1.865)	*0.151* (-2.10 to 15.40)
Postmenopausal	26.30 (18.75 to 36.55) n = 8	20 (17.1 to 34.7) n = 5	*0.404* (0.649 to 2.385)	37.9 (24.0 to 41.0) n = 3	25.2 (8.1 to 29.2) n = 3	*0.371* (0.189 to 18.375)	*0.540* (-21.40 to 25.50)
BMI < 25 (kg/m^2)	31.0 (15.5 to 45.2) n = 18	20.5 (19.5 to 27) n = 10	*0.289* (0.800 to 1.998)	37.9 (28.4 to 44.6) n = 9	26.9 (25.2 to 38.0) n = 5	*0.06* (0.987 to 1.503)	*0.643* (-11.60 to 16.40)
BMI 25.00-29.99 (kg/m^2)	27 (24.30 to 32.4) n = 9	20 (16 to 28) n = 7	*0.058* (0.9808 to 2.39)	33.85 (28.0 to 42.95) n = 8	23.25 (16.3 to 27.5) n = 8	*0.04* (1.047 to 3.8968)	*0.386* (-7.30 to 16.90)
BMI ≥ 30 (kg/m^2)	22.35 (19.2 to 30.80) n = 10	18.65 (13.1 to 24) n = 10	*0.257* (0.8413 to 1.7541)	41.65 (41.3 to 42.0) n = 2	29.0 (22.85 to 32.45) n = 4	*0.217* (0.366 to 4.853)	*0.086* (-15.50 to 27.30)

[1] Values are medians (interquartile range).
[2]Generalized linear model of log transformed data.
[3]Analysed with a Wilcoxon two-sample test.
Graves' disease, GD. 25(OH)D, 25-hydroxyvitamin D (reference range = 32-80 ng/ml); BMI, body mass index. BMI is calculated as weight in kilograms divided by height in meters squared (kg/m^2). Subjects were classified into one of the following categories: BMI < 18.50 kg/m^2: underweight, BMI = 18.50-24.99 kg/m^2: normal weight, BMI = 25.00-29.99 kg/m^2: overweight, BMI = 30.00-34.99 kg/m^2: obesity. (5)

Table 4.4.2 compares the frequency (%) of baseline 25(OH)D levels according to menopausal status and BMI.

Black and white patients

Among premenopausal females more black patients (58.6%) compared to white patients (31.3%), had decreased 25(OH)D levels, but this difference failed to reach statistical significance (p = 0.079).

White patients and controls

A higher proportion of premenopausal controls (78.6%) had a low 25(OH)D level compared to patients (31.3%) (p = 0.014). The small number of subjects in the postmenopausal subgroups made it difficult to reach definite conclusions. It was only in the overweight group (BMI = 25-29.99 kg/m^2), where increased BMI had a significant impact on 25(OH)D levels. In this group, all the controls had a low 25(OH)D (100%) compared to 25% of patients (p = 0.025).

Black patients and controls

Significantly more premenopausal controls (86.4%) had a decreased 25(OH)D level compared to patients (58.6%) (p = 0.030). There was no significant difference in 25(OH)D levels between the postmenopausal patients and controls. Likewise, no significant difference in 25(OH)D levels was found between patients and controls in the different BMI categories.

Table 4.4.2. Frequency (%) of subjects at baseline according to 25-hydroxyvitamin D levels, menopausal status, and BMI

	Black Females n = 40	**Black Controls** n = 39	**p-value[1] Black patients vs. Black controls**	**White Females** n = 20	**White Controls** n = 20	**p-value[1] White patients vs. White controls**	**p-value[2] GD: Black- vs. White patients**
Premenopausal	n = 29	n = 22		n = 16	n = 14		
Low	17 58.6%	19 86.4%		5 31.25%	11 78.57%		
Normal	12 41.4%	3 13.6%		11 68.75%	3 21.43%		
High	0	0	*0.03*	0	0	*0.014*	*0.079* (F)
Postmenopausal	n = 8	n = 5		n = 3	n = 3		
Low	6 75%	3 60%		1 33.3%	3 100%		
Normal	2 25%	2 40%		2 66.7%	0		
High	0	0	*0.808*	0	0	*0.157*	*0.491* (F)

Table 4.4.2. (Continued)

	Black Females n = 39	**Black Controls** n = 39	**p-value[1] Black patients vs. Black controls**	**White Females** n = 20	**White Controls** n = 20	**p-value[1] White patients vs. White controls**	**p-value[2] GD: Black- vs. White patients**
BMI < 25	n = 18	n = 10		n = 9	n = 5		
Low	9 50%	8 80%		4 44.4%	3 60%		
Normal	9 50%	2 20%		5 55.6%	2 40%		
High	0	0	*0.241*	0	0	*0.317*	*1.000 (F)*
BMI 25.00-29.99	n = 9	n = 7		n = 8	n = 8		
Low	6 66.7%	6 85.7%		2 25%	8 100%		
Normal	3 33.3%	1 14.3%		6 75%	0		
High	0	0	*0.398*	0	0	*0.025*	*0.153* (F)
BMI ≥ 30	n = 10	n = 10		n = 2	n = 4		
Low	8 80%	8 80%		0	3 75%		
Normal	2 20%	2 20%		2 100%	1 25%		
High	0	0	*0.808*	0	0	*0.3173*	*0.091* (F)

Footnote (Table 4.4.2.)
[1]Cochran-Mantel-Haenszel p-value.
[2]Chi-square p-value, except where indicated by (F) for Fisher's exact method due to sparse cells.
Graves' disease, GD. 25(OH)D, 25-hydroxyvitamin D (reference range = 32-80 ng/ml); BMI, body mass index. BMI is calculated as weight in kilograms divided by height in meters squared (kg/m^2). Subjects were classified into one of the following categories: BMI < 18.50 kg/m^2: underweight, BMI = 18.50-24.99 kg/m^2: normal weight, BMI = 25.00-29.99 kg/m^2: overweight, BMI = 30.00-34.99 kg/m^2: obesity. (5)

Discussion: *25-hydroxyvitamin D (25(OH)D) levels*

Irrespective of menopausal status or BMI, the black patients and black- and white controls tended to have lower 25(OH)D levels compared to white patients with GD. Median 25(OH)D levels of black patients and black- and white controls were also below the lower limit of normal (i.e. 25(OH)D insufficiency).

The association between GD and 25(OH)D remains controversial. In earlier studies it was shown that the 25(OH)D levels were normal in white females suffering from hyperthyroidism (24, 25). A recent meta-analysis, including different ethnic groups from across the globe, showed that patients with GD from Europe were unlikely to have 25(OH)D deficiency, while non-European populations were more likely to be 25(OH)D deficient (26). This study postulated that good socio-economic status may be the reason for the normal 25(OH)D levels observed in European countries. A large genetic study from Sweden found that patients with GD did have lower 25(OH)D levels compared to healthy controls (27). It has also been shown that in non-European ethnic groups the 25(OH)D levels may be reduced in hyperthyroid patients (28-30). In a Japanese study it was shown that the 25(OH)D levels of newly diagnosed females with GD were significantly lower 25(OH)D when compared to healthy controls (30). A publication from Nigeria also confirmed low 25(OH)D levels in patients with GD (31). An earlier meta-analysis also showed that a low 25(OH)D level may increase the risk of developing GD (26).

Vitamin D deficiency is a world-wide problem (32). Vitamin D deficiency affects all ages across the globe with the Middle East having the lowest levels observed (32). Vitamin D deficiency also affects affluent countries, e.g. the USA (33). In the 2011-2012 NHANES III, more than a third of the participants above the age of 20 years were 25(OH)D deficient (33). Being black and a poor education were found to be risk factors. Vitamin D deficiency is common in Africa (34). A meta-analysis performed to evaluate the 25(OH)D status of African subjects confirmed that the prevalence of 25(OH)D deficiency is high across the continent (34). It must be kept in mind that age, BMI and ethnicity plays a role in 25(OH)D levels and that darker pigmented skin may pre-dispose to 25(OH)D deficiency (35, 36). This was confirmed by a study reviewing the 25(OH)D status across the globe (32). It is of interest that this study concluded that seasonality had little or no effect on 25(OH)D levels. The authors postulated that in countries at risk, e.g. with long winters, fortified food may prevent 25(OH)D deficiency. In a South African study performed in Johannesburg, it was however found that seasonality played a role in 25(OH)D deficiency (36).

Bouillon suggests that a cut-off for 25(OH)D deficiency be set at 20ng/ml (37). Nine black patients, one white patient, four black controls and two white controls had 25(OH)D levels below 20ng/ml.

The low 25(OH)D levels found in black patients and controls may be explained on ethnic grounds, but the reason for the low 25(OH)D levels found in white controls remain unclear. The white controls were healthy community dwelling females from the Free State province. Patients and controls were also matched according to season, age, and BMI, ruling out the effect of these factors. Information on other factors that influence 25(OH)D levels for example out of door activities, clothing coverage, vitamin D supplements, physical activity, etc., were not included and may have shed some light on the difference in 25(OH)D levels when comparing white patients and –controls .

4.5 **1,25-dihydroxyvitamin D ($1,25(OH)_2D$) measurements**

Black and white patients

The median 1,25-dihydroxyvitamin D ($1,25(OH)_2D$) levels for black and white subjects were well within the reference range, 45.0 ng/L (35.0 to 64.0) and 39.0 ng/L (33.5 to 51.0) respectively and did not differ significantly (Table 4.5.1.). Among white premenopausal subjects the median $1,25(OH)_2D$ level was higher compared to postmenopausal subjects, while median levels of $1,25(OH)_2D$ did not seem to be affected by menopausal status in the black group. This is the opposite of what was noted with 25(OH)D levels. With increasing BMI, the median $1,25(OH)_2D$ levels of the black females also decreased progressively, as was seen with the 25(OH)D levels.

White patients and controls

The median $1,25(OH)_2D$ level of the white controls (81.0 ng/L [65.0 to 135.0]) was raised slightly above the upper limit of normal (≤ 67 ng/L). Patients had significantly lower levels of $1,25(OH)_2D$ (39.0 ng/L [33.5 to 51.0]) in comparison to the control group (81.0 ng/L [65.0 to 135.0]) ($p = 0.0001$). The premenopausal controls had significantly higher (87.0 ng/L [67.0 to 202.0]) $1,25(OH)_2D$ levels compared to the respective patient subgroup (42.0 ng/L [35.0 to 53.0]) ($p < 0.0001$). The $1,25(OH)_2D$ levels did not differ significantly between the postmenopausal subgroups. Normal weight controls had a median $1,25(OH)_2D$ level significantly higher (83.0 ng/L [67.0 to 91.0]) than the patient subgroup (39.0 ng/L [35.0 to 48.0]) ($p = 0.011$). The control subjects did have higher $1,25(OH)_2D$ levels than patients at every BMI category.

Black patients and controls

The median $1,25(OH)_2D$ level of the black patient group (45.0 ng/L [35.0 to 64.0]) was within the normal reference range (N = 19.9-67 ng/L), but was significantly lower compared to black controls (155 ng/L [91 to 217]) ($p < 0.0001$). Premenopausal black patients had a significantly lower median $1,25(OH)_2D$ level (45.0

ng/L [37.0 to 61.0]) although still within the normal reference range, compared to healthy premenopausal controls (176.5 ng/L [114 to 217]) ($p < 0.0001$). Postmenopausal controls tended to have higher $1,25(OH)_2D$ levels than patients, but this difference did not reach statistical significance. An increase in BMI was associated with decreasing $1,25(OH)_2D$ levels in patients and controls. There was a significant difference between every group at every BMI cut-off, with black controls persistently having higher $1,25(OH)_2D$ levels. In all BMI categories $1,25(OH)_2D$ levels were significantly higher in controls than in patients (BMI < 25 kg/m^2: $p = 0.003$; BMI 25-29.99 kg/m^2: $p = 0.002$; BMI > 30 kg/m^2: $p = 0.003$).

Table 4.5.1. Baseline levels of 1,25-dihydroxyvitamin D according to menopausal status and BMI[1]

	Black Females n = 39	**Black Controls** n = 39	**p-value (95% confidence limits for ratio)[2] Black patients vs. Black controls**	**White Females** n = 20	**White Controls** n = 20	**p-value (95% confidence limits for ratio)[2] White patients vs. White controls**	**p-value (95% confidence limits for median differences)[3] GD: Black- vs. White patients**
$1,25(OH)_2D$							
All subjects	45.0 (35.0 to 64.0) n = 35	155 (91 to 217) n = 26	*< 0.0001* (0.224 to 0.404)	39.0 (33.5 to 51.0) n = 20	81.0 (65.0 to 135.0) n = 17	*< 0.0001* (0.261 to 0.539)	*0.156* (-20.00 to 3.00)
Premenopausal	45.0 (37.0 to 61.0) n = 27	176.5 (114 to 217) n = 22	*< 0.0001* (0.202 to 0.394)	42.0 (35.0 to 53.0) n = 17	87.0 (67.0 to 202.0) n = 14	*< 0.0001* (0.23 to 0.519)	*0.366* (-16.00 to 6.00)
Postmenopausal	46.5 (30.5 to 76.5) n = 8	82.5 (62 to 120) n = 4	*0.198* (0.059 to 2.447)	32.0 (11.0 to 39.0) n = 3	42.0 (38.0 to 49.0) n = 3	*0.267* (0.109 to 2.875)	*0.153* (-72.00 to 15.00)
BMI < 25 (kg/m^2)	48.5 (37.0 to 67.0) n = 18	213.5 (74 to 220) n = 10	*0.0003* (0.203 to 0.548)	39.0 (35.0 to 48.0) n = 9	83.0 (67.0 to 91.0) n = 5	*0.011* (0.179 to 0.702)	*0.129* (-33.00 to 3.00)
BMI 25.0-29.9 (kg/m^2)	45.0 (38.0 to 61.0) n = 9	119.5 (91 to 181) n = 6	*0.002* (0.252 to 0.616)	39.0 (23.5 to 50.0) n = 8	78.0 (54.5 to 121.0) n = 8	*0.091* (0.156 to 1.212)	*0.248* (-34.00 to 11.00)
BMI ≥ 30 (kg/m^2)	38.0 (24.0 to 57.5) n = 8	147 (93 to 172) n = 10	*0.003* (0.105 to 0.514)	53.0 (35.0 to 59.0) n = 3	187.0 (54.5 to 313.0) n = 4	*0.134* (0.034 to 2.519)	*0.540* (-29.00 to 40.00)

Footnote (Table 4.5.1.)
[1] Values are medians (interquartile range).
[2]Generalized linear model of log transformed data.
[3]Analysed with a Wilcoxon two-sample test.
Graves' disease, GD. $1,25(OH)_2D$, 1,25-dihydroxyvitamin D (reference range 19.9-67ng/L). BMI, body mass index. BMI is calculated as weight in kilograms divided by the height in meters squared (kg/m^2) and is used to classify patients into the following categories: BMI < 18.50 kg/m^2: underweight, BMI = 18.50-24.99 kg/m^2: normal weight, BMI = 25.00-29.99 kg/m^2: overweight, BMI = 30.00-34.99 kg/m^2: obesity.

The frequency (%) of subjects at baseline according to their $1,25(OH)_2D$ concentrations is shown in Table 4.5.2.

Black and white patients

The $1,25(OH)_2D$ levels across different thresholds (low, normal, or high), and menopausal status (pre- or postmenopausal), did not differ significantly between the two groups of patients. The same was true for different BMI categories.

White patients and controls

A significantly greater proportion of premenopausal white controls (71.4%) had a high $1,25(OH)_2D$ level compared to white patients (6.3%) ($p = 0.002$). In the postmenopausal subgroup $1,25(OH)_2D$ levels did not differ significantly between patients and controls. When stratified according to BMI, no significant differences were found between white patients and -controls.

Black patients and controls

A significantly greater proportion of premenopausal controls (86.4%) had a high $1,25(OH)_2D$ level compared to the patient group (14.8%) ($p < 0.0001$). In the postmenopausal subgroup $1,25(OH)_2D$ levels also did not differ significantly between patients and controls. In all three BMI categories a significantly greater proportion of controls had high $1,25(OH)_2D$ levels compared to patients.

Table 4.5.2. Frequency (%) of subjects at baseline according to 1,25-dihydroxyvitamin D, menopausal status, and BMI

	Black Females n = 39	**Black Controls** n = 39	**p-value[1] Black patients vs. Black controls**	**White Females** n = 20	**White Controls** n = 20	**p-value[1] White patients vs. White controls**	**p-value[2] GD: Black- vs. White patients**
Premenopausal	n = 27	n = 22		n = 16	n = 14		
Low	2 7.4%	0		2 12.5%	0		
Normal	21 77.8%	3 13.6%		13 81.25%	4 28.57%		
High	4 14.8%	19 86.4%	*< 0.0001*	1 6.25%	10 71.43%	*0.002*	*0.736* (F)
Postmenopausal				n = 3	n = 3		
Low	1 12.5%	0		1 33.33%	0		
Normal	4 50%	1 25%		2 66.67%	3 100%		
High	3 37.5%	3 75%	*0.18*	0	0	*0.317*	*0.509* (F)

Table 4.5.2. (Continued)

	Black Females n = 39	**Black Controls** n = 39	**p-value[1] Black patients vs. Black controls**	**White Females** n = 20	**White Controls** n = 20	**p-value[1] White patients vs. White controls**	**p-value[2] GD: Black- vs. White patients**
BMI < 25	n = 18	n = 10		n = 8	n = 5		
Low	0	0		1 12.5%	0		
Normal	14 77.8%	2 20%		7 87.5%	2 40%		
High	4 22.2%	8 80%	*0.01*	0	3 60%	*0.074*	*0.162* (F)
BMI 25.00-29.99	n = 9	n = 6		n = 8	n = 8		
Low	1 11.1%	0		2 25%	0		
Normal	6 66.7%	0		5 62.5%	3 37.5%		
High	2 22.2%	6 100%	*0.022*	1 12.5%	5 62.5%	*0.1025*	*1.0000* (F)
BMI ≥ 30							
Low	2 25%	0		0	0		
Normal	5 62.5%	2 20%		3 100%	2 50%		
High	1 12.5%	8 80%	*0.026*	0	2 50%	*0.157*	*1.0000* (F)

Footnote (Table 4.5.2.)
[1]Cochran-Mantel-Haenszel p-value.
[2]Chi-square p-value, except where indicated by (F) for Fisher's exact method due to sparse cells.
Graves' disease, GD.

Discussion: *1,25-dihydroxyvitamin D (1,25(OH)$_2$D) measurements*

In this study patients with GD, regardless of ethnicity, had median 1,25(OH)$_2$D levels that were significantly lower compared to controls. Furthermore, among black subjects with GD, median 1,25(OH)$_2$D levels declined with increasing BMI.

Low dietary calcium intake stimulates the production of 1,25(OH)$_2$D (38). In a systematic review by Balk et al. it was shown that South African calcium intake was low (39). It has also been shown that dietary calcium intake of black South African females are significantly lower when compared to white females (40). This may play a role in the different 1,25(OH)$_2$D levels, although nonsignificant, observed between black and white patients.

It must be noted that 1,25(OH)$_2$D plays an important role in immune modulation by making the immune system more "tolerable" and explaining why low levels are associated with an increased risk of development of GD (28). It has also been shown that a mutation of the vitamin D receptor, to which 1,25(OH)$_2$D binds and has it effects, may predispose to the development of GD (41). Low levels of 1,25(OH)$_2$D may predispose a susceptible individual to develop GD (27).

In a number of studies hyperthyroidism was associated with decreased 1,25(OH)$_2$D levels (13, 25, 42). This reduced 1,25(OH)$_2$D occurs despite normal 25(OH)D levels (15). The reduced 1,25(OH)$_2$D levels are due to reduced renal 1-alpha-hydroxylase activity and increased metabolic clearance (25, 42). The increases in serum calcium levels, especially observed in black patients, could also explain the suppressed 1,25(OH)$_2$D levels (43).

It has been shown before that there is an inverse relationship between obesity and 25(OH)D and 1,25(OH)$_2$D levels (44, 45). It does however seem that this association may be dependent on ethnicity. A US study evaluated the relationship between body fat and vitamin D status from the NHANES III data in euthyroid individuals (46). This NHANES III (1988-1994) study found that the inverse relationship between body fat percentage and vitamin D status was strong in white females, but weak in black females. It also found that this inverse relationship was stronger in younger patients. A northern European study did find an inverse

relationship between 1,25$(OH)_2$D status and increasing BMI in the population studied (47). A more recent Turkish study also confirmed a negative relationship between body fat and vitamin D status (48). A South African study could not confirm a relationship between body fat and vitamin D status in the black population studied (36). Research performed in Kwa-Zulu Natal, South Africa, investigating vitamin D status in older African and Indian patients, conversely showed an increase in vitamin D levels with increasing adiposity (49).

4.6 **Measurements of selected markers of inflammation**

Black- and white patients

The median level of tumour necrosis factor alpha (TNFα) in black patients (2.47 pg/ml [1.96 to 3.26]) was significantly higher compared to that of white patients (1.92 pg/ml [1.51 to 2.29]) ($p = 0.022$) (Table 4.6.1.). The median erythrocyte sedimentation rate (ESR) was also significantly higher in black females (21.0 mm in the 1st hour [14.0 to 42.0]) compared to white females (8 mm in 1st hour [5.0 to 12.0]) ($p = 0.002$) while the median total white cell count, median levels of IL-6, and CRP did not differ significantly between the two groups of patients.

White patients and controls

The inflammatory markers for both patients and controls were within the normal reference range and there were no significant differences between the two groups.

Black patients and controls

Although still within reference range (0.0-6.4 pg/ml) in both groups, the IL-6 values of black patients (3.50 pg/ml [2.50 to 5.60]) were significantly higher than in black controls (2.7 pg/ml [2.2 to 4.2] ($p = 0.037$).

The median TNFα value was higher in the black patients with hyperthyroidism compared to the controls but was still within reference range and no significant difference was demonstrated.

Table 4.6.1. Selected markers of inflammation at baseline[1]

	Black Females n = 39	**Black Controls** n = 39	**p-value (95% confidence limits for ratio)[2] Black patients vs. Black controls**	**White Females** n = 20	**White Controls** n = 20	**p-value (95% confidence limits for ratio)[2] White patients vs. White controls**	**p-value (95% confidence limits for median differences)[3] GD: Black- vs. White patients**
WCC (cells x 10^9)	5.92 (4.33 to 7.22) n = 39	Not done		6.28 (5.35 to 7.34) n = 16	Not done		*0.313* (-0.560 to 1.680)
IL-6 (pg/ml)	3.50 (2.50 to 5.60) n = 37	2.7 (2.2 to4.2) n = 27	*0.037* (1.024 to 2.053)	2.60 (1.40 to 5.0) n = 18	2.4 (1.2 to 3.0) n = 17	*0.488* (0.609 to 2.709)	*0.114* (-2.00 to 0.40)
TNFα (pg/ml)	2.47 (1.96 to 3.26) n = 22	1.88 (1.58 to 2.25) n = 11	*0.073* (0.960 to 2.111)	1.92 (1.51 to 2.29) n = 13	1.6 (1.36 to 1.92) n = 13	*0.152* (0.908 to 1.693)	*0.022* (-1.34 to -0.04)
CRP (mg/L)	5.35 (1.85 to 11.20) n = 16	Not done		4.75 (1.50 to 8.25) n = 4	Not done		*0.705* (-10.30 to 5.80)
ESR (mm in 1st hour)	21.0 (14.0 to 42.0) n = 30	Not done		8.0 (5.0 to 12.0) n = 9	Not done		*0.002* (-30.00 to -6.00)

[1] Values are medians (interquartile range). [2]Generalized linear model of log transformed data.

Footnote (Table 4.6.1.: Continued)
[3]Analysed with a Wilcoxon two-sample test. Graves' disease, GD. WCC, white cell count (reference range 4-10 cells x 10^9); IL-, interleukin 6 (reference range = 0.0-6.4pg/ml); TNFα, tumour necrosis factor alpha (reference range = 1.47-5.93 pg/ml); CRP, C-reactive protein (reference range = 0.0-5.0 mg/L); ESR, erythrocyte sedimentation rate (reference range 0-31mm in 1st hour).

Table 4.6.2 compares the frequency (%) of subjects at baseline according to the respective inflammatory marker concentrations.

Black and white patients

No significant differences were noted comparing the frequency (%) of black with white subjects at baseline according to the respective reference ranges of selected inflammatory markers.

White patients and controls

The median WCC, IL-6-, and TNFα levels did not differ significantly when categorized according to their respective reference ranges.

Black patients and controls

The median WCC, IL-6-, and TNFα levels also did not differ significantly when ranked according to their respective reference ranges.

Table 4.6.2. Frequency (%) of subjects at baseline according to the respective reference ranges for selected inflammatory markers

	Black Females n = 39	**Black Controls** n = 39	**p-value[1] Black patients vs. Black controls**	**White Females** n = 20	**White Controls** n = 20	**p-value[1] White patients vs. White controls**	**p-value[2] GD: Black- vs. White patients**
WCC (cells x 10^9)	n = 39	Not done		n = 16	Not done		
Low	5 12.8%			0			
Normal	33 84.6%			15 93.7%			
High	1 2.6%			1 6.25%			*0.287* (F)
IL-6 (pg/ml)	n = 37	n = 27		n = 18	n = 17		
Low	0	0		0	0		
Normal	29 78.4%	26 96.3%		15 83.3%	15 88.24%		
High	8 21.6%	1 3.7%	*0.071*	3 16.7%	2 11.76%	*0.763*	*1.0000* (F)
TNFα (pg/ml)	n = 22	n = 11		n = 13	n = 13		
Low	1 4.55%	1 9.09		3 23.1%	5 38.46%		
Normal	20 90.9%	10 91%		10 76.9%	8 61.54%		
High	1 4.55%	0	*0.317*	0	0	*0.228*	*0.274* (F)

Footnote (Table 4.6.2.)
[1]Cochran-Mantel-Haenszel p-value.
[2]Chi-square p-value, except where indicated by (F) for Fisher's exact method due to sparse cells.
Graves' disease, GD. WCC, white cell count (reference range 4-10 cells x 10^9); IL-, interleukin 6 (reference range = 0.0-6.4pg/ml); TNFα, tumour necrosis factor alpha (reference range = 1.47-5.93 pg/ml); CRP, C-reactive protein (reference range = 0.0-5.0 mg/L); ESR, erythrocyte sedimentation rate (reference range 0-31mm in 1st hour).

Discussion: *Inflammatory markers*

Although median TNFα and ESR levels were overall significantly higher in black females compared to their white counterparts. The levels of inflammatory markers were normal in 77% or more of subjects from both ethnic groups were within the normal reference ranges. The results of previous studies on inflammatory markers in subjects with thyrotoxic GD have produced contradicting results (50-54). These authors reported TNFα and IL-6 levels to be normal, both increased and only one increased.

Although within the normal reference range, the median IL-6 level found in black patients was significantly higher compared to black controls. This was not seen between the white patients and their controls. IL-6 may be associated with hyperthyroidism, but recent evidence would suggest that IL-6 may play a role in the development of GD (55). In a recent meta-analysis by Imani et al. it was suggested that an IL-6 polymorphism may be a risk factor for the development of GD (55).

TNFα has also recently been shown to play a role in the development of GD and not only forms part of the inflammatory process (56). A recent meta-analysis reviewed whether TNFα polymorphisms could increase an individual's risk of developing GD. It was found that a specific polymorphism of the TNFα gene increased individuals of European descent's risk of developing GD, but not Asians. Although not statistically significant, the median TNFα levels in both patient groups were higher compared to controls.

4.7 Leptin and IGF-1 concentrations

Black and white patients

As expected, median leptin levels in both ethnic groups increased with increasing BMI (Table 4.7.1.). The levelling off seen among black patients in the obesity class I and II categories could be due to the small number of participants in these categories. In general, median leptin

levels tended to be higher in black- than white patients, but it only reached statistical significance in the overweight category (BMI = 25.00-29.99 kg/m^2). In this category the median leptin level for black females was 28.3 ng/ml [17.9 to 30.5] compared to 16.1 ng/ml [7.9 to 19.85] in white females (p = 0.043).

The median IGF-1 level in white patients (107.0 ng/ml [94.0 to 215.0]) were not statistically different from that in black patients (83.6 ng/ml [33.6 to 141.5]).

White patients and controls

No significant differences could be detected in median leptin levels between the white patients and controls. The IGF-1 level of white patients (107.0 ng/ml [94.0 to 215.0]) was significantly lower (p = 0.05) than white controls (203.5 ng/ml [132.50 to 236]).

Black patients and controls

Median leptin levels did not differ significantly between black females with GD and controls. The median IGF-1 level of black patients (83.6 ng/ml [33.6 to 141.5]) was below the lower limit of normal (≥ 107ng/ml) and was significantly lower than that of the control group (132 ng/ml [98 to 193]) (p = 0.001).

Table 4.7.1. Leptin and IGF-1 levels[1]

	Black Females n = 39	**Black Controls** n = 39	**p-value (95% confidence limits for ratio)[2] Black patients vs. Black controls**	**White Females** n = 20	**White Controls** n = 20	**p-value (95% confidence limits for ratio)[2] White patients vs. White controls**	**p-value (95% confidence limits for median differences)[3] GD: Black- vs. White patients**
Leptin (ng/ml)	16.3 (6.7 to 33.95) n = 36	24 (7.6 to 26.8) n = 9	*0.389* (0.617 to 3.257)	14.4 (5.5 to 24.2) n = 20	14.6 (9.50 to 22.30) n = 11	0.254 (0.454 to 1.257)	*0.326* (-12.50 to 2.90)
Leptin(ng/ml) according to BMI							
BMI < 25 (kg/m^2)	6.2 (4.3 to 14.9) n = 17	5.4 (1.7 to 7.6) n = 3	*0.355* (0.428 to 7.964)	6.9 (5.0 to 13.8) n = 9	7.7 (5.25 to 9.9) n = 4	0.962 (0.378 to 2.740)	*0.59* (-3.30 to 8.30)
BMI = 25.00-29.99 (kg/m^2)	28.3 (17.9 to 30.5) n = 9	25.4 (24 to 26.8) n = 3	*0.819* (0.451 to 2.585)	16.1 (7.9 to 19.85) n = 8	19.5 (16.7 to 22.3) n = 5	0.175 (0.165 to 1.582)	*0.0433* (-23.10 to 0.40)
BMI = 30.00-34.99 (kg/m^2)	48.15 (26.0 to 65.55) n = 4	n = 0	Not comparable	35.0 (25.0 to 45.0) n = 2	18.9 (14.6 to 23.2) n = 2	Insufficient matched pairs for analysis	*0.643* (-54.60 to 37.80)
BMI ≥ 35.00 (kg/m^2)	40.55 (32.6 to 67.4) n = 6	30.3 (23.8 to 77.5) n = 3	Insufficient matched pairs for analysis.	45.6 n = 1	n = 0	Not comparable	*0.617* (-41.00 to 16.30)
IGF-1(ng/ml)	83.6 (33.6 to 141.5) n = 32	132 (98 to 193) n = 27	*0.001* (0.356 to 0.738)	107.0 (94.0 to 215.0) n = 15	203.5 (132.50 to 236) n = 16	0.05 (0.426 to 1.00)	*0.108* (-7.60 to 85.40)

[1] Values are medians (interquartile range).
[2]Generalized linear model of log transformed data.

[3]Analysed with a Wilcoxon two-sample test.
Graves' disease, GD. BMI, body mass index. BMI < 25 kg/m^2: leptin reference range =0.2-45.8 ng/ml; BMI = 25.00-29.9 kg/m^2: leptin reference range = 3-65.7 ng/ml; BMI = 30.00-34.99 kg/m^2: leptin reference range = 8.1-79.1 ng/ml; BMI ≥ 35.00 kg/m^2: leptin reference range =11.9-137.4 ng/ml; IGF-1, insulin-like growth factor 1 (reference range = 107.8-246.7 ng/ml).

Tables 4.7.2 and 4.7.3 compares the frequency (%) of subjects at baseline according to the leptin and IGF-1 categories.

Black and white patients

When comparing the proportion of black and white patients within different strata of leptin and IGF-1 reference ranges (low, normal, or high), there were no significant differences between the two groups. This was also true when controlling for BMI.

White patients and controls

When comparing the proportion of white subjects and controls within different strata of leptin and IGF-1 reference ranges (low, normal, or high), there were no significant differences between the two groups. This was also true for leptin levels at different BMI categories. More white patients (53.3%) had a decreased IGF-1 level compared to the control group (12.5%) ($p = 0.025$).

Black patients and controls

When comparing the proportion of black subjects and controls within different strata of leptin and IGF-1 reference ranges (low, normal, or high), there were no significant differences between the two groups. This was also true for leptin levels at different BMI categories. However, a greater proportion of black patients (56.3%) had a low IGF-1 level compared to controls. The proportion was almost double that which was found in the control group (29.6% to $p = 0.01$).

Table 4.7.2. Frequency (%) of subjects at baseline according to the respective reference ranges of Leptin

	Black Females n = 39	**Black Controls** n = 39	**White Females** n = 20	**White Controls** n = 20
Leptin				
BMI				
BMI < 25				
Normal	17 100%	3 100%	8 100%	4 100%
BMI 25.00-29.99				
Normal	9 100%	3 100%	8 100%	5 100%
BMI 30.00-34.99				
Low	1 25%	0		
Normal	2 50%	0	2 100%	2 100%
High	1 25%	0		
BMI ≥ 35.00				
Normal	6 100%	3 100%	0	1 100%

Table 4.7.3. Frequency (%) of subjects at baseline according to the respective reference ranges of IGF-1

	Black Females n = 39	**Black Controls** n = 39	**p-value[1] Black patients vs. Black controls**	**White Females** n = 20	**White Controls** n = 20	**p-value[1] White patients vs. White controls**	**p-value[2] GD: Black- vs. White patients**
IGF-1	n=32						
Low	18 56.3%	8 29.6%		8 53.3%	2 12.5%		
Normal	13 40.6%	15 55.65		6 40%	12 75%		
High	1 3.1%	4 14.8%	*0.01*	1 6.7%	2 12.5%	*0.025*	*1.0000 (F)*

[1]Cochran-Mantel-Haenszel p-value.
[2]Chi-square p-value, except where indicated by (F) for Fisher's exact method due to sparse cells.
Note: Where no p-value displayed: No p-value generated.
Graves' disease, GD.

Discussion: *Leptin and IGF-1*

Except for the overweight BMI category, median leptin levels did not differ significantly between the two patient groups. Also, when comparing leptin levels of patients and controls no significant differences could be detected. An increase in median leptin with increasing BMI was observed in all four groups.

Leptin levels directly correlate with adipose tissue mass and when body fat decreases, leptin levels are reduced (57, 58). Ethnic differences in leptin levels do exist with African American and black South African women having higher levels of leptin than their white counterparts of comparable BMI (Chantler *et al.*, 2012, Ruhl *et al.*, 2004, Van der Merwe *et al.*, 1999). A publication investigating the effect of hyperthyroidism on leptin levels found that the levels were increased during hyperthyroidism and returned to normal after achieving euthyroidism (59). However in a German study it was found that leptin levels remained unchanged during hyperthyroidism (60). A more recent study showed leptin levels to be reduced with newly diagnosed hyperthyroidism (61). A question that needs to be answered is: what is the temporal relationship between GD development and leptin levels? It must be kept in mind that although the patients in this study were matched according to age, BMI and ethnicity, the patients were seen during the thyrotoxic stage of GD. It would be interesting to review leptin levels after recovery from GD to determine what the effect of GD was on pre-hyperthyroidism leptin levels.

Although no significant differences in median IGF-1 levels were found between the two patient groups in this study, the IGF-1 level of both patient groups were below the lower limit of normal. The IGF-1 level of black- and white patients was significantly lower compared to their respective controls.

IGF-1 may reflect nutritional status and thus the catabolic state associated with hyperthyroidism (62). The effect of hyperthyroidism on IGF-1 levels remains controversial. In a study evaluating IGF-1 levels in newly diagnosed hyperthyroid patients it was found that up to 20% of newly diagnosed patients had a decreased IGF-1 level (63). A study evaluating IGF-1 levels in hyperthyroid adolescents did not detect any IGF-1 abnormalities (64). Raised IGF-1 levels were found in hyperthyroid patients in a study evaluating the effects of thyroid hormone on bone metabolism (65). This study included patients with either GD or multinodular goitre, but no differences in the IGF-1 levels were observed between the two conditions. In a study on rats it was shown that insulin growth factor binding proteins

(IGFBPs) were increased during hyperthyroidism and that this may alter the availability of IGF-1 (66). In a study on humans it was shown that IGFBP-3 is normal during hyperthyroidism, but that IGFBP-1 is increased (67). On the contrary an earlier study showed that IGFBP-3 is increased during hyperthyroidism (68). It has been shown that IGF-1 and its receptor, IGF-1R, play a role in especially Graves' orbitopathy (69, 70). It has also been shown that reduced IGF-1 may be associated with more severe hyperthyroidism (63).

4.8 **Summary and conclusions**

Differences in the risk of development of osteoporosis and subsequent fragility fractures do exist between different populations and ethnic groups (71). A recent study from the USA showed that up to 90% of osteoporotic fractures occur in white women, with less than 10% occurring in black- and Hispanic women combined (72). Important differences and similarities in skeletal health and fracture risk between black- and white South African women have been identified over the past 3 decades (73-75). In black populations from the USA, it has been documented that the skeleton is relatively protected against the negative effects of endocrinopathies like hyperparathyroidism (76, 77). This raises the question whether the same holds true for South African women?

Important differences and similarities had been detected in the biochemical measurements studied in black- and white South African patients with GD.

Black patients with GD had lower PTH levels along with higher calcium levels when compared to white patients. This difference, along with a significantly higher urine DPD/creatinine ratio in black patients, may signal a more severe detrimental effect of GD on the skeleton of black females.

The 25(OH)D levels of the black patients and controls were lower than in white patients, which may reflect an ethnic difference. However, white controls also had lower 25(OH)D levels than the white patients. Patients and controls were matched according to BMI, age and seasonality and these factors should not have influenced results. Socio-economic differences may explain the differences between black- and white patients, but not white patients and -controls.

It was expected that the $1,25(OH)_2D$ levels would be decreased, but levels were within the reference range for both patient groups, while it was raised in the control groups. This raises

the question if patients with GD did not have a relative $1,25(OH)_2D$ deficiency? This warrants further investigation.

Another interesting fact to keep in mind is the effect of IGF-1 on $1,25(OH)_2D$ levels (62). It is known that IGF-1 stimulates $1,25(OH)_2D$ production. The IGF-1 levels were reduced in both patient groups and this may have had a detrimental effect on the $1,25(OH)_2D$ levels. The actual reason for the $1,25(OH)_2D$ levels observed are not clear and further investigation is warranted.

Although most of the selected inflammatory markers were within the normal reference ranges, the IL-6 values were higher in the black patients compared to their controls. The effect of hyperthyroidism on inflammatory markers and the role of inflammatory markers in hyperthyroidism needs to be clarified further.

Leptin levels did not differ significantly between patients and controls for both ethnic groups, but it must be kept in mind that patients entered the study due to a disease state associated with loss of -muscle and fat mass. The pre-disease leptin levels may have looked different. The IGF-1 was reduced in both patient groups and may reflect nutritional status. The small number of subjects in the control groups makes direct comparison impossible.

It seems that from a biochemical perspective hyperthyroidism due to GD had a negative impact on bone metabolism of black South African females and when considering variables such as PTH, calcium, and urine DPD/creatinine ratio, these women may more severely affected compared to white females suffering from GD.

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Chapter 5

Results and Discussion: Dual energy X-ray Absorptiometry - Bone Mineral Density

5.1 **Introduction**

There is a lack of normative BMD data for South African females of all ethnic groups. Current guidelines from the International Society of Clinical Densitometry (ISCD) suggests that when generating a T-score a uniform Caucasian female database should be used for both females and males from all ethnic groups (1). If normative data for Z-scores of a population exist, these population specific Z-scores should be used. The patient's self-reported ethnicity should be used when deciding on which Z-score database is to be used. For Z scores in this study, normative data from National Health and Nutrition Examination Survey III (NHANESIII) were used and a Z-score $\leq$ -2.0 was considered as decreased and defined as low bone mass. The white female data base supplied by Hologic® was used as reference population.

In this chapter the results of dual-energy X-ray absorptiometry (DXA) BMD investigations obtained at baseline, and at 6 and 12 months from subjects are reported and discussed. The DXA measurements of patients with GD at different times are compared with controls at baseline.

5.2 **Bone mineral density at baseline**

Actual bone mineral density (BMD) in g/cm^2 at baseline as determined at selected sites is shown in Table 5.2.1.

Black- and white patients

Among black female patients with GD, the median BMD was significantly greater at the left femoral neck (0.78 g/cm^2 [0.70 to 0.88]) compared to white female patients (0.73 g/cm^2 [0.64 to 0.79]) (p = 0.033). The median BMD tended to be greater (but not significantly so) at all other sites in black compared to white patients except in the distal 3rd of the left forearm and the femoral neck (as noted above). The forearm site in black females had a borderline significant lower median BMD (0.62 g/cm^2 [0.51 to 0.66]) in comparison to white subjects (0.66 g/cm^2 [0.60 to 0.73]) (p = 0.049).

White patients and –controls

The median bilateral average of total hip BMD of patients (0.85 g/cm^2 [0.77 to 0.91]) was significantly lower compared to that of controls (1.01 g/cm^2 [0.92 to 1.1]) (p = 0.017). There was also a trend towards a lower median BMD (0.82 g/cm^2 [0.77 to 0.91]) at the left total hip in the patient group compared to controls (0.94 g/cm^2 [0.83 to 1.04]), but the difference did not reach statistical significance (p = 0.051). Likewise, at the distal 3rd of the left forearm patients had a lower median BMD (0.66 g/cm^2 [0.60 to 0.73]) compared to controls (0.71 g/cm^2 [0.66 to 0.75] but this difference also failed to reach statistical significance (p = 0.078).

Black patients and -controls

No significant differences were found at baseline when comparing the actual BMD of black patients with controls.

Table 5.2.1. Actual bone mineral density (g/cm^2) at baseline[1]

	Black Females with GD n = 39	**Black Controls** n = 39	**p-value (95% confidence limits for ratio)[3] Black patients vs. Black controls**	**White Females with GD** n = 19	**White Controls** n = 17	**p-value (95% confidence limits for ratio)[3] White patients vs. White controls**	**p-value (95% confidence limits for median differences)[2] GD: Black- vs. White patients**
Lumbar Spine	0.91 (0.83 to 1.02)	1.02 (1.0 to 1.08)	*0.633* (0.95 to 1.09)	0.91 (0.85 to 1.02)	1.11 (0.97 to 1.4)	*0.21* (0.51 to 1.17)	*0.695* (-0.048 to 0.087)
Left femoral neck	0.78 (0.70 to 0.88)	0.82 (0.75 to 0.89)	*0.472* (0.91 to 1.04)	0.73 (0.64 to 0.79)	0.77 (0.72 to 0.88)	*0.131* (0.80 to 1.03)	*0.033* (-0.151 to -0.006)
Left total hip	0.88 (0.81 to 0.95)	0.95 (0.86 to 1.01)	*0.139* (0.89 to 1.02)	0.82 (0.72 to 0.90)	0.94 (0.83 to 1.04)	*0.051* (0.79 to 1.0)	*0.069* (-0.134 to 0.005)
Bilateral average of total hip	0.88 (0.82 to 0.97)	0.93 (0.85 to 1.02)	*0.161* (0.81 to 1.04)	0.85 (0.76 to 0.90)	1.01 (0.92 to 1.1)	*0.017* (0.74 to 0.97)	*0.138* (-0.131 to 0.025)
Right femoral neck	0.79 (0.67 to 0.89)	0.81 (0.75 to 0.91)	*0.402* (0.90 to 1.04)	0.74 (0.63 to 0.82)	0.81 (0.70 to 0.86)	*0.315* (0.84 to 1.06)	*0.126* (-0.143 to 0.016)
Right total hip	0.88 (0.80 to 0.99)	0.95 (0.85 to 1.03)	*0.195* (0.90 to 1.02)	0.87 (0.76 to 0.92)	0.91 (0.84 to 0.99)	*0.131* (0.84 to 1.03)	*0.252* (-0.121 to 0.036)
Left forearm distal 3rd	0.62 (0.51 to 0.66)	0.64 (0.59 to 0.67)	*0.150* (0.87 to 1.02)	0.66 (0.60 to 0.73)	0.71 (0.66 to 0.75)	*0.078* (0.83 to 1.01)	*0.049* (-0.001 to 0.107)

[1] Values are medians (interquartile range). [2] Analysed with a Wilcoxon two-sample test. [3] Generalized linear model of log transformed data.

The frequency (%) of normal and decreased Z-scores obtained at baseline from black and white patients as well as from their respective controls are shown and compared in Table 5.2.2.

Black- and white patients

Significantly more black female patients (43.6%) had a low Z-score at the lumbar spine compared to their white counterparts (15%; p = 0.028). More white female patients (20%) had a low Z-score at the level of the left femoral neck compared to black female patients (2.6%; p = 0.04). There was no significant difference between the two groups at other sites measured.

White patients and –controls

No significant differences were detected when comparing the Z-scores of white patients with white controls.

Black patients and –controls

Significant differences in the frequency of decreased Z-scores were found between black patients and black controls at the radius and the whole body. Decreased Z-scores at the left total forearm site were significantly more frequent in the patient group (38.5%) compared to controls (7.4%; p = 0.016). The same was true for the left forearm distal 3rd with 25.6% of patients having had a decreased Z-score compared to 3.7% in the control group (p = 0.049). Significantly more subjects also had a decreased Z-score of the ultra-distal radius (30.8%) compared to controls (3.7%; p = 0.028). Decreased whole body Z-scores were also more frequent in subjects (30.6%) compared to controls (0%; p = 0.004).

Table 5.2.2. Frequency (%) of subjects at baseline according to Z-score categories at selected anatomical sites

	Black Females with GD n = 39	**Black Controls** n = 27	**p-value[1] Black patients vs. Black controls**	**White Females with GD** n = 20	**White Controls** n = 17	**p-value[1] White patients vs. White controls**	**p-value[2] GD: Black- vs. White patients**
Lumbar spine							
Normal	22 56.4%	21 77.8%		17 85.0%	16 94.1%		
Decreased	17 43.6%	6 22.2%	*0.211*	3 15.0%	1 5.9%	*0.564*	*0.0281*
Left femoral neck		n = 26					
Normal	38 97.4%	26 100%		16 80.0%	16 94.1%		
Decreased	1 2.6%	0	*0.479*	4 20.0%	1 5.9%	*0.317*	*0.0408* (F)
Left total hip							
Normal	36 92.3%	26 100%		17 85.0%	17 100%		
Decreased	3 7.7%	0	*0.221*	3 15.0%	0	*0.157*	*0.397 (F)*

Table 5.2.2. (Continued)

	Black Females with GD n = 39	**Black Controls** n = 27	**p-value[1] Black patients vs. Black controls**	**White Females with GD** n = 20	**White Controls** n = 17	**p-value[1] White patients vs. White controls**	**p-value[2] GD: Black- vs. White patients**
Bilateral average of total hip							
Normal	35 89.7	9 100%		17 85.0%	10 100%		
Decreased	4 10.3	0	*0.317*	3 15.0%	0	*0.317*	*0.679* (F)
Right femoral neck		n = 27					
Normal	35 89.7%	27 100%		17 85.0%	17 100%		
Decreased	4 10.3%	0	*0.117*	3 15.0%	0	*0.157*	*0.679* (F)
Right total hip							
Normal	36 92.3%	27 100%		17 85.0%	17 100%		
Decreased	3 7.7%	0	*0.221*	3 15.0%	0	*0.157*	*0.398* (F)

Table 5.2.2. (Continued)

	Black Females with GD n = 39	**Black Controls** n = 27	**p-value[1] Black patients vs. Black controls**	**White Females with GD** n = 20	**White Controls** n = 17	**p-value[1] White patients vs. White controls**	**p-value[2] GD: Black- vs. White patients**
Left total forearm							
Normal	24 61.5%	25 92.6%		14 77.8%	17 100%		
Decreased	15 38.5%	2 7.4%	*0.016*	4 22.2%	0	*0.083*	*0.227*
Left forearm distal 3rd							
Normal	29 74.4%	26 96.3%		16 88.9%	17 100%		
Decreased	10 25.6%	1 3.7%	*0.049*	2 11.1%	0	*0.157*	*0.303* (F)
Ultra-distal left forearm							
Normal	27 69.2%	26 96.3%		14 77.8%	17 100%		
Decreased	12 30.8%	1 3.7%	*0.028*	4 22.2%	0	*0.083*	*0.504*
Whole body score	(n = 36)			(n = 19)			
Normal	25 69.4%	27 100%		15 79.0%	17 100%		
Decreased	11 30.6%	0	*0.005*	4 21.0%	0	*0.083*	*0.452*

[1]Cochran-Mantel-Haenszel p-value. [2]Chi-square p-value, except where indicated by (F) for Fisher's exact method due to sparse cells. Z-scores ≤ -2.0 was defined as abnormal.

The median Z-scores at baseline were compared between patient groups (Table 5.2.3).

Black- and white patients

The median Z-score of black patients at the lumbar spine (-1.8 [-2.7 to -1.0]) was significantly lower compared to that of white patients (-0.95 [-1.55 to 0.1]) (p = 0.007). The median Z-score of black patients at the distal 3rd left forearm were lower (-0.9 [-2.5 to -0.2]) in comparison to white patients (-0.25 [-1.1 to 0.8]), but this just failed to reach significance (p = 0.051). No significant differences were detected at the other sites measured.

Table 5.2.3. Median Z-scores at baseline: Patients[1]

	Black Females with GD n = 39	**White Females with GD** n = 20	**p-value (95% confidence limits for median differences)[2] GD: Black- vs. White patients**
Lumbar Spine	-1.8 (-2.7 to -1.0)	-0.95 (-1.55 to 0.1)	*0.007* (0.3 to 1.6)
Left femoral neck	-1.0 (-1.4 to -0.2)	-0.85 (-1.45 to -0.2)	*0.873* (-0.7 to 0.5)
Left total hip	-0.9 (-1.3 to -0.3)	-0.9 (-1.15 to -0.15)	*0.854* (-0.5 to 0.4)
Bilateral average of total hip	-0.8 (-1.3 to -0.4)	-0.65 (-1.15 to -0.2)	*0.791* (-0.5 to 0.5)
Right femoral neck	-0.9 (-1.5 to -0.3)	-0.65 (-1.25 to -0.05)	*0.742* (-0.6 to 0.7)
Right total hip	-0.7 (-1.3 to -0.2)	-0.45 (-1.2 to -0.1)	*0.619* (-0.5 to 0.6)
Left forearm distal 3rd	-0.9 (-2.5 to -0.2)	-0.25 (-1.1 to 0.8)	*0.051* (0.0 to 1.8)

[1] Values are medians (interquartile range). [2] Analysed with a Wilcoxon two-sample test.

Table 5.2.4. compares the median Z-scores at baseline between patients and their controls.

White patients and –controls

The median Z-score of the bilateral average of total hip site of white patients (-0.5 [-1.1 to -0.1]) was significantly lower when compared to white controls (0.9 [0.0 to 1.6]) (p = 0.014).

Black patients and –controls

No significant differences were observed comparing the median Z-scores of black patients with black controls at baseline.

Table 5.2.4. Median Z-scores at baseline: Patients and Controls[1]

	Black Females with GD n = 39	**Black Controls** n = 27	**p-value (95% confidence limits for mean differences)[2] Black patients vs. Black controls**	**White Females with GD** n = 19	**White Controls** n = 17	**p-value (95% confidence limits for mean differences)[2] White patients vs. White controls**
Lumbar Spine	-1.8 (-2.7 to -1.0)	-1.0 (-1.9 to -0.5)	*0.297* (-0.827 to 0.26)	-0.8 (-1.5 to 0.3)	-0.2 (-1.0 to 0.4)	*0.156* (-1.406 to 0.243)
Left femoral neck	-1.0 (-1.4 to -0.2)	-0.3 (-0.8 to -0.1)	*0.386* (-0.59 to 0.23)	-0.8 (-1.4 to -0.1)	0.1 (-1.1 to 0.5)	*0.113* (-1.492 to 0.172)
Left total hip	-0.9 (-1.3 to -0.3)	-0.05 (-0.8 to 0.5)	*0.062* (-0.762 to 0.019)	-0.9 (-1.1 to -0.1)	0.0 (-0.9 to 1.1)	*0.039* (-1.717 to -0.049)
Bilateral average of total hip	-0.8 (-1.3 to -0.4)	0.1 (-0.8 to 0.5)	*0.058* (-1.405 to 0.025)	-0.5 (-1.1 to -0.1)	0.9 (0.0 to 1.6)	*0.014* (-2.169 to -0.311)
Right femoral neck	-0.9 (-1.5 to -0.3)	-0.3 (-1.0 to -0.1)	*0.386* (-0.59 to 0.233)	-0.6 (-1.2 to 0.0)	-0.3 (-1.2 to 0.3)	*0.397* (-1.124 to 0.467)
Right total hip	-0.7 (-1.3 to -0.2)	-0.3 (-0.8 to 0.4)	*0.103* (-0.71 to 0.068)	-0.4 (-1.2 to 0.0)	0.0 (-0.6 to 0.8)	*0.124* (-1.260 to 0.166)
Left forearm distal 3rd	-0.9 (-2.5 to -0.2)	-0.5 (-1.5 to 0.3)	*0.053* (-1.36 to 0.008)	0.1 (-1.1 to 0.8)	0.7 (-0.1 to 1.0)	*0.081* (-1.88 to 0.121)

[1] Values are medians (interquartile range). [2] Generalized linear model of log transformed data.

The frequency (%) of subjects according to T-score categories at baseline for black- and white patients as well as for their respective controls, are shown and compared in Table 5.2.5. Only patients > 50 years of age were included. Osteopenia was diagnosed when the T-score was ≤ -1.0 but >-2.5 and osteoporosis when the T-score was ≤ -2.5.

The number of patients and controls was limited due to the age limitation. The highest prevalence of GD occurs between the ages of 30 to 50 years and this may explain the limited number of patients above the age of 50 years that presented and were included (2).

Black and white patients

There were nine black and 3 white patients above the age of 50 years where T-scores could be determined and analysed. There were no statistical differences between the two racial groups at any of the sites measured.

White patients and controls

There were only 3 subjects in each group over the age of 50 years. There were no significant differences in the T-score values comparing white patients and controls at baseline.

Black patients and controls

T-scores could be compared between nine black patients and 6 black controls. There were no significant differences between the two groups observed at any site measured.

Table 5.2.5. Frequency (%) of subjects older than 50 years at baseline according to T-score categories at selected anatomical sites

	Black Females with GD n = 9	**Black Controls** n = 6	**p-value[1] Black patients vs. Black controls**	**White Females with GD** n = 3	**White Controls** n = 3	**p-value[1] White patients vs. White controls**	**p-value[2] GD: Black- vs. White patients**
Lumbar spine							
Osteoporosis	3 33.3%	1 16.7%		0	0		
Osteopenia	3 33.3%	5 83.3%		1 33.3%	0		
Normal	3 33.3%	0	*0.564*	2 66.7%	3 100%	*0.317*	*0.727* (F)
Left femoral neck							
Osteoporosis	2 22.2%	0		0	0		
Osteopenia	3 33.3%	3 50%		2 66.7%	0		
Normal	4 44.4%	3 50%	*0.371*	1 33.3%	3 100%	*0.157*	*1.0* (F)
Left total hip							
Osteoporosis	1 11.1%	0		0	0		
Osteopenia	3 33.3%	1 16.7%		2 66.7%	0		
Normal	5 55.6%	5 83.3%	*0.117*	1 33.3%	3 100%	*0.157*	*0.659* (F)

Table 5.2.5. (Continued)

	Black Females with GD n = 9	**Black Controls** n = 6	**p-value[1] Black patients vs. Black controls**	**White Females with GD** n = 3	**White Controls** n = 3	**p-value[1] White patients vs. White controls**	**p-value[2] GD: Black- vs. White patients**
Bilateral average of total hip							
Osteoporosis	2 22.2%	0		0	0		
Osteopenia	2 22.2%	0		2 66.7%	0		
Normal	5 55.6%	4 100%	*0.18*	1 33.3%	3 100%	*0.157*	*0.509* (F)
Right femoral neck							
Osteoporosis	3 33.3%	0		0	0		
Osteopenia	2 22.2%	3 50%		2 66.7%	2 66.7%		
Normal	4 44.4%	3 50%	*0.264*	1 33.3%	1 33.3%		*0.545* (F)
Right total hip							
Osteoporosis	2 22.2%	0		0	0		
Osteopenia	2 22.2%	1 16.7%		2 66.7%	0		
Normal	5 55.6%	5 83.3%	*0.174*	1 33.3%	3 100%	*0.157*	*0.509* (F)

Table 5.2.5. (Continued)

	Black Females with GD n = 9	**Black Controls** n = 6	**p-value[1]** **Black patients vs. Black controls**	**White Females with GD** n = 3	**White Controls** n = 3	**p-value[1]** **White patients vs. White controls**	**p-value[2]** **GD: Black- vs. White patients**
Left total forearm							
Osteoporosis	4 44.4%	1 16.7%		1 33.3%	0		
Osteopenia	3 33.3%	3 50%		0	0		
Normal	2 22.2%	2 33.3%	*0.18*	2 66.7%	3 100%	*0.317*	*0.545* (F)
Left forearm distal 3rd							
Osteoporosis	6 66.7%	1 16.7%		1 33.3%	0		
Osteopenia	0	2 33.3%		0	0		
Normal	3 33.3%	3 50%	*0.102*	2 66.75	3 100%	*0.317*	*0.523* (F)
Ultra-distal left forearm							
Osteoporosis	4 44.4%	1 16.7%		1 33.3%	0		
Osteopenia	3 33.3%	3 50%		0	0		
Normal	2 22.2%	2 33.3%	*0.18*	2 66.7%	3 100%		*0.545* (F)
Whole body score							
Osteoporosis	6 66.7%	1 16.7%		0	0		
Osteopenia	1 11.1%	4 66.6%		1 33.3%	0		
Normal	2 22.2%	1 16.7%	*0.0833*	2 66.7%	3 100%		*0.118* (F)

[1]Cochran-Mantel-Haenszel p-value. [2]Chi-square p-value, except where indicated by (F) for Fisher's exact method due to sparse cells. T-scores: Normal > -1.0; Osteopaenia -1.0 to -2.4; Osteoporosis ≤ -2.5.

Discussion: *Baseline DXA measurements*

Among females with GD, a greater proportion of black patients (43.6%) had a median Z-score of the lumbar spine ≤ 2.0 compared to white patients (15%; p = 0.028). A greater proportion of white patients compared to black patients had a Z-score ≤ -2.0 at the left femoral neck (20.0% vs. 2.6% respectively) (p = 0.041).

It is, however, hard to ignore the fact that in white women with GD actual median BMD (g/cm^2) of the left femoral neck was significantly lower than in their black counterparts (p = 0.033). Although not reflected by the Z-scores, the BMD (g/cm^2) at the distal 3rd of the radius was significantly lower in black patients when compared to white patients (p = 0.049).

Among women older than 50 years, median T-scores did not differ significantly between the two groups of patients.

Differences in BMD do exist between African American women and black South African Women (3). In a study by Daniels *et al.* investigating geometric differences between the two population groups, it was shown that black women from the U.S.A. had greater geometric measurements of the femur compared to black South African women (3). Compared to black women from the U.S.A. the Z-scores may be decreased, but the absolute BMD of the black- and white South African women with GD were comparable. Higher BMD values of the lumbar spine and femur have been shown in premenopausal black women from the U.S.A. compared to their white counterparts (4, 5).

Ethnic differences in BMD between healthy black and white South African females do exist (6-9). In the early 1990s Daniels *et al.* showed that the peak femoral bone density of healthy black females where higher when compared to white females (9). A study by Chantler *et al.* found that hip BMD was higher in premenopausal black females, but that lumbar spine BMD was lower when compared to whites (6). A recent study from Cape Town found that the lumbar spine BMD of black premenopausal females were equal or lower than that found in their white counterparts (7). In the same study the lumbar BMD of black- and white females were the same and it also found the hip BMD to be higher in black females irrespective of menopausal status. This raises the question whether the finding of lower lumbar spine- and hip BMD found were due to GD or rather ethnic differences?

While almost 80% of black controls at baseline had a normal lumbar spine Z-score compared to only 56% of black patients, this difference was not significant. White controls tended to

have a higher BMD when compared to white patients at all sites measured, but it only reached significance in the bilateral hip average measurement ($p = 0.017$). This finding may indicate ethnic differences rather than differences due to GD.

It has previously been shown that hyperthyroidism affects both trabecular- and cortical bone (10). The spine is rich in trabecular bone while the distal radius and proximal femur are predominantly rich in cortical bone (11). The negative effect of hyperthyroidism on BMD at the hip and spine have been confirmed by others (12). The Nord-Trøndelag Health Study 1995–1997 (HUNT 2) confirmed that a suppressed TSH also correlates with a low BMD of the distal radius (13). Interestingly in a study by Segna *et al.* it was shown that hyperthyroidism had a detrimental effect on the hip BMD, but not the spine (14). In a northern European study it was shown that fracture risk was almost doubled in hyperthyroid subjects when compared to controls (15). The study showed that the main sites contributing to the increased fracture rates were the spine and distal radius, especially in subjects above the age of 50 years. This doubling of fracture risk associated with hyperthyroidism was also observed in a Scottish study (16).

In the current study it seems that ethnic differences may explain some of the differences observed. However, some differences observed may be due to GD. A greater proportion of black patients had decreased Z-scores at the left forearm distal 3rd of the radius compared to controls although median BMD at this site did not differ significantly. The opposite was observed in white subjects and controls although the numbers were extremely low. Median bilateral average of total hip BMD of white patients was significantly lower compared to white controls. These differences may be due to GD.

5.3 **Vertebral fracture assessment: Baseline**

Vertebral fracture assessment (VFA) was performed according to the Genant system and using the software supplied by Hologic (17). The vertebrae included in the analysis were from the fourth thoracic vertebra (T4) to the 4th lumbar vertebra (L4).

The accuracy of the placement of the vertebral tracer was confirmed by the investigator. According to the Genant system a vertebral fracture can either be a wedge-, biconcave- or crush deformity. The fractures are then graded according to height loss: Normal / no fracture

< 20% height loss; Mild fracture 20-24.9% height loss; Moderate fracture 25-40% height loss; Severe fracture > 40% height loss.

Black- and white patients

Except for the 8th thoracic vertebra (T8) no significant differences were found between the two groups at any of the other sites measured (Appendix 5A). At the level of T8 (Table 5.3.1.), more white female patients (15.8%) had radiological evidence of any degree of biconcave deformity when compared to black patients (5.6%; p = 0.369). The clinical significance of this finding, however, is unclear.

White patients and controls

No significant difference in the frequency of vertebral fractures, according to the Genant system, could be identified between white patients and controls. For additional comparisons of VFA, see Appendix 5B.

Black patients and controls

Contrary to expectation significantly more black control subjects (compared to subjects with GD) had biconcave deformities at the level of T9 (p = 0.034), T11 (p = 0.01) and T12 (p = 0.016) (5.3.2.). For additional comparisons of VFA see Appendix 5C.

Table 5.3.1. Frequency (%) of patients with evidence of a biconcave deformity vertebral fracture at T8 at baseline

	Black Females with GD n = 36	**White Females with GD** n = 19	**p-value***
T8 biconcave deformity			
Normal	34 94.4%	16 84.2%	
Mild	0	3 15.8%	
Moderate	2 5.6%	0	
Severe	0	0	*0.037* (F)

* Chi-square p-value, except where indicated by (F) for Fisher's exact method due to sparse cells.
Fractures were graded according to height loss: normal / no fracture < 20% height loss; mild fracture = 20-24.9% height loss; moderate fracture = 25-40% height loss; severe fracture > 40% height loss.

Table 5.3.2. Frequency (%) of black patients and controls with evidence of a biconcave deformity vertebral fracture at T9, 11 & 12 at baseline

	Black Females with GD n = 37	**Black Controls** n = 25	**p-value***
T9 biconcave deformity	n = 37	n = 25	
Normal	34 91.9%	20 80%	
Mild	3 8.1%	2 8%	
Moderate	0	2 8%	
Severe	0	1 4%	*0.034*
T11 biconcave deformity			
Normal	37 100%	20 80%	
Mild	0	2 8%	
Moderate	0	3 12%	
Severe	0	0	*0.01*
T12 biconcave deformity			
Normal	37 100%	19 76%	
Mild	0	3 12%	
Moderate	0	3 12%	
Severe	0	0	*0.016*

* Cochran-Mantel-Haenszel p-value.

The vertebral fracture assessment (VFA) are reported according to the Genant classification (17): Normal < 20% height loss; Mild fracture = 20-24.9% height loss; Moderate fracture = 25-40% height loss; Severe fracture > 40% height loss.

Discussion: V*ertebral fracture assessment at baseline*

In total, 57 clinically important vertebral deformities were observed in black patients, and 31 deformities in white patients with GD. Eight deformities were observed in black postmenopausal patients with GD and four in white postmenopausal patients. Black subjects had significantly more moderate to severe deformities at the level of T8 on VFA than their white counterparts. This finding is limited to T8 and the clinical relevance thereof is questionable especially in view of the finding of more frequent vertebral deformities on VFA assessment in black control subjects compared to black females with GD.

In a 2015 study by Conradie *et al.* it was shown that black euthyroid females suffered vertebral fractures at the same rate as their white counterparts (8). In the early 1990s, Daniels and colleagues showed that radial- and spinal bone mineral density were equal between healthy black- and white South African females (9). The same group however showed that black South African females had a higher femoral BMD when compared to white females. Studies in 2012 and 2014 showed that black premenopausal women had lower or equivalent BMD values of the spine and increased BMD values at the hip irrespective of menopausal status (6, 7). In this current study black female patients tended to have an increased frequency of decreased Z-scores of the AP-spine which is in keeping with the earlier studies. The comparable fracture rates on VFA, only one significant difference detected, is also in line with the previous studies reported. Once again this illustrates that the difference in the Z-scores of the AP-spine may be due to ethnic differences rather than GD.

The reason why black controls had more radiologically important fractures on VFA is unclear. Hyperthyroidism is associated with decreased BMD at the hip, spine and distal radius (12-14). Black controls did have significantly lower 25-hydroxyvitamin D (25(OH)D) (20 ng/ml [16 to 27]) levels when compared to black patients (27.0 ng/ml [20.5 to 40.0]; $p = 0.021$). This was especially true for premenopausal black female controls. Premenopausal black euthyroid controls had a statistically significant lower 25(OH)D level (20.1 ng/ml [16 to 24]) when compared to black patients (28.4 ng/ml [20.5 to 40.0]; $p = 0.013$). Vitamin D deficiency is associated with increased fracture risk (18, 19). At this stage it is important to note that a 25(OH)D level of less than 32 ng/ml, although indicating reduced levels in white women, may not be indicative of reduced levels in black women yet (20). Powe *et al.* showed that in African Americans a decreased 25(OH)D level is accompanied by a decreased Vitamin D-binding protein. This decrease in Vitamin D-binding protein leads to an increase

of bioavailable 25(OH)D levels in African Americans comparable to their white counterparts. In a 2011 study investigating the relationships between 25(OH)D levels and other factors influencing bone health it was found that a level of 20 ng/ml may be more indicative of decreased 25(OH)D levels in non-Hispanic black Americans (21). However, the median 25(OH)D levels of the black controls in the current study was 20 ng/ml, which is the threshold identified as low and associated with increased fracture risk. (Table 4.4.1.)

5.4 **Rate of recovery of BMD at 6- and 12 months**

Participating patients received an appointment card with a 6-month follow-up date at the endocrine clinic. A diary of the given date was also generated. To improve follow-up compliance patients were phoned one week in advance to remind them of the up-coming visit. If patients failed to attend, they were contacted weekly for 4 weeks after the given date. Patients who followed-up at 6 months went through the same process at 12 months. Patients who did not attend at 6 months, but followed up later, were re-scheduled for the 12-month visit. Factors that influenced follow-up included death (1 black patient died before the 6 month follow up visit), pregnancy (1 black patient became pregnant 3 months after entering the study), logistical problems (e.g. no transport to attend the clinic) etc. The follow-up rate for black patients was 49% at 6 months and 46% at 12 months. At 6 months 60% of white patients followed-up and at 12 months 55%.

Table 5.4.1 compares the thyroid-stimulating hormone- (TSH) and thyroxine levels (T_4) 6 months after therapy between black- and white patients. The median TSH level of white patients (4.63 mIU/L [1.24 to 32.69]) was like that of black patients (3.15 mIU/L [0.02 to 10.4]) at 6 months ($p = 0.336$). Similarly, T_4 levels of the white patients were not significantly different to those of black patients (16 pmol/L [10 to 17] compared to 13.6 pmol/L [3.57 to 24.45]; $p = 0.247$). It must be noted that the number of subjects is small, making it unlikely that small differences will be significant.

Table 5.4.1. Median TSH and T_4 levels at 6 months[1]

	Black Females with GD	**White Females with GD**	**p-value (95% confidence limits for median differences)[2] GD: Black- vs. White patients**
TSH (mIU/L)	n = 9	n = 8	
Baseline	0.01 (0.01 to 0.01)	0.01 (0.01 to 0.01)	
6 months	3.15 (0.02 to 10.4)	4.63 (1.24 to 32.69)	*0.336* (-6.95 to 27.52)
T_4 (pmol/L)	n = 8	n = 7	
Baseline	99.8 (34.4 to 100)	41.3 (26.6 to 56)	
6 months	13.6 (3.57 to 24.45)	16 (10 to 17)	*0.247* (-15.2 to 67)

[1] Values are medians (interquartile range).
[2] Statistical method: Wilcoxon two-sample test.
TSH, thyroid-stimulating hormone (reference range = 0.27-4.20 mIU/L); T4, thyroxine (reference range = 12.0-22.0) pmol/L.

Table 5.4.2 depicts the differences in BMD between black and white patients at 6 and 12 months. The patients included here are those who were followed up at 6 and 12 months.

At baseline, the median BMD of the left femoral neck was significantly higher in the group of black females (0.78 g/cm^2 [0.70 to 0.88]) compared to their white counterparts (0.73 g/cm^2 [0.64 to 0.79]; p = 0.033). After 6 months the black subjects had a median BMD of 0.80 g/cm^2 (0.70 to 0.91) compared to 0.72 g/cm^2 (0.63 to 0.77) in the white subjects (p = 0.031). At 12 months the difference was even more with a median BMD of the left femoral neck 0.87 g/cm^2 (0.76 to 0.98) in black subjects compared to 0.74 g/cm^2 (0.55 to 0.79) in white subjects (p = 0.012).

At baseline and at 6 months the median BMD of the right femoral neck did not differ significantly between the two groups. However, at 12 months a significant difference became

apparent with a median BMD of 0.88 g/cm^2 (0.76 to 1.01) in black patients compared to a median BMD 0.76 g/cm^2 (0.59 to 0.88) in white subjects (p = 0.031).

The BMD of the white subjects at the distal 3rd of the left forearm was significantly higher at baseline when compared to black subjects. This difference however disappeared at 6- and 12-month follow-up with no significant differences between the two groups at 6- and 12-month follow-ups.

Table 5.4.2. Actual bone mineral density (g/cm^2) of patients at 6- and 12 months[1]

	Black Females with GD	**White Females with GD**	**p-value (95% confidence limits for median differences)[2] GD: Black- vs. White patients**
Lumbar Spine			
Baseline	0.91 (0.83 to 1.02) n = 39	0.91 (0.85 to 1.02) n = 20	*0.695* (-0.048 to 0.087)
6 months	0.95 (0.88 to 1.16) n = 18	1.08 (0.97 to 1.21) n = 12	*0.319* (-0.09 to 0.25)
12 months	1.07 (0.98 to1.19) n = 17	0.98 (0.81 to 1.16) n = 11	*0.337* (-0.26 to 0.09)
Left femoral neck			
Baseline	0.78 (0.70 to 0.88) n = 39	0.73 (0.64 to 0.79) n = 20	*0.033* (-0.151 to -0.006)
6 months	0.80 (0.70 to 0.91) n = 18	0.72 (0.63 to 0.77) n = 12	*0.031* (-0.22 to -0.01)
12 months	0.87 (0.76 to 0.98) n = 17	0.74 (0.55 to 0.79) n = 11	*0.012* (-0.23 to -0.06)
Left total hip			
Baseline	0.88 (0.81 to 0.95) n =39	0.82 (0.72 to 0.90) n = 20	*0.069* (-0.134 to 0.005)
6 months	0.90 (0.81 to 0.98) n = 18	0.83 (0.74 to 0.87) n = 12	*0.099* (-0.18 to 0.03)
12 months	0.97 (0.87 to 1.07) n = 17	0.86 (0.77 to 0.93) n = 11	*0.860* (-0.27 to 0.04)
Bilateral average of total hip			
Baseline	0.88 (0.82 to 0.97) n = 39	0.85 (0.76 to 0.90) n = 20	*0.138* (-0.131 to 0.025)
6 months	0.89 (0.83 to 0.99) n = 18	0.83 (0.75 to 0.89) n = 12	*0.189* (-0.18 to 0.04)
12 months	0.96 (0.87 to 1.06) n = 17	0.87 (0.77 to 0.97) n = 11	*0.138* (-0.25 to 0.04)

Table 5.4.2. (Continued)

	Black Females	**White Females**	**p-value (95% confidence limits for median differences)[2] GD: Black- vs. White patients**
Right femoral neck			
Baseline	0.79 (0.67 to 0.89) n = 39	0.74 (0.63 to 0.82) n = 20	*0.126* (-0.143 to 0.016)
6 months	0.82 (0.67 to 0.92) n = 18	0.76 (0.62 to 0.79) n = 12	*0.083* (-0.20 to 0.03)
12 months	0.88 (0.76 to 1.01) n = 17	0.76 (0.59 to 0.88) n = 11	0.030 (-0.28 to -0.00)
Right total hip			
Baseline	0.88 (0.80 to 0.99) n = 39	0.87 (0.76 to 0.92) n = 20	*0.252* (-0.121 to 0.036)
6 months	0.87 (0.83 to 1.00) n = 18	0.84 (0.75 to 0.92) n = 12	*0.330* (-0.17 to 0.07)
12 months	0.96 (0.88 to 1.04) n = 17	0.88 (0.76 to 1.02) n = 11	*0.165* (-0.24 to 0.04)
Left forearm distal 3rd			
Baseline	0.62 (0.051 to 0.66) n = 39	0.66 (0.60 to 0.73) n = 20	*0.0493* (-0.001 to 0.107)
6 months	0.57 (0.49 to 0.64) n = 18	0.61 (0.53 to 0.69) n = 12	*0.230* (-0.04 to 0.14)
12 months	0.57 (0.54 to 0.61) n = 17	0.63 (0.56 to 0.68) n = 11	*0.194* (-0.03 to 0.11)

[1] Values are medians (interquartile range).
[2] Statistical method used: Analysed with a Wilcoxon two-sample test.

Change in BMD (g/cm^2) after 12 months in black- vs. white subjects

Table 5.4.3 reviews the changes in BMD from baseline to 12 months after therapy at selected sites. At 12 months 17 out of 39 black patients followed-up and 11 out of 20 white patients.

There were no significant differences between the two subject groups when comparing the change in median BMD 12 months after initiation of therapy for GD.

Table 5.4.3. Change in BMD (g/cm^2): Black- and white patients who were available at baseline and at 12 months[1]

	Black Females with GD **n = 17**	**White Females with GD** **n = 11**	**p-value** **(95% confidence limits for median differences)[2]** **GD: Black- vs. White patients**
Lumbar Spine			
Baseline	1.02 (0.96 to 1.17)	0.92 (0.84 to 1.08)	
12 months	1.11 (1.04 to 1.19)	0.92 (0.80 to 1.17)	
Change	0.07 (-0.03 to 0.11)	0.04 (0.02 to 0.06)	*0.514* (-0.09 to 0.06)
Left femoral neck			
Baseline	0.78 (0.71 to 0.88)	0.69 (0.56 to 0.79)	
12 months	0.87 (0.76 to 0.98)	0.74 (0.55 to 0.79)	
Change	0.05 (-0.01 to 0.09)	0.01 (-0.02 to 0.02)	*0.301* (-0.09 to 0.03)
Left total hip			
Baseline	0.87 (0.81 to 0.96)	0.81 (0.66 to 0.89)	
12 months	0.97 (0.87 to 1.07)	0.86 (0.77 to 0.93)	
Change	0.07 (0.01 to 0.11)	0.05 (0.02 to 0.12)	*0.832* (-0.06 to 0.05)

Table 5.4.3. (Continued)

	Black Females n = 17	**White Females n = 11**	**p-value (95% confidence limits for median differences)[2] GD: Black- vs. White patients**
Bilateral average of total hip			
Baseline	0.88 (0.82 to 0.98)	0.85 (0.66 to0.91)	
12 months	0.96 (0.87 to 1.06)	0.87 (0.77 to 0.97)	
Change	0.05 (0.01 to 0.10)	0.04 (0.01 to 0.11)	*0.869* (-0.06 to 0.05)
Right femoral neck			
Baseline	0.79 (0.71 to 0.93)	0.76 (0.59 to 0.82)	
12 months	0.88 (0.76 to 1.01)	0.76 (0.59 to 0.88)	
Change	0.04 (0.00 to 0.09)	0.03 (-0.02 to 0.08)	*0.556* (-0.074 to 0.04)
Right total hip			
Baseline	0.88 (0.81 to 0.99)	0.88 (0.66 to 0.93)	
12 months	0.96 (0.88 to 1.04)	0.88 (0.76 to 1.02)	
Change	0.06 (0.02 to 0.10)	0.02 (-0.00 to 0.10)	*0.410* (-0.07 to 0.04)
Left forearm distal 3rd			
Baseline	0.60 (0.54 to 0.63)	0.66 (0.59 to 0.73)	
12 months	0.57 (0.57 to 0.61)	0.63 (0.56 to 0.68)	
Change	0.00 (-0.03 to 0.02)	-0.01 (-0.05 to 0.00)	*0.279* (-0.04 to 0.02)

[1] Values are medians (interquartile range).
[2] Statistical method used: Analysed with a Wilcoxon two-sample test.

BMD (g/cm^2) in subjects at 12 months vs. controls at baseline

Table 5.4.4 compares the BMD of patients that followed up at 12 months with their controls at baseline. Seventeen black patients were followed-up at 12 months and were ideally matched with 15 black controls at baseline according to age, body mass index (BMI) and

seasonality. Only 10 white patients had a complete set of data at 12 months and were matched accordingly.

White patients and controls:

Twelve months after the initiation of therapy for GD the median bilateral average total hip BMD (0.90 g/cm^2 [0.77 to 0.97]) remained significantly lower in the patient group compared to the control group (1.02 g/cm^2 [0.94 to 1.10]; p = 0.037). At baseline the median BMD of the distal 3rd of the left forearm did not differ significantly between the white subjects and controls, but 12 months after therapy the white subjects had a significantly lower BMD at this site when compared to the control group (p = 0.016).

Black patients and –controls:

Twelve months after the initiation of therapy for GD, median BMD in black subjects remained overall comparable to that of control subjects and no statistically significant differences were found.

Table 5.4.4. Actual BMD (g/cm^2): Patients with GD at 12 months compared to their controls at baseline[1]

	Black Females with GD	Black Controls	p-value (95% confidence limits for ratio)[2] Black patients vs. Black controls	White Females with GD	White Controls	p-value (95% confidence limits for ratio)[2] White patients vs. White controls
Lumbar Spine	1.07 (0.98 to 1.19)	1.02 (0.99 to 1.07)	*0.11* (0.98 to 1.19)	1.07 (0.89 to 1.16)	1.16 (0.95 to 1.40)	*0.191* (0.35 to 1.27)
Left femoral neck	0.87 (0.76 to 0.98)	0.83 (0.74 to 0.91)	*0.725* (0.89 to 1.18)	0.75 (0.64 to 0.79)	0.86 (0.76 to 0.89)	*0.098* (0.74 to 1.03)
Left total hip	0.97 (0.87 to 1.07)	0.95 (0.86 to 1.01)	*0.943* (0.86 to 1.15)	0.89 (0.78 to 0.93)	1.02 (0.85 to 1.08)	*0.227* (0.73 to 1.09)
Bilateral average of total hip	0.96 (0.87 to 1.06)	0.88 (0.85 to 0.96)	*0.873* (0.75 to 1.29)	0.90 (0.77 to 0.97)	1.02 (0.94 to 1.10)	*0.037* (0.77 to 0.99)
Right femoral neck	0.88 (0.76 to 1.01)	0.90 (0.69 to 0.91)	*0.115* (0.98 to 1.20)	0.78 (0.66 to 0.88)	0.84 (0.71 to 0.86)	*0.37* (0.78 to 1.10)
Right total hip	0.96 (0.88 to 1.04)	0.95 (0.85 to 1.05)	*0.369* (0.95 to 1.13)	0.90 (0.77 to 1.02)	0.96 (0.86 to 1.03)	*0.396* (0.79 to 1.11)
Left forearm distal 3rd	0.57 (0.54 to 0.61)	0.64 (0.59 to 0.67)	*0.077* (0.82 to 1.01)	0.63 (0.57 to 0.68)	0.72 (0.69 to 0.78)	*0.016* (0.78 to 0.97)

[1] Values are medians (interquartile range). [2] Generalized linear model of log transformed data.

Change of BMD from baseline to 6 months versus change from 6-12 months

Table 5.4.5 compares the change in BMD from baseline to 6 months versus the change from 6-12 months in the patient groups. Only patients that were followed up at 6 and 12 months were included in this analysis. It must be noted that the numbers were small, and therefore no statistically significant differences were observed.

Table 5.4.5. Change in BMD (g/cm^2) in black and white patients who were followed up at baseline, 6 and 12 months[1]

	Black Females with GD n = 11	**p-value[2] GD: 0-6 vs. 6-12**	**White Females with GD n = 7**	**p-value[2] GD: 0-6 vs. 6-12**
Lumbar Spine				
0-6 months	-0.042 (-0.07 to 0.00)		0.0715 (0.09 to 0.11)	
6-12 months	0.114 (0.04 to 0.17)	*0.156* (-0.16 to 0.26)	-0.0185 (-0.07 to 0.04)	*0.125* (-0.203 to -0.002)
Left femoral neck				
0-6 months	-0.002 (-0.02 to 0.03)		-0.002 (-0.01 to 0.02)	
6-12 months	0.012 (0.0 to 0.05)	*0.898* (-0.12 to 0.10)	0.009 (-0.0 to 0.02)	*0.578* (-0.097 to 0.078)
Left total hip				
0-6 months	0.012 (0.0 to 0.06)		0.008 (-0.09 to 0.07)	
6-12 months	0.022 (0.01 to 0.06)	*0.831* (-0.06 to 0.09)	0.033 (-0.01 to 0.05)	*1.000* (-0.166 to 0.064)
Bilateral average of total hip				
0-6 months	0.039 (-0.01 to 0.06)		0.016 (-0.01 to 0.06)	
6-12 months	0.022 (-0.0 to 0.04)	*0.765* (-0.05 to 0.08)	0.026 (0.0 to 0.03)	*0.938* (-0.067 to 0.064)

Table 5.4.5. (Continued)

	Black Females with GD n = 11	**p-value[2] GD: 0-6 vs. 6-12**	**White Females with GD n = 11**	**p-value[2] GD: 0-6 vs. 6-12**
Right femoral neck				
0-6 months	0.014 (-0.02 to 0.08)		0.001 (-0.01 to 0.02)	
6-12 months	0.025 (-0.01 to 0.08)	*0.967* (-0.04 to 0.06)	0.024 (-0.0 to 0.1)	*0.578* (-0.134 to 0.11)
Right total hip				
0-6 months	0.016 (-0.02 to 0.06)		0.026 (-0.02 to 0.05)	
6-12 months	0.026 (0.01 to 0.05)	*0.916* (-0.05 to 0.10)	0.019 (0.0 to 0.05)	*0.938* (-0.075 to 0.069)
Left forearm distal 3rd				
0-6 months	0.0005 (-0.02 to 0.02)		-0.0165 (-0.02 to -0.01)	
6-12 months	0.0135 (-0.02 to 0.03)	*0.547* (-0.02 to 0.07)	0.013 (-0.02 to 0.02)	*0.438* (-0.032 to 0.047)

[1] Values are medians (interquartile range).
[2] Statistical method used: Analysed with a Wilcoxon two-sample test.

Discussion: *Rate of recovery of BMD at 6- and 12 months*

Among black patients the median BMD at the left femoral neck was significantly greater at baseline and at 6- and 12 months compared to that of white patients. This difference remained significant despite both black- and white patient groups being euthyroid at 6 months already. At the right femoral neck, the median BMD of black patients was also higher compared to that of white patients at all three time points, but only reached statistical significance at 12 months. Median BMD at both left and right femoral neck areas for both patient groups at 12 months did not differ significantly in comparison to their respective controls at baseline. These observations along with the patient differences at 12 months may reflect subtle ethnic differences in BMD at the femoral neck rather than differences induced by GD.

It must be noted that the bilateral average of the total hip BMD was significantly lower at baseline in white patients compared to white controls. This difference remained at 12 months. No significant differences were observed at this site between black patients and –controls at baseline and 12 months. This finding may illustrate a GD effect rather than an ethnic difference i.e. greater bone loss due to GD observe in the total hip area of white patients.

At baseline, the left forearm distal 3rd median BMD of the white patients was significantly higher compared to that of the black patients. This difference was no longer significant at 6- and 12 months. It must be noted that the number of subjects were reduced at the 6- and 12-month visits and this may influence the outcome. There was also no significant difference in the rate of recovery at this site comparing black- and white female patients. Among white patients at baseline median BMD at the left forearm distal 3rd of was lower compared to white controls, but this difference failed to reach statistical significance. At 12 months, however, the difference in median BMD at the left forearm distal 3rd among white patients and controls became highly significant. There were no significant differences between the black patients and their controls at 12 months at this site. This intra-ethnic difference between white patients and –controls may also reflect a GD effect, rather than an ethnic difference.

An increase in BMD was observed 12 months after therapy in both patient groups. The BMD does recover after treatment for hyperthyroidism (12). In a study evaluating the effect of treatment of hyperthyroidism on BMD, it was shown that at 3 years the BMD of patients with previous hyperthyroidism were comparable to healthy controls (12). It does however seem that initially, especially within the first 12 months after therapy, the recovery of the BMD is

not complete (22). The findings reported in this thesis support this observation – at least for the distal 3rd of the forearm in white but not in black subjects. It also seems that menopausal status influences the recovery rate, with postmenopausal women not recovering completely from the hyperthyroid state (23). Thyroid surgery has been shown to be associated with a rapid increase of the BMD (24). In a recent publication it was also shown that radio-active iodine therapy was associated with a delayed BMD recovery when compared to surgery (25). Despite the recovery of BMD the fracture risk may persist for up to 5 years after the diagnosis of hyperthyroidism (26).

Although not observed in the current study, previous researchers found an increased risk for fracture at especially the spine, hip and forearms of patients suffering from hyperthyroidism (15, 27, 28). This fracture risk was increased up to three-fold in patients who had received radio-active iodine (15). Most of the patients included in the current study received radio-active iodine as part of their treatment regimen. Thyroid surgery and the use of anti-thyroid drugs have been shown to be associated with a decreased fracture risk (26, 29).

5.5 **Summary and conclusions**

In the current study it seems that ethnic differences may explain some of the differences observed. However, some differences observed may be due to GD.

More black patients had decreased Z-scores of the AP-spine when compared to white patients. The same was not observed when comparing black patients with their controls and this finding may be indicative of ethnic differences rather GD induced differences. It is important to remember differences do exist when comparing the BMD of black South African women with that of African American women.

Black patients had decreased Z-scores at the radius when compared to controls. The BMD at the hip of white patients were lower than that found in controls. These differences may be due to GD.

5.6 **References**

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Chapter 6

Results and Discussion: Dual-energy X-ray Absorptiometry - Body Composition

6.1 **Introduction**

In this chapter the results of body composition measurements as determined by Dual-energy X-ray absorptiometry (DXA) on black and white women newly diagnosed with GD are compared and discussed.

6.2 **Body composition: Black- and white patients**

The median BMI of black and white females at baseline were 25 (21 to 32) kg/m^2 and 25 (21 to 28.5) kg/m^2 respectively and did not differ significantly (p = 0.344) (Table 4.1.1). The percentage body fat per anatomical region at baseline for black- and white females with GD and their respective controls are shown in Table 6.2.1.

Black- and white patients

Comparing the percentage fat distribution between black- and white patients suffering from GD, no significant differences were observed between the two groups at baseline. The arm- and leg fat tended to be higher in black patients when compared to whites, but it did not reach statistical significance.

White patients and –controls

White females with GD had significantly higher head fat percentage (18.4%) compared to white controls (18.1%) (p= 0.049) at baseline. No other significant differences were observed.

Black patients and controls

Black female controls had significantly higher head fat percentage (22.6%) compared to black patients (18.4%; p < 0.0001) at baseline. No other significant differences were observed.

Table 6.2.1. Percentage (%) body fat per anatomical region for black and white female subjects at baseline[1]

Site (%)	**Black Females with GD** n = 38	**Black Controls** n = 27	**p-value (95% confidence limits for ratio)[3] Black patients vs. Black controls**	**White Females with GD** n = 20	**White Controls** n = 17	**p-value (95% confidence limits for ratio)[3] White patients vs. White controls**	**p-value (95% confidence limits for median differences)[2] GD: Black- vs. White patients**
Left arm fat	42.7 (34.8 to 52.4)	46.6 (34.4 to 54.1)	*0.878* (0.88 to 1.11)	41.6 (33.4 to 51.8)	39.7 (33.9 to 42.6)	*0.211* (0.95 to 1.26)	*0.647* (-9.1 to 4.8)
Right arm fat	41.15 (33.1 to 51.9)	44.7 (32.2 to 51.4)	*0.904* (0.87 to 1.13)	39.5 (32.4 to 47.7)	34.8 (33 to 40.9)	*0.199* (0.94 to 1.29)	*0.567* (-9.1 to 4.7)
Trunk fat	35.8 (28.2 to 43.6)	33.9 (28.5 to 43.4)	*0.901* (0.87 to 1.13)	32 (24.2 to 39.6)	28.4 (24.7 to 33.8)	*0.464* (0.90 to 1.24)	*0.192* (-10.2 to2.0)
Left leg fat	48.45 (44.5 to 54.3)	47 (42.1 to 50.1)	*0.208* (0.97 to 1.12)	44.7 (38.4 to 51.4)	44.5 (38.7 to 46.9)	*0.239* (0.96 to 1.15)	*0.066* (-7.8 to 0.4)
Right leg fat	47.8 (43.8 to 53)	45.5 (40.9 to 50.8)	*0.150* (0.98 to 1.13)	45.6 (38.2 to 51.2)	43.8 (38.6 to46.4)	*0.377* (0.95 to 1.14)	*0.105* (-7.8 to 0.8)

Table 6.2.1. (Continued)

Site (%)	**Black Females with GD** n = 39	**Black Controls** n = 27	**p-value (95% confidence limits for ratio)[3] Black patients vs. Black controls**	**White Females with GD** n = 20	**White Controls** n = 17	**p-value (95% confidence limits for ratio)[3] White patients vs. White controls**	**p-value (95% confidence limits for median differences)[2] GD: Black- vs. White patients**
Subtotal fat	41.2 (36 to 48.1)	40.8 (36.1 to 49.2)	*0.523* (0.9 to 1.1)	37.65 (32.9 to 47)	36 (32.2 to 40.2)	*0.117* (0.98 to 1.21)	*0.196* (-7.8 to 1.5)
Head fat	18.4 (18.2 to18.8)	22.6 (19.4 to 23)	*< 0.0001* (0.8 to 0.90)	18.4 (18.2 to 18.7)	18.1 (17.8 to 22.4)	*0.049* (0.90 to 1.00)	*0.774* (-0.3 to 0.2)
Total fat	39.8 (34.5 to 46.6)	38.9 (35 to 47.7)	*0.583* (0.9 to 1.1)	36.4 (30.8 to 45.4)	34.9 (30.4 to 37.8)	*0.93* (0.72 to 1.36)	*0.185* (-8.2 to 1.6)

[1] Values are medians (interquartile range).[2] Analysed with a Wilcoxon two-sample test. [3] Generalized linear model of log transformed data.

Table 6.2.2 compares the differences in body composition by body compartment between black- and white patients with GD. This comparison includes fat and lean mass. There were no significant differences in body composition between the two groups of patients at baseline.

Table 6.2.2. Body composition by compartment at baseline[1]

Site	**Black Females with GD** **n = 39**	**White Females with GD** **n = 20**	**p-value** **(95% confidence limits for median differences)[2]** **GD: Black- vs. White patients**
BMI (kg/m^2)	25 (21 to 32) n = 39	25 (21 to 28.5) n = 20	*0.344* (-5.00 to 2.00)
Total lean mass (g)	36349 (31094 to 40064) n = 38	38561 (36429 to 43191) n = 20	*0.069* (-281.60 to 6364.20)
Total fat mass (g)	25867 (17271 to 33849) n = 38	24967 (18014 to 32479) n = 20	*0.806* (-7109.30 to 5275)
Trunk fat (%)	35.8 (28.2 to 43.6) n = 37	32 (24.3 to 39.7) n = 20	*0.185* (-8.20 to 1.60)

[1] Values are medians (interquartile range). [2] Analysed with a Wilcoxon two-sample test.

Discussion: *Body composition at baseline*

In this study no significant differences in body composition were observed at baseline between black- and white females suffering from GD. Head fat content differed significantly between black patients and black controls, as well as white patients and white controls, but did not differ between white patients and black patients. Although not statistically significant, the arm- and leg fat of black patients tended to be higher when compared to white patients.

Previous studies showed ethnic differences in body composition between healthy black and white South African females (1-3). Chantler found that black premenopausal females had significantly lower visceral adipose tissue (VAT) compared to white premenopausal females (1). The reverse was true for subcutaneous adipose tissue (SAT) with black females having significantly more SAT compared to white females. Similar results were obtained in an earlier study by Goedecke *et al.* (2). More recently it was shown that black South African females tended to have more leg fat but less trunk fat compared to white females (4).

Hyperthyroidism is associated with changes in body composition which not only includes loss of bone- and lean mass, but also fat mass (5, 6). It must be kept in mind that patients were enrolled while suffering from GD, and not before contracting this condition. Thus, patients were matched with controls during the disease state and not when healthy. This may explain the absence of differences between the patients and controls at baseline as they were matched during the disease state and while being healthy.

6.3 **Changes in body composition between baseline and 6- and 12 months**

6.3.1 ***Rate of change in weight and body mass index from baseline to 6 and 12 months: The Patients***

Table 6.3.1 compares the median body weight (kg) of black- and white patients at baseline, 6- and 12 months. It also compares the rate of change in body weight from baseline to 6 months, 6- to 12 months, and baseline- to 12 months. The BMI of black- and white patients who followed up at 12 months were also compared, along with the Fat Mass Index (FMI) at baseline, 6- and 12 months. The comparison of body weight at 6- and 12 months between patient groups was done for all patients where data was available at specific time points, whereas the percentage change was only calculated for patients who had data available at both time points.

Among black female patients a progressive increase in median body weight from baseline to 12 months after treatment was observed while in white patients median body weight dropped somewhat at 6 months followed by a period of weight gain. Although not statistically significant ($p = 0.564$) median body weight of black patients tended to be less at baseline (64.8kg [55 to 76.3]) compared to that of white patients (69.5kg [60.3 to 74]). At 6- and 12 months the median body weight of black patients were higher compared to that of white

patients. Although these differences in body weight were not statistically significant it was of clinical importance.

Comparing the rate of change of in body weight at different time points no statistically significant differences were observed between black and white patients, but black patients had a clinically important increase (14.8% [(9.7 to 27.2]) from baseline to 12 months compared to white patients (7.63% [0 to 13.5]; p = 0.139).

The median BMI did not differ significantly between the two patient groups at 12 months although the difference was clinically meaningful.

Although the median FMI of black patients tended to be higher at all time points, it did not reach statistical significance. The FMI increased in both ethnic groups from baseline up to 12 months.

Table 6.3.1. Body weight (kg) at baseline, 6- and 12 months and change (%) in body weight, BMI at 12 months and FMI at baseline, 6- and 12 months[1]

Parameter	**Black Females with GD**	**White Females with GD**	**p-value (95% confidence limits for median differences)[2] GD: Black- vs. White patients**
Weight (kg)			
Baseline	64.8 (55 to 76.3) n = 39	69.5 (60.3 to 74) n = 20	*0.564* (-6 to 10.4)
6 months	73.4 (61 to 81) n = 18	66.5 (63.8 to 73) n = 12	*0.611* (-15 to 9)
12 months	80.4 (63 to 98) n = 16	71 (55 to 85) n = 11	*0.444* (-27 to 10)
Change in weight (%)			
Baseline-6 months	3 (0 to 10.9) n = 18	0.69 (0 to 4.29) n = 12	*0.326* (-8.806 to 1.852)
6-12 months	2.1 (0 to 12.1) n = 10	3.1 (0 to 11) n = 7	*0.921* (-12.069 to 8.138)
Baseline-12 months	14.8 (9.7 to 27.2) n = 16	7.63 (0 to 13.5) n = 11	*0.138* (-16.888 to 1.852)
BMI (kg/m^2)	33.6 (27.8 to 35.1) n = 17	26.7 (20.5 to 31.6) n = 11	*0.078* (-13.10 to 1.040)

Table 6.3.1. (Continued)

Parameter	**Black Females with GD**	**White Females with GD**	**p-value (95% confidence limits for median differences)[2] GD: Black- vs. White patients**
Fat Mass Index (kg/m^2)			
Baseline	9.87 (7.1 to 14.3) n = 38	8.63 (6.4 to 12.5) n = 20	*0.326* (-3.682 to -1.181)
6 months	11.7 (10.0 to 16.3) n = 16	9.49 (5.7 to 13.6) n = 11	*0.114* (-6.176 to 1.387)
12 months	14.33 (9.3 to 21.1) n = 15	9.71 (5.4 to 15.6) n = 11	*0.073* (-9.854 to -4.543)

[1] Values are medians (interquartile range). [2] Analysed with a Wilcoxon two-sample test.

Table 6.3.2 compares the rate of change of BMI from baseline to 6- and 12 months within each patient group. Patient numbers are limited since only patients who were evaluated at 12 months were included.

Black patients

Comparing the BMI of black patients at baseline (25.5 kg/m^2 [22 to 36]) with the BMI at 6 months (27.3 kg/m^2 [24.8 to 33.7]) a significant increase of 4.3% (p = 0.048) was found. The same was observed when comparing the BMI at baseline (26 kg/m^2 [22 to 32]) with the BMI at 12 months (33.6 kg/m^2 [27.8 to 35.1]), with a significant increase of 13.2% from baseline (p = 0.0002). An increase was observed from 6 to 12 months, but it was not statistically significant.

White patients

A significant increase of 1.96% (p = 0.042) in BMI from baseline (25.5 kg/m^2 [21 to 28.5] to 6 months (24.9 kg/m^2 [21.8 to 28.7]) was observed in white patients. Comparing the BMI at baseline (26 kg/m^2 [20 to 28]) with the BMI at 12 months (26.7 kg/m^2 [20.5 to 31.6]) a significant increase of 8.7% (p = 0.01) was observed. A small increase was observed from 6-12 months, but it was not statistically significant.

Table 6.3.2. BMI (kg/m^2) at baseline, 6- and 12 months and change (%) in BMI[1]

Parameter	**Black Females with GD**	**p-value (95% confidence limits)[2]**	**White Females with GD**	**p-value (95% confidence limits)[2]**
Baseline-6 months	n = 18		n = 12	
Baseline (kg/m^2)	25.5 (22 to 36)		25.5 (21 to 28.5)	
6 months (kg/m^2)	27.3 (24.8 to 33.7)		24.9 (21.8 to 28.7)	
Change (%)	4.3 (-0.5 to 10.5)	*0.048* (14.805 to 25)	1.96 (-0.08 to 5.3)	*0.042* (5.381 to 10.345)
6-12 months	n = 11		n = 7	
6 months (kg/m^2)	27.1 (23.2 to 33.6)		24.8 (21.4 to 28.5)	
12 months (kg/m^2)	28.3 (23.6 to 34.5)		26.7 (22 to 31.6)	
Change (%)	2.5 (0 to 12.1)	*0.078* (12.1 to 30.451)	3.1 (0 to 10.9)	*0.188*
Baseline-12 months	n = 17		n = 11	
Baseline (kg/m^2)	26 (22 to 32)		26 (20 to 28)	
12 months (kg/m^2)	33.6 (27.8 to 35.1)		26.7 (20.5 to 31.6)	
Change (%)	13.2 (8 to 24.5)	*0.0002* (28.519 to 79.615)	8.7 (0 to 14.5)	*0.01* (14.462 to 34.867)

[1] Values are medians (interquartile range).[2] Analysed with a Wilcoxon two-sample test.

6.3.2 ***Rate of change of body composition per compartment from baseline to 6 & 12 months: The Patients***

The body composition at 6- and 12 months compared to baseline between black and white female patients who were available for follow up at 6- and 12 months were compared. Only patients with follow up at 6 or 12 months were compared to their baseline values.

There were no significant differences in the change from baseline to 6 months between black- and white female patients (Appendix 6A). There were also no significant differences in the change from baseline to 12 months between black- and white female patients (Appendix 6B).

6.3.3 ***Comparison of body composition of patients at 6- and 12 months vs. controls at baseline***

Table 6.3.3 compares the BMI, FMI, and body composition by compartment of patients and controls at baseline, and at 6 and 12 months.

White patients and controls

The baseline data shown includes all the patients and controls at enrolment into the study. There were no significant differences between the white patients and their controls at baseline.

The follow-up data compares only patients who were followed up at 6 and 12 months with their controls at baseline. There were no significant differences between white patients at 6 and 12 months and baseline controls in body composition.

Black patients and controls

The baseline data shown includes all the black patients and controls at enrolment into the study. The median BMI at baseline for black women with GD and healthy black control subjects were 25 (21 to 32) kg/m^2 and 29 (22 to 34) $kg/m^{2,}$ respectively. While this difference was not statistically significant ($p = 0.373$) it was clinically meaningful.

A progressive increase in BMI was found in black patients from baseline (therapy) and it was statistically significant at 12 months. At 12 months the median BMI of black patients (33.6 kg/m^2 [27.8 to 35.1]) was significantly higher compared to controls (29 kg/m^2 [23.0 to 38.0]; $p = 0.029$).

At 6 months after commencement of therapy, black patients had a significantly greater fat mass (29502 g [23769 to 38790]) compared to the black controls (28020 g [19375 to 35324]; p = 0.038). This difference was maintained at 12 months. At 12 months after therapy the black patients had significantly greater fat mass (36067 g [20759 to 51377]) compared to black controls (26096 g [20950 to 33807]; p = 0.009).

After 6 months the percentage trunk fat did not differ significantly between black patients and the control group but at 12 months patients had significantly more trunk fat (43.2%) compared to controls (34.5%; p = 0.024). Percentage total fat at 12 months followed the same pattern. Black patients had a significantly higher percentage total fat (45.6%) compared to controls (39.3%; p = 0.009).

Although the median FMI of black patients (9.87 kg/m^2 [7.11 to 14.28]) was lower than controls at baseline (11.09 kg/m^2 [7.86 to 13.63]) it failed to reach statistical significance (p = 0.892). The median FMI of black patients (11.7 kg/m^2 [9.96 to 16.35]) was significantly higher (p = 0.039) at 6 months compared to controls (11.36 kg/m^2 [7.86 to 13.63]). The same was observed at 12 months with black patients (14.33 kg/m^2 [9.35 to 21.11]) having a significantly higher FMI (p = 0.011) compared to black controls (11.1 kg/m^2 [8.0 to 12.88]).

Table 6.3.3. BMI (kg/m^2) and body composition per compartment for patients and controls at baseline, and 6 and 12 months[1]

Parameter	**Black Females with GD**	**Black Controls**	**p-value (95% confidence limits for ratio)[2] Black patients vs. Black controls**	**White Females with GD**	**White Controls**	**p-value (95% confidence limits for ratio)[2] White patients vs. White controls**
BMI (kg/m^2)						
Baseline	25 (21 to 32) n = 39	29 (22 to 34) n = 28	*0.3735* (0.94 to 1.02)	25 (21 to 29) n = 19	26 (24 to 28) n = 17	*0.200* (0.90 to 1.02)
6 months	27.3 (24.8 to 33.7) n = 18	29.0 (22.5 to 34.0) n = 16	*0.5278* (0.94 to 1.13)	24.9 (21.8 to 28.7) n = 12	26 (23 to 28) n = 11	*0.959* (0.91 to 1.11)
12 months	33.6 (27.8 to 35.1) n = 17	29.0 (23.0 to 38.0) n = 15	*0.0286* (1.01 to 1.22)	28.24 (22 to 31.6) n = 10	25.5 (23 to 28) n = 10	*0.116* (0.98 to 1.19)

Table 6.3.3. (Continued)

Parameter	**Black Females with GD**	**Black Controls**	**p-value (95% confidence limits for ratio)[2] Black patients vs. Black controls**	**White Females with GD**	**White Controls**	**p-value (95% confidence limits for ratio)[2] White patients vs. White controls**
Total lean mass (g)						
Baseline	36349 (31095 to 40064) n = 38	37975 (35025 to 42909) n = 27	*0.67* (0.93 to 1.05)	38616 (36261 to 43999) n = 19	38644 (37737 to 44084) n = 17	*0.570* (0.90 to 1.06)
6 months	38976 (33334 to 43094) n = 16	37471 (33772 to 38655) n = 14	*0.154* (0.97 to 1.19)	40667 (35972 to 44243) n = 11	38530 (35513 to 43330) n = 10	*0.576* (0.90 to 1.20)
12 months	40920 (34185 to 50200) n = 15	39131 (36790 to 43502) n = 14	*0.211* (0.97 to 1.13)	42749 (39280 to 48037)	38694 (38316 to 43330)	*0.161* (0.96 to 1.22)

Table 6.3.3. (Continued)

Parameter	**Black Females with GD**	**Black Controls**	**p-value (95% confidence limits for ratio)[2] Black patients vs. Black controls**	**White Females with GD**	**White Controls**	**p-value (95% confidence limits for ratio)[2] White patients vs. White controls**
Total fat mass (g)						
Baseline	25868 (17271 to 33849) n = 38	25483 (20590 to 35324) n = 27	*0.583* (0.91 to 1.18)	25068 (19046 to 33758) n = 19	23697 (18401 to 28392) n = 17	*0.120* (0.93 to 1.71)
6 months	29502 (23769 to 38790) n = 16	28020 (19375 to 35324) n = 14	*0.038* (1.01 to 1.44)	25221 (15746 to 34691) n = 11	27662 (20775 to 30237) n = 10	*0.363* (0.74 to 2.14)
12 months	36067 (20759 to 51377) n = 15	26096 (20950 to 33807) n = 14	*0.009* (1.08 to 1.54)	28111 (20005 to 39880) n = 10	23336 (15984 to 29572) n = 10	*0.122* (0.87 to 2.75)

Table 6.3.3. (Continued)

Parameter	**Black Females with GD**	**Black Controls**	**p-value (95% confidence limits for ratio)[2] Black patients vs. Black controls**	**White Females with GD**	**White Controls**	**p-value (95% confidence limits for ratio)[2] White patients vs. White controls**
Trunk fat (%)						
Baseline	35.8 (28.2 to 44) n = 37	33.9 (28.5 to 43.4) n = 27	*0.9013* (0.87 to 1.13)	32.2 (25.6 to 40.4) n = 19	28.4 (24.7 to 33.8) n = 17	*0.464* (0.90 to 1.24)
6 months	40.15 (34.4 to 41.4)	37.5 (29.1 to 43.4)	*0.0743* (0.99 to 1.26)	33.2 (23.2 to 40.4)	33.75 (26.6 to 38.8)	*0.984* (0.86 to 1.16)
12 months	43.2 (38.8 to 47.7)	34.5 (29.9 to 37.6)	*0.0239* (1.02 to 1.30)	34.15 (25.0 to 46.2)	27.95 (24.4 to 33.7)	*0.065* (0.99 to 1.41)

Table 6.3.3. (Continued)

Parameter	**Black Females with GD**	**Black Controls**	**p-value (95% confidence limits for ratio)[2] Black patients vs. Black controls**	**White Females with GD**	**White Controls**	**p-value (95% confidence limits for ratio)[2] White patients vs. White controls**
Total fat (%)						
Baseline	39.8 (34.5 to 46.6) n = 37	38.9 (35 to 47.7) n = 27	*0.583* (0.94 to 1.12)	37.2 (31.5 to 45.7) n = 19	34.95 (30.4 to 37.8) n = 16	*0.9297* (0.72 to 1.36)
6 months	42.7 (39.8 to 46.7)	40.5 (35.0 to 47.7)	*0.108* (0.98 to 1.19)	40.1 (28.5 to 44) n = 11	35.1 (32.7 to 43) n = 9	*0.5145* (0.83 to 1.11)
12 months	45.6 (40.1 to 50.1)	39.3 (35.2 to 42.5)	*0.009* (1.04 to 1.23)	37.35 (30.7 to 47.7) n = 10	32.7 (29.8 to 36.9) n = 9	*0.384* (0.91 to 1.25)

Table 6.3.3. (Continued)

Site	**Black Females with GD**	**Black Controls**	**p-value (95% confidence limits for ratio)[2] Black patients vs. Black controls**	**White Females with GD**	**White Controls**	**p-value (95% confidence limits for ratio)[2] White patients vs. White controls**
Fat Mass Index (kg/m^2)						
Baseline	9.87 (7.1 to 14.3) n = 38	11.09 (7.9 to 13.6) n = 27	*0.892* (0.88 to 1.15)	8.75 (6.4 to 12.5) n = 19	8.84 (7.1 to 10.1) n = 17	*0.341* (0.93 to 1.22)
6 months	11.7 (10.0 to 16.3) n = 16	11.36 (7.9 to 13.6) n = 14	*0.039* (1.01 to 1.39)	9.49 (5.7 to 13.6) n = 11	9.88 (7.4 to 11.1) n = 10	*0.972* (0.81 to 1.24)
12 months	14.33 (9.3 to 21.1) n = 15	11.1 (8.0 to 12.9) n = 14	*0.011* (1.07 to 1.52)	10.28 (6.6 to 15.6) n = 10	8.15 (6.7 to 9.8) n = 10	*0.138* (0.93 to 1.58)

[1] Values are medians (interquartile range). [2]Generalized linear model of log transformed data.

Discussion: *BMI, FMI, and body composition per compartment for patients and controls at baseline, and 6 and 12 months*

When evaluating the differences in the change in body composition between black- and white patients with thyrotoxicosis, there were no significant differences at baseline or at 6- and 12 months.

Both black and white women gained weight during the 12 months following successful treatment for GD. The body weight did not differ significantly between black and white women at any time point.

At disease presentation median BMI did not differ significantly between white patients with GD and healthy white controls indicating that patients and controls were well-matched for BMI. Body composition per compartment at baseline also did not differ significantly between white patients and controls. Median BMI of white patients increased at 6 and 12 months after treatment for GD but was still comparable with controls at baseline (Small sample size). Six and 12 months after definitive treatment for GD body composition in white patients were still comparable to that of control subjects and did not change significantly. This indicates that hyperthyroidism due to GD in white women did not alter body composition – at least not in this BMI category.

Significant differences were detected between black patients and healthy controls at 6 and 12 months. After definitive treatment for GD the median BMI of black patients increased from the overweight category to obesity class I. One year after definitive treatment for GD the median BMI of black patients was significantly greater compared to controls. All the other measurements of fatness (total fat mass, percentage total fat, and percentage trunk fat also increased progressively in black patients and differed significantly from controls after 12 months. The transition of the hyperthyroid to the euthyroid state in black female patients altered body composition compared to euthyroid healthy control subjects (different from what was found in white female patients). Lean mass also increased but did not differ significantly from black controls.

The FMI (Fat mass in kg/ length in m^2) (7) did not differ between the two patient groups at baseline, 6- and 12 months, although the value was higher in the black women through-out. The FMI did not differ significantly between white patients and their controls at any time point. A progressive increase was observed in FMI of the black patients after treatment. This

indicates an increase in relative body fat content. The advantage of FMI is that it is not influenced by fat free mass (7).

Recovery from thyrotoxicosis has been shown to be associated with increases in lean-, bone- and fat mass (5, 8). These changes have been shown to be associated with increases in BMI (9, 10). In an earlier study it was shown that patients recovering from hyperthyroidism may be prone to the development of obesity (11). This study confirms this finding at least in black women. This was confirmed by others (12). It was also implied that treatment modality for GD may impact on body composition during the recovery period. Results in this regard are, however, controversial. Previously it was found that the increase in fat mass observed after treatment was associated with the use of radio-active iodine therapy rather than carbimazole (11, 13). Recently it has been shown that the effects are actually comparable (14). Although not significant, the median duration of GD in black patients were 6 months compared to 3 months ($p = 0.079$) in white patients before presentation (Table 4.1.1). This difference in duration may explain the differences in recovery between black- and white patients.

6.4 **Summary and conclusions**

Although the rate of weight gain and increase in median BMI after definitive treatment for GD were comparable between black and white women, black women were more prone to obesity after recovery and this was accompanied by a change in body composition in the latter.

6.5 **References**

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Chapter 7

Bone Histomorphometry

7.1 **Introduction**

In this chapter the bone histology performed on consenting black female patients are analysed and discussed. Bone biopsies were not performed on white female patients as there were deemed to be enough information available in the literature.

7.2 **Selection of patients for bone histomorphometry**

Histomorphometry data on white females with GD are already available and described in the literature (1). Thus, bone histomorphometry was only performed in a subset of black patients enrolled in this study. Black females with active GD, with a DXA bone mineral density Z-score of ≤ -2 standard deviations (SD) (low bone mass) or a T-score of ≤ -2.5 SD (osteoporosis) at any of the measured skeletal sites and/ or a prevalent fragility fracture at the time of diagnosis of hyperthyroidism, underwent iliac crest biopsies, after informed consent was obtained. The type of metabolic disease and more specifically the presence of any mineralization defects, including overt osteomalacia, were assessed histologically.

7.3 **Technical difficulties and notes when interpreting the bone histomorphometry**

7.3.1 ***Technical difficulties***

The double labelling of the bone specimens with tetracycline allows for interpretation of dynamic bone parameters. The tetracycline labelling was unfortunately not optimal and blurred in some of the biopsy specimens with a negative effect on the accuracy of the calculations. The tetracycline labelling may have been influenced negatively by factors such as patient compliance and decreased binding of the tetracycline. The impaired binding to the bone forming surface may have been tetracycline type- or dose dependant. Dynamic bone parameters in some of the study subjects must thus be interpreted with caution as noted later in the text. A discrepancy between the calculated volumetric bone mass (histologically) and areal bone mass (DXA scanning) was observed. Standard DXA uses two-dimensional projection techniques and measures areal density (g/cm^2) and does not measure true volumetric bone mass**.**

7.3.2 ***Terminology***

7.3.2.1 *Mineralization Lag Time* (MLT): Can only be objectively calculated if double tetracycline labels are clearly visible. An MLT exceeding 120 days is indicative of overt osteomalacia.

7.3.2.2 *Osteomalacia* (OM): OM should ideally be based on dynamic assessment and calculation of MLT. Lesser degrees of decreased mineralization do occur and parameters to quantify this include osteoid volume (Osteoid Volume/ Bone Volume [OV/ BV]) and surface (Osteoid Surface/ Bone Surface [OS/ BS]), reduced mineral volume and osteoid seam width. Reduced mineralization in many of the subjects, due to the defective labelling, is based on a best calculated delayed MLT (if possible) and /or the presence of excess osteoid and reduced mineral volume.

7.4 **Results**

7.4.1 **Total histomorphometry cohort**

7.4.1.1 *Clinical characteristics*

Seventeen patients were eligible for bone histomorphometry, but only 10 study patients consented to undergo a bone biopsy at baseline. The baseline characteristics of the 10 study patients who underwent a bone biopsy for bone histomorphometry are tabulated in Table 7.4.1. The mean age of the cohort was 43 ± 12 years with a minimum age of 29 years and a maximum age of 63 years. The mean body mass index (BMI) was 24 ± 5 kg/m^2, five patients were of normal weight, one patient was underweight, and four patients were overweight or obese. Four of the patients were postmenopausal. None of the females, pre- or postmenopausal, sustained clinical fragility fractures of the spine or long bones. The indication for an iliac biopsy was based on the presence of a DXA bone mineral density (BMD) in keeping with a low bone mass (Z-score ≤ -2 SD) in premenopausal subjects or osteoporosis (T-score ≤ -2.5 SD) in postmenopausal subjects at one or more measured sites in nine of the 10 patients. Five of the subjects demonstrated morphometric vertebral fractures on lateral vertebral assessment with DXA and this was the only indication for an iliac crest biopsy in a single patient (Patient 4). Eighty percent (4/5) of the morphometric fractures were mild compression (< 25%).

Table 7.4.1. Clinical characteristics of histomorphometry cohort

Clinical features	**Histomorphometry cohort (n=10)**										
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**Mean (± SD)**
Age (years)	**51**	30	34	45	29	**56**	**63**	27	**45**	47	43 (12)
BMI (kg/m^2)	**21**	22	15	32	27	**20**	**24**	21	**32**	25	24 (5)
Menopause	**Yes**	No	No	No	No	**Yes**	**Yes**	No	**Yes**	No	
Clinical fracture Spine Long bones	**None** **None**	None None	None None	None None	None None	**None** **None**	**None** **None**	None None	**None** **None**	None None	

BMI: Body mass index: weight in kilograms divided by the height squared in meters (BMI = kg/m^2), was used to classify patients into the following categories: < 18.50: underweight, 18.50-24.99: normal weight, 25.00-29.99: overweight, 30.00-34.99: obesity class 1, 35.00-39.99: obesity class 2 and ≥ 40.00: obesity class 3 (2). SD: Standard deviation. Data for postmenopausal females noted in bold.

7.4.1.2 *Biochemistry*

Biochemical findings of the histomorphometry cohort are shown in table 7.4.2. All patients were biochemically thyrotoxic at the time of their bone biopsy with free T_4 and free T_3 levels markedly elevated. Mean free T_3 and T_4 for the cohort were increased 4-5 times above the upper limit of the reference range. All the patients had a suppressed thyroid-stimulating hormone (TSH) level that confirmed the primary nature of the thyrotoxicosis.

The mean 25-hydroxyvitamin D (25(OH)D) level (26 $\pm$ 12 ng/ml) was below the lower limit of the reference range with values being low in 7 of the 10 females (< 32 ng/ml). The mean 1,25-dihydroxyvitamin D (1,25$(OH)_2$D), and the serum calcium- and parathyroid hormone (PTH) levels were all within the reference range. The PTH level was elevated in a single patient in whom a decreased 25(OH)D and an increased 1,25$(OH)_2$D were present.

Bone turnover was assessed with biochemical markers of bone formation (bone specific serum alkaline phosphatase (BAP) and serum osteocalcin) and bone resorption (urinary-deoxypyridinoline to creatinine ratio). The serum BAP was elevated in all four postmenopausal females and in 4 of the 6 premenopausal subjects. Osteocalcin, on the other

hand, was only marginally elevated in a single premenopausal patient in the presence of a normal BAP. Biochemistry findings indicated markedly elevated resorption in 9 of the 10 patients with the mean for urinary-deoxypyridinoline to creatinine 4 times the upper limit of the normal range.

Table 7.4.2. Biochemical findings in histomorphometry cohort

Biochemistry	**Histomorphometry cohort (n=10)**										
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**Mean (±SD)**
T_3 (mIU/L)	**30**	49	44	51	47	**38**	**17**	23	**NA**	45	38 (12)
T_4 (pmol/L)	**99.6**	>100	>100	>100	>100	**>100**	**41.3**	51.7	**24.7**	>100	81 (30)
TSH (mIU/L)	**0.01**	0.01	0.01	0.01	0.01	**0.01**	**0.01**	0.01	**0.01**	0.01	0.01
25(OH)D (ng/ml)	**15.4**	15.2	12	34.1	25.6	**15.5**	**27.7**	40	**22.7**	47.5	26 (12)
$1,25(OH)_2D$ (ng/L)	**26**	30	29	37	61	**82**	**53**	88	**NA**	51	51 (23)
Calcium (mmol/L)	**2.36**	2.29	2.39	2.65	2.54	**2.14**	**2.23**	2.38	**2.28**	2.54	2.38 (0.2)
PTH (pg/ml)	**37.0**	52.0	36.0	14.1	13.2	**89.3**	**53.5**	56.4	**35.7**	24.4	41 (23)
BAP (µg/L)	**43.4**	74.5	23.4	14.1	15.7	**32.0**	**34.3**	42.0	**32.4**	44.1	36 (17)
Osteocalcin (ng/ml)	**45.4**	28.5	34.1	12.3	44.6	**36.2**	**11.0**	24.4	**48.3**	34.3	32 (13)
U- DPD/ Creat (nmol/mmol)	**54.5**	24.8	29.2	31.5	29.7	**26.9**	**19.4**	29.7	**4.5**	45.7	30 (14)
IGF-1 (ng/ml)	**49**	49	61	136	144	**< 20**	**51**	138	**203**	79	101 (56)
Leptin (ng/ml)	**6.2**	5.7	1.8	51.5	28.9	**4.7**	**5.5**	16.0	**79.6**	4.2	20 (26)
Interleukin 6 (pg/ml)	**2.0**	9.1	5.6	4.0	3.9	**16.4**	**3.0**	2.8	**3.8**	2.2	5 (4)

Footnote (Table 7.4.2.)
T_4, thyroxine (reference range = 12.0-22.0 pmol/L); T_3, tri-iodothyronine (reference range = 3.1-6.8 pmol/L); TSH, thyroid-stimulating hormone (reference range = 0.27-4.20 mIU/L); Calcium (reference range = 2.15-2.50 mmol/L); PTH: parathyroid hormone (reference range = 15-65 pg/ml); Urine DPD to Creat. Ratio, urinary deoxypyridinoline (expressed as a ratio to urinary creatinine) (reference range = 3.0-7.4 nmol/mmol); 25(OH)D: 25-hydroxyvitamin D (reference range = 32-80 ng/ml); $1,25(OH)_2D$, 1,25-dihydroxyvitamin D (reference range = 19.9-67 ng/L); IL-6: interleukin 6 (reference range = 0.0-6.4 pg/ml); BAP, serum bone specific alkaline phosphatase (premenopausal reference range = 3-19 µg/L; postmenopausal reference range = 6-26 µg/L); Leptin, (according to BMI):< 25 (0.2-45.8); 25.00-29.99 (3-65.7); 30.00-34.99 (8.1-79.1); ≥ 35.00 (11.9-137.4); IGF-1, insulin-like growth factor 1 (reference range = 107.8-246.7 ng/ml); Osteocalcin, (premenopausal reference range = 6.5-42.3 ng/ml, postmenopausal reference range = 5.4-59.1 ng/ml). SD: Standard deviation. NA: Not available. Data for postmenopausal females noted in bold.

7.4.1.3 *DXA determined BMD and morphology findings*

7.4.1.3.1 Densitometry

Table 7.4.3 reports the densitometry findings of the histomorphometry cohort. In the group the lowest mean Z-scores (pre- and postmenopausal females) were reported at the trabecular rich lumbar spine region. A BMD measurement in keeping with osteoporosis at the lumbar spine was present in all the postmenopausal females. One postmenopausal woman had a BMD in keeping with osteoporosis at both proximal femoral sites. Low lumbar spine bone mass was present in four of the six premenopausal females whereas none of the premenopausal females had a low BMD at the proximal femur (Z-scores were all greater than -2SD) (See 7.5 *Discussion* for a possible explanation). All the postmenopausal females had a low BMD (Z-scores all less than -2SD) at the lumbar spine and three of the four had a low BMD at the distal radius.

Table 7.4.3. Densitometry findings in histomorphometry cohort

Densitometry	**Histomorphometry cohort (n=10)**										
	1	**2**	**3**	4	**5**	**6**	**7**	**8**	**9**	**10**	**Mean (± SD)**
Lumbar BMD											
T-score	**-3.1**	NA	NA	NA	NA	**-3.0**	**-3.5**	NA	**-2.6**	NA	-3.05 (0.4)
Z-score	**-3.1**	-2.7	-2.1	-1.7	-2.7	**-2.7**	**-2.6**	-1.8	**-3.0**	-4.3	-2.7 (0.7)
Total Hip											
T-score	**-2.4**	NA	NA	NA	NA	**-1.8**	**-2.6**	NA	**-1.1**	NA	-2.0(0.7)
Z-score	**-2.1**	-1.4	-0.8	-0.8	-0.9	**-1.4**	**-1.7**	-1.2	**-1.4**	-1.9	-1.4(0.4)
Femoral Neck											
T-score	**-2.2**	NA	NA	NA	NA	**-2.4**	**-2.7**	NA	**-1.3**	NA	-2.1 (0.60)
Z-score	**-1.8**	-1.4	-1.1	-1.0	-1.1	**-1.8**	**-1.7**	-1.4	**-1.5**	-1.5	-1.4 (0.3)
Distal 3rdforearm											
T-score	**-5.8**	NA	NA	NA	NA	**-4.5**	**-5.2**	NA	**-1.1**	NA	-4.1 (2.1)
Z-score	**-5.0**	-1.4	-0.5	0.4	-1.2	**-3.5**	**-3.7**	-2.5	**-0.5**	-0.9	-1.9 (1.7)

Proximal hip and forearms scores limited to left side assessment. If the patient ≥ 50 years of age, the T- and Z-score is reported. If the patient < 50 years of age, the Z-score is reported. NA: not applicable. Postmenopausal data tabulated in bold.

7.4.1.3.2 Vertebral morphology (VFA)

Five females had vertebral deformities based on the semi quantitative Genant system (3) (Table 7.4.4.). Mild compression deformities were present in 4 cases. One patient (patient 2) had two mild deformities which were clinically significant. Moderate deformities were only noted in one premenopausal patient with normal BMD (patient 4). It is important to note that a single mild deformity may not be associated with an increased risk of fracture and that only 2 or more mild deformities are considered clinically important (4).

Table 7.4.4. Vertebral Fracture Assessment (VFA)

Patient	Morphometric Fractures
1	Normal
2	Mild wedge deformities T6 and L1
3	Normal
4	Moderate wedge deformities T4 and T6
5	Normal
6	Mild wedge deformity T7
7	Mild biconcave deformity T10
8	Normal
9	Mild biconcave deformity L4
10	Normal

Vertebral fracture assessment (VFA) was performed according to the Genant system and using the software supplied by Hologic (3). According to the Genant system a vertebral fracture can either be a wedge-, biconcave- or crush deformity. The fractures are then graded according to height loss: Normal/ no fracture < 20% height loss; Mild fracture = 20-24.9% height loss; Moderate fracture = 25-40% height loss; Severe fracture > 40% height loss. 50% of the histomorphometry cohort had a vertebral deformity according to the Genant system. Only one participant had a clinically important deformity with height loss of ≥ 25%.

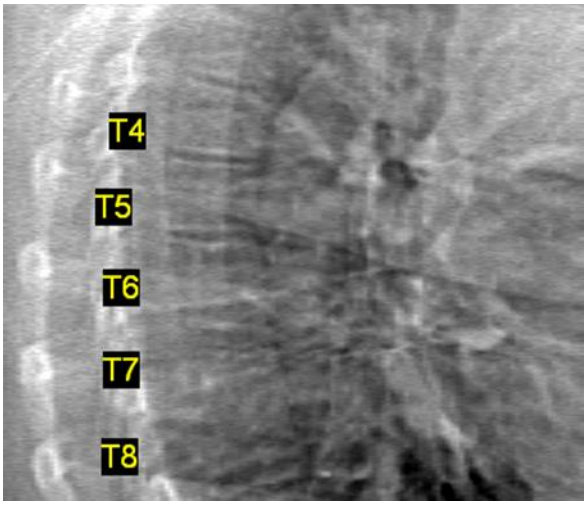
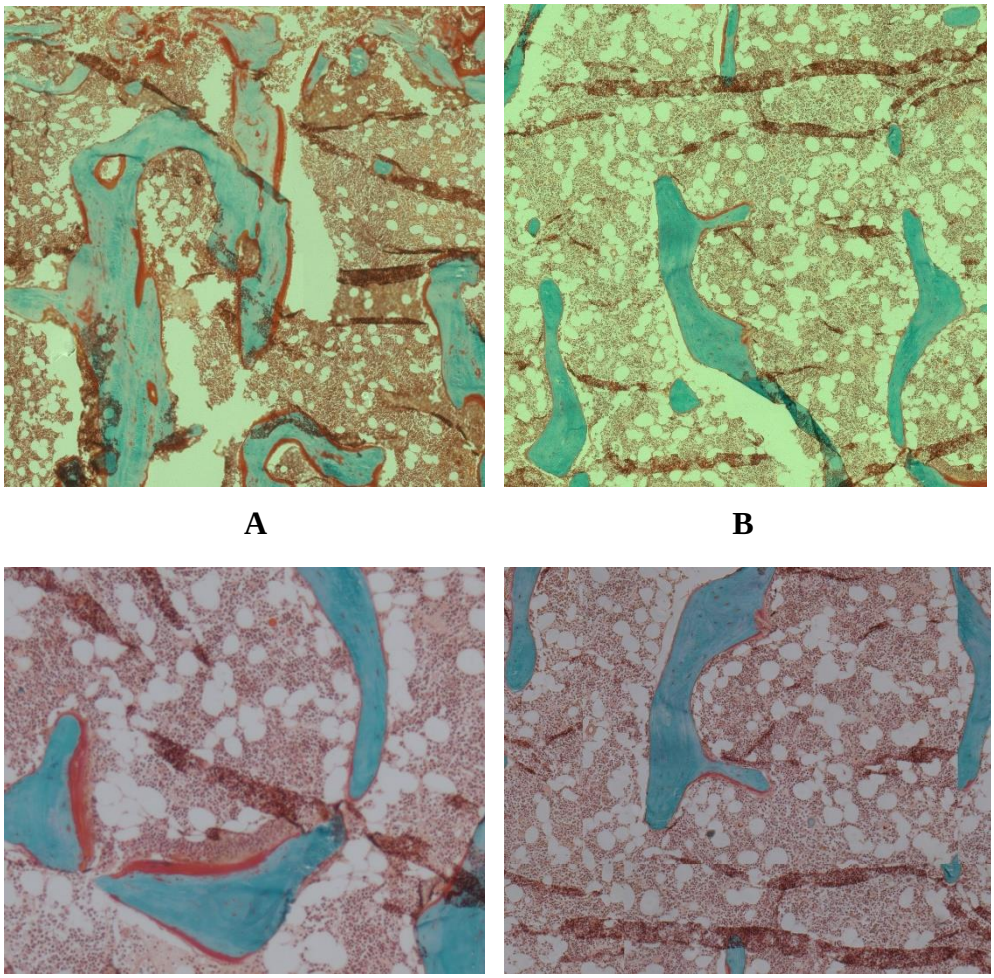


Figure 7.1: Vertebral morphology of Patient 4. Moderate (25-40% height loss) wedge deformities of T4 and T6.

7.4.1.4 Bone histomorphometry

An abundance of osteoid was noted in most biopsy specimens (Table 7.4.5.). The mean values for relative osteoid volume (OV/TV), total osteoid volume (OV/BV), total osteoid

surface (OS/BS) and the absolute osteoid thickness (O.Th) were increased and the absolute values elevated in 8 of the 10 patients (patient 4: normal OV/TV and OV/BV; patient 8:borderline normal OV/TV). The mean values for the resorptive- (ES/BS) and osteoclastic resorptive surfaces (Oc.S/BS) were increased and the absolute values for these measures increased in all but 3 patients (patients 4, 9 and 10) and 1 patient (patient 10) respectively.



A **B**

Figure 7.2: Histomorphometry in women with thyrotoxicosis. A: Abundance of osteoid. Abundant osteoid noted in 8 of the 10 women with thyrotoxicosis and associated with an increased bone turnover state in 7 of the 8 women. B: Normal histomorphometry present in 2 of the histomorphometry study cohort.

Table 7.4.5. Histomorphometry parameters

Histology	**Histomorphometry cohort (n=10)**											
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**Mean (±SD)**	**Normal Range***
BV/TV (%)	**21.9**	22.1	22.6	27.1	17.6	**20.8**	**22.8**	11.4	**13.3**	21.3	20 (4.7)	
OV/TV (%)	**4.12**	4.1	1.48	0.59	1.87	**1.33**	**0.99**	0.79	**1.71**	1.7	1.9 (1.2)	0.45±0.4
OV/BV (%)	**18.8**	18.5	6.5	2.2	10.6	**6.4**	**4.3**	6.9	**12.8**	8.0	9.5 (5.6)	<4
OS/BS (%)	**67.6**	34.8	26.6	11.3	45.3	**45.4**	**28.7**	25.4	**51.5**	44.4	38.1 (16.0)	17.9± 6.8
O. Th (mcm)	**31.2**	48.1	24.8	18.7	26.1	**17.0**	**21.6**	17.6	**18.2**	19.7	24.3 (9.5)	9.5±0.3
SDOS	**1.68**	1.09	0.76	0.40	0.91	**1.00**	**0.59**	0.57	**1.19**	1.10	0.91 (0.4)	0.53± 0.3
ES/BS (%)	**11.3**	8.4	6.8	5.1	7.8	**10.3**	**8.4**	11.3	**5.6**	3.1	7.8 (2.7)	4.3±1.6
OcS/BS (%)	**2.58**	1.91	1.55	2.66	1.83	**3.78**	**2.33**	1.88	**2.11**	0.68	2.1 (0.8)	0.53±0.5

BV: Bone volume. TV: Tissue volume. OV: Osteoid volume. OS: Osteoid surface. BS: Bone surface. O. Th.: Osteoid thickness. SDOS: Surface density osteoid seams. ES: Endosteal. BS: Bone surface. Oc. S: Osteoclast surface. BV/TV %: Total Bone volume. OV/TV %: Relative Osteoid Volume. OV/BV %: Total Osteoid Volume. OS/BS: Total osteoid surface. ES/BS %: Total resorptive surfaces. Oc. S/BS %: Osteoclastic resorptive surfaces. Data for postmenopausal females noted in bold. (5)

The correlation between biochemical markers of bone formation/ resorption and histomorphometry parameters are shown in table 7.4.6. The correlation between serum osteocalcin and total osteoid surface is shown in Fig. 7.3.

Table 7.4.6. Correlation (Pearson correlation coefficient) between biochemical markers of bone resorption/ formation and bone histomorphometry parameters

Parameter	OC	Correlation	BAP	Correlation	U DPD	Correlation	25(OH)D	Correlation	$1,25(OH)_2D$	Correlation
OV/TV (%)	0.5	Moderate	0.7	Moderate	0.4	Weak	**-0.5**	Moderate	**-0.6**	Moderate
OV/BV (%)	0.6	Moderate	0.6	Moderate	0.2	Weak	**-0.4**	Weak	**-0.4**	Weak
OS/BS (%)	0.8	Moderate	0.2	Weak	0.3	Weak	**-0.3**	Weak	**-0.1**	Weak
O. Th (mcm)	0.1	Weak	0.7	Moderate	0.1	Weak	**-0.5**	Moderate	**-0.6**	Moderate
SDOS	0.8	Moderate	0.4	Weak	0.4	Weak	**-0.4**	Weak	**-0.3**	Weak
ES/BS (%)	0.1	Weak	0.2	Weak	0.1	Weak	**-0.4**	Weak	0.3	Weak
OcS/BS (%)	**-0.1**	Weak	**-0.2**	Weak	**-0.2**	Weak	**-0.5**	Moderate	0.2	Weak

Table 7.4.6 (Continued)

Parameter	Ca	Correlation	PTH	Correlation
OV/TV (%)	**-0.2**	Weak	0.0	None
OV/BV (%)	**-0.2**	Weak	0.0	None
OS/BS (%)	**-0.3**	Weak	0.1	Weak
O. Th (mcm)	**-0.1**	Weak	0.0	None
SDOS	**-0.3**	Weak	0.0	None
ES/BS (%)	**-0.5**	Moderate	0.6	Moderate
OcS/BS (%)	**-0.5**	Moderate	0.6	Moderate

Urinary deoxypyridinoline expressed as a ratio to urinary creatinine (U DPD); Serum bone specific alkaline phosphatase (BAP); Osteocalcin (OC); TV: Tissue volume. OV: Osteoid volume. OS: Osteoid surface. BS: Bone surface. O. Th.: Osteoid thickness. SDOS: Surface density osteoid seams. ES: Endosteal. BS: Bone surface. Oc. S: Osteoclast surface. BV/TV %: Total Bone volume. OV/TV %: Relative Osteoid Volume. OV/BV %: Total Osteoid Volume. OS/BS: Total osteoid surface. ES/BS %: Total resorptive surfaces. Oc. S/BS %: Osteoclastic resorptive surfaces. Negative correlations in bold. Weak correlation: 0 to < 0.5 or -0.5 < to 0. Moderate correlation: 0.5 to < 1.0 or -1.0 < to -0.5.

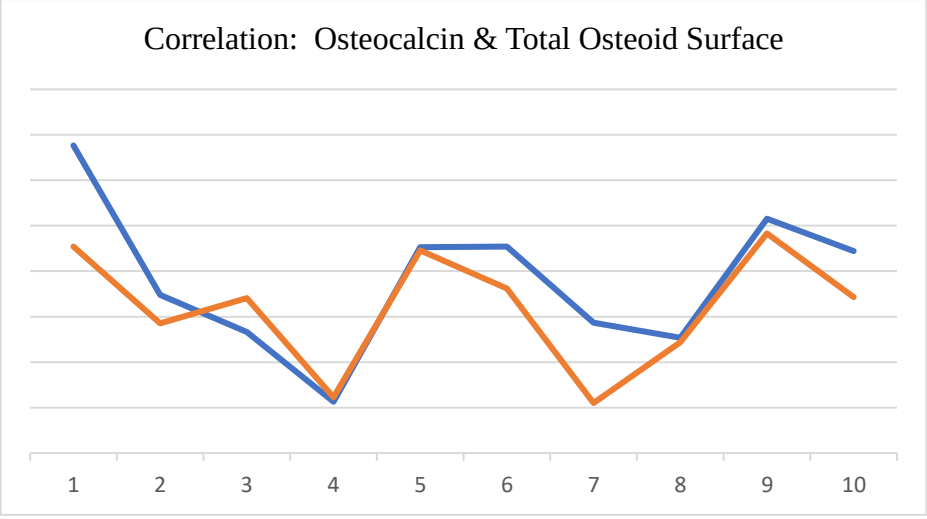


Figure 7.3. The correlation (Pearson) between serum osteocalcin (--) and total osteoid surface (--) was moderately positive.

7.4.2 **Individual Patient Histomorphometry Data**

Table 7.4.7 reviews the bone histomorphometry of the individual patients.

Table 7.4.7. Individual histomorphometry

Patient	Duration of Symptoms (Months)	Histomorphometry comment(s)	Impression
1	6	Normal bone volume (Bone Volume/ Tissue Volume [BV/ TV]). Increased resorption parameters. Excess osteoid. MLT not calculated (poor labelling, inaccurate).	High turnover Abundant osteoid
2	7	Normal bone volume (Bone Volume/ Tissue Volume [BV/ TV]). Increased resorption parameters. Excess osteoid. MLT calculated to be high (Caution: poor labelling, inaccurate).	High turnover Abundant osteoid
3	3	Normal bone volume (Bone Volume/ Tissue Volume [BV/ TV]). Resorptive parameters at upper end of normal. Slightly increased osteoid parameters. MLT could not be calculated.	Normal to slightly high turnover bone state. Slightly increased osteoid.
4	2	Normal to high bone volume (Bone Volume/ Tissue Volume [BV/ TV]). Normal to high resorptive parameters. Normal osteoid parameters. MLT normal.	Normal bone histomorphometry.
5	4	Reduced bone volume (Bone Volume/ Tissue Volume [BV/ TV]). High resorptive parameters. Excess osteoid. MLT normal (Interpreted with caution).	High turnover Abundant osteoid
6	1	Normal bone volume (Bone Volume/ Tissue Volume [BV/ TV]). High resorptive parameters. Excess osteoid. MLT not calculated.	High turnover Abundant osteoid
7	12	Normal to high bone volume (Normal to high Bone Volume/ Tissue Volume [BV/TV]). High resorptive parameters. Slightly increased osteoid surface parameters. MLT not calculated.	High turnover bone. Slightly increased osteoid.

Table 7.4.6. (Continued)

Patient	Duration of Symptoms (Months)	Histomorphometry comment(s)	Impression
8	8	Markedly reduced bone volume for age (Markedly reduced Bone Volume/ Tissue Volume [BV/TV] for age). Slightly increased bone turnover. Normal osteoid volume and surface parameters. MLT not calculated.	Slightly elevated bone turnover. Normal osteoid.
9	1	Low bone volume (Low Bone Volume/ Tissue Volume [BV/TV]). Slightly elevated bone turnover. Excess osteoid. MLT not calculated.	Slightly elevated bone turnover. Abundant osteoid.
10	12	Normal bone volume (Normal Bone Volume/ Tissue Volume [BV/TV]). Normal bone turnover. Excess osteoid. MLT not calculated.	Normal bone turnover. Increased osteoid.

7.5 **Discussion**

Ten black hyperthyroid females consented to undergo iliac crest biopsies for histomorphometric evaluation at diagnosis of GD. Females were pre-selected for histological assessment based on the presence of either documented low bone mass (Z-score ≤ -2 SD) in premenopausal females or evidence of OP in postmenopausal females (T-score ≤ -2.5 or presence of either a vertebral or hip fracture). The sub-cohort consisted of relatively young females with a mean age of 43 ± 12 years, which included 6 premenopausal females. In this cohort (10 black female patients), histomorphometric assessment at baseline, prior to the introduction of any anti-thyroid therapy, documented increased bone mineral resorption and an increase in unmineralized osteoid in most study patients. An explanation for the increased osteoid volume could be explained by an increased bone turnover or reduced mineralization.

DXA Bone Mineral Density

In premenopausal black females who underwent bone histomorphometry, the most pronounced bone loss occurred at the trabecular rich spine, whereas the mean DXA Z-score was normal at both the total hip and distal radius, sites known to contain more cortical bone.

Only one of the six premenopausal females had low bone mass at the total hip region. BMD in the osteoporotic range was present at the lumbar spine in all the postmenopausal females and in three of the four females at the distal radius site. The lowest Z-scores in postmenopausal females were observed at the distal radius although the values were also low at the lumbar spine. In this small cohort, significantly lower trabecular BMD was thus present in both pre- and postmenopausal females. Distal radius findings significant of cortical bone loss was also evident in most older females.

Black females are regarded as genetically protected against osteoporosis, but recent South African (SA) studies have questioned this dogma (6-8). Higher BMD and lower fracture rates have been consistently reported in African American women in studies conducted in the United States of America (USA) (9, 10). On the African continent, studies have also noted higher hip BMD in black females compared to white females, but consistently documented similar vertebral BMD in both ethnic groups (11-15). The lower spinal BMD noted at baseline in this small sample of pre- and postmenopausal black females with GD may thus not necessarily reflect more pronounced trabecular bone loss due to hyperthyroidism. The lower spinal BMD may merely reflect the lower spinal BMD documented in otherwise healthy black SA females. The higher BMD found in black females may mask the extent of bone loss at the proximal femur- and hip regions at the onset of disease.

Biochemical assessment of bone turnover and mineralization

In this study, the increase in the bone resorptive marker (urine DPD to creatinine ratio) was also more pronounced and more consistent throughout the study cohort compared to the bone formation parameters. Mean urinary DPD was increased fourfold in accordance with the literature documenting a marked increase in bone resorption in the hyperthyroid state, in some studies up to 7-8 times compared to age and sex matched controls (16-19).

Markers of bone formation revealed less consistent results in our study. Bone specific alkaline phosphatase (BAP) was elevated in 80% (8/10) of our study cohort. This is in accordance with data reported in the literature (18, 20). The median BAP levels were marginally elevated in postmenopausal females and 1.5 times elevated in premenopausal females. Osteocalcin, which was also expected to be raised in the setting of hyperthyroidism, was only marginally elevated in one premenopausal woman. The discrepancy in the change

in bone formation markers in our study may be explained by potential different contributors to the raised levels of either BAP or osteocalcin. In the setting of overt untreated hyperthyroidism bone remodelling is significantly accelerated. This may result in a lagging of optimal mineralization of newly formed osteoid and may, over and above a net loss of bone mass, also result in suboptimal mineralization of bone as encountered in patients with hyperparathyroidism. Alkaline phosphatase, an enzyme present in osteoblasts, is a good marker of osteomalacia and is often markedly elevated in such a setting. The disproportionate elevation in BAP compared to osteocalcin may imply that the elevation in BAP is not only due to increased bone formation, as part of the activated remodelling cycle, but may also be indicative of defective mineralization.

Vitamin D deficiency

The 25(OH)D levels were below 20 ng/mL in four of the females in the histomorphometry group, but none of the patients had a level below 12 ng/mL usually required for the development of osteomalacia. Serum calcium, PTH and $1,25(OH)_2D$ levels were normal in all females arguing against abnormal vitamin D and calcium homeostasis potentially contributing to skeletal demineralization in the cohort.

Serum levels of 25(OH)D, $1,25(OH)_2D$ and other metabolites of vitamin D have been studied in patients with hyperthyroidism by various investigators (21-26). Some studies have documented normal serum levels of 25(OH)D, whereas other studies have shown subnormal to significantly lower serum levels of 25(OH)D compared to controls (22-25). In a single study conducted on Indian subjects with hyperthyroidism, severe vitamin D deficiency was documented in 30% of patients (27). No data on vitamin D status in black females with thyrotoxicosis have been reported in the literature thus far.

Histomorphometry

This is the first study evaluating bone histomorphometry in SA black females with GD.

A previous South African study evaluated iliac crest histomorphometry in normal black- and white adults (28). In comparing the results of normal black females with those of black females with GD from this study, it was found that the mean bone volume of patients was higher, except for normal black females between the ages of 20 to 30 years. Interestingly, the

same was observed when comparing black female patients with normal white females. The mean total osteoid surface was more than double in black female patients in comparison to normal black females with the highest value in the age group 51-60 years. The same pattern was observed for total osteoid volume, relative osteoid volume, osteoid thickness and total resorptive surfaces, with black female patients having higher mean values compared to normal black South African females.

An abundance of osteoid was present in six of the 10 females who underwent bone histology. This is in keeping with decreased bone mineralization and a tendency towards osteomalacia with the median osteoid seam width increased more than three-fold the upper range of normal. Two of the females had slightly elevated osteoid surface and volume parameters. In two females osteoid was present in normal amounts with no evidence of poor mineralization in the biopsy specimen. The accumulation of excess osteoid is known to occur in states of high bone turnover such as primary or secondary hyperparathyroidism and hyperthyroidism (Silverberg et al, 1989, Rao et al 1993; Mosekilde en Mensen 1978, Moore et al 2009). Shortening of the remodelling cycle limits the available time for the mineralization phase. It is this lag in mineral deposition that is responsible for the accumulation of excess osteoid. Histological features suggestive of osteomalacia should lead to consideration of severe vitamin D deficiency. This is not necessarily the case as demonstrated in our patient cohort. Correction of the hyperthyroid state should revert the abnormal histomorphometry to normal without the need for exogenous vitamin D.

The correlation coefficient was calculated to determine the relationship between different variables. It was expected that a strong relationship would be seen especially between markers of bone resorption and histomorphometry. However, the correlations were moderate at most.

The results of this study were limited by the small sample size and difficulties with the tetracycline labelling process. A repeat of the bone histomorphometry 1 year after treatment and normalization of the thyroid function would have clarified the effect of GD on bone health.

To conclude, in black SA females, hyperthyroidism does affect bone metabolism. The effects are characterized by accelerated bone turnover, predominantly stimulation of bone resorption and demineralization as evidenced by abundant osteoid on histology.

7.6 **References**

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Chapter 8

Combined Discussion, Conclusions and Recommendations

8.1 **Introduction**

Ethnic differences in bone health have been demonstrated across the globe (1). Differences in bone health and fracture risk also exist between the different ethnic groups found in South Africa (2-5). It has been shown that the vertebral fracture risk between black- and white South African women are comparable, but that black South African women suffer less femur fractures (2, 5). In certain non-South African black populations it has been shown that the skeleton is relatively protected against the detrimental effects of endocrinopathies like hyperparathyroidism (6, 7). The main objective of the study was to assess and compare indices of bone health in black and white South African women with GD at the time of diagnosis, and at 6 and 12 months after commencement of treatment for GD.

This thesis describes the bone health of black and white female patients diagnosed with GD in a South-African setting. Forty consecutive and consenting black females between the ages of 25 and 65 years of age with confirmed GD who attended the Endocrine Clinic at UAH were included in this study. These patients were matched with healthy black females according to age (±5 years), categories of BMI and seasonality. Twenty consecutive and consenting white females presenting at the same endocrine clinic with confirmed GD disease were also included in the study. The white female patients were also matched with healthy white controls according to the same criteria.

Predefined bone-related biochemical markers were obtained at baseline from black- and white female patients and their respective controls. Comparisons were made between black and white female patients as well as between each ethnic group and their respective controls. DXA was performed at baseline, and after 6 months and 12 months of treatment. BMD and body composition-related variables obtained at the different time-points were studied. Comparisons were made at each time point between black and white female patients as well as between each ethnic group and their respective controls. It is important to keep in mind that the reference population used for the calculation of categories of BMD and body composition results was the NHANES III female Caucasian data base (8). Double

tetracycline-labelled bone histomorphometry was performed in a subset of consenting black patients fulfilling predefined criteria. Since a bone histomorphometry study had already been performed in Scandinavian women suffering from hyperthyroidism, histomorphometry was not repeated in white patients in this study (9).

Both the patient groups were comparable at baseline regarding demographic characteristics, except for smoking and mean blood pressure. Forty percent of white patients were current smokers compared to none of the black patients. The mean blood pressure of black patients was slightly but significantly higher compared to white patients. No significant differences were observed between the two ethnic groups of patients in terms of thyroid function and thyroid autoimmunity. The number of postmenopausal patients in both groups was limited which is in keeping with the age distribution associated with GD (10). It is most likely coincidental that none of the patients in either group had evidence of Graves' orbitopathy, especially in the group of white women where 40% of patients were current smokers. This is of interest as it has been shown that current smoking not only increases the risk of development of GD, but smoking is also associated with an increased risk of the development of Graves' orbitopathy (11).

8.2 **Biochemistry**

The median PTH level of black patients at baseline was suppressed well below the lower limit of the reference range and was also significantly lower than median PTH levels in controls as well as in white patients. Among white patients median PTH levels were also significantly decreased compared to white controls but were still within the reference range. The suppressed PTH level observed in black patients was accompanied by hypercalcemia. The median calcium level of white patients was normal. Markers of bone formation as well as –resorption was increased: Overall median BAP and osteocalcin levels, both markers of increased osteoblast activity, were significantly greater in black and white patients compared to their respective controls, while median levels of urine DPD, a marker of bone resorption, were also significantly greater in patients from both ethnic groups compared to their respective controls. Among black patients the median PTH level was significantly lower and the median urine DPD level significantly greater than in white patients. The median BAP and osteocalcin levels did not differ significantly between the ethnic groups. Based on these biochemistry findings it may be concluded that GD increased bone turnover significantly in

women of both ethnic groups, but that in black patients, GD had a more pronounced effect on bone resorption than on bone formation compared to white patients.

Both black patients and black control subjects were 25(OH)D deficient at baseline as was evident from median 25(OH)D levels which were below the laboratory cut-off value of 32 ng/ml. The median 25(OH)D level of white patients was within the normal range while the median 25(OH)D in white control subjects also indicated vitamin D deficiency. Although the median levels of 25(OH)D in black and white patients were discordant, the difference in median 25(OH)D levels were not significant. Median 25(OH)D levels in both black and white control subjects were significantly lower compared to their respective patient groups. Median 25(OH)D levels were also significantly lower in both black and white premenopausal control subjects compared to patients. BMI category did not explain differences in median 25(OH)D levels apart from white patients in the pre-obese category who had 25(OH)D levels significantly higher than in controls.

It is important to note that the laboratory cut-offs used for 25(OH)D levels, were based on laboratory reference ranges. These reference ranges were obtained from northern hemisphere epidemiological studies and were not based on normative data for the South African- or even the Free State population. Investigation is required to determine the normal 25(OH)D reference ranges for the Free State population. The difference in 25(OH)D levels observed between the patient groups may be due to GD, but it may also reflect ethnic differences. Until this is clarified, the question regarding supplementation remains unanswered.

Median 1,25$(OH)_2$D levels in patients and controls were strikingly different and often the converse from what was observed for 25(OH)D. Among black and white patients median 1,25$(OH)_2$D) levels were within the reference range and did not differ significantly between the groups. The median 1,25$(OH)_2$D levels of both black and white control subjects however were above the upper limit of the reference range, and in both ethnic groups significantly greater in controls compared to patients. The same pattern was also evident in premenopausal patients and their respective controls. Across all BMI categories median 1,25$(OH)_2$D levels in black controls were above the upper limit of normal and was also significantly higher in control subjects compared to black patients. Although median 1,25$(OH)_2$D levels in white control subjects in all BMI categories were above the upper limit of normal these levels did not differ significantly between white patients and -controls. This suggests interference of

GD in the conversion 25(OH)D to 1,25$(OH)_2$D (12, 13). The increased levels of calcium observed, especially in black patients, may explain the discrepancy in 1,25$(OH)_2$D levels observed (14). It is well-known that calcium intake by South Africans, especially black South Africans, are low (15). Low calcium intake is a well-known inducer of increased 1α-hydroxylase (16). This may explain the discrepancy seen in 1,25$(OH)_2$D levels between especially black patients and –controls.

The normal to high 1,25$(OH)_2$D levels observed in black patients and –controls, as well as white controls is intriguing. Firstly, if the 25(OH)D levels of black patients and –controls are truly low, it is difficult to explain the normal, and even high levels of 1,25$(OH)_2$D observed. Thus: a low substrate, but a normal product. Second, white patients had normal levels of 25(OH)D accompanied by normal levels of 1,25$(OH)_2$D. This while white controls had reduced 25(OH)D levels accompanied by high 1,25$(OH)_2$D. Black patients and –controls also had reduced 25(OH)D levels accompanied by normal- and high 1,25$(OH)_2$D levels, respectively. Finally, normative data for the Free State population is lacking and ethnic differences may be reflected in the results found.

The inflammatory markers, TNFα and IL-6, in both patient groups were within the respective laboratory reference ranges and not significantly different from their respective controls. The small numbers of participants must be taken in account. The median TNFα level in black patients was however significantly higher compared to the level found in white patients. This was accompanied by a significantly higher erythrocyte sedimentation rate (ESR) in black compared to white patients. These findings may indicate a higher pro-inflammatory state associated with GD in black patients. Increased TNFα levels have also been associated with increased osteoclastogenesis and bone resorption (17).

Leptin levels were evaluated as markers of body composition. Median levels of leptin, irrespective of BMI, did not differ significantly among patients of both ethnic groups and their respective controls.

IGF-1 was examined as a growth factor that may be involved in muscle physiology (18). Median IGF1 levels did not differ significantly between black and white patients. Median IGF1 levels in patients of both ethnic groups were, however, below the lower limit of the reference range and were also significantly lower in patients than in their respective controls.

The thyroid influences IGF1, IGF1-receptors as well as insulin-like growth factor–binding proteins (IGFBPs) independent of growth hormone (19). Decreased IGF1 may be indicative of the severity of GD (20). Martin *et al.* showed that there was no correlation between IGF1 levels and the presence or severity of Graves' orbitopathy. It has been suggested that anti-bodies influencing the IGF1-receptor may rather play a role in the development of Graves' orbitopathy (21).

8.3 **Bone mineral density**

Among black and white women with GD the median BMD (g/cm^2) measured at all sites of interest were lower, but not statistically so, at baseline compared to the respective controls. The exception was the average of the median of both total hip BMDs (g/cm^2) which was significantly lower in white patients compared to controls. This is indicative of a GD effect. Among white patients median BMD of the left femoral neck at baseline was also significantly lower compared to black patients. There was no difference at this site between white patients and controls and the difference is thus an ethnic difference rather than a GD induced difference. Based on BMD measurements it can be concluded that the impact of GD in black and white females at diagnosis of the condition was minimal, although white patients had lower total hip BMD than controls.

Among black females with GD median Z-scores (at sites of interest) also did not differ significantly compared to healthy black control subjects at baseline. The same was true for white women except for the average of the median bilateral hip and median left total hip Z-scores. The median Z-score at the lumbar spine of black patients was significantly lower than that of white patients. None of the median Z-scores in either of the ethnic groups were below -2.0. A significantly greater proportion of black females with GD compared to healthy black control subjects had low Z-scores (≤ -2.0 SD) at the left forearm and the whole body. The proportion of white females with GD and with low Z-scores did not differ significantly from that of white control subjects with decreased Z-scores at any of the sites measured. Based on Z-score measurements at baseline it can be concluded that GD in black women did have a significant negative effect on BMD for age at the left forearm and whole body. These findings were discordant with actual BMD measurements as were discussed in the previous paragraph. BMD Z-scores in white women showed that GD had a significant negative impact

on the actual bilateral average of the total hip as well as the left total hip areas which were in approximate agreement with actual BMD measurements as shown above.

In the postmenopausal group of patients, the proportion of subjects in the different T-score categories (normal, osteopaenia, and osteoporosis) did not differ significantly among the two patient groups or among patients and their respective controls, but because of the small group numbers, interpretation of the results must be treated with caution.

Patients with GD underwent follow-up DXA-scans at 6- and 12 months after commencement of definitive therapy for GD. All patients received RAI. Six months after therapy thyroid function test results were comparable between the two groups of patients and thyroid function had reverted from a hyperthyroid to a euthyroid state.

The BMD (g/cm^2) of black patients that were followed up at 12 months were compared with their controls at baseline. Except for the right femoral neck and the left distal radius, the BMD of black patients was higher at all sites measured 12 months after therapy for GD compared to black controls. This difference was not statistically significant, but opposite from what was found at baseline. Comparing the actual BMD of white patients with controls 12 months after therapy, the bilateral average of total hip of white patients remained significantly lower compared to white controls. At baseline, the actual BMD of the left distal forearm of white patients was lower, although non-significant, compared to white controls. Twelve months after therapy the actual BMD of white patients at this site was significantly lower compared to controls. This may indicate a sustained effect of GD-induced bone loss.

The difference in BMD between black- and white patients at the left femoral neck was maintained at 6- and 12 months with white patients having a significantly lower BMD at this site throughout. Although not observed at baseline, at 12 months the BMD of the right femoral neck of white patients was significantly lower compared to black patients. This difference may once again signal an ethnic difference instead of a GD-induced difference as this difference was not found when comparing white patients with controls at 12 months. In white patients BMD at this site did not recover as well as was the case in black patients. The mild BMD deficit observed between black- and white patients at baseline at the left distal forearm had been diminished at 12 months. No difference in the rate of recovery of BMD

was observed between the first and the second six-month periods at any of the sites within each ethnic group.

Assessing vertebral fractures using DXA, more white female patients had evidence of any degree of important deformity when compared to black females at the level of the T8 vertebrae. No other significant differences were observed between the two patient groups. The clinical significance of this finding is unclear. No significant differences in the frequency of vertebral fractures were observed between white patients and controls. Interestingly more black control subjects had important deformities at the level of T9 compared to black women suffering from GD. Once again, the clinical significance is unclear.

The effects of GD on BMD as determined by DXA differed between black and white women according to the measurement (actual BMD, Z-scores, or T-scores), anatomical site of interest, and timing of the investigation.

8.4 **Body composition**

The median BMI at baseline of both black and white females with GD was at the lower threshold of overweight (25.5 kg/m^2) and did not differ significantly. Neither percentage fat per anatomical region nor body composition per compartment differed significantly between the two groups at baseline.

Black women gained a median of 14.8% of their baseline weight during the 12 months following RAI compared to 7.6% in white women. Percentage weight gain during this period did not differ significantly between the groups but was clinically meaningful in both black and white patients. It should be noted that about half of subjects dropped out of the study by 12 months follow up. The final median BMI recorded after 12 months were 33.6 kg/m^2 and 28.2 kg/m^2 in black and white patients respectively and did not differ significantly between the two groups. The median BMI in black patients, however, moved up one category (from overweight to obesity class I). The corresponding percentage change in BMI after 12 months was +13.2% and +8.7%. These changes were significantly different from baseline for each group. In both patient groups the largest increase in BMI took place during the second 6-month period. The fat mass index (FMI) did not differ significantly between the two groups

of patients at baseline nor after 12 months. FMI increased from baseline to 12 months in both groups, but the increase was only significant in black women. The advantage of FMI over BMI to define obesity status is that FMI is independent of lean mass status (22).

The increase in median BMI in black patients over 12 months was accompanied by a significant increase in total fat mass, percentage total fat, percentage trunk fat, and an increase in FMI. The latter changes were not significant in white patients. Lean mass (representing mostly muscle mass) increased in both groups during the 12 months after RAI treatment but the increase was not significant in any of the groups.

In summary, some interesting observations were made regarding ethnic differences in the regaining of weight after RAI therapy. The body composition of black and white females was remarkably similar at the time of diagnosis of GD. The duration of GD also did not differ significantly between the two groups, but 40% of white women were smokers compared to none of the black women. The large proportion of smokers in the white group of patients may explain the disproportionate weight gain in black women as well as the difference in body composition between the two groups 12 months after RAI. The treatment modality used seems to may influence weight and body composition changes (23-25). Previously it had been found that the increase in fat mass observed after treatment was associated with the use of radio-active iodine therapy rather than carbimazole (23, 24). Recently however, these differences have been disputed as the fat gain has been shown to be comparable (26). Similarly, results of weight gain studies in surgically treated compared to radio-active iodine treatment have been reported. In an earlier study it was shown that there were no differences in the weight gained when comparing surgical intervention versus radio-active iodine (27). However, a more recent study showed that the weight gain observed after treatment for hyperthyroidism was more in patients receiving surgery compared to other modalities (25).

8.5 **Bone histomorphometry**

Ten consenting black patients with confirmed structural bone disease underwent biopsies at baseline before therapy. Structural bone disease was defined as low bone mass, incident fractures or vertebral fractures (VFA).

The osteoid parameters were increased which is suggestive of osteomalacia. It must be noted that the tetracycline labelling was sub-optimal and an explanation for the increased osteoid

volume could be increased bone turnover or reduced mineralization. The latter may have been due to 25(OH)D deficiency. There were increased parameters of bone resorption and increased bone turnover. This was supported by increased markers of bone resorption and was associated with increased markers of bone formation. However, it seems that the bone formation was unable to compensate for bone resorption and thus the abnormal bone histomorphometry.

The bone histomorphometry was suggestive of a high bone turnover state.

8.6 **Strengths**

The strengths of the study comprise the homogenous populations included, no differences regarding thyroid function and auto-immune markers at baseline, as well as patients and controls being well-matched according to age, BMI, and seasonality. Also, only a single DXA operator performed the scans during the study.

8.7 **Weaknesses**

The weaknesses of the study were the number of participants included – small sample size. An increased number of subjects may have detected smaller differences. Also, only white patients were smoking and there was no control for risk factors (smoking, parity, etc).

No biochemical marker follow-up at 6- and 12 months in order to evaluate improvement/ recovery was performed. DXA was performed at 6- and 12 month intervals after treatment, which in this cohort was radio-active iodine. The ideal would have been to perform DXA at 6- and 12 months after normalization of the thyroid function. Longterm follow-up (e.g.: 5 years) in order to evaluate the resolution of the condition and the impact on fracture risk was also not performed. A longer follow up of body composition after treatment would also have been helpful in order to determine whether there is an exaggerated recovery in black females or a recovery to the normal mean.

A small number of patients were included for bone histology. There were technical issues with the tetracycline-labeling making exact diagnosis and conclusions difficult. Follow-up histomorphometry in order to determine resolution of the metabolic bone disease found in

black patients is lacking.

8.8 **Conclusion**

Ethnicity does play a role in bone health in different population groups in South Africa (28). Biomarkers of bone turnover in black- and white South African women, with GD, confirmed an increase in bone resorption and –formation. The levels of markers of bone resorption were significantly higher in black patients, confirming that the bone health of black women is not protected against the detrimental effects of GD on bone health.

In comparing the BMD of black- and white South African women with GD with each other, as well as healthy controls, it was shown that ethnic- as well as disease related differences do exist. Once again it was shown that black women are not protected against the bone complications of this endocrinopathy. The rate of recovery after treatment was comparable between the two patient groups.

The body composition of both patient groups was comparable at baseline and increased significantly in both groups up to 12 months after treatment. Although not statistically different, black patients went from the overweight category to obesity class 1 after treatment, while white patients with GD remained in the overweight category during the period 12 months after therapy.

Histomorphometry, in a sub-set of black South African women suffering from GD, confirmed accelerated bone turnover, predominant stimulation of bone resorption and histological evidence of demineralization as evidenced by abundant osteoid on histology.

This thesis showed that the impact of GD on the bone health of black and white women diagnosed with GD varied according to the modality of investigation used to study different aspects of the interaction between GD and bone health. It is concluded that GD has detrimental but partially reversible effects on the bone health of black South African women.

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Appendix Chapter 3

Methodology

Appendix 3A Advertisement for Healthy Volunteers (English)

UNIVERSITY OF THE FREE STATE
UNIVERSITEIT VAN DIE VRYSTAAT
YUNIVESITHI YA FREISTATA
UFS·UV
HEALTH SCIENCES
GESONDHEIDSWETENSKAPPE

The Division of Endocrinology, at Universitas Hospital, is conducting a study on ***BONE HEALTH IN PATIENTS SUFFERING FROM HYPERTHYROIDISM***.

As part of the study healthy black and white female volunteers are needed.

If you are:

- ☑ A black or white female between the ages of 25 and 65 years.
- ☑ Not Smoking.
- ☑ Not using more than 2 units of alcohol per day.

[1Unit = 1 Glass of wine (200ml)/ 25ml Strong liquor/ 1 Can of Beer (340ml)]

- ☑ Not using medication concerning bone health (e.g.: Fosamax®; Hormone replacement therapy; etc.).
- ☑ Not having a history of thyroid disease.
- ☑ Interested in knowing about the status of your thyroid and bone health.

By volunteering for the study you may be helping to benefit the wider community through the development of new and improved management strategies for patients suffering from thyroid disease. In return for your time your thyroid and bone health will be evaluated for free.

If you are interested in participating, please contact Wimpie de Lange at Universitas Hospital:

051-4053154

071-6839977 (*Short* = 6728)

delangew@ufs.ac.za

205 Nelson Mandela Drive/Rylaan, Park West/Parkwes, Bloemfontein 9301, South Africa/Suid-Afrika
P.O. Box/Posbus 339, Bloemfontein 9300, South Africa/Suid-Afrika, T: +27(0)51 401 9111, www.ufs.ac.za

Appendix 3A Advertisement for Healthy Volunteers (Afrikaans)

UNIVERSITY OF THE FREE STATE
UNIVERSITEIT VAN DIE VRYSTAAT
YUNIVESITHI YA FREISTATA
UFS·UV
HEALTH SCIENCES
GESONDHEIDSWETENSKAPPE

Die Endokrien Afdeling by Universitas Hospitaal beplan ‘n studie oor ***BEEN GESONDHEID IN PASIËNTE WAT LY AAN HIPERTIREOSE.***

As deel van die studie word gesonde wit en swart vroulike vrywilligers benodig.

As u:

- ☑ ‘n Swart of wit vrou tussen die ouderdomme van 25 en 65 jaar is.
- ☑ Nie rook nie.
- ☑ Nie meer as 2 eenhede alkohol per dag gebruik nie..

[1Eenheid = 1 Glas of wyn (200ml)/ 25ml Sterk alkohol/ 1 Blikkie bier (340ml)]

- ☑ Nie medikasie gebruik war been gesondheid beïnvloed nie(e.g.: Fosamax®; Hormonn vervangings terapie; ens.).
- ☑ Nie ‘n geskiedenis van tiroïedsiekte het nie.
- ☑ Geïntereseerd is oor die status van u tiroïed en been gesondheid.

Deur u vrywillige deelname sal u bydra tot die gemeenskap deur die ontwikkeling van nuwe en verbeterde behandeling strategieë vir pasiënte wat ly aan hipertireose. In ruil vir u tyd sal u skildklier en been gesondheid gratis evalueer word.

As u belangstel, kontak asb. vir Wimpie de Lange by Universitas Hosppitaal.

If you are interested in participating, please contact Wimpie de Lange at Universitas Hospital:

051-4053154

071-6839977 (*Kort* = 6728)

delangew@ufs.ac.za

205 Nelson Mandela Drive/Rylaan, Park West/Parkwes, Bloemfontein 9301, South Africa/Suid-Afrika
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Appendix 3B Diagnostic criteria for thyroid storm

Diagnostic Criteria for Thyroid Storm: The Wartofsky Score

Thermoregulatory dysfunction		**Cardiovascular dysfunction**	
Temperature (ºC)		Tachycardia	
99-99.9 (37.2-37.7)	5	99-109	5
100-100.9 (37.8-38.3)	10	110-119	10
101-101.9 (38.4-38.8)	15	120-129	15
102-102.9 (38.9-39.4)	20	130-139	20
103-103.9 (39.5-39.9)	25	≥140	25
≥104.0 (>40)	30	Congestive heart failure	
Central nervous system effects		Mild	5
Mild	10	Pedal oedema	
Agitation		Moderate	10
Moderate		Bibasilar rales	
Delirium	20	Severe	15
Psychosis		Pulmonary oedema	
Extreme lethargy		Atrial fibrillation	10
Severe		**Precipitant history**	
Seizure	30	Negative	0
Coma		Positive	10
Gastrointestinal-hepatic dysfunction			
Moderate			
Diarrheal	10		
Nausea/vomiting			
Abdominal pain			
Severe	20		
Unexplained jaundice			

* A score of 45 or more is highly suggestive of thyroid storm; a score of 25 to 44 supports the diagnosis; and a score below 25 makes thyroid storm unlikely (1).

Appendix 3C: Endocrine Annual Report – 2011 (Excerpt)

3. BINNEPASIëNT GETALLE

Statistiek van die saal

	2011**	**2010**	**2009**	**2008**
Toelatings	208 (357 volgens suster Du Plooy se statistieke)	283 (323* volgens Sr Du Plooy se stats)	237	244
Sterftes	1	2 (4 volgens Sr Du Plooy se stats)	2	4
Opsommings	79	164	230	209
Saalkonsultasies	65	98	138	110
Ongevalle konsultasies	48	58	68	39

**** 33 weke**

Uiteensetting van saal-opnames:

Toestande waarmee pasiënte opgeneem is:	**Getal: 2011**
Hipertireose ***(Hyperthyroidism)***	116
Hipotireose	5
Cushing's	15
Addison's	5
Feochromositoom	10
Hipertensie	13

Insulinoom	8 (4 pasiënte, waarvan 2 elk 3 keer opgeneem is)
Hipofisêre probleme	9
Vertraagde groei/ puberteit/ Hipogonadisme	12
Osteoporose	1
Paratiroïede	10
MEN sindroom	1
Diabetes	126
Ander	26
Totaal	**357**

Appendix 3D Patient **i**nformation leaflet (English)

English: Patient Info: Hyperthyroidism

Welcome at Universitas Hospital's Department of Endocrinology.

We would like you to participate in a study to collect information about the effects of GD on bone- and fat metabolism.

1. What is Hyperthyroidism?

Hyperthyroidism is a condition associated with Hyperactivity of the Thyroid gland.

2. What are the causes of Hyperthyroidism?

There are multiple causes for Hyperthyroidism, but the more common causes include: GD (Autoimmune Disorder); Toxic Thyroid Adenoma (Hyperactive nodule in the Thyroid) & Multi-nodular Goitre (Multiple Hyperactive nodules in the Thyroid).

3. What are the problems associated with Hyperthyroidism?

Hyperthyroidism is associated with many problems, which include the following:

- Heat intolerance;
- Atrial Fibrillation (Heart Dysrhythmia);
- Hypertension;
- Osteoporosis;
- Infertility &
- Death.

4. What treatment options are available for Hyperthyroidism?

Treatment options include oral medication, surgery, and radiation. The treatment best suited for your type of Hyperthyroidism will be determined and discussed with you during admission.

- Anti-thyroid drugs are safe and easy to use. The drugs included are: Carbimazole, Methimazole and Propylthiouracil. The most common side-effect is a skin rash. A sore throat might be indicative of infection due to white cell involvement.

- The doctor must be notified immediately if you develop a skin rash or sore throat while being treated with Neomercazole. If you cannot reach the doctor immediately, stop your medication. Please contact the doctor to arrange emergency blood investigations. Thyroid nodules can also be treated with radio-active iodine or anti-thyroid drugs.
- There is a good chance that your thyroid will end up being under-active, but this is easily treated with thyroxine tablets.
- Radioactive iodine cannot be given if you are pregnant or breastfeeding and it is recommended that you avoid pregnancy in the 6 months after taking radioactive iodine, because this may damage the thyroid of the unborn child. After this period, it will be safe to fall pregnant. If you are pregnant, drugs can be given to control your thyroid condition.
- In the first week after treatment, you should avoid close contact with any child or person. This means that you should not hold them close to you. You can still be in the same room as them.
- There is a good chance that your thyroid will end up being under-active, but this is easily treated with thyroxine tablets.

5. What is the purpose of the study?

- The study will look at all patients with Graves 'disease that are admitted to Universitas Hospital.
- It is expected that this research will improve our understanding of the effect of Graves' disease on bone and will help in the future care of patients like yourself.
- All information about you will be collected, including results of blood tests and scans of the thyroid, bones, and heart.
- Your treatment will not differ at all from current treatment, except that information will be collected, stored, and analyzed later.
- Blood will also be stored, that may be used in further investigation of your thyroid disease later.
- At all times, your confidentiality will be protected and at no time will you be identified in any publications that may result from this research.

6. How often should I visit the clinic after finishing treatment?

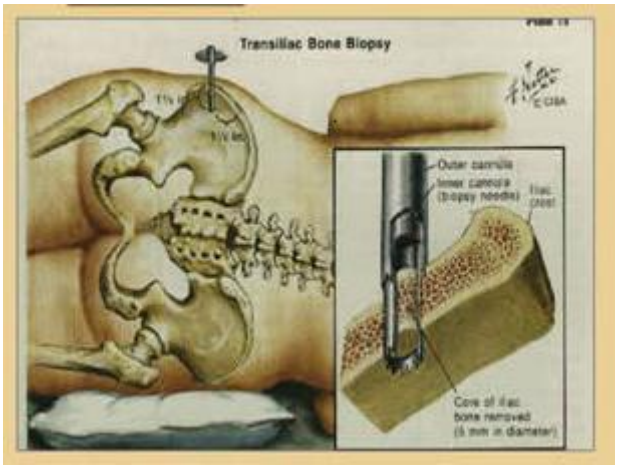
- If you agree to participate in the study, you will be re-admitted after two weeks for four days.
- You will be followed-up at Out-Patients, Universitas Hospital, at intervals of 6 weeks, 3 months and 6 monthly. After 6- and 12-months new information will be obtained and stored.

7. What special investigations will be performed during the study?

- Thyroid uptake scans. 2 scans [Technetium and iodine] done by Nuclear medicine, will involve your thyroid being scanned to show the activity of your thyroid cells. This helps us to confirm the diagnosis and to plan the most effective dose of radioactive iodine.
- DXA bone scan allows us to measure the density of your bones. Thyroid disease can cause thinning of the bones and this scan can help us assess this effect. It will be repeated at 6 months.
- DXA-scan for evaluation of body composition.
- Echocardiogram ultrasound and electrocardiogram. These tests the function of the heart.
- Several blood tests, to check the activity of your thyroid, liver function tests and lipid profiles, in addition to routine tests.
- Blood will be stored and used only to investigate Graves' thyroid disease. The blood taken for the evaluation of the cytokines (TNF-α & IL-6), leptin and IGF-1 will be stored at Ampath Laboratories' National Reference Laboratory in Centurion. The blood samples will be batched to reduce the cost of the study.
- Bloods taken for $1,25(OH)_2$ Vitamin D and IGF-1 will be stored for later evaluation when more funds become available.
- Bone biopsy – A bone biopsy will be performed if you give consent.

  On days 1 to 3 of your admission you will receive a course of antibiotics (if you are not allergic to tetracycline), which will be repeated on days 18 to 20.

  A bone biopsy will be performed on day 25 under local anesthetic. The procedure will be performed by a specialist orthopedic surgeon.



If the biopsy result is abnormal, we would like to repeat the biopsy, with your consent, after one year to observe the effect of treatment.

The following complications can occur (1 in 2000 persons (2)):

Pain (Most common);
Bleeding (Rare);
Lateral cutaneous nerve injury (Very rare);
Wound infection (Very rare);
Fractures (Very rare) &
Osteomyelitis (= infection of Bone, Exceedingly rare).

- No-one else will have access to the blood and no financial benefit to the investigator will follow.

8. <u>What are the advantages of taking part in the study?</u>

The results of this study will help us to better understand the complications of GD. This will help us to improve the management of patients suffering from GD. It will help people from South-Africa and world-wide.

9. <u>Is the study safe?</u>

- The study will not influence your management in any way.
- The safety of radio-active iodine has been discussed.

- Confidentiality is guaranteed.

10. Is taking part in the study compulsory?

- No, if you decide not to take part it will not influence your management in any way.
- If you decide to take part initially, but decide to withdraw later, you only need to inform the investigator. (See phone number at end.)

11. Will the information be confidential and secure?

- Yes, all data will be stored in a secure cabinet in the Department of Medicine.
- Publications will not identify individuals. No outside person will have access to your name and the results.
- The required Ethics approval from the University of Free State Ethics Committee has been obtained prior to the commencement of the study.
- Participation in the study is free. No fee or costs will be paid to the patients.

Contact Person:

Dr. W de Lange. Department Internal Medicine, Universitas Hospital, Bloemfontein.

Cell: 071-6839977

Appendix 3D Patient information leaflet (Afrikaans)

Afrikaans: Pasiënt Inligting: Hipertireose

Welkom by Universitas Hospitaal se Departement Endokrinologie.

Ons wil graag hê u moet deelneem aan ‘n studie waar ons die effek van Graves se siekte op been- en vet metabolisme ondersoek.

1. Wat is Hipertireose?

Hipertireose is ‘n toestand wat gekenmerk word deur hiper-/ ooraktiwiteit van die Tiroïed (Skildklier).

2. Wat is die oorsaak van Hipertireose?

Daar is veelvuldige oorsake vir Hipertireose, maar die mees algemene oorsake sluit die volgende in:

- Graves se siekte (Autoïmmuun Toestand);
- Toksiese Tiroïed adenoom (Ooraktiewe nodule in die Tiroïed) &
- Multi-Nodulêre Goiter (Veelvuldige ooraktiewe massas i.d. Tiroïed).

3. Wat is die Komplikasies (Nagevolge) van Hipertireose?

Hipertireose word gekenmerk deur veelvuldige nagevolge, wat die volgende insluit:

- Hitte-intoleransie (-onverdraagsaamheid);
- Atriale Fibrillasie (Hart disritme);
- Hipertensie;
- Osteoporose;
- Infertiliteit (Onvrugbaarheid) &
- Dood.

4. Watter Behandeling is beskikbaar vir Hipertireose?

Verskeie behandelingsopsies bestaan vir Hipertireose. Dit sluit in orale medikasie, chirurgie en bestraling. Die geskikte behandeling sal bepaal en bespreek word met u na gelang van die oorsaak van u hipertireose.

- Anti-tiroïed middels is gewoonlik veilig en maklik om te neem. Die middels is Karbimazool, Methimazole, asook Propieltiourasiel. Die algemeenste newe-effek is 'n veluitslag. Die lewer en die witbloedselle kan aangetas word, maar dit is baie skaars. 'n Infeksie soos 'n seer keel, dui op witbloedsel aantasting.
- Indien u Neomerkasool gebruik moet enige veluitslag of seerkeel so gou as moontlik aan die dokter rapporteer word. As daar 'n rede is waarom u nie die dokter dadelik kan kontak wanneer u 'n veluitslag of seerkeel het nie, stop die medikasie dadelik. Kontak die dokter sodat bloedtoetse gedoen kan word.
- Tiroïed nodules kan ook behandel word met radio-aktiewe jodium of anti-tiroïed middels.
- In die eerste week nadat u radio-aktiewe jodium ontvang het, moet u noue kontak met enige kind of persoon vermy. Dit beteken u mag hulle nie vashou nie. U mag wel in dieselfde kamer wees. Studies tot op hede gedoen, toon geen verhoogde risiko van enige vorm van kanker na radio-aktiewe jodium toediening nie.
- U kan nie radio-aktiewe jodium ontvang wanneer u swanger is of borsvoed nie. Dit word aanbeveel dat u swangerskap vermy vir 6 maande nadat u radio-aktiewe jodium ontvang het, omdat dit die tiroïed van die ongebore kind mag beskadig. Na hierdie periode is dit veilig om swanger te raak. Wanneer u swanger is met hipotireose, word middels gebruik om die tiroïed se funksie te beheer.
- Daar is 'n goeie kans dat u tiroïed op die ou end onder-aktief sal wees, maar dit is maklik behandelbaar met tiroksien-tablette.

4. **Wat is die doel van die studie?**

- In die studie sal gekyk word na pasiënte met 'n ooraktiewe tiroïed wat in Universitas Hospitaal toegelaat word.
- Ons poog in hierdie studie om die effek van Graves se siekte op been beter te verstaan, en in die toekoms te help met die behandeling van pasiënte soos uself.

- Alle inligting met betrekking tot u bloedtoetse, tiroïed-flikkergramme, beenflikkergramme en hartondersoeke sal bymekaar gehou word.

- U behandeling sal glad nie verskil van die huidige behandeling nie, behalwe dat die inligting versamel en geberg word en later analiseer sal word.

- Bloed sal ook geberg word vir verdere ondersoeke van u tiroïed op 'n latere stadium.

- Vertroulikheid sal ten alle tye behou word en op geen stadium sal u geïdentifiseer word in enige publikasie wat mag volg uit hierdie navorsing nie.

5. Hoe dikwels sal u die hospitaal besoek?

- Indien u toestemming tot deelname aan die studie verleen sal u heropgeneem word na twee weke vir 4 dae.

- U sal 'n afspraak by Buitepasiënte, Universitas Hospitaal, kry na 6 weke, 3 mde., 6 mde. en dan 6 maandeliks. Na 6 en 12 mde. sal meer inligting versamel en geberg word.

6. Die volgende toetse sal gedoen word gedurende die studie:

- Tiroïed opname (tegnesium en jodium) word deur Kerngeneeskunde gedoen. Hiermee word die tiroïed ondersoek om die aktiwiteit van die tiroïedselle te bepaal. Dit help ons om die diagnose te bevestig en om die mees effektiewe dosis radio-aktiewe jodium uit te werk.

- DXA-studie waarmee die digtheid van u bene gemeet word. Tiroïedsiekte veroorsaak verdunning van die bene (osteoporose) en die DXA help ons om dit te beoordeel. Dit word herhaal na 6, 12 en 18 mde.

- DXA-ondersoek ter beoordeling van u liggaam-samestelling.

- Echokardiogram, Ultraklank en EKG. Hierdie toets die funksie van die hart.

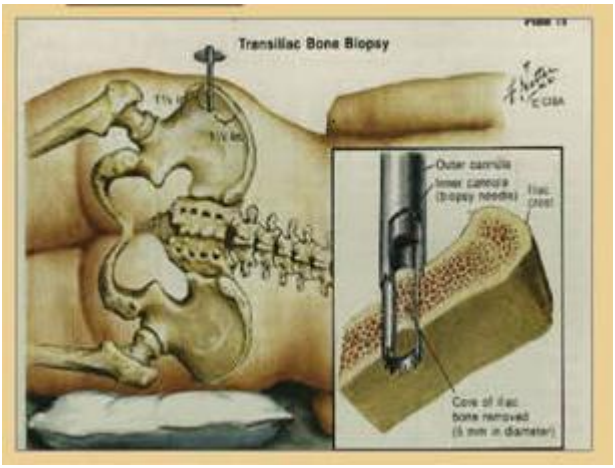
- Beenbiopsie – Indien u toestem tot deelname aan die studie sal ‘n beenbiopsie uitgevoer word.

Op dag 1 tot 3 van u opname sal u ‘n kursus anti-biotika ontvang (indien u nie allergies vir tetrasikliene nie), wat dan weer herhaal word op dag 18 tot 20.

Op dag 25 sal u ‘n trans-iliale beenbiopsie ondergaan. Die prosedure vind plaas onder lokale verdowing en word uitgevoer deur ‘n spesialis van die departement Ortopedie.

Na die biopsie sal u vir 4 ure geobserveer word, waarna u kan huistoe gaan.

(Sien diagram vir posisie van die biopsie)



Indien die biopsie abnormaal is, sal ons graag dit wil herhaal, met u toestemming, een jaar na behandeling om die verandering waar te neem.

Die volgende komplikasies kom soms voor (1 in 2000 persone (2)):

Pyn (Mees algemeen);
Bloeding (Skaars);
Laterale kutane oppervlakkige senuwee besering (Baie Skaars);
Wond Infeksie (Baie Skaars);
Fraktuur (Baie Skaars) &
Osteomiëltis (=Infeksie v.d. beenweefsel; Baie skaars)

- 'n Hele aantal bloedtoetse nl. die vlak van die tiroïed hormoon, lewer funksies, lipiedprofiel en roetine toetse.

- Bloed sal geberg word om Graves se tiroïed siekte te ondersoek. Niemand anders het toegang tot die bloed nie. Bloed vir die sitokiene (TNF-α en IL-6), leptien en IGF-1 sal gestoor word by Ampath laboratoriums se Nasionale Verwysings Laboratorium in Centurion. Die doel hiervan is om die hele reeks ondersoeke gelyk te doen in ‘n poging om koste te bespaar.

Die bloede vir 1,25(OH)2 Vitamien D en IGF-1 sal gestoor word vir latere evaluering wanneer meer fondse beskikbaar is.

8. Wat is die voordele van insluiting in die studie?

Met die resultate van die studie sal ons Graves se siekte en die komplikasies beter verstaan. Dit sal ons help om ons sorg vir pasiënte met hierdie toestand te verbeter. Dit sal mense in hierdie land en die res van die wêreld help.

9. Is die studie veilig?

- Hierdie studie sal nie u versorging op enige manier beïnvloed nie.
- Die veiligheid van radio-aktiewe jodium is reeds bespreek.
- U vertroulikheid is gewaarborg.

10. Is dit verpligtend dat u deelneem?

Nee, as u verkies om nie deel te neem nie, sal u versorging sonder voorbehoud op die normale manier voortgaan.

As u deelneem en later besluit om te onttrek, stel u die ondersoeker in kennis en dit sal gereël word. (Telefoonnommers onderaan beskikbaar)

11. Is die inligting vertroulik en veilig?

Ja, alle data word gestoor in 'n kluis by die Dept. Interne Geneeskunde.

Individue word nie identifiseer in publikasies nie. Geen buitestaander het toegang tot u naam en resultate nie.

Die studie word aan die Etiese Komitee van die Universiteit van die Vrystaat voorgelê vir goedkeuring, voordat dit 'n aanvang neem.

Deelname in die studie is gratis. Daar is geen betaling gemaak aan die pasiënte.

Daar is geen finansiële voordeel vir die navorser in die studie nie.

Kontak Persoon:

Dr. W de Lange. Departement Interne Geneeskunde, Universitas Hospitaal, Bloemfontein.

Sel.: 071-6839977

Appendix 3E Consent for participation in study (English)

Title: **Bone health in GD: A comparison of black and white South African women.**

I, [NAME IN BLOCK LETTERS] .....

Have read the attached explanation of this study and have discussed it with Dr. Wimpie de Lange.

I understand what the study entails.

I am willing to take part in the study.

Signed: ..... Date: .....

I, Wimpie de Lange, have explained the purpose of the study to .....

Signed: ..... Date: .....

Witness 1: .....

Signed: ..... Date: .....

Witness 2: .....

Signed: ..... Date: .....

Appendix 3E Consent for participation in study (Afrikaans)

Titel: **Bone health in GD: A comparison of black and white South African women.**

Ek, [NAAM in BLOKLETTERS] ..... het die aangehegde verduideliking van hierdie studie gelees en bespreek met Dr. Wimpie de Lange.

Ek verstaan wat die studie behels.

Ek is gewillig om deel te neem.

Geteken: ..... Datum: .....

Ek, Wimpie de Lange, het die aard en die doel van die studie aan ..... verduidelik.

Geteken: ..... Datum: .....

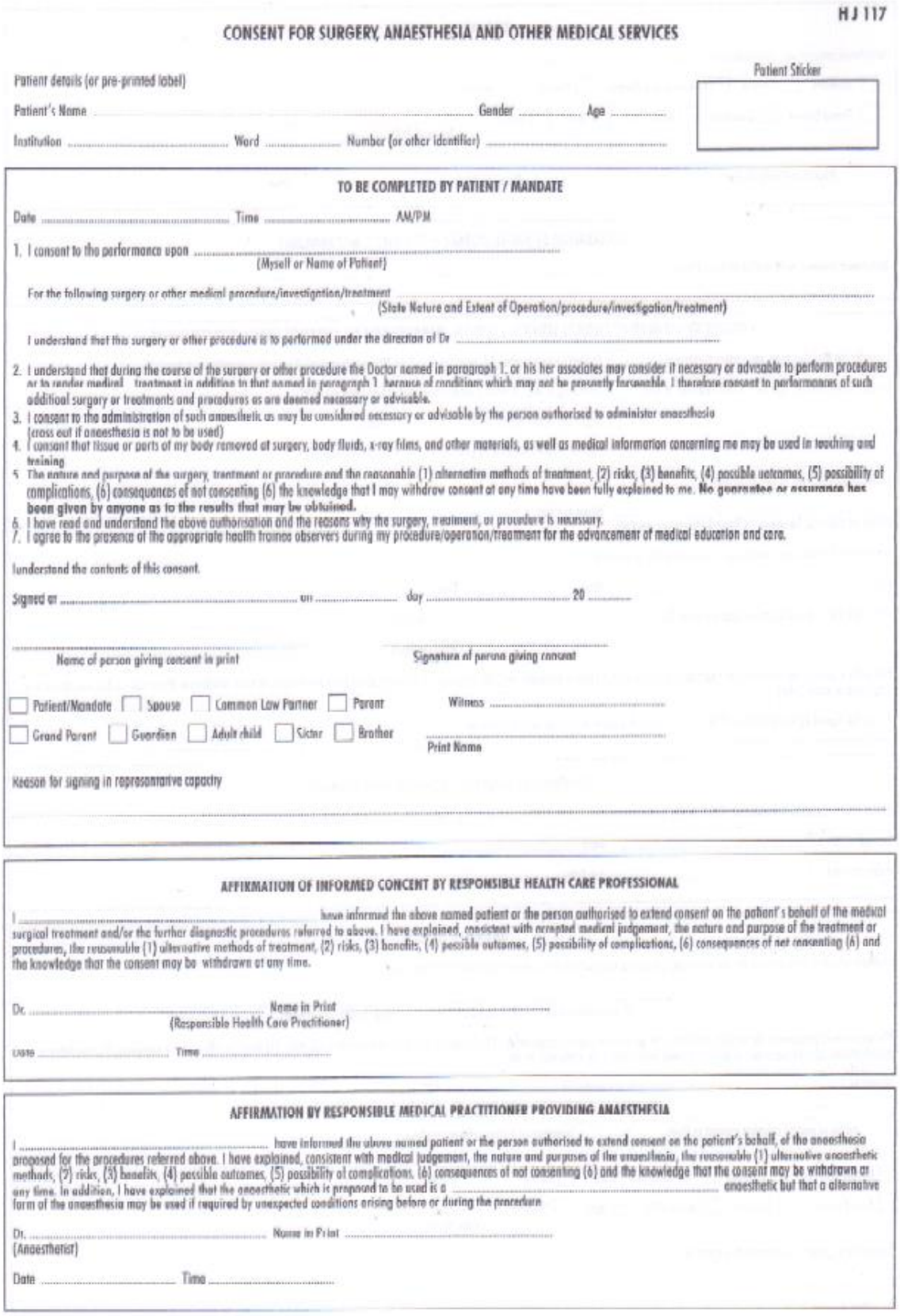
Getuie 1: .....

Geteken: ..... Datum: .....

Getuie 2: .....

Geteken: ..... Datum: .....

Appendix 3F Consent for participation in study: Bone Biopsy (English)



HJ 117

CONSENT FOR SURGERY, ANAESTHESIA AND OTHER MEDICAL SERVICES

Patient details (or pre-printed label)

Patient Sticker

Patient's Name ..... Gender ..... Age .....

Institution ..... Ward ..... Number (or other identifier) .....

TO BE COMPLETED BY PATIENT / MANDATE

Date ..... Time ..... AM/PM

1. I consent to the performance upon .....
(Myself or Name of Patient)

For the following surgery or other medical procedure/investigation/treatment .....
(State Nature and Extent of Operation/procedure/investigation/treatment)

I understand that this surgery or other procedure is to performed under the direction of Dr .....

2. I understand that during the course of the surgery or other procedure the Doctor named in paragraph 1, or his her associates may consider it necessary or advisable to perform procedures or to render medical treatment in addition to that named in paragraph 1 because of conditions which may not be presently foreseeable. I therefore consent to performances of such additional surgery or treatments and procedures as are deemed necessary or advisable.
3. I consent to the administration of such anaesthetic as may be considered necessary or advisable by the person authorised to administer anaesthesia (cross out if anaesthesia is not to be used)
4. I consent that tissue or parts of my body removed at surgery, body fluids, x-ray films, and other materials, as well as medical information concerning me may be used in teaching and training
5. The nature and purpose of the surgery, treatment or procedure and the reasonable (1) alternative methods of treatment, (2) risks, (3) benefits, (4) possible outcomes, (5) possibility of complications, (6) consequences of not consenting (6) the knowledge that I may withdraw consent at any time have been fully explained to me. **No guarantee or assurance has been given by anyone as to the results that may be obtained.**
6. I have read and understand the above authorisation and the reasons why the surgery, treatment, or procedure is necessary.
7. I agree to the presence of the appropriate health trainee observers during my procedure/operation/treatment for the advancement of medical education and care.

I understand the contents of this consent.

Signed at ..... on ..... day ..... 20 .....

....
Name of person giving consent in print — Signature of person giving consent

☐ Patient/Mandate ☐ Spouse ☐ Common Law Partner ☐ Parent ☐ Grand Parent ☐ Guardian ☐ Adult child ☐ Sister ☐ Brother

Witness .....

.....
Print Name

Reason for signing in representative capacity .....

AFFIRMATION OF INFORMED CONCENT BY RESPONSIBLE HEALTH CARE PROFESSIONAL

I ..... have informed the above named patient or the person authorised to extend consent on the patient's behalf of the medical surgical treatment and/or the further diagnostic procedures referred to above. I have explained, consistent with accepted medical judgement, the nature and purpose of the treatment or procedures, the reasonable (1) alternative methods of treatment, (2) risks, (3) benefits, (4) possible outcomes, (5) possibility of complications, (6) consequences of not consenting (6) and the knowledge that the consent may be withdrawn at any time.

Dr. ..... Name in Print .....
(Responsible Health Care Practitioner)

Date ..... Time .....

AFFIRMATION BY RESPONSIBLE MEDICAL PRACTITIONER PROVIDING ANAESTHESIA

I ..... have informed the above named patient or the person authorised to extend consent on the patient's behalf, of the anaesthesia proposed for the procedures referred above. I have explained, consistent with medical judgement, the nature and purposes of the anaesthesia, the reasonable (1) alternative anaesthetic methods, (2) risks, (3) benefits, (4) possible outcomes, (5) possibility of complications, (6) consequences of not consenting (6) and the knowledge that the consent may be withdrawn at any time. In addition, I have explained that the anaesthetic which is proposed to be used is a ..... anaesthetic but that a alternative form of the anaesthesia may be used if required by unexpected conditions arising before or during the procedure.

Dr. ..... Name in Print .....
(Anaesthetist)

Date ..... Time .....

Appendix 3F (Continued)

TELEPHONIC CONSENT

Telephonic consent was obtained from .....

☐ Mandate ☐ Spouse ☐ Common Law Partner ☐ Parent Witness .....

☐ Grand Parent ☐ Guardian ☐ Adult Child ☐ Sister ☐ Brother .....
Name in Print

....
Physician/Social Worker Name in Print Date

DECLARATION BY SOCIAL WORKER IF CONSENT IS NOT AVAILABLE

Telephonic consent could not be obtained from .....

CONSENT BY THE HEAD OF CLINICAL SERVICES / CLINICAL MANAGER ON CALL / HEAD OF HEALTH ESTABLISHMENT

Considering that this is an emergency treatment / ☐ Patient's age less than 14 years (medical) or 18 years (surgical) if parents / guardian not available

Irreparable damage can result if not attended to and: ☐ According to the Children's Amendment Act (43 of 1976 art 8A)

☐ According to the Mental Health Act (Act 17 Of 2002)

☐ Patient /mandate uable to give consent for the above procedure

I hereby give consent to this procedure

..... Name in Print .....
Head of Clinical Services / Clinical Manager on Call

If telefonically discussed, Ihereby give consent to this procedure*

I, Dr ..... Date ..... Time .....

Discussed the case and obtained consent from Dr. ..... Witness .....

Name in Print .....

NB! Before presenting a case to the Head of Clinical Services / Clinical Manager on Call, documented attempts must have been made to trace relatives or the social worker must have been employed to trace family

* Must be signed by Head of Clinical Services / Clinical Manager on Call as soon as possible

STATEMENT OF INTERPRETER (WHERE APPROPRIATE)

I have interpreted the information above to the patient to the best of my ability and in a way I believe he/she can understand

Signed ..... Date .....

Name in Print ..... Job Title .....

INFORMED REFUSAL

I hereby refuse to give consent for the following surgery or other medical procedure/investigation/treatment .....

.....
(State Nature and Extend of Operation/procedure/investigation/treatment)

The nature and purpose of the surgery, treatment or procedure and the reasonable (1) alternative methods of treatment, (2) risks, (3) benefits, (4) possible outcomes, (5) possibility of complications, (6) consequences of not consenting have been fully explained to me.

Signed at ..... on day of ..... 20.....

....
Name of person refusing consent in Print Signature of person refusing consent

☐ Patient/Mandate ☐ Spouse ☐ Common Law Partner ☐ Parent Witness .....

☐ Grand Parent ☐ Guardian ☐ Adult child ☐ Sister ☐ Brother .....
Print Name

Reason for signing in representative capacity

.....

Appendix 3G Control consent for participation in study (English)

Title: **Bone health in GD: A comparison of black and white South African women.**

I, [NAME IN BLOCK LETTERS] .....

Have read the attached explanation of this study and have discussed it with Dr. Wimpie de Lange.

I understand what the study entails.

I am willing to take part in the study.

Signed: ..... Date: .....

I, Wimpie de Lange, have explained the purpose of the study to .....

Signed: ..... Date: .....

Witness 1: .....

Signed: ..... Date: .....

Witness 2: .....

Signed: ..... Date: .....

Appendix 3G Control consent for participation in study (Afrikaans)

Titel: Bone health in GD: A comparison of black and white South African women.

Ek, [NAAM in BLOKLETTERS] ..... het die aangehegde verduideliking van hierdie studie gelees en bespreek met Dr. Wimpie de Lange.

Ek verstaan wat die studie behels.

Ek is gewillig om deel te neem.

Geteken: ..... Datum: .....

Ek, Wimpie de Lange, het die aard en die doel van die studie aan ..... verduidelik.

Geteken: ..... Datum: .....

Getuie 1: .....

Geteken: ..... Datum: .....

Getuie 2: .....

Geteken: ..... Datum: .....

Appendix 3H Control information leaflet (Afrikaans)

Afrikaans: Kontrole Inligting: Hipertireose

Welkom by Universitas Hospitaal se Departement Endokrinologie.

Ons wil graag hê u moet deelneem aan ‘n studie waar ons die effek van Graves se siekte op been- en vet metabolisme ondersoek.

1. Wat is Hipertireose?

Hipertireose is ‘n toestand wat gekenmerk word deur hiper-/ ooraktiwiteit van die Tiroïed (Skildklier).

2. Wat is die oorsaak van Hipertireose?

Daar is veelvuldige oorsake vir Hipertireose, maar die mees algemene oorsake sluit die volgende in:

a. Graves se siekte (Autoïmmuun Toestand);

b. Toksiese Tiroïed adenoom (Ooraktiewe nodule in die Tiroïed) &

c. Multi-Nodulêre Goiter (Veelvuldige ooraktiewe massas i.d. Tiroïed).

3. Wat is die Komplikasies (Nagevolge) van Hipertireose?

Hipertireose word gekenmerk deur veelvuldige nagevolge, wat die volgende insluit:

- Hitte-intoleransie (-onverdraagsaamheid);
- Atriale Fibrillasie (Hart disritme);
- Hipertensie;
- Osteoporose;
- Infertiliteit (Onvrugbaarheid) &
- Dood.

4. Wat is die doel van die studie?

- In die studie sal gekyk word na pasiënte met 'n ooraktiewe tiroïed wat in Universitas Hospitaal toegelaat word.

- Gedurende die studie wil ons kyk na die been-gesondheid van pasiënte wat ly aan Graves se siekte, en hoe dit vergelyk met die van ‘n gesonde individu soos u.

- Ons poog in hierdie studie om die effek van Graves se siekte op been beter te verstaan, en in die toekoms te help met die behandeling van die pasiënte.

- Alle inligting met betrekking tot u bloedtoetse, tiroïed-flikkergramme, beenflikkergramme en hartondersoeke sal bymekaar gehou word.

- Vertroulikheid sal ten alle tye behou word en op geen stadium sal u geïdentifiseer word in enige publikasie wat mag volg uit hierdie navorsing nie.

5. Hoe dikwels sal u die hospitaal besoek?

- U sal die hospitaal eenmalig besoek vir ‘n reeks ondersoeke.

- Indien daar enige abnormaliteite is sal dit aan u geopenbaar word en kan u verder hanteer word deur ons of u eie geneesheer.

6. Die volgende toetse sal gedoen word gedurende die studie:

- DXA-studie waarmee die digtheid van u bene gemeet word. Graves se siekte veroorsaak verdunning van die bene (osteoporose) en die DXA help ons om dit te beoordeel.

- DXA-studie ter beoordeling van u liggaam-samestelling.

- ‘n Reeks bloedondersoeke sal uitgevoer word. Dit sluit die volgende in:

TSH, T3 & T4
Serum Osteocalcin
Urinary Deoxypyridinoline
25(OH) Vitamin D
$1,25(OH)_2$ Vitamin D

Parathyroid Hormone
Leptin, TNFα & IL-6

- Bloed sal ook getrek word en gestoor word vir latere evaluasie.

- Bloed sal net geberg word om Graves se tiroïed siekte te ondersoek. Niemand anders het toegang tot die bloed nie. Bloed vir die sitokiene (TNF-α en IL-6), leptien en IGF-1 sal gestoor word by Ampath laboratoriums se Nasionale Verwysings Laboratorium in Centurion. Die doel hiervan is om die hele reeks ondersoeke gelyk te doen in ‘n poging om koste te bespaar.

Die bloede vir 1,25(OH)2 Vitamien D en IGF-1 sal gestoor word vir latere evaluering wanneer meer fondse beskikbaar is.

7. Wat is die voordele van insluiting in die studie?

Met die resultate van die studie sal ons Graves se siekte en die komplikasies beter verstaan. Dit sal ons help om ons sorg vir pasiënte met hierdie toestand te verbeter. Dit sal mense in hierdie land en die res van die wêreld help.

8. **Is die inligting vertroulik en veilig?**

- Deelname is vrywilliglik.

- U vertroulikheid is gewaarborg. Alle data word gestoor in 'n kluis by die Dept. Interne Geneeskunde.

- Individue word nie geïdentifiseer in publikasies nie. Geen buitestaander het toegang tot u naam en resultate nie.

- Die studie word aan die Etiese Komitee van die Universiteit van die Vrystaat voorgelê vir goedkeuring, voordat dit 'n aanvang neem.

- Deelname in die studie is gratis. Daar is geen betaling gemaak aan die pasiënte.

- Daar is geen finansiële voordeel vir die navorser in die studie nie.

Kontak Persoon:

Dr. W de Lange. Departement Interne Geneeskunde, Universitas Hospitaal, Bloemfontein. Sel.: 071-6839977

Appendix 3H Control information leaflet (English)

English: Control Info: Hyperthyroidism

Welcome at Universitas Hospital's Department of Endocrinology.

We would like you to participate in a study to collect information about the effects of GD on bone- and fat metabolism.

1. What is Hyperthyroidism?

Hyperthyroidism is a condition associated with Hyperactivity of the Thyroid gland.

2. What are the causes of Hyperthyroidism?

There are multiple causes for Hyperthyroidism, but the more common causes include: GD (Autoimmune Disorder); Toxic Thyroid Adenoma (Hyperactive nodule in the Thyroid) & Multi-nodular Goitre (Multiple Hyperactive nodules in the Thyroid).

3. What are the problems associated with Hyperthyroidism?

Hyperthyroidism is associated with many problems, which include the following:

- Heat intolerance;
- Atrial Fibrillation (Heart Dysrhythmia);
- Hypertension;
- Osteoporosis;
- Infertility &
- Death.

4. What is the purpose of the study?

- The study will look at all patients with GD that are admitted to Universitas Hospital.

- During the study we want to compare the effects of GD on bone health in patients suffering from the disease, to normal healthy individuals like yourself.
- It is expected that this research will improve our understanding of the effect of Graves' disease on bone and will help in the future care of patients like yourself.
- All information about you will be collected, including results of blood tests and scans of the thyroid, bones, and heart.
- Your treatment will not differ at all from current treatment, except that information will be collected, stored, and analyzed later.
- Blood will also be stored, that may be used in further investigation of your thyroid disease later.

5. <u>How often should I visit the clinic after finishing treatment?</u>

- You will have to visit the hospital once.
- If any abnormalities are found, you will be notified, and further treatment will be arranged.

6. <u>What special investigations will be performed during the study?</u>

- DXA bone scan allows us to measure the density of your bones. Thyroid disease can cause thinning of the bones and this scan can help us assess this effect. It will be repeated at 6 months.
- DXA-scan for evaluation of body composition.
- Several blood tests will be performed, namely:

  TSH, T3 & T4
  Serum Osteocalcin
  Urinary Deoxypyridinoline
  25(OH) Vitamin D
  $1,25(OH)_2$ Vitamin D
  Parathyroid Hormone
  Leptin, TNFα & IL-6

- Blood will be taken and stored for later evaluation.
- Blood will be stored and used only to investigate Graves' thyroid disease. The blood taken for the evaluation of the cytokines (TNF-α & IL-6), leptin and IGF-1 will be stored at Ampath Laboratories' National Reference Laboratory in Centurion. The blood samples will be batched to reduce the cost of the study.

Bloods taken for $1,25(OH)_2$ Vitamin D and IGF-1 will be stored for later evaluation when more funds become available.

- No-one else will have access to the blood and no financial benefit to the investigator will follow.

7. <u>What are the advantages of taking part in the study?</u>

The results of this study will help us to better understand the complications of GD. This will help us to improve the management of patients suffering from GD. It will help people from South-Africa and world-wide.

8. <u>Will the information be confidential and secure?</u>

- Yes, all data will be stored in a secure cabinet in the Department of Medicine.
- Confidentiality is guaranteed.
- Publications will not identify individuals. No outside person will have access to your name and the results.
- The required Ethics approval from the University of Free State Ethics Committee has been obtained prior to the commencement of the study.
- Participation in the study is free. No fee or costs will be paid to the patients.
- There is no financial advantage to the researcher.

<u>Contact Person:</u>

Dr. W de Lange. Department Internal Medicine, Universitas Hospital, Bloemfontein.

Cell: 071-6839977

Appendix 3I Patient questionnaire

Patient Questionnaire, Results- and Management Sheet:

Patient No.: _____ **Date:**

Investigator:

Consent: **Y / N**

Info leaflet: **Y / N**

1. **Demographic Data:**

D.O.B [Year]:

Pregnant: Y☐ N☐ Employed: Y☐ N☐

Education: Pre-/Postmenopausal:

Town: Contact Number:

2. **Clinical Data:**

History:

Main Symptoms:	
	Duration:
CVS: Ischemia Y☐ N☐ By-pass Y☐ N☐	Heart Failure Y☐ N☐
Intermittent Claudication Y☐ N☐	Other:
RF: Smoker: Y☐ N☐ Pack years:	DM:Y☐ N☐ Hpt :Y☐ N☐ Family:Y☐ N☐
Family History Thyroid Disease: Y☐ N☐	Family History of Osteoporosis: Y☐ N☐
Symptoms of Compression: Y☐ N☐	
Previous use of: Thyroxine Y☐ N☐ Duration: Dosage:	Previous use of: Neomercazole: Y☐ N☐ Duration: Dosage:
Other:	
Medication on Admission:	

Examination:

General:	Height: cm Mass: kg BMI:
Thyroid: Size: Nature:	Bruit: Y☐ N☐ Cervical lymphnodes: Y☐ N☐
Skin: Y☐ N☐ Hair loss: Y☐ N☐	Nail abnormalities: Y☐ N☐
CNS: Emotional lability:Y☐ N☐	Psychosis: Y☐ N☐
Reflexes: Normal☐ Increased☐ Decreased☐	Other:
CVS / KVS: Pulse: /min	BP Supine: mmHg Stand: mmHg
Other:	Resp:
GIT:	
Eyes: DiseaseY☐N☐ Exopthalmos: Y☐ N☐	Hertl:L R Opthalmoplegia: Y☐ N☐
Corneal Disease: Y☐ N☐	Optic Neuropathy: Y☐ N☐
Visual Acuity: L R Pinhole:L R	Lid lag: Y☐ N☐ Lid swelling: Y☐ N☐
Possible Thyroid Crisis: Y☐N☐Impending☐	Actual Score:

4. Special Investigations

Full Blood Count:	
Haemoglobin (Hb):	
Mean Corpuscular Volume (MCV):	
White cell Count:	
Platelets:	
Other	
Urea & Electrolytes, Calcium, Magnesium & Phosphate:	
Na	
K	
Urea	
Creatinine	
Corrected Calcium	
Magnesium	
Phosphate	
Liver enzyme tests:	
Billirubin	
Total Proteins	
Albumin	
AST	
ALT	
ALP	
GGT	
TSH, T3 & T4:	
TSH	
FT4	
T3	
Thyroid Antibody Titres:	
Thyroid Stimulating Hormone Receptor	
Thyroglobulin	
Thyroxine peroxidase	
β-Human Chorionic Gonadotropin:	
Fasting blood glucose:	
Lipids*:*	***Pro-BNP:***
Total Cholesterol	
Triglycerides	***CRP:***
HDL	
LDL	
ECG:	***Echocardiogram:***
Rhythm:	LA Size:
Rate:	RA Size:
Axis:	LVH: Y☐ N☐
Other:	Diastolic Dysfunction: Y☐ N☐
	RVPSP:
	Other:
Chest X-Rays:	

CT / MRI Orbit: Y☐ N☐	
Technetium Scan:	
Single nodule☐ / Multiple nodules☐ / Diffuse☐ / Normal ☐	Nodule: Cold☐ Warm☐
I^{123} Uptake Scan:	
Percentage uptake: 6hrs: 24hrs:	Treatment dose given:
Thyroid ultrasound: Y☐ N☐	
Single nodule☐ / Multinodular☐ / Diffuse☐ Cyst☐ / Normal☐	FNA: Y☐ N☐ Result:

DXA Bone scan:				
	BMD ($g/cm^{2)}$	T-Score	Z-Score	
Spine :				
L1				
L2				
L3				
L4				
L5				
Total :				
Hip :				
Neck :				
Trochanter :				
Inter :				
Total				

Additional Special Investigations:

Serum Osteocalcin:	
Serum Bone Alkaline Phosphatase:	
Urinary Deoxypyridinoline:	
Parathyroid Hormone:	
25(OH) Vitamin D:	$1,25(OH)_2$ Vitamin D:
Leptin:	
TNFα:	
IL-6:	IGF-1:
Double Tetracycline-Labelled Bone Histomorphometry: Y☐ N☐	*Comment:*
DXA measurement of Intra-abdominal Fat: Y☐ N☐	*Comment:*

5. Management

1st Line:	*Factors affecting choice:*
Carbimazole☐	Patient ☐
Radioactive Iodine ☐	Pathology ☐
Surgery ☐	Compression ☐
Lugoll's Iodine ☐	Cosmetic ☐
Steroids ☐	Convenience ☐
Symptomatic ☐	Pregnancy ☐
	Compliance ☐
2nd Line: Y☐ N☐	*What:*
Propranolol: Y☐ N☐	*Dosage:*
Other drugs: Y☐ N☐	*Specify:*

6. Initial Outcomes:

Length of Admission (Days):	
Days in Multi / High Care:	
Death (In Hospital):	Y☐ N☐

7. **Management of Eye Disease:**

None ☐ / Steroids ☐ / Surgery ☐ / Radiotherapy☐	*Details:*

8. **Follow-up Form: 6 weeks, 6- & 12 months**

Patient No.: _____

6 Weeks ☐ TSH & T4

6 Months ☐ TSH & T4 DXA for BMD DXA for Body Composition

12 Months ☐ TSH & T4 DXA for BMD DXA for Body Composition
Bone Histomorphometry

Thyroid Function:	
TSH	
T4	
DXA measurement of bone mineral density:	*Comment:*
DXA measurement of Intra-abdominal Fat: Y☐ N☐	*Comment:*
Treatment:	*Dosage:*
Propranolol: ☐	
Neomercazole: ☐	
Eltroxin: ☐	
Other: ☐	*Specify:*
Double Tetracycline-Labelled Bone Histomorphometry: Y☐ N☐	*Comment:*

Appendix 3J Control questionnaire

Control Group Study Questionnaire & Investigation Results Document:

1. **Demographic Data:** Control No.: _____

D.O.B [Year]:

Pregnant: Y☐ N☐ Employed: Y☐ N☐

Race: B☐ W☐ M☐ Education:

Town: Contact Number:

2. **Osteoporosis & Previous Fracture History:**

Family History of Osteoporosis: Y☐ N☐

Diagnosis of Osteoporosis : Y☐ N☐ Diagnosis : DXA☐ Fracture☐

Previous Fracture : Y☐ N☐ Fragility☐ Trauma☐ Site :

3. **Medical History:**

Smoking: Y☐ N☐ Stopped: Y☐ N☐ Pack Years:

Glucocorticoids: Y☐ N☐ Stopped: Y☐ N☐ Dosage:

Rheumatoid Arthritis: Y☐ N☐

Alcohol: Y☐ N☐ Units per day:

Other :

4. **Clinical Data:**

Length: Weight: BMI:

5. DXA Result:

DXA Bone scan:				
	BMD ($g/cm^{2)}$	T-Score	Z-Score	
Spine :				
L1				
L2				
L3				
L4				
L5				
Total :				
Hip :				
Neck :				
Trochanter :				
Inter :				
Total				

6. Special Investigations:

TSH:	T4: T3:
Serum Osteocalcin:	
Serum Bone Alkaline Phosphatase:	
Urinary Deoxypyridinoline:	
Parathyroid Hormone:	
25(OH) Vitamin D:	$1,25(OH)_2$ Vitamin D:
Leptin:	
TNFα:	
IL-6:	IGF-1:

Appendix 3K Ethics approval

UNIVERSITY OF THE FREE STATE
UNIVERSITEIT VAN DIE VRYSTAAT
YUNIVESITHI YA FREISTATA
UFS UV

Research Division
Internal Post Box G40
☎(051) 4052812
Fax (051) 4444359

E-mail address: StraussHS@ufs.ac.za

Ms H Strauss

2012-05-29

REC Reference nr 230408-011
IRB nr 00006240

DR W DE LANGE
DIVISION OF ENDOCRINOLOGY
DEPARTMENT OF INTERNAL MEDICINE
FACULTY OF HEALTH SCIENCES
UFS

Dear Dr De Lange

ECUFS NR 49/2012
PROJECT TITLE: BONE HEALTH IN GRAVES' DISEASE: A COMPARISON OF BLACK AND WHITE SOUTH AFRICAN WOMEN

- You are hereby kindly informed that the Ethics Committee approved the above project at the meeting held on 22 May 2012 after the revised Information Leaflet was submitted.
- Committee guidance documents: Declaration of Helsinki, ICH, GCP and MRC Guidelines on Bio Medical Research. Clinical Trial Guidelines 2000 Department of Health RSA; Ethics in Health Research: Principles Structure and Processes Department of Health RSA 2004; Guidelines for Good Practice in the Conduct of Clinical Trials with Human Participants in South Africa, Second Edition (2006); the Constitution of the Ethics Committee of the Faculty of Health Sciences and the Guidelines of the SA Medicines Control Council as well as Laws and Regulations with regard to the Control of Medicines.
- Any amendment, extension or other modifications to the protocol must be submitted to the Ethics Committee for approval.
- The Committee must be informed of any serious adverse event and/or termination of the study.
- A progress report should be submitted within one year of approval of long term studies and a final report at completion of both short term and long term studies.
- Kindly refer to the ECUFS reference number in correspondence to the Ethics Committee secretariat.

Yours faithfully

.....
PROF WH KRUGER
CHAIR: ETHICS COMMITTEE

Cc Prof WF Mollentze

Appendix 3L Approval by Dr.N.R.J. van Zyl (Executive officer: UAH)

Department of
Health
FREE STATE PROVINCE

22 March 2012

Dr. W de Lange
Endocrinology Department
Universitas Academic Hospital

Dear Dr de Lange

RESEARCH PROJECT: BONE HEALTH IN GRAVES DISEASE: A COMPARISON OF BLACK AND WHITE SOUTH AFRICAN WOMEN

Herewith permission for the mentioned project to be done at Universitas Academic Hospital on the following conditions:

1. The research should not expose the users and the Department to any avoidable harm.
2. Annual progress reports should be submitted and also a research report at the end of the research process.
3. Reporting of Adverse Events related to the research process must be done within 48 hours of discovery.
4. There shall be provision for obtaining informed consent from all patients/staff where appropriate.
5. Briefing sessions should be conducted with all stakeholders prior to commencement and at the end of the study to provide feedback where appropriate.
6. That approval is obtained from the Ethics Committee.

HEAD: CLINICAL SERVICES: DR NRJ VAN ZYL
Private Bag X20660, Bloemfontein, 9300. Tel. No.: 051-4052866,
Fax: 051-4053500, Room 1077, First Floor, Universitas Academic Hospital
Email: vanzylnr@universitas.fs.gov.za

Appendix 3L (Continued)

The Chief Executive Officer must be notified if the findings of the project will be published and a research report needs to be sent to the Head Clinical Services as soon as the study is completed.

DR NRJ VAN ZYL
2012 -03- 22
HEAD: CLINICAL SERVICES
UNIVERSITAS ACADEMIC HOSPITAL

Yours sincerely

DR NIC R J VAN ZYL
HEAD: CLINICALSERVICES
UNIVERSITAS ACADEMIC HOSPITAL

HEAD: CLINICAL SERVICES: DR NRJ VAN ZYL
Private Bag X20660, Bloemfontein, 9300. Tel. No.: 051-4052866,
Fax: 051-4053500, Room 1077, First Floor, Universitas Academic Hospital
Email: vanzylnr@universitas.fs.gov.za

Appendix 3M Matching of Black Patients with Controls

Obs	Match Number	Case/ Control	BMI New Lab	Obs	Match Number	Case/ Control	BMI New Lab
1	1	Case	38	69	16	Control	30
2	1	Control	36	72	17	Case	26
5	2	Case	21	73	17	Case	21
6	2	Case	19	74	17	Control	21
7	2	Control	23	77	18	Case	37
10	3	Case	22	78	18	Control	39
11	3	Case	26	81	19	Case	47
12	3	Control	26	82	19	Control	46
16	4	Case	21	83	20	Case	27
17	4	Case	15	84	20	Case	27
18	4	Control	19	85	20	Control	29
21	5	Case	36	86	21	Case	20
22	5	Control	32	87	21	Control	23
25	6	Case	21	88	22	Case	22
26	6	Case	20	89	22	Control	20
27	6	Control	22	90	23	Case	32
30	7	Case	38	91	23	Control	38
31	7	Control	39	92	24	Case	54
34	8	Case	44	93	24	Control	44
35	8	Control	43	94	25	Case	26
38	9	Case	32	95	25	Case	25
39	9	Case	31	96	25	Control	27
40	9	Control	30	97	26	Case	23
43	10	Case	29	98	26	Control	22
44	10	Case	24.7	99	27	Case	24
45	10	Control	29	100	27	Control	22
46	11	Case	25	101	28	Case	19
47	11	Control	29	102	28	Case	19
50	12	Case	24.8	103	28	Control	21
51	12	Case	24				
52	12	Control	29				
56	13	Case	28				
57	13	Control	28				
60	14	Case	29				
61	14	Control	32				
64	15	Case	24.99				
65	15	Control	20				
68	16	Case	32				

References

1. Burch HB, Wartofsky L. Life-threatening thyrotoxicosis. Thyroid storm. Endocrinol Metab Clin North Am. 1993;22(2):263-77.
2. Revell PA. Histomorphometry of bone. Journal of clinical pathology. 1983;36(12):1323-31.
3. Genant HK, Wu CY, van Kuijk C, Nevitt MC. Vertebral fracture assessment using a semiquantitative technique. J Bone Miner Res. 1993;8(9):1137-48.

Appendix Chapter 5

Results and data analysis of Dual-energy X-ray Absorptiometry investigations

Appendix 5A Frequency (%) of subjects with evidence of a vertebral deformity according to VFA: Black- and white patients at baseline

	Black Females with GD	**White Females with GD**	**p-value***
T4 wedge deformity	(n = 30)	(n = 11)	
Normal	26 86.6%	10 90.9%	
Mild	2 6.7%	0	
Moderate	2 6.7%	1 9.1%	
Severe	0	0	*1.000* (F)
T5 wedge deformity	(n = 32)	(n = 16)	
Normal	25 78.1%	15 93.8%	
Mild	4 12.5%	1 6.2%	
Moderate	3 9.4%	0	
Severe	0	0	*0.5826 (F)*
T6 wedge deformity	(n = 34)	(n = 17)	
Normal	30 88.2%	14 82.3%	
Mild	3 8.8%	1 5.9%	
Moderate	0	2 11.8%	
Severe	1 3%	0	*0.2596* (F)

*: Chi-square p-value, except where indicated by (F) for Fisher's exact method due to sparse cells. The vertebral fracture assessment (VFA) are reported according to the Genant classification (3): Normal < 20% height loss; Mild fracture = 20-24.9% height loss; Moderate fracture = 25-40% height loss; Severe fracture > 40% height loss.

Appendix 5A (Continued)

	Black Females with GD	**White Females with GD**	**p-value***
T7 wedge deformity	(n = 36)	(n = 18)	
Normal	29 80.5%	16 88.9%	
Mild	5 13.9%	2 11.1%	
Moderate	2 5.6%	0	
Severe	0	0	*0.8506* (F)
T8 wedge deformity	(n = 36)	(n = 19)	
Normal	33 91.7%	17 89.5%	
Mild	1 2.8%	2 10.5%	
Moderate	2 5.5%	0	
Severe	0	0	*0.3593* (F)
T9 wedge deformity	(n = 37)	(n = 19)	
Normal	35 94.6%	18 94.7%	
Mild	2 5.4%	1 5.3%	
Moderate	0	0	
Severe	0	0	*1.0000* (F)

Appendix 5A (Continued)

	Black Females with GD	**White Females with GD**	**p-value***
T10 wedge deformity	(n = 37)	(n = 20)	
Normal	37 100%	18 90.0%	
Mild	0	1 5.0%	
Moderate	0 0.0%	1 5.0%	
Severe	0	0	*0.1190* (F)
T11 wedge deformity			
Normal	36 97.3%	19 95.0%	
Mild	1 2.7%	0	
Moderate	0	1 5.0%	
Severe	0	0	*0.5827* (F)
T12 wedge deformity			
Normal	34 91.9%	18 90%	
Mild	2 5.4%	1 5.0%	
Moderate	1 2.7%	1 5.0%	
Severe	0	0	*1.0000* (F)

Appendix 5A (Continued)

	Black Females with GD	**White Females with GD**	**p-value***
L1 wedge deformity	(n = 37)	(n = 20)	
Normal	36 97.3%	19 95.0%	
Mild	1 2.7%	0	
Moderate	0	1 5.0%	
Severe	0	0	*0.5827* (F)
L2 wedge deformity			
Normal	37 100%	20 100%	
Mild	0	0	
Moderate	0	0	
Severe	0	0	
L3 wedge deformity			
Normal	37 100%	20 100%	
Mild	0	0	
Moderate	0	0	
Severe	0	0	
L4 wedge deformity			
Normal	36 97.3%	20 100%	
Mild	1 2.7%	0	
Moderate	0	0	
Severe	0	0	*1.0000* (F)

Appendix 5A (Continued)

	Black Females with GD	**White Females with GD**	**p-value***
T4 biconcave deformity	(n = 30)	(n = 11)	
Normal	29 96.7%	11 100%	
Mild	0	0	
Moderate	1 3.3%	0	
Severe	0	0	*1.0000* (F)
T5 biconcave deformity	(n = 32)	(n = 16)	
Normal	31 96.9%	15 93.7%	
Mild	0	1 6.3%	
Moderate	1 3.1%	0	
Severe	0	0	*0.5603* (F)
T6 biconcave deformity	(n =34)	(n = 17)	
Normal	33 97.1%	15 88.2%	
Mild	1 2.9%	2 11.8%	
Moderate	0	0	
Severe	0	0	*0.2547* (F)

Appendix 5A (Continued)

	Black Females with GD	**White Females with GD**	**p-value***
T7 biconcave deformity	(n = 36)	(n = 18)	
Normal	33 91.7%	16 88.9%	
Mild	2 5.5%	1 5.6%	
Moderate	1 2.8%	1 5.6%	
Severe	0	0	*1.0000* (F)
T8 biconcave deformity	(n = 36)	(n =19)	
Normal	34 94.4%	16 84.2%	
Mild	0	3 15.8%	
Moderate	2 5.6%	0	
Severe	0	0	*0.0369* (F)
T9 biconcave deformity	(n = 37)	(n =19)	
Normal	34 91.9%	17 89.5%	
Mild	3 8.1%	2 10.5%	
Moderate	0	0	
Severe	0	0	*1.0000* (F)

Appendix 5A (Continued)

	Black Females with GD	**White Females with GD**	**p-value***
T10 biconcave deformity	(n = 36)	(n = 20)	
Normal	31 83.8%	18 90.0%	
Mild	5 13.5%	2 10.0%	
Moderate	1 2.7%	0 0.0%	
Severe	0	0	*1.0000* (F)
T11 biconcave deformity	(n = 37)	(n = 20)	
Normal	37 100%	20 100%	
Mild	0	0	
Moderate	0	0	
Severe	0	0	
T12 biconcave deformity			
Normal	37 100%	19 95.0%	
Mild	0	1 5.0%	
Moderate	0	0	
Severe	0	0	*0.3509* (F)

Appendix 5A (Continued)

	Black Females with GD	**White Females with GD**	**p-value***
L1 biconcave deformity	(n = 37)	(n = 20)	
Normal	37 100%	19 95.0%	
Mild	0	1 5.0%	
Moderate	0	0	
Severe	0	0	*0.3509* (F)
L2 biconcave deformity			
Normal	36 97.3%	20 100%	
Mild	1 2.7%	0	
Moderate	0	0	
Severe	0	0	*1.0000* (F)
L3 biconcave deformity			
Normal	36 97.3%	20 100%	
Mild	1 2.7%	0	
Moderate	0	0	
Severe	0	0	*1.0000* (F)

Appendix 5A (Continued)

	Black Females with GD	**White Females with GD**	**p-value***
L4 biconcave deformity	(n = 37)	(n = 20)	
Normal	36 97.3%	20 100%	
Mild	1 2.7%	0	
Moderate	0	0	
Severe	0	0	*1.0000* (F)
T4 crush deformity	(n = 30)	(n = 11)	
Normal	30 100%	11 100%	
Mild	0	0	
Moderate	0	0	
Severe	0	0	
T5 crush deformity	(n = 32)	(n =16)	
Normal	32 100%	16 100%	
Mild	0	0	
Moderate	0	0	
Severe	0	0	
T6 crush deformity	(n = 34)	(n =17)	
Normal	34 100%	17 100%	
Mild	0	0	
Moderate	0	0	
Severe	0	0	

Appendix 5A (Continued)

	Black Females with GD	**White Females with GD**	**p-value***
T7 crush deformity	(n = 36)	(n = 18)	
Normal	36 100%	18 100%	
Mild	0	0	
Moderate	0	0	
Severe	0	0	
T8 crush deformity	(n = 36)	(n = 19)	
Normal	36 100%	19 100%	
Mild	0	0	
Moderate	0	0	
Severe	0	0	
T9 crush deformity	(n = 36)	(n = 19)	
Normal	37 100%	19 100%	
Mild	0	0	
Moderate	0	0	
Severe	0	0	
T10 crush deformity	(n = 37)	(n = 20)	
Normal	37 100%	20 100%	
Mild	0	0	
Moderate	0	0	
Severe	0	0	

Appendix 5A (Continued)

	Black Females with GD	**White Females with GD**	**p-value***
T11 crush deformity	(n = 37)	(n = 20)	
Normal	37 100%	19 95.0%	
Mild	0	1 5.0%	
Moderate	0	0	
Severe	0	0	*0.3509* (F)
T12 crush deformity			
Normal	37 100%	20 100%	
Mild	0	0	
Moderate	0	0	
Severe	0	0	
L1 crush deformity			
Normal	36 97.3%	20 100%	
Mild	1 2.7%	0	
Moderate	0	0	
Severe	0	0	*1.0000* (F)
L2 crush deformity			
Normal	37 100%	20 100%	
Mild	0	0	
Moderate	0	0	
Severe	0	0	

Appendix 5A (Continued)

	Black Females with GD	**White Females with GD**	**p-value***
L3 crush deformity	(n = 37)	(n = 20)	
Normal	37 100%	20 100%	
Mild	0	0	
Moderate	0	0	
Severe	0	0	
L4 crush deformity			
Normal	34 91.9%	20 100%	
Mild	3 8.1%	0	
Moderate	0	0	
Severe	0	0	*0.5448* (F)

Appendix 5B Frequency (%) of subjects with evidence of a vertebral deformity according to VFA: White patients and controls at baseline

	White Females with GD	**White Controls**	**p-value***
T4 wedge deformity	n = 10	n = 5	
Normal	10 100%	5 100%	
Mild	0	0	
Moderate	0	0	
Severe	0	0	
T5 wedge deformity	n = 15	n = 10	
Normal	15 100%	10 100%	
Mild	0	0	
Moderate	0	0	
Severe	0	0	
T6 wedge deformity	n = 16	n = 10	
Normal	13 81.25%	10 100%	
Mild	1 6.25%	0	
Moderate	2 12.50	0	
Severe	0		*0.1797*

Where no p-value supplied, the cells are equal. *: Cochran-Mantel-Haenszel p-value. The vertebral fracture assessment (VFA) are reported according to the Genant classification (3): Normal < 20% height loss; Mild fracture = 20-24.9% height loss; Moderate fracture = 25-40% height loss; Severe fracture > 40% height loss.

Appendix 5B (Continued)

	White Females with GD	**White Controls**	**p-value***
T7 wedge deformity	n = 17	n = 10	
Normal	15 88.2%	10 100%	
Mild	2 11.8%	0	
Moderate	0	0	
Severe	0	0	*0.1573*
T8 wedge deformity	n = 18		
Normal	16 88.9%	10 100%	
Mild	2 11.1%	0	
Moderate	0	0	
Severe	0	0	*0.3173*
T9 wedge deformity	n = 18		
Normal	17 94.4%	10 100%	
Mild	0	0	
Moderate	1 5.6%	0	
Severe	0	0	*0.3173*

Appendix 5B (Continued)

	White Females with GD	**White Controls**	**p-value***
T10 wedge deformity	n = 19	n = 10	
Normal	18 94.7%	10 100%	
Mild	1 5.3%	0	
Moderate	0	0	
Severe	0	0	*0.3173*
T11 wedge deformity			
Normal	18 94.7%	10 100%	
Mild	0	0	
Moderate	1 5.3%	0	
Severe	0	0	*0.3173*
T12 wedge deformity			
Normal	17 89.4%	10 100%	
Mild	1 5.3%	0	
Moderate	1 5.3%	0	
Severe	0	0	*0.3173*

Appendix 5B (Continued)

	White Females with GD	**White Controls**	**p-value***
L1 wedge deformity	n = 19	n = 10	
Normal	19 100%	10 100%	
Mild	0	0	
Moderate	0	0	
Severe	0	0	
L2 wedge deformity			
Normal	19 100%	10 100%	
Mild	0	0	
Moderate	0	0	
Severe	0	0	
L3 wedge deformity			
Normal	19 100%	10 100%	
Mild	0	0	
Moderate	0	0	
Severe	0	0	
L4 wedge deformity			
Normal	19 100%	10 100%	
Mild	0	0	
Moderate	0	0	
Severe	0	0	

Appendix 5B (Continued)

	White Females with GD	**White Controls**	**p-value***
T4 biconcave deformity	n = 10	n = 5	
Normal	10 100%	5 100%	
Mild	0	0	
Moderate	0	0	
Severe	0	0	
T5 biconcave deformity	n = 15	n = 10	
Normal	15 100%	10 100%	
Mild	0	0	
Moderate	0	0	
Severe	0	0	
T6 biconcave deformity	n = 16	n = 10	
Normal	15 93.8%	10 100%	
Mild	1 6.2%	0	
Moderate	0	0	
Severe	0	0	

Appendix 5B (Continued)

	White Females with GD	**White Controls**	**p-value***
T7 biconcave deformity	n = 17	n = 10	
Normal	15 88.2%	10 100%	
Mild	1 5.9%	0	
Moderate	1 5.9%	0	
Severe	0	0	*0.1797*
T8 biconcave deformity	n = 18		
Normal	15 83.3%	10 100%	
Mild	3 16.7%	0	
Moderate	0	0	
Severe	0	0	*0.1573*
T9 biconcave deformity	n = 18		
Normal	17 94.4%	10 100%	
Mild	1 5.6%	0	
Moderate	0	0	
Severe	0	0	

Appendix 5B (Continued)

	White Females with GD	**White Controls**	**p-value***
T10 biconcave deformity	n = 19	n = 10	
Normal	18 94.7%	10 100%	
Mild	1 5.3%	0	
Moderate	0	0	
Severe	0	0	
T11 biconcave deformity			
Normal	19 100%	10 100%	
Mild	0	0	
Moderate	0	0	
Severe	0	0	
T12 biconcave deformity			
Normal	18 94.7%	10 100%	
Mild	1 5.3%	0	
Moderate	0	0	
Severe	0	0	

Appendix 5B (Continued)

	White Females with GD	**White Controls**	**p-value***
L1 biconcave deformity	n = 19	n = 10	
Normal	18 94.7%	10 100%	
Mild	1 5.3%	0	
Moderate	0	0	
Severe	0	0	
L2 biconcave deformity			
Normal	19 100%	10 100%	
Mild	0	0	
Moderate	0	0	
Severe	0	0	
L3 biconcave deformity			
Normal	19 100%	10 100%	
Mild	0	0	
Moderate	0	0	
Severe	0	0	

Appendix 5B (Continued)

	White Females with GD	**White Controls**	**p-value***
L4 biconcave deformity	n = 19	n = 10	
Normal	19 100%	10 100%	
Mild	0	0	
Moderate	0	0	
Severe	0	0	
T4 crush deformity	n = 10	n = 5	
Normal	10 100%	5 100%	
Mild	0	0	
Moderate	0	0	
Severe	0	0	
T5 crush deformity	n = 15	n = 5	
Normal	15 100%	10 100%	
Mild	0	0	
Moderate	0	0	
Severe	0	0	
T6 crush deformity	n = 16	n = 10	
Normal	16 100%	10 100%	
Mild	0	0	
Moderate	0	0	
Severe	0	0	

Appendix 5B (Continued)

	White Females with GD	**White Controls**	**p-value***
T7 crush deformity	n = 17	n = 10	
Normal	17 100%	10 100%	
Mild	0	0	
Moderate	0	0	
Severe	0	0	
T8 crush deformity	n = 18		
Normal	18 100%	10 100%	
Mild	0	0	
Moderate	0	0	
Severe	0	0	
T9 crush deformity			
Normal	18 100%	10 100%	
Mild	0	0	
Moderate	0	0	
Severe	0	0	
T10 crush deformity	n = 19		
Normal	19 100%	10 100%	
Mild	0	0	
Moderate	0	0	
Severe	0	0	

Appendix 5B (Continued)

	White Females with GD	**White Controls**	**p-value***
T11 crush deformity	n = 19	n = 10	
Normal	19 100%	10 100%	
Mild	0	0	
Moderate	0	0	
Severe	0	0	
T12 crush deformity			
Normal	19 100%	10 100%	
Mild	0	0	
Moderate	0	0	
Severe	0	0	
L1 crush deformity			
Normal	19 100%	10 100%	
Mild	0	0	
Moderate	0	0	
Severe	0	0	
L2 crush deformity			
Normal	19 100%	10 100%	
Mild	0	0	
Moderate	0	0	
Severe	0	0	

Appendix 5B (Continued)

	White Females with GD	**White Controls**	**p-value***
L3 crush deformity	n = 19	n = 10	
Normal	19 100%	10 100%	
Mild	0	0	
Moderate	0	0	
Severe	0	0	
L4 crush deformity			
Normal	19 100%	10 100%	
Mild	0	0	
Moderate	0	0	
Severe	0	0	

Appendix 5C Frequency (%) of subjects with evidence of a vertebral deformity according to VFA: Black patients and controls at baseline

	Black Females with GD	**Black Controls**	**p-value***
T4 wedge deformity	n = 30	n = 7	
Normal	26 86.7%	6 85.7%	
Mild	2 6.7%	0	
Moderate	2 6.6%	0	
Severe	0	1 14.3%	*0.5961*
T5 wedge deformity	n = 32	n = 9	
Normal	25 78.1%	8 88.9%	
Mild	4 12.5%	1 11.1%	
Moderate	3 9.4%	0	
Severe		0	*0.1573*
T6 wedge deformity	n = 34	n = 12	
Normal	30 88.2%	9 75%	
Mild	3 8.8%	2 16.7%	
Moderate	0	1 8.3%	
Severe	1 3%	0	*0.7029*

*: Cochran-Mantel-Haenszel p-value. The vertebral fracture assessment (VFA) are reported according to the Genant classification (3): Normal < 20% height loss; Mild fracture = 20-24.9% height loss; Moderate fracture = 25-40% height loss; Severe fracture > 40% height loss.

Appendix 5C (Continued)

	Black Females with GD	**Black Controls**	**p-value***
T7 wedge deformity	n = 36	n = 18	
Normal	29 80.6%	16 88.9%	
Mild	5 13.8%	0	
Moderate	2 5.6%	2 11.1%	
Severe	0	0	*0.7868*
T8 wedge deformity		n = 21	
Normal	33 91.7%	15 71.4%	
Mild	1 2.8%	3 14.3%	
Moderate	2 5.5%	2 9.5%	
Severe	0	1 4.8%	*0.0532*
T9 wedge deformity	n = 37	n = 25	
Normal	35 94.6%	24 96%	
Mild	0	0	
Moderate	2 5.4%	1 4%	
Severe	0	0	*0.8415*

Appendix 5C (Continued)

	Black Females with GD	**Black Controls**	**p-value***
T10 wedge deformity	n = 37	n = 25	
Normal	37 100%	23 92%	
Mild	0	1 4%	
Moderate	0	1 4%	
Severe	0	0	*0.0578*
T11 wedge deformity			
Normal	36 97.3%	23 92%	
Mild	1 2.7%	1 4%	
Moderate	0	1 4%	
Severe	0	0	*0.3363*
T12 wedge deformity			
Normal	34 91.9%	23 92%	
Mild	2 5.4%	0	
Moderate	1 2.7%	2 8%	
Severe	0	0	*0.7851*

Appendix 5C (Continued)

	Black Females with GD	**Black Controls**	**p-value***
L1 wedge deformity	n = 37	n = 25	
Normal	36 97.3%	25 100%	
Mild	1 2.7%	0	
Moderate	0	0	
Severe	0	0	*0.4795*
L2 wedge deformity			
Normal	37 100%	23 92%	
Mild	0	0	
Moderate	0	1 4%	
Severe	0	1 4%	*0.1797*
L3 wedge deformity			
Normal	37 100%	25 100%	
Mild	0	0	
Moderate	0	0	
Severe	0	0	
L4 wedge deformity			
Normal	36 97.3%	24 96%	
Mild	1 2.7%	0	
Moderate	0	1 4%	
Severe	0	0	*0.6547*

Appendix 5C (Continued)

	Black Females with GD	**Black Controls**	**p-value***
T4 biconcave deformity	n = 30	n = 7	
Normal	29 96.7%	7 100%	
Mild	0	0	
Moderate	1 3.3%	0	
Severe	0	0	
T5 biconcave deformity	n = 32	n = 9	
Normal	31 96.9%	9 100%	
Mild	0	0	
Moderate	1 3.1%	0	
Severe	0	0	
T6 biconcave deformity	n = 34	n = 12	
Normal	33 97.1%	11 91.7%	
Mild	1 2.9%	1 8.3%	
Moderate	0	0	
Severe	0	0	*1.0000*

Appendix 5C (Continued)

	Black Females with GD	**Black Controls**	**p-value***
T7 biconcave deformity	n = 36	n = 18	
Normal	33 91.7%	18 100%	
Mild	2 5.6%	0	
Moderate	1 2.7%	0	
Severe	0		*0.2743*
T8 biconcave deformity		n = 21	
Normal	34 94.4%	18 85.7%	
Mild	0	1 4.8%	
Moderate	2 5.6%	2 9.5%	
Severe	0	0	*0.0956*
T9 biconcave deformity	n = 37	n = 25	
Normal	34 91.9%	20 80%	
Mild	3 8.1%	2 8%	
Moderate	0	2 8%	
Severe	0	1 4%	*0.0338*

Appendix 5C (Continued)

	Black Females with GD	**Black Controls**	**p-value***
T10 biconcave deformity	n = 37	n = 25	
Normal	31 83.8%	21 84%	
Mild	5 13.5%	2 8%	
Moderate	1 2.7%	0	
Severe	0	2 8%	*0.2039*
T11 biconcave deformity			
Normal	37 100%	20 80%	
Mild	0	2 8%	
Moderate	0	3 12%	
Severe	0	0	*0.0099*
T12 biconcave deformity			
Normal	37 100%	19 76%	
Mild	0	3 12%	
Moderate	0	3 12%	
Severe	0	0	*0.0156*

Appendix 5C (Continued)

	Black Females with GD	**Black Controls**	**p-value***
L1 biconcave deformity	n = 37	n = 25	
Normal	37 100%	23 92%	
Mild	0	1 4%	
Moderate	0	1 4%	
Severe	0	0	*0.1317*
L2 biconcave deformity			
Normal	36 97.3%	24 96%	
Mild	1 2.7%	1 4%	
Moderate	0	0	
Severe	0	0	*0.8084*
L3 biconcave deformity			
Normal	36 97.3%	23 92%	
Mild	0	1 4%	
Moderate	1 2.7%	1 4%	
Severe	0	0	*0.5688*

Appendix 5C (Continued)

	Black Females with GD	**Black Controls**	**p-value***
L4 biconcave deformity	n = 37	n = 25	
Normal	36 97.3%	24 96%	
Mild	1 2.7%	0	
Moderate	0	1 4%	
Severe	0	0	*0.6547*
T4 crush deformity	n = 30	n = 7	
Normal	30 100%	7 100%	
Mild	0	0	
Moderate	0	0	
Severe	0	0	
T5 crush deformity	n = 32	n = 9	
Normal	32 100%	9 100%	
Mild	0	0	
Moderate	0	0	
Severe	0	0	
T6 crush deformity	n = 34	n = 12	
Normal	34 100%	12 100%	
Mild	0	0	
Moderate	0	0	
Severe	0	0	

Appendix 5C (Continued)

	Black Females with GD	**Black Controls**	**p-value***
T7 crush deformity	n = 36	n = 18	
Normal	36 100%	18 100%	
Mild	0	0	
Moderate	0	0	
Severe	0	0	
T8 crush deformity		n = 21	
Normal	36 100%	21 100%	
Mild	0	0	
Moderate	0	0	
Severe	0	0	
T9 crush deformity	n = 37	n = 25	
Normal	37 100%	25 100%	
Mild	0	0	
Moderate	0	0	
Severe	0	0	
T10 crush deformity			
Normal	37 100%	25 100%	
Mild	0	0	
Moderate	0	0	
Severe	0	0	

Appendix 5C (Continued)

	Black Females with GD	**Black Controls**	**p-value***
T11 crush deformity	n = 37	n = 25	
Normal	37 100%	25 100%	
Mild	0	0	
Moderate	0	0	
Severe	0	0	
T12 crush deformity			
Normal	37 100%	25 100%	
Mild	0	0	
Moderate	0	0	
Severe	0	0	
L1 crush deformity			
Normal	36 97.3%	25 100%	
Mild	0	0	
Moderate	0	0	
Severe	1 2.7%	0	*0.3173*
L2 crush deformity			
Normal	37 100%	25 100%	
Mild	0	0	
Moderate	0	0	
Severe	0	0	

Appendix 5C (Continued)

	Black Females with GD	**Black Controls**	**p-value***
L3 crush deformity	n = 37	n = 25	
Normal	37 100%	25 100%	
Mild	0	0	
Moderate	0	0	
Severe	0	0	
L4 crush deformity	n = 34		
Normal	34 91.9%	25 100%	
Mild	3 8.1%	0	
Moderate	0	0	
Severe	0	0	*0.1573*

Appendix Chapter 6

Results: Dual-energy X-ray Absorptiometry - Body Composition

Appendix 6A Rate of change of body composition per compartment from baseline to 6 & 12 months: The Patients[1]

Site	**Black Females with GD** **n = 39**	**White Females with GD** **n = 20**	**p-value[2]** **(95% confidence limits)** **White - Black**
BMI			
Baseline	25.5 (22; 36) n = 18	25.5 (21; 28.5) n = 12	
6 months	27.3 (24.8; 33.7)	24.90 (21.79; 28.74)	
Change (%)	4.28 (-0.54; 10.48)	1.96 (-0.08; 5.28)	*0.4717* (-8.40; 2.56)
Total lean mass			
Baseline	34812 (30543; 38731) n = 15	37255 (34113; 38921) n = 11	
6 months	37284 (33122; 41975)	400667 (35972; 44243)	
Change (%)	7.79 (4.01; 15.88)	8.04 (2.66; 16.53)	*0.8968* (-8.46; 6.57)

Appendix 6A (Continued)

Site	**Black Females with GD** **n = 39**	**White Females with GD** **n = 20**	**p-value[2]** **(95% confidence limits)** **White - Black**
Total fat mass			
Baseline	27051 (19188; 41270)	26759 (16983; 34161)	
6 months	28898(23417; 39803)	25222 (15746; 34691)	
Change (%)	7.7 (-1.62; 14.32)	2.91 (-5.74; 8.9)	*0.1535* (-15.32; -6.17)
Trunk fat (%)			
Baseline	39.65 (31.8; 47.7) n = 14	33.5 (25.6; 43.3) n = 11	
6 months	40.85 (34.8; 41.5)	33.20 (23.2; 40.4)	
Change (%)	-1.96 (-9.01; 7.45)	-4.43 (-9.63; 6.93)	*0.5470* (-12.59; 5.92)

Appendix 6A (Continued)

Site	**Black Females with GD** **n = 39**	**White Females with GD** **n = 20**	**p-value[2]** **(95% confidence limits)** **White - Black**
Total fat (%)			
Baseline	42.5 (36.4; 50.9) n = 15	40 (31.5; 45.7) n = 11	
6 months	44.1 (39.70; 47.2)	40.1 (28.5; 44)	
Change (%)	-1.93 (-3.92; 2.31)	-3.72 (-9.44; 6.91)	*0.3370* (-9.47; -2.13)

[1] Values are medians (interquartile range).
[2] Analysed with a Wilcoxon two-sample test.

Appendix 6B Body composition of patients: Percentage change from baseline to 12 months of those who followed up[1]

Site	**Black Females with GD** **n = 39**	**White Females with GD** **n = 20**	**p-value[2]** **(95% confidence limits)** **White - Black**
	n = 39	n = 20	
BMI			
Baseline	25.5 (22; 32) n = 17	26 (20; 28) n = 11	
12 months	33.6 (27.8; 35.1) n = 17	26.7 (20.5; 31.6) n = 11	
Change (%)	13.2 (7.97; 24.47) n = 17	8.67 (0.0; 14.46) n = 11	*0.2126* (-15.52; 3.41)
Total lean mass			
Baseline	36155 (30999; 42215) n = 15	37211 (36261; 38616) n = 11	
12 months	40920 (34185; 50200) n = 15	42715 (39279; 48037) n = 11	
Change (%)	8.98 (5.32; 20.42) n = 15	11.65 (6.93; 15.14) n = 11	*0.8559* (-6.97; 6.48)

Appendix 6B (Continued)

Site	**Black Females with GD** **n = 39**	**White Females with GD** **n = 20**	**p-value[2]** **(95% confidence limits)** **White - Black**
Total fat mass			
Baseline	26432 (19125; 33849) n = 15	21752 (16481; 33758) n =11	
12 months	36066 (20759; 51377) n = 15	27403 (13926; 39879) n = 11	
Change (%)	26.68 (1.22; 41.25) n = 15	7.7 (5.03; 25.98) n = 11	*0.2035* (-36.21; 6.55)
Trunk fat (%)			
Baseline	37.1 (30.9; 45.5) n = 14	26.5 (19.9; 43.3) n = 11	
12 months	43.2 (38.8; 47.7) n = 15	32.5 (20.1; 46.2) n = 11	
Change (%)	7.66 (-1.9; 16.71) n = 15	6.87 (-7.22; 13.39) n = 11	*0.5841* (-22.42; 10.99)

Appendix 6B (Continued)

Site	**Black Females with GD** **n = 39**	**White Females with GD** **n = 20**	**p-value[2]** **(95% confidence limits)** **White - Black**
Total fat (%)			
Baseline	40.2 (35.4; 49.4) n = 15	32.5 (26.8; 46.4) n = 11	
12 months	45.6 (40.1; 50.1) n = 15	33.4 (26.6; 47.7) n = 11	
Change (%)	2.16 (-1.74; 12.19) n = 15	3.25 (-5.54; 10.6) n = 11	*0.5680* (-17.72; 7.04)

[1] Values are medians (interquartile range).
[2] Analysed with a Wilcoxon two-sample test.