Research in the Division of Endocrinology
Aims & Objectives

- To create and publish new scientific knowledge

- To explore the practical application of theoretical knowledge and thereby improve:
  - patient care
  - teaching
  - techniques, technologies, further research

- To improve insights and enrich an individual’s ability:
  - critical analysis; QC
  - systematic, mechanistic thinking
  - problem solving
Research Culture

OSTEOPOROSIS

DIABETES
Research Culture

OSTEOPOROSIS

DIABETES

Descriptive & Mechanistic
Research Culture

OSTEOPOROSIS

Descriptive & Mechanistic

Clinical & Basic

DIABETES
Research Culture

OSTEOPOROSIS
- Descriptive & Mechanistic
- Clinical & Basic

DIABETES

Bedside
Bench
Examples of BB&B Research

- Glucocorticoid Osteoporosis
- HIV, ARVs and Skeletal Health
- Acanthosis Nigricans
- Diabetic Bone Disease
- Dietary Protein and Bone Health
- Stress Fracture in Athletes
- Hypervitaminosis A and Bone
# PREVALENCE OF STEROID INDUCED-OSTEOPOROSIS

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Steroid Therapy</th>
<th>Non-steroid Therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total</strong></td>
<td>27/59 (46%)</td>
<td>12/51</td>
</tr>
<tr>
<td>Premenopausal</td>
<td>15/34 (44%)</td>
<td>4/24</td>
</tr>
<tr>
<td>Postmenopausal</td>
<td>5/11 (45%)</td>
<td>1/5</td>
</tr>
<tr>
<td>Males</td>
<td>7/14 (50%)</td>
<td>7/22</td>
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</table>

Nortje et al
Pathogenesis of GIOP

↑ Acute Bone Resorption

↓ Chronic

↓ BONE MASS / STRENGTH

↓ Chronic Bone Formation

↑ Early
Pathogenesis of GIOP

- ↓ Gut Ca Absorption
- ↑ Renal Ca Excretion
- ↑ PTH
- ↑ PTH ACTIV
- ▼ OB-PTHR
- ↓ cAMP-PDE
- ↑ IL-6 synthesis
- ↑ PGE2
- ↑ OC apoptosis

↑ Acute Bone Resorption

↓ BONE MASS / STRENGTH

↓ Chronic Bone Formation

↑ Early
Pathogenesis of GIOP

↓ Gut Ca Absorption

↑ Renal Ca Excretion

↓ Lh.Fsh
↓ Gonadal Steroidogenesis
↓ Acth,Dhea,A
Abn Vit D

↑ PTH

↑ PTH ACTIV

↑ OB-PTHR
↓ cAMP-PDE

↑ IL-6 synthesis
↑ PGE2
↑ OC apoptosis

↑ Acute Bone Resorption
↓ Chronic

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Pathogenesis of GIOP

↑ Gut Ca Absorption

↑ Renal Ca Excretion

↑ PTH

↑ PTH ACTIV

↑ OB-PTHR

↓ cAMP-PDE

↑ RANKL
↓ OPG
↓ IL-6 rec

GC effects on bone cells

↑ Acute Bone Resorption

↓ Chronic

↓ BONE MASS / STRENGTH

↓ Chronic Bone Formation

↑ Early

↓ LH.FSH
↓ Gonadal Steroidogenesis
↓ ACTH,DHEA,A Abn Vit D

↓ IL-6 synthesis
↑ PGE2
↑ OC apoptosis

Growth factors
Myopathy
Pathogenesis of GIOP

↑ Gut Ca Absorption
↑ Renal Ca Excretion

↑ PTH

↑ PTH ACTIV

↑ OB-PTHR
d↓ cAMP-PDE

↑ Acute Bone Resorption
↓ Chronic

↑ RANKL
↓ OPG
↓ IL-6 rec

GC effects on bone cells

↓ LH, FSH
↓ Gonadal Steroidogenesis
↓ ACTH, DHEA, A
Abn Vit D

↓ BONE MASS / STRENGTH

↓ Chronic Bone Formation
↑ Early

Direct effects of GCs on bone cells

Growth factors
Myopathy

↑ IL-6 synthesis
↑ PGE2
↑ OC apoptosis
Direct effects of GCs on Osteoblasts/Preosteoblasts

- Intracellular Signal Transduction
- Cell Fate: Proliferation, Differentiation,
  Trans-differentiation, Senescence, Apoptosis
Growth Factors

Autophosphorylation of receptor

P- = Tyrosine phosphorylation to activate proteins

P- = Tyrosine phosphorylation

Ras

Raf

MEK

ERK

PTP’ase / GCs

Nucleus

Mitosis
Osteoblast Proliferation

![Graph showing Osteoblast Proliferation](image)

- **% cpm**
  - TPA
  - Dex+TPA

- **VO$_4$**

- **n=8, P < 0.005**
Bone Mineral Density (g/cm$^2$)

C  S  SV  V

SE

p<0.01  C vs S
p<0.005  SV vs S

Eagar et al
Dynamic Formation Parameters

Eagar et al
Glucocorticoids

↑ Tyr phosphatases
↓ MAPK/ERK activity
↓ Ob proliferation
↓ Bone formation
↓ BMD
↓ Bone strength
MKP-1 induction correlates with ERK dephosphorylation

MKP-1 overexpression in MG-63 human osteoblasts abrogates proliferation

10% FCS

1µM Dex - 7h

MKP-1 →

P- ERK

Hulley et al 2007
Cell fate and the bone–fat axis

- cbfa-1
- PPARγ

- Transdifferentiation
- Apoptosis

- PPARγ
Influence of GCs on cell fate and the bone – fat axis
Examples of BB&B Research

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- HIV, ARVs and Bone
- Acanthosis Nigricans
- Diabetes and Skeletal Health
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Causes of a low BMD in HIV

- Direct effects of the Disease
- Associated Osteoporosis Risk factors
- Effects of HIV Treatment

- Innes et al
- Andersson et al
- Eagar et al
ARVs & Osteoblasts

- Direct infection and apoptosis
  
  Wang et al AIDS RHV 2002

- Para / autocrine effects of TNF-α on osteoblast apoptosis
  

- MSC → Adipocytes

- Osteoblasts
Proliferation of ADSC from Visceral Depots

Data 3: Prolif 100% Visc 1% FCS

Data 4: Prolif 100% Visc 10% FCS

Greyling et al 2012
Proliferation of ADSC from Subcutaneous Depots

Data 1: Prolif 100% S/C 1% FCS

Data 2: Prolif 100% S/C 10% FCS

Greyling et al, 2012
Examples of BB&B Research

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THE PREVALENCE AND CLINICAL SIGNIFICANCE OF ACANTHOSIS NIGRICANS IN DIABETIC AND NON-DIABETIC WOMEN OF MIXED ANCESTRY

Hoffman et al

4 Phases of study:
1. Determine prevalence in adult female population and association with plasma glucose, obesity and hypertension (385 patients)
2. Determine prevalence of AN in diabetic females and association with components of the metabolic syndrome, micro- and macrovascular complications (250 patients)
3. Determine association of AN (+) T2DM patients with the degree of insulin resistance and presence and distribution of body fat, PCOS and NAFLD (85 patients)
4. Investigate the cellular pathway activated in AN patients

Progress
- Approved by Ethics Committee; HC funding
- Submitted for PhD application
- Phase 1 recruitment completed (ready for statistical analysis)
- Funding obtained for phase 4
Insulin Resistance: Defects in Signalling
Intracellular Insulin Signalling

Diagram showing the intracellular insulin signalling pathway, including the insulin receptor, IRS proteins, SHc, Grb-2, SOS, SHP-2, PI 3-Kinase, and GLUT4. The pathway involves mitogenic and metabolic processes.

Mitogenic

Metabolic
Phase IV
To assess possible pathophysiological mechanisms involved in the development of AN in patients with T2DM

- Standard punch biopsy from area of AN, from area of apparent normal skin, from control subject
- Separate epidermis from dermis by dispase treatment; liberate keratinocytes from epidermis with trypsin; plate on a collagen-coated tissue culture petri.
- Use isolated keratinocytes and fibroblasts to assess:
  - *Cellular proliferation* (ex vivo in absence of potent mitogens, and in vitro following treatment with 20% FBS or insulin)
  - *Rate of apoptosis* (TUNEL staining)
  - Contribution of the MAPK/ERK pathway in mediating the cellular proliferation of these cell (inhibitors like UO126, phospho-ERK etc)
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<td>Clinical risk factors for the development of osteoporosis (<em>RB, PhD</em>)</td>
<td>Micro- and macrovascular complications of diabetes in white and mixed race populations</td>
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<td>Risk factors for hip fractures (<em>DvP, PhD</em>)</td>
<td>The lung in DM (<em>MS, PhD</em>)</td>
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<td>Body fat distribution and OP</td>
<td>Diabetic bone disease (<em>FSH MD</em>)</td>
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<td>Endemic fluorosis</td>
<td>Diabetic diarrhoea</td>
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<td>Osteoporosis in men</td>
<td>Lipid and lipoprotein profiles in diabetes</td>
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<td>Season, latitude and Vit D activation</td>
<td>Measurement of microalbuminuria in diabetes</td>
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<td>Exercise induced OP in women (<em>KM, PhD</em>)</td>
<td>Measurement of serum ketone bodies in diabetes, employing a point-of-care device.</td>
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<td>BMD in anorexia nervosa (<em>RE, MSc</em>)</td>
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<td>BMD and fracture in Black and White South African women (<em>MC, PhD</em>)</td>
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<td>Statins and bone (<em>FM, PhD</em>)</td>
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### CURRENT STUDIES

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<td>• Hyperglycaemic emergencies at secondary level: Defining the metabolic crisis of DKA</td>
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<tr>
<td>➢ VO₄</td>
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<tr>
<td>• Others</td>
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Diabetes in Pregnancy

Rademan, Conradie et al

1. **Clinical patient care**
   We have established a multidisciplinary team directed at optimal tertiary management of pregnant diabetics presenting to the TBAH over the last 3 years and include a team of dedicated obstetricians, diabetologists, a dietitian and a nurse educator.

2. **Provincial Government of Western Cape Guidelines**
   Instrumental in revising guidelines for care of pregnant diabetics in the Western Cape. Directed mainly at primary and secondary care facilities and medical staff.

   **PROVINCIAL GOVERNMENT WESTERN CAPE**
   **DIABETES IN PREGNANCY**
   Guideline for the management of diabetes and its complications from pre-conception to the postnatal period

3. **Clinical Research**
   Incidence of Type 2 diabetes mellitus in women following a diagnosis of Gestational Diabetes.
### CURRENT STUDIES

**OSTEOPOROSIS**
- Incidence of fractures in four population groups in South Africa
- Molecular biology of stem cell differentiation into fat and bone cells
- Drugs and osteoporosis
  - ARV
  - AI
  - VO<sub>4</sub>
- Others

**DIABETES**
- Acanthosis nigricans
- TZDs and bone
- Diabetes in pregnancy
- Prospective audit of hyperglycaemic emergencies presenting at a tertiary level (TBAH): Prevalence and nature of KPD
- Hyperglycaemic emergencies at secondary level: Defining the metabolic crisis of DKA
Aim:

- Prospective Audit of Hyperglycaemic Emergencies presenting to TAH.
- Describe the incidence of DKA in existing type 1 and 2 patients.
- Investigate and compare the prevalence of KPD in different ethnic groups (white, black, mixed race and Indian).
Hyperglycaemic emergencies at secondary level: Defining the metabolic crisis of DKA

Aim:

- To characterize hyperglycaemic emergencies at a secondary level care facility in the Western Cape (Cape Winelands East / Boland Overberg), and to

- Evaluate the use of a validated point-of-care device to measure serum ketones (β-OHB) in defining the metabolic crisis of DKA.

- Sub-analysis of patients presenting with features of ketosis-prone Diabetes (KPD).