1. Persistent elevation of BHCG-levels in a patient with stage 5D Chronic Kidney Disease

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Introduction and aim: A 24 year old female with Stage 5D chronic kidney disease (CKD) presents with persistent positive BHCG levels. BHCG elevation causes include pregnancy, pituitary production, hydatidiform mole, persistent trophoblast disease, malignancies and false positive hCG. Variants of the hCG molecule has been identified and includes sulfated hCG, hyperglycosylated hCG, hCG free β-subunit and hyperglycosylated hCG free β-subunit. Different hCG fractions are generated depending on the disease process. Isolated cases of falsely raised BHCG in both men and women with CKD have been reported. Origin of hCG has been attributed to pituitary production, coupled with CKD impairing metabolism and clearance of hCG.

Methodology: Samples were tested by the NHLS Universitas Academic Laboratory using the Cobas e 601 analyzer (Roche) for intact human chorionic gonadotropin and the β-subunit. This immunoassay detects the holo-hormone, nicked forms of hCG, β-core fragment and free β-subunit. A qualitative urine test, detecting total hCG was negative, while a laboratory urine analysis had a positive result. Analysis on different instruments and serial dilutions excluded assay interference from human antimouse antibodies (HAMA), heterophilic antibodies or a high dose hook effect. Imaging studies including magnetic resonance imaging (MRI) brain, pelvis, computed tomography (CT) chest and pelvic ultra sound was performed. Diagnostic laparoscopy was refused by the patient due to her ongoing peritoneal dialysis.

Results: Serial BHCG results show persistent elevation. Imaging studies yielded no explanation to HCG production.

Conclusion: Elevated BHCG could be attributed to a tumour of gestational or non-gestational origin, failed pregnancy, familial elevated hCG, reduced clearance and degradation of hCG in CKD or changes of hCG associated with CKD. Immunoassay interference should be considered when patient results and clinical presentation differs. False positive test results may subject patients to unnecessary investigation and it may prevent patients from qualifying for organ transplantation.

2. Hypoglycaemia associated with multicentric Castleman disease

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The causes of severe hypoglycaemia with suppressed insulin levels are relatively few. Non-islet-cell tumour hypoglycaemia needs to be considered in the absence of chronic renal disease, chronic liver disease, drugs e.g. alcohol, inanition and hypocortisolism. We describe a case of recurrent, severe, unprovoked hypoglycaemic episodes in a 47-year-old HIV positive male who was subsequently diagnosed with multicentric Castleman disease (MCD). MCD is classified as a lymphoproliferative disorder. Several mechanisms can be postulated to explain the hypoglycaemia, but the most likely in our patient was the secretion of insulin-like growth factor 2 (IGF-2) and IGF-2 precursors which can bind to insulin receptors. We have enlisted the help of a laboratory in the United Kingdom to measure IGF-2 levels. We are also seeking assistance for further staining to be done on the histological specimens. To the best of our knowledge this is the first report from Africa and only the second case worldwide documenting hypoglycaemia as a manifestation of MCD.

3. Primary carcinoid tumour of the testis: A Case report

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Carcinoid tumours are neuroendocrine tumours that may arise from various sites, mostly the GI tract and bronchopulmonary system. Carcinoid tumour of the testis is a very rare disease and accounts for less than 1% of all testicular neoplasms. As far as the rest of the male genitourinary system is concerned, it can also occur in the kidney, bladder and prostate. We present a case of a 53 year old man presenting with the incidental finding of a testicular tumour and histologically proven carcinoid (positive staining for synaptophysin and chromogranin) with low grade features. He did not display features of the carcinoid syndrome. Further tests including chest radiograph, chest CT, Tectrotide scan and urinary 5-HIAA, were done and were negative. He was not hypogonadal as has been described before in the literature. The largest series to date identified 29 cases of primary testicular carcinoid from 7 academic institutions and had a mean age of 36 years. Most patients presented with either a testicular mass or swelling and secondly as an incidental finding. Only two patients had the carcinoid syndrome. Mitotic features were rarely seen in the primary carcinoid tumours. The importance of follow-up in these cases are stressed as they can present with late metastases, especially in atypical carcinoid.

4. A retrospective analysis of thyroid disease in pregnancy at Chris Hani Baragwanath Academic Hospital, Soweto, South Africa

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Background: Thyroid disease in pregnancy is a frequently encountered clinical challenge. The reported prevalence of hyperthyroidism is 0.1-0.4% of all pregnancies, with Graves’ disease accounting for the majority of cases
The prevalence of hypothyroidism is estimated to be 2.3-3.5%, of which overt hypothyroidism accounts for 0.3-0.5% and subclinical hypothyroidism 2-3%, in iodine-sufficient areas. The importance of detecting and managing thyroid disease in pregnancy is necessitated by the adverse outcomes of untreated disease for both mother and foetus. To date, there have been no studies in South Africa evaluating the prevalence and spectrum of thyroid disease in pregnancy.

**Objectives:** To evaluate the prevalence and spectrum of thyroid disease during pregnancy at Chris Hani Baragwanath Academic Hospital (CHBAH). In addition, to assess the underlying causes, control and management thereof, and the maternal and neonatal outcomes using clinical and biochemical criteria.

**Methods:** A retrospective review of the 88 patients with thyroid disorders, who attended the Antenatal Endocrine Clinic, during the period January 2004 to April 2008, was undertaken. All patients had been referred by the ten antenatal clinics in Soweto as well as the Endocrine Clinic at CHBAH. All underwent an initial assessment, and were monitored frequently by a team comprising obstetricians, endocrinologists and a nurse educator. Delivery records were obtained where available. Thyroid function tests were performed on the neonates at 48 hours of age.

**Results:** Eighty-eight women with thyroid disorders were managed at the clinic. Their mean age was 29 years and the mean duration of gestation 19.8 weeks at first visit. Sixty (68%) were hyperthyroid, 21 (24%) hypothyroid, and 7 (8%) had endemic (colloid) goiter. The majority of the hyperthyroid patients had Graves’ disease and most of the hypothyroid patients were due to post I-131 ablation for Graves’ disease. Sixty two percent of the Graves’ disease patients and 56% of those with hypothyroidism were rendered euthyroid prior to delivery. The majority of babies were delivered vaginally. Overall, there was only one fatal maternal outcome. There were four intra-uterine fetal deaths and one delivered vaginally. Overall, there was only one fatal maternal outcome.

**Conclusion:** This is the first report in Africa analyzing the spectrum and outcomes of thyroid disease in pregnancy.

**6. Localization of parathyroid adenoma in patient with MEN1 syndrome: a case study.**

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Multiple endocrine neoplasia Type 1 (MEN1) is a disease that is inherited as an autosomal dominant disorder with approximately 100% of patients having Parathyroid tumours by the age of 50 years. Our patient is a 37 year old male diagnosed with MEN1 syndrome, confirmed with the delC174 mutation and received a total parathyroidectomy with autotransplant in his R forearm in 1998. He presented with an inappropriately elevated PTH (PTH 299.4 pmol/L) with an enlarging mass on his R forearm in the region of the previous parathyroid autotransplant. Sonar and MRI neck showed a nodule in the area of the R thyroid lobe, but this was not confirmed on MIIB scan. He did however have a parathyroid adenoma in his R forearm on MIBI scan. He was lost to follow-up and presented four months later with symptoms of hypocalcaemia (Cor.Ca 2.78mmol/L, and PTH 299.4pmol/L). Immunoassay interference through either
the effect of heterophilic antibodies or human anti animal antibodies (HAMA’s) and a high dose hook effect were excluded by Chemical Pathology. A working parathyroid autotransplant was confirmed with a PTH ratio of 6.17 between the transplanted arm and the contralateral arm. An ischemic blockade maneuver could not exclude the presence of parathyroid tissue outside the grafted arm. The remarkable variation of the parathyroid hormone levels was ascribed to the superficial nature of the parathyroid autotransplant and the nodule found in the neck is likely the source of PTH outside of the transplant. We describe the methods used to localize the presence of the parathyroid adenoma.

7. Characteristics and outcome of surgically treated pituitary tumours at Inkosi Albert Luthuli Central Hospital, Durban, South Africa.

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Aims: To describe the clinical, biochemical, radiological and histological features and to determine the outcome of all patients with pituitary tumours treated surgically at Inkosi Albert Luthuli Central Hospital in Durban over a 5 year period.

Research design: Retrospective chart review from 2008 to 2012. Clinical, biochemical and radiologic data were collected before and one year after surgery. Histopathology findings and peri-operative complications were recorded.

Results: 70 patients were included (age 44.8±14.9 years, 55.7% female). Headache and visual disturbance were the predominant presenting symptoms (84.1% and 78.3%). Most tumours were macroadenomata (97.1%). Trans-sphenoidal surgery was employed in 63 patients (90%). A single procedure was required in 55.7% patients, two procedures in 30% and up to six in others. Complete resection was achieved in 9 patients (12.8%), residual tumour post-surgery was found in 48 (68.6%) and no change in tumour size occurred in 13 patients (18.6%). Medical therapy was used in 22 (31.4%) and radiotherapy in 13 (18.6%). The commonest pathology was non-functional adenoma in 33 (47.1%) and 29 (41.4%) were secretory tumours. Overall mortality was 4.3%. The commonest surgical complication was CSF leak [10%; n=7]. New post-surgical pituitary hypofunction occurred in 62 (88.6%). The outcome at one year was similar to that on discharge.

Conclusions: Patients presenting to IALCH had large tumours, and complete resection was achieved in a minority. There was a low overall mortality but high rate of post-surgical pituitary hypofunction.

8. A case of bone pain in a patient with previous thyroid cancer

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Background: The presentation of a patient with lytic bone lesions raises suspicions of primary bone cancer, multiple myeloma or metastatic disease from underlying breast cancer or thyroid cancer. Other aetiologies such as parathyroid cancer remain an unlikely possibility.

Case presentation: A 77 year old Caucasian female presented with a history of worsening heartburn and back pain. Six years prior to this she underwent a total thyroidectomy for a thyroid malignancy. She also gave a history of previous “parathyroid adenoma” resection. Clinical examination revealed epigastric tenderness abdominally and tenderness over her thoracic vertebrae. X-rays showed a lytic lesion in the thoracic spine (T10). Computerized tomography confirmed the lytic lesion and noted a 12x14mm mass in the right lower lobe of the lung. Laboratory investigations revealed a low thyroglobulin. Serum adjusted-calcium 2.98mmol/L (2.20-2.50 mmol/L) and PTH 1589pg/ml (15-65pg/ml). Parathyroid 99Tc-sestaMIBI had no uptake. Venous sampling revealed elevated PTH in the azygous vein. Partial pneumonectomy and T10 vertebral resection were performed and histology confirmed metastatic parathyroid carcinoma, although she was eucalcaemic immediately post-operatively, the PTH remained elevated [555pg/ml]. Gallium 68 dotatate PET/CT failed to show any uptake post operatively. Hypercalcemia is currently managed with a calcium mimetic agent.

Conclusion: The incidence and prevalence of parathyroid carcinoma remains low. It is responsible for less than 5% of all cases of primary hyperparathyroidism [1]. Parathyroid malignancy is difficult to differentiate from a benign adenoma, however, the degree of hypercalcemia, the presence of brown tumours or the degree of elevation of parathyroid levels is suggestive. Definitive diagnosis is made histologically.

Parathyroid carcinoma usually occurs in primary hyperparathyroidism, but may occur in secondary or tertiary hyperparathyroidism. Similarly, parathyroid adenomas are associated with the development of thyroid cancers [2]. Cure is dependent on an “en bloc” resection of the entire tumour and restoration of eucalcaemia. Metastatic disease with resultant hypercalcemia is difficult to manage. Chemotherapeutic agents lack efficacy. Bisphosphonates remain a treatment for hypercalcemia. Calcium sensing receptor agonists, whilst efficacious remain costly.

Despite medical treatment post-surgery long term survival remains 40-86% at 5 years and in the event of a recurrence, survival is 0% at 5 years [2-5].

9. Primary hyperparathyroidism presenting in adolescence can mimic Rickets: 2 case reports

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Introduction: Primary Hyperparathyroidism (PHPT) is a relatively common endocrine disorder with a reported incidence of 21.6 per 100 000. The prevalence in children is not known but it is thought to be uncommon and the clinical presentation in this subgroup of patients are not well described.

Case Report 1: A 17 year old boy developed pain in both hips. His GP noted bilateral genu valgus which worsened
and he eventually stopped schooling. Bilateral osteotomies were performed at the local hospital and follow-up revealed malunion which prompted referral to the endocrine unit. Examination revealed short stature, pectus carinatum, Harrison’s sulcus, kyphoscoliosis and rachitic rosary. Corrected calcium was 3.02 mmol/l and PTH 134.6 pmol/l. Radiological findings were compatible with rickets. Sestamibi scan showed a large left inferior parathyroid adenoma. Bone mineral density (BMD) Z-score -5.0 at hip; -4.9 at the spine. Left parathyroid adenomectomy yielded a benign 3.5g adenoma. The postoperative course was complicated by hungry bone syndrome (HBS). Skeletal deformities improved over the following year and he returned to school.

**Case Report 2:** A 13 year old boy developed pain in both legs and two months later a curvature to his lower limbs. Examination revealed a windswept deformity of the lower limbs with genu valgus deformity of the left and varus deformity of the right knee. Corrected calcium was 3.4 mmol/l and PTH 131.1 pmol/l. Radiological features were suggestive of rickets. BMD scan showed a Z score of -2.4 at the hip and -4.8 at the lumbar spine. Sestamibi scan showed increased focal uptake on the right side of the neck. A unilateral neck exploration a right superior parathyroid lesion was resected and confirmed to be an adenoma. Latent tetany developed on day 2 post-surgery and postoperative PTH levels were undetectable. A1 follow-up 1 month later he remained eucalcaemic on calciferol and calcium supplementation.

**Conclusion:** PHPT occurring in adolescence may have a clinical presentation almost identical to that of rickets. All patients presenting with skeletal deformities including a rickets phenotype, must have serum calcium and phosphate levels measured as part of the diagnostic workup.

**10. Extensive diffuse normolipaemic xanthomatosis**

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Diffuse normolipaemic plane xanthoma (DNPX) is a rare form of xanthoma usually associated with an underlying haematological or inflammatory condition, which may present many years prior to the onset of systemic illness. We present a case of this uncommon condition occurring in association with a monoclonal gammopathy of unknown significance (MGUS).

A 74-year-old male presented with diffuse, yellow-orange skin discoloration involving his face, neck, arms, chest and back, of a few months duration. There was no history of medication use or systemic illness. Initial laboratory tests including a fasting lipogram were normal. A skin biopsy showed groups of lipid laden foamy histiocytes in the dermis and perivascular areas in keeping with DNPX. The erythrocyte sedimentation rate was 76 mm/hr and an IgG kappa monoclonal band was demonstrated on serum protein electrophoresis. The urine was negative for Bence-Jones protein. A bone marrow aspirate and trephine biopsy showed a variably cellular bone marrow with <10% plasma cells suggestive of MGUS. Also called generalised plane xanthomatosis, DNPX is a rare form of xanthoma which occurs as a macular yellow-orange or yellow-brown discolouration and is most commonly seen over the trunk and sides of the neck in a symmetrical distribution. These xanthomata were originally thought to occur idiosyncratically due to their association with normal lipid levels, however many of these patients subsequently developed dysglobulinaemias and paraproteinaemias. In addition to MGUS, multiple myeloma, adult T-cell lymphoma, cryoglobulinaemia, leukaemia, eosinophilia granulomatosis, rheumatoid arthritis, Takayasu’s arteritis and even colon carcinoma have all been described associated with the condition. This case illustrates the occurrence of diffuse normolipaemic plane xanthoma in association with a MGUS and highlights the necessity of searching for potentially serious underlying systemic conditions.

**11. A clinical description of a family with paraganglioma in South Africa**

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**Background:** Although the clinical characteristics, functionality and pattern of inheritance of paraganglioma in the Western world are well described, there is a dearth of information outlining these features in South Africa. We therefore wished to describe a family from the Eastern Cape with three generations affected by presence of paraganglioma.

**Methods:** The clinical characteristics of the index patient and the affected first and second-degree relatives were described. The unaffected relatives were traced by interviewing the affected family and found in the Western and Eastern Cape. The affected relatives were assessed for functionality by screening for fractionated urine metanephrine, normetanephrine. The patients with positive histology for paraganglioma were subjected to immunocytochemistry with succinyl-dehydrogenase B antibodies. DNA was extracted from the affected and the unaffected relatives for analysis of the succinyl dehydrogenase gene.

**Results:** The index subject was an 18-year-old female, affected by bilateral carotid body tumours in whom it was ascertained to be non-functional. The MIBG scan showed no other abnormal neuro-endocrine tissue. Histology was positive for paraganglioma, staining positive for chromogranin A and synaptophysin. Her father was affected by bilateral carotid body tumours, but was never subjected to surgery. Her deceased grandfather was also affected by bilateral carotid body tumours, but this was never confirmed histologically. Her father’s brother had a glomus tumour excised. A further 17 relatives were screened and found to have no clinical evidence of paraganglioma.

**Conclusions:** Although this is a very rare condition, awareness of the devastating cosmetic result and potential functionality are warranted. Immunocytochemistry has a pivotal role in identifying the culprit genes.
12. Pregnancy outcomes in women with pregestational diabetes attending Groote Schuur hospital 2011-2012

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Objective: This study aimed to describe the outcomes of pregnancy in patients with pregestational diabetes attending Groote Schuur Hospital

Methods: A retrospective audit was undertaken of all women who attended Groote Schuur Hospital Obstetric unit with pregestational diabetes from 1 September 2010 to 31 August 2011. Routinely collected information at booking and during the rest of pregnancy was entered onto a data abstraction form.

Results: 229 women were included, 35 with type 1 diabetes (Type 1DM) and 194 with type 2 diabetes (Type 2DM). The Type 2 DM group was older (33±6yrs vs 28±6yrs) and fewer were nulliparous (57.1% vs 18.1%) than the Type 1DM group. Gestational age at booking was similar in both groups: Type 2DM (15±7 weeks), Type 1DM (13±7 weeks) as was the rate of HIV infection (10% and 9%), but mean booking HbA1c was higher in the Type 1DM group (9±2% vs 7±2%, p <0.05). On preliminary analysis, the miscarriage rate was 4 times higher in the Type 1DM group (8.6% vs 2%) and perinatal mortality rates were 5% and 2.5% in Type 1 and 2 DM groups respectively. There were 5 foetal anomalies (n=1 Type 1DM and n=4 Type 2DM pregnancies). Rates of macrosomia were similar in the 2 groups Type 1DM (9%) and Type 2DM (10%).

Conclusion: This retrospective audit conducted in a service providing specialised care to disadvantaged women with pregestational diabetes demonstrates poor outcomes. Improvement of preconception care and during pregnancy is essential to improve pregnancy outcomes.

13. Diabetic nephropathy in a tertiary care clinic in South Africa, a cross-sectional study.

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Objective: The aim of this study was to determine the prevalence of micro- or macroalbuminuria among type 1 and type 2 diabetic patients and to examine the relationship with diabetes control parameters: haemoglobin A1C (HbA1c), blood pressure (BP) and lipids.

Design: Analytical cross-sectional study.

Setting and subjects: The study consisted of 754 patients with either type 1 or type 2 diabetes mellitus, attending a diabetic clinic at the Kalafong Hospital in Pretoria, South Africa. Data were extracted from the electronic database of the Kalafong Diabetes Clinic of all patients who attended the clinic from January to December 2012.

Outcome measures: Micro- or macroalbuminuria and estimated glomerular filtration rate (eGFR). A logistic regression analysis was performed to determine the relationship between microalbuminuria and diabetic control parameters.

Results: The patients (N=754) were predominantly black (91.1%) and female (62.9%) with a mean age of 57.2 (±14.9) years old. The majority of the 754 patients (66.6%) had type 2 diabetes. The median duration of diabetes was 11 years. Of all patients, 88.9% had HbA1c > 7%, and 81% had low-density lipoprotein (LDL) cholesterol ≥1.8 mmol/l. High BP was diagnosed in 79.2% of the patients, of which 75.1% were receiving angiotensin-converting enzyme(ACE) inhibitors. Of the study population 66.4% had a normal urine albumin-to-creatinine ratio (ACR). The median recorded urine ACR for both sexes was 1.3 mg/mmol. The eGFR of the patients ranged between 1.99 and 430.56 with a median of 102.3 ml/min/1.73m². Overall prevalence of micro- or macroalbuminuria was 33.6%. Logistic regression revealed that HbA1c, duration of diabetes, systolic BP, male sex and triglycerides predicted microalbuminuria.

Conclusion: The prevalence of micro- or macroalbuminuria in this study falls within the ranges of what has been previously reported in Africa. In all patients, HbA1c and duration of diabetes were the strongest predictors of microalbuminuria, and age was the strongest predictor of a low eGFR. Diabetes was poorly controlled, making the progression to end-stage renal failure a real concern in these patients.

14. The effect of mobile screening and treatment feedback on HbA1c and complications screening in primary care clinics of the Tshwane district

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Aim: To determine the effect of a mobile screening and expert feedback on glycaemic control (HbA1c) and the extent of screening for complications in the diabetic population of the Tshwane district

Methods: A cluster-randomised trial was conducted in 12 primary care clinics in the Tshwane district. Data regarding treatment and complication evaluation was obtained from the clinical records of diabetic patients for the previous 12 months. All clinics received training regarding the national guidelines for diabetes. Patients randomised to the active arm of the study had HbA1c, LDL-cholesterol and urine albumin tested, as well as a foot exam and eye fundoscopy and visual acuity screening. Written treatment feedback was given by a specialist Physician in conjunction with a Family Physician. Patients randomised to the control arm also had their HbA1c tested and the results were shared with the clinic. A second 12-month review of their clinical records was done to observe the effect on HbA1c and screening outcome as well as treatment changes implemented.

Results: A total of 599 diabetic patients attending these clinics for review were consecutively interviewed and clinically examined. The mean age was 58 years and 80.5% had a body mass index [BMI] ≥ 25kg/m². Sixty-eight percent of patients were female. When evaluating the mean HbA1c between year 1 and year 2 [ 8.7% (±2.4); 8.6% (±2.2)], no statistically significant change (p=0.4) was observed. Using McNemar’s test, we found significant increases in only HbA1c and albumin tests requested in the control arm. In the intervention group, screening for complications (HbA1c, LDL-cholesterol, Eyes, Feet, Renal, Cardiovascular) increased to nearly 100% as the
mobile screening team conducted the tests (McNemar’s Chi² ranging from 194.1-300; all p-values < 0.001)

Conclusion: Changes in the mean HbA1c between round 1 and round 2 were not observed. The proportion screened for complications increased dramatically when a dedicated mobile team, using structured diabetes care guidelines, were actively conducting the screening. New methods of care, such as mobile screening with a dedicated team at primary care should be explored for complications screening and for improving glycaemic control.

POSTERS: BASIC SCIENCES/LABORATORY

1. Does the ATM protein play a role in the myocardial pathology associated with obesity and insulin resistance?

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Background: Ataxia-telangiectasia (A-T) is an autosomal recessive disorder caused by mutations in the ATM (ataxia-telangiectasia mutated) gene. ATM, is a 350kDa serine/threonine protein kinase belonging to the family of phosphatidylinositol-3 kinase like kinases (PIKK) and has a large number of substrates. ATM can be localized to the nuclear, cytosolic or mitochondrial compartments of a cell. A-T patients have either no or very low expression of ATM, display a very high incidence of insulin resistance or type 2 diabetes mellitus and are more susceptible to ischaemic heart disease. ATM is activated by insulin, hypoxia, DNAstrand breaks or oxidative stress and has been implicated in cancer, metabolic disorders, low anti-oxidant defence and atherosclerosis. Because of scant information on (i) the role of ATM in signalling cascades especially in the heart, (ii) possible cardiovascular effects of ATM and (iii) evidence that obesity may alter the expression of ATM, we aimed to investigate the expression and impact of ATM in the heart in the context of obesity-induced insulin resistance.

Methods: Wistar rats were rendered obese and insulin resistant by feeding a diet supplemented with sugar and fat for 16 weeks. Glucose tolerance, fasting insulin and glucose levels were determined and, after sacrifice, body weight and intra-peritoneal fat weight of the animals. Ventricular cardiomyocytes were prepared by perfusion-digestion and insulin responses measured using accumulation of [3H]2-deoxyglucose. The specific ATM inhibitor KU60019 was used to manipulate activity of the protein. Expression of ATM was determined by Western blotting and commercially available antibodies. Cardiac mitochondria were prepared by differential centrifugation and their oxidative capacity measured using a Clark-type electrode. Cardiac mitochondria were prepared by differential centrifugation and their oxidative capacity was determined by Western blotting and commercially available antibodies. Cardiac mitochondria were prepared by differential centrifugation and their oxidative capacity was determined by Western blotting and commercially available antibodies. Cardiac mitochondria were prepared by differential centrifugation and their oxidative capacity was determined by Western blotting and commercially available antibodies. Cardiac mitochondria were prepared by differential centrifugation and their oxidative capacity was determined by Western blotting and commercially available antibodies. Cardiac mitochondria were prepared by differential centrifugation and their oxidative capacity was determined by Western blotting and commercially available antibodies.

Results: We demonstrated for the first time that: (i) expression of ATM is down regulated in the heart in obesity/insulin resistance; (ii) Inhibition of ATM in cardiomyocytes attenuates insulin-stimulated glucose uptake; (iii) ATM is expressed in AECs; (iv) ATM is localized to myocardial mitochondria and (v) its expression is down regulated in mitochondria from hearts of obese animals that also display mitochondrial dysfunction.

Conclusion: ATM is a potential role player and drug target in the development of an obesity related or diabetic cardiomyopathy.

2. Anti-diabetic and phytochemical analysis of extracts of Sutherlandia frutescens: Regulation of macrophage differentiation by Sutherlandia frutescens.

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Sutherlandia frutescens is a medicinal plant indigenous to South Africa, which is used to treat various conditions, including type 2 diabetes. The macrophage lineage is a common component in type 2 diabetes and the other indicated conditions. Sub-sets of this lineage are thought to play a functional role in disease progression. THP-1 cells have become one of the most widely used human cell lines to investigate the function and regulation of monocytes and macrophages. In order to explore the role of S. frutescens on macrophage differentiation and function in disease treatment, we have measured the response of THP1 cells to aqueous and organic extracts from a single sample of plant material, previously shown by us to be effective in targeting type 2 diabetes in a rat model system. Macrophages interact with bacteria and their products through CD14, a pattern recognition surface receptor operating in conjunction with the human Toll-like family of receptors. CD14 is also involved in the response to phorbol 12-myristate 13-acetate (PMA) which induces differentiation of the less mature suspension cells into adherent macrophages. Using flow cytometry, we have shown that after 48 hour and 72 hour treatments, hot aqueous S. frutescens significantly increases the expression of the activated macrophage receptor CD14 on THP-1 cells induced to differentiate by PMA. Additionally THP-1 cells treated for 48 and 72 hours with both S. frutescens and PMA have increased adherence and altered cell surface morphology compared to those treated with PMA alone. We hypothesize that S. frutescens, having had no effect on THP-1 cell phenotype after 24 hours, acts upon later specific patterns of macrophage differentiation and activation. Further research is needed to confirm these findings by analyzing other CD macrophage lineage markers, and to extend the study to primary cultures of macrophages isolated from human peripheral blood mononuclear cells.

3. Cardiovascular disease in hyperalphalipoproteinaemia due to mutations in endothelial lipase

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High-density lipoprotein-cholesterol (HDLC) has historically been associated with protection against cardiovascular disease, with a resultant interest in the development of agents that raise HDLC. However, such interventions have failed to translate into cardiovascular benefit in stage II clinical trials. Investigations now examine specific effects of proteins involved in HDL metabolism. Endothelial lipase (EL) is a unique lipase, discovered in 1999, which acts primarily to catabolise...
phospholipids on HDL without dissociation of apolipoproteins. The impact of mutations in the coding gene, LIPG, on HDLC levels, and their effects on cardiovascular outcomes are of interest.

Patients with unusually high HDLC (>2.5mmol/L) were found to have known and novel mutations in LIPG. These mutations were sought among a random sample of 600 patients with HDLC levels ranging from 1.2 - 2.5mmol/L using RT-PCR and high resolution melting. Where a mutation was found, including those with HDLC ≥2.5mmol/L, the presenting fasting lipogram and clinical information were reviewed and data relating to cardiovascular outcomes were analysed. Of the 18 individuals with mutations in LIPG as well as HDLC levels ≥1.6mmol/L in males and ≥1.8mmol/L in females, eight had had cardiac intima/media thickness measurement, and four of these were at increased risk (≥1mm). Two of the 18 had symptoms or signs of peripheral vascular disease, two had angina pectoris, two had xanthelasmas, eight had arcus cornea1es, one had suffered a cardiovascular accident (CVA), one went on to undergo coronary artery bypass graft and suffered a CVA, while one suffered three CVAs subsequent to initial presentation. Hyperalphalipoproteinaemia associated with mutations in LIPG is thus not globally protective against cardiovascular disease. Further investigation should be undertaken to determine the HDL species and function, to understand the significance of HDL changes, and to decide about therapeutic strategies relating to manipulation of LIPG or EL.

4. The Prevalence of Lectin-Like Low Density Lipoprotein Receptor-1 (Lox-1) K167n polymorphism in hyperlipidaemic patients in the South African Population

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Introduction: Coronary artery disease (CAD) is a major contributor to morbidity and mortality worldwide. CAD is characterised by the development of atherosclerosis due to the retention and oxidative modification of low density lipoprotein (OxLDL) within the sub-endothelium. Recently genetic risk factors associated with the disease have been reported. A lectin-like oxidised low density lipoprotein receptor (LOX-1) has been identified as an OxLDL receptor on the endothelial cells. LOX-1 is encoded by oxidized low-density lipoprotein (lectin-like) receptor-1 (OLR1) gene, mapped to chromosome 12p13. A single-nucleotide polymorphism (G501C) which leads to an amino acid substitution (lysine to asparagine) at position 167 (K167N) has been identified. This polymorphism has been associated with coronary artery disease. There is a high prevalence of hyperlipidaemia and CAD in South Africa, however, there is as yet no data on the prevalence of LOX-1 (K167N) gene polymorphism in the South African population. Aim: To determine the prevalence of LOX-1 (K167N) polymorphism in hyperlipidaemic and familial hypercholesterolemia (FH) patients.

Materials and methods: 84 hyperlipidaemic patients, 20 treatment naïve FH patients at Charlotte-Maxeke Hospital (CMH) and 49 ‘healthy’ control participants from Wits medical school and NHLS were recruited for the study. Lipogram was measured and DNA extracted. RFLP based PCR was performed to determine the LOX-1 (K167N) polymorphism. Results: There was a statistically significant difference in the N allele frequency between the hyperlipidaemic (0.65) and the FH patient (0.82) group (p=0.03), but there was no difference in the N allele frequency for either of these groups with the control subjects (0.66).

Conclusion: These data suggests that FH patients may carry other gene variants than those identified in the LDL receptor gene that may exacerbate progression to CAD, which is highly prevalent in this population.

5. Establishing a viable streptozotocin-induced type 2 diabetic rat model

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Background: The global prevalence of type 2 diabetes (T2D) is escalating rapidly. Streptozotocin (Stz), a selective b-cell toxin is widely used to experimentally induce T2D in rodents. Stz is a robust alkylating agent that disrupts glucose transport, glucokinase activity and disintegrates multiple DNA strands. We previously established 40 mg/ml Stz as the optimal dose for inducing T2D. The present study assessed the period, viz., 7 or 14 days post-Stz injection, required to induce a T2D rat model. Weight, water intake, food consumption, blood glucose and glucose tolerance were assessed.

Methods: Forty 3-month-old male Wistar rats were randomly assigned to 4 groups: control-7 day (n = 10), Stz-7 day (n = 10), control-14 day (n = 10) and Stz-14 day (n = 10). The control rats were injected intraperitoneally with citrate buffer whereas the Stz rats were injected with 40 mg/kg of Stz. All rats were maintained on a standard rodent diet and provided with drinking water ad libitum. Weight, water intake, food intake, urine output, random blood glucose, fasting (4 hour) glucose concentrations and oral glucose tolerance tests (OGTTs; after 16h overnight fasting) were performed.

Results: At 7 days, Stz rats had higher fluid intake (p = 0.002), urine output (p = 0.001) and glucose intolerance (p = 0.003) compared to control rats. At 14 days, Stz rats had reduced body weights (p = 0.022), increased fluid (p < 0.0001) and food (p = 0.008) intake, elevated 4hr fasting blood glucose concentrations (p = 0.032) and glucose intolerance (p = 0.001) relative to control rats.

Conclusion: At 7 days post-Stz (40 mg/ml) injection, some diabetogenic traits emerged in rats. However, at 14 days post-Stz injection at 40 mg/ml, a more viable T2D rat model was established characterized by elevated fasting glucose concentrations concomitant with glucose intolerance.
Sutherlandia frutescens. Exposure of insulin resistant human liver cell cultures to a hot aqueous extract of S. frutescens has been shown to significantly stimulate glucose uptake, reduce lipid accumulation, and reduce hepatic glucose production. The object of this study is to evaluate the effectiveness of aqueous and organic extracts of S. frutescens as anti-diabetic therapeutic agents and identify potential active phytocompounds.

S. frutescens extracts from a single sample of plant material collected at a site in the Karoo were prepared from air dried leaves and shoots by hot aqueous, cold aqueous, 100% and 80% ethanol and 100% and 80% methanol extraction. Palmitate or a combination of insulin and fructose was used to generate an in vitro model of insulin resistance in Chang liver cells and HepG2 hepatocytes to compare untreated control, insulin resistant and S. frutescens treated insulin resistant cultures. Induction of insulin resistance and its prevention by S. frutescens was assessed by quantifying glucose uptake, gluconeogenesis and intracellular lipid accumulation in the cell cultures. Active phytocompounds within the extracts were identified using TripleTOF LC-MS.

The insulin resistant cells took up significantly less glucose, secreted more glucose into the culture medium and accumulated more intracellular lipid than the control cultures. Simultaneous treatment with each of the S. frutescens extracts significantly reduced the development of these insulin resistance parameters. The hot aqueous extract totally prevented development of all the parameters.

Triple TOF LC-MS analysis of the extracts identified several compounds previously shown to have anti-diabetic activity recorded in the anti-diabetic and anticancer database (DIACAN) published by the Bioinformatics Centre, Kerala Agricultural University, India. In addition phytocompounds not previously shown to be anti-diabetic were identified. The pattern of phytocompounds found in each extract differed and we have identified common compounds and extract-specific compounds.

Thus we have identified the hot aqueous extract of S. frutescens as the most effective anti-diabetic preparation and the phytocompounds detected will be analysed with a view to identifying critical compounds for diabetes therapy.

7. An investigation into the anti-obesity properties of polar and non-polar fractions of Cyclopia in 3T3-L1 adipocytes

Aim: The aim of this study was to evaluate the anti-obesity properties of polar and non-polar fractions of three Cyclopia species (Cyclopia subternata, Cyclopia intermedia and Cyclopia maculata) in 3T3-L1 adipocytes.

Methods: Methanol extracts of the three Cyclopia species were fractionated using liquid-liquid fractionation with butanol: water to divide the extracts into their polar and non-polar fractions. Differentiated 3T3-L1 adipocytes were treated with six liquid-liquid fractions at four different concentrations for 24 hours, and their effect on intracellular lipid accumulation, cell viability and glucose uptake was determined using the Oil Red O, triglyceride quantification, MTT, ATP, and 2-deoxy-[3H]-D-glucose assays, respectively.

Results: High performance liquid chromatography (HPLC) quantification of the fractions confirmed that mangiferin, isomangiferin, hesperidin and eriocitrin were the major phenolic compounds in Cyclopia extracts, with the non-polar fractions more enriched in these phenolic compounds compared to their polar counterparts. The polar fractions of C. subternata and C. maculata, and a non-polar fraction of C. intermedia decreased intracellular lipid accumulation as measured by Oil Red O staining and triglyceride content, in a dose-response manner. None of these fractions significantly affected cell viability as demonstrated by MTT activity, however, the non-polar C. maculata and polar C. intermedia fractions significantly lowered ATP content.

Conclusion: Compared to other Cyclopia fractions investigated in this study, the non-polar C. intermedia fraction was the most bioactive, showing the highest activity for lipid reduction, without being cytotoxic. Future studies are aimed at confirming the anti-obesity properties of the non-polar C. intermedia fraction using the db/db mouse model, and further fractionation of the bioactive fraction using high performance counter current chromatography (HPCCC).

8. Incidence of macroprolactinaemia in samples received at NHLS Universitas Laboratory

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Introduction and aim: Biologically inactive Macroprolactin is a high molecular mass complex of monomeric Prolactin and immunoglobulin (IgG). If present in a patient sample it is a cause of measured hyperprolactinaemia and diagnostic confusion, which can lead to unnecessary testing in absence of disease as the routine prolactin assay detects monomeric and oligomeric forms of prolactin. Sample treatment by Polyethylene Glycol (PEG) removes macroprolactin by precipitation. This is a simple, inexpensive and widely used
Poster Presentations

Objective: A high-fat diet, particularly high in saturated fat, predisposes individuals to obesity, insulin resistance, beta-cell dysfunction and type 2 diabetes (DM2). This study investigated the effects of a high fat and sucrose diet (HFDS) and exercise training on pancreatic islet function and apoptosis in C57BL/6 mice. Methods: C57BL/6 mice were divided into 4 groups: HFDS (High fat diet 20% carbohydrate, 20% proteins, 60% fat) and control diet (C: 70% carbohydrate, 20% proteins, 10% fat) HFDS + training (HFDS + tr: treadmill running at 20% peak treadmill velocity for 30 minutes, five days per week) and control diet trained (C + tr). After 8 weeks, glucose and insulin tolerance was assessed, and pancreatic islets were isolated for the determination of islet cell death and viability, and control groups (P<0.05).

Conclusion: For the first time that 8 weeks exercise training ameliorated the deleterious effects of the HFDS on insulin sensitivity, GSIS, and pancreatic viability, and maintained endogenous antioxidant enzyme content. These findings suggest that exercise training may be beneficial to prevent the adverse metabolic effects associated with consuming a western diet. Further human studies are encouraged to test this hypothesis.

10. Differences in alkaline phosphatase modification and activity by adipocytic and osteoblastic differentiation of mesenchymal stromal cells.

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Alkaline phosphatase (ALP) has an established role in bone mineralisation, but is also present in many other tissues. Previously it has been found that increased ALP activity and expression has been detected in immortalised preadipocyte cell lines during adipogenesis, though little is known about activity during adipogenesis in primary cells. In the present study, mesenchymal stromal cells (MSCs) were isolated from bone marrow and adipose depots of rats and differentiated towards either an adipocytic or osteoblastic lineage. It was found that ALP activity was detected in all cells undergoing adipogenesis and osteogenesis, with greater ALP activity in bone marrow-derived MSCs (bmmMSCs) compared to adipocyte-derived MSCs (ADSCs). Greater ALP activity was also observed in the ADSCs at the earlier stages of adipogenic differentiation compared to the later stages, while activity progressively increased during adipogenesis in bmmMSCs. ALP activity was present in bmmMSCs undergoing osteogenesis, as well as in ADSCs, but at a much reduced level.

Tissue nonspecific ALP (TNAP) is the ALP isozyme present in bone, and also in other tissues such as kidney and liver. These different isoforms are distinguished from each other based on the glycosylation state of the protein. As glycosylation may have a profound effect on the function of proteins and TNAP is known to be essential for mineralisation by osteoblast and involved in lipid accumulation during adipogenesis, we wished to examine whether the glycosylation state of the enzyme would be different when MSCs are differentiation into either an osteoblastic or adipocytic phenotype. Consequently TNAP was purified from bmmMSCs differentiated towards either an osteoblastic or an adipocytic phenotype and were analysed by gel electrophoresis (HYDRA/SYS electrophoresis system). Glycosylation of the enzyme was confirmed by using wheat germ lectin, which inhibits migration of any ALP isoenzymes that are glycosylated. It was found that the glycosylation pattern detected in the cells during adipogenesis was different to that of cells undergoing osteoblastogenesis. This indicates that TNAP glycosylation might modulate the function of the enzyme and requires further investigation.