Poster Presentations
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1 Experience with I131MIBG in two patients with carcinoid heart disease

[1] ***Weinreich C,*** [1] ***Ross IL,*** [2] ***Kotze T,*** [1] ***Levitt NS***
[1] Division of Endocrinology, University of Cape Town, Cape Town, South Africa
[2] Division of Nuclear Medicine in Department of Radiation Medicine, Groote Schuur Hospital, Cape Town, South Africa
Correspondence to: Dr Carsten Weinreich, e-mail: carsten.weinreich@uct.ac.za

Introduction: About 50% of patients with carcinoid syndrome will develop cardiac involvement. We present our experience of two patients with carcinoid syndrome and cardiac involvement.

Case1: A 57 year old male presented with a three year history of intermittent diarrhoea, nausea, intense pruritis and unprovoked episodes of cutaneous flushing. Three months prior to presentation he developed symptoms of right heart failure. 5HIAA levels at diagnosis were 820.8 µmol/24hr (10.4-41).

Case 2: A 39 year old female was managed for an amoebic liver abscess by percutaneous drainage and metronidazole in 2005. A follow up CT scan in 2007 revealed a new lesion in the liver. Fine needle aspiration cytology of this lesion confirmed a metastatic carcinoid tumour. Her carcinoid symptoms had started a year earlier. In 2009 she developed right heart failure. The chromogranin A and urinary 5-H-IAA levels were markedly elevated, > 585U/l (0–23) and 899 µmol/24 hr respectively in 2007. Without treatment the urinary 5-H-IAA has risen to 1975 µmol/24 hr in 2009. Both patients had echocardiographic evidence for carcinoid heart disease including severe tricuspid regurgitation and thickened valves. Patient 2 also had severe pulmonary stenosis and regurgitation. Both patients received 300 mCi of I131 MIBG therapy after positive 123MIBG uptake scans.

Outcomes: Patient 1 died of IVC obstruction two weeks following therapeutic MIBG. Patient 2 had a more favourable response to treatment which includes twice daily octreotide (100µg bd). At six months follow up there was a marked improvement in carcinoid and cardiac symptoms with no deterioration in echo findings. The urinary 5HIAA levels fell by 30%.

2 True hermaphroditism in a South African black adolescent

Gordon D, Shires R
Division of Endocrinology, Chris Hani Baragwanath Hospital and University of the Witwatersrand, Johannesburg, South Africa
Correspondence to: Dr Debbie Gordon, e-mail: debbiegordon@global.co.za

Introduction: True hermaphroditism (TH), or OT-DSD (ovotesticular disorder of sexual development), is a rare condition with a worldwide prevalence of 1: 20000 but commoner in sub-Saharan Africans, affecting 1:10000 population. Ambiguous external genitalia and/or hypospadias are usually evident at birth and later on gynaecomastia is frequent. Cryptorchidism, inguinal hernia and intermittent haematuria may also occur.

Case report: A 14 year old scholar, with male gender identity, had a one year history of bilateral breast development. At the age of two, a hypospadias had been repaired. Vision and sense of smell were normal. There was no history of drug ingestion. Family history was unremarkable. Examination was that of a tall adolescent with eunuchoidal body proportions and marked bilateral gynaecomastia. The penis was small and bound by a chordee. The scrotum appeared to contain a single large gonad (length 4 cm).

Investigations: Hormonal studies: Testosterone 1.5nmol/L, oestradiol 226 pmol/L, FSH 2IU/L, LH 1.4/L. *Karyotype* (50 lymphocytes) was 46,XX. *Molecular analysis* showed the SRY gene (Yp) to be absent. A post contrast CT scan showed a well-defined cystic structure between the bladder and rectum.

He underwent bilateral mastectomies. Two weeks later, the large cystic structure, a blind-ending vagina was removed. The scrotum was explored and a large L sided ovotestis and small R sided testis were removed. The patient adapted well psychologically and the oestradiol level fell to < 92 pmol/L. He was started on testosterone replacement therapy. Six months later testicular prostheses were implanted.

Discussion and conclusion: TH or OT-DSD is a relatively common disorder of sexual development in black sub-Saharan Africans in whom the genotype is nearly always 46,XX. In the absence of the Y chromosome and SRY sequences in XX OT-DSD, explanations to account for the existence of testicular tissue have been aided by recent advances in our knowledge of the molecular mechanisms involved in testicular differentiation.

3 Assessing math literacy skills in type 1 diabetic children and their caregivers

[1] ***Moosa FY,*** [2] ***Segal D***
[1] Department of Paediatrics, Chris Hani Baragwanath Hospital, Johannesburg, South Africa
[2] Department of Paediatric Endocrinology, Charlotte-Maxeke Johannesburg Academic Hospital, Johannesburg, South Africa
Correspondence to: Dr Fatima Yakoub Moosa, e-mail: Fatima.moosa@wits.ac.za

Objectives: To assess the level and application of mathematical skills in type 1 diabetic children and their primary care-givers attending the paediatric diabetic clinics at the Chris Hani Baragwanath and Charlotte- Maxeke Johannesburg Academic Hospitals.

Methods: A face-to-face structured interview and questionnaire was used to obtain demographic data, current or highest level of education, duration of diabetes, last HbA1c, insulin regimen, meal plan, presence of existing diabetic complications, number of documented hypoglycaemic and diabetic keto-acidosis episodes. The same questionnaire was used to assess the basic level of mathematical skills in each individual and the application of these skills in diabetes management. This took the form of problem solving exercises and a scoring system was used to determine if these numerical skills correlated with the current grade or the highest level of education achieved by each participant.

Results: 53% of children tested underscored, implying that their basic math skills were inadequate for their current grade. When these skills were applied to diabetes related tasks, they scored even lower. The majority of these patients also had higher HbA1c levels, showing an association between diabetes control and poor numeracy skills.

Conclusions: Poor math literacy impacts negatively on diabetes management and metabolic control.

4 Hyperglycaemic crisis: clinico-laboratory features and outcomes of admissions for hyperosmolar versus non-hyperosmolar ketoacidosis

Ekpebegh CO

Walter Sisulu University/Nelson Mandela Academic Hospital, Mthatha, South Africa

Correspondence to: Dr C Ekpebegh, e-mail chuksekpebegh@yahoo.com

Objective: To compare the presenting clinical features, serum biochemistry parameters and outcomes of admissions for hyperglycaemic hyperosmolar keto-acidosis (HHKA) in comparison with hyperglycaemic non hyperosmolar keto-acidosis (HNHKA).

Methods: Retrospective review of medical records over the two year period of 2008 and 2009.

Results: There were 24 and 97 admissions respectively for HHDKA and HNHDKA. The mean ages of patients (per admissions) were 40.3 ± 20.8 years for HHDKA and 40.4 ± 18.8 years for HHDKA, $p < 0.05$. HNHDKA admissions were about equally represented by persons with known type 1, known type 2 and newly diagnosed diabetes while admissions for HHDKA were predominantly by persons newly diagnosed with diabetes. Unconsciousness at presentation occurred in 77.3% of HHDKA versus 37.3% of HNHDKA, $p < 0.05$. Mortality rate was 37.5% of HHDKA versus 13.4% of HHDKA admissions.

Conclusions: Patients with HHKA had comparable ages as those with HNHKA. Admissions for HHKA in comparison with HNHKA were more likely to be associated with new onset diabetes, altered level of consciousness and intra-hospital mortality.

5 A patient with hypercalcaemia and a lung mass

[1] ***Kemp T,*** [2] ***van Wyk J,*** [1] ***Ascott-Evans B***

[1] Endocrinology and Metabolism Unit, Tygerberg Hospital, Stellenbosch University, South Africa

[2] Department of Pathology, Tygerberg Hospital, Stellenbosch University, South Africa

Correspondence to: Dr Tanja Kemp, e-mail: kemp.tanja@gmail.com

Hyperparathyroidism can occasionally present with unusual complications as the initial manifestation of the disease. We describe here a young man who presented with hypercalcaemia and a very large mass in his chest cavity, which was confirmed on histology to be a brown tumour. On further investigations he was found to have a single parathyroid adenoma which was subsequently surgically removed, with normalisation of his serum calcium level.

6 The association of Apo CIII, beta-3 adrenergic receptor and TNF-alpha polymorphisms with lipodystrophy in HIV-positive patients receiving anti-retroviral therapy

Tlomatsana TF, Naran NH, Crowther NJ

Department of Chemical Pathology, NHLS and the University of the Witwatersrand, Johannesburg, South Africa

Correspondence to: Francis Tlomatsana, e-mail: francis.tlomatsana@adcock.com

Introduction: Since the introduction of highly active antiretroviral therapy, body shape changes and metabolic abnormalities have increasingly been observed in HIV-positive patients. Lipodystrophy is one of the most prominent conditions which is characterised by both lipoatrophy and lipohypertrophy. The aim of this study was to determine the possible association of ApoCIII, beta-3 adrenergic receptor and TNF-alpha gene polymorphisms with the presence of lipodystrophy.

Method: 209 HIV-positive subjects were recruited from Helen Joseph and Charlotte Maxheke Hospitals. Lipodystrophy was identified via patient self-reports on body fat changes using a questionnaire. Anthropometric data was recorded and genomic DNA extracted. A PCR-based RFLP technique was used to screen for ApoCIII (-T455C and -C482T), beta-3 (-T64A) and TNF-alpha (-G238A and -G308A) gene polymorphisms.

Results: Lipodystrophy was observed in 34% of the subjects and was characterised by lower weight (64 ± 11.4) and BMI (24 ± 4.0) compared to subjects without lipodystrophy (69 ± 14.9, 26 ± 5.6, $p < 0.05$). Subjects with lipodystrophy had smaller waist and hip sizes ($p < 0.05$). Lipodystrophy subjects had a significantly higher ($p < 0.05$) glucose level than subjects without lipodystrophy. The frequency for the TNF-alpha variant allele, -308A was significantly higher in individuals with lipodystrophy (25%) compared to those without lipodystrophy (13%; $p < 0.05$). The allele frequencies for the ApoCIII, beta-3 adrenergic receptor and TNF alpha -238 polymorphisms were similar between patients with and without lipodystrophy. The distribution for all the polymorphisms was within Hardy Weinberg equilibrium.

Conclusion: Lipodystrophy in this patient cohort is characterised by lipoatrophy and the presence of the variant A allele at the TNF alpha -308 locus.

7 Increased fatty acid supply induces the hexosamine biosynthetic pathway in heart cells

Harris R, Essop MF
Department of Physiological Sciences, Stellenbosch University, South Africa
Correspondence to: Rinah Harris, e-mail: 13946560@sun.ac.za

Elevated circulating free fatty acids during obesity may interfere with myocardial signalling cascades thereby contributing to the onset of insulin resistance. Furthermore, increased flux through the hexosamine biosynthetic pathway is strongly associated with the onset of insulin resistance and type 2 diabetes. Here higher O-GlcNAc production (HBP end-product) may post-translationally modify target proteins, thereby perturbing its function. For this study, we hypothesised that high fatty acid supply activates the HBP thereby providing a mechanism whereby excess fatty acid availability may lead to insulin resistance. To investigate our hypothesis, rat cardiac-derived H9c2 myoblasts were exposed to 250 µM oleic acid for 24, 48 and 72 hours, respectively ± an HBP pharmacologic inhibitor (40 µM DON). The degree of HBP activation was subsequently determined by flow cytometric analysis (O-GlcNAc as primary antibody; PE-Texas Red as secondary antibody). We found that overall O-GlcNAcylation was elevated after 24 hours oleic acid exposure, further increasing at the 48 and 72 hour experimental time points. Intriguingly, DON administration blunted this effect. Our study shows that elevated fatty acid supply increases HBP flux in heart cells. We propose that such HBP activation modifies target proteins within the insulin signalling cascade thereby contributing to the onset of myocardial insulin resistance and type 2 diabetes.

8 Increased hexosamine biosynthetic pathway flux upregulates myocardial acetyl-coA gene expression

Imbriolo J, Essop MF
Department of Physiological Sciences, Stellenbosch University, South Africa
Correspondence to: Jamie Imbriolo, e-mail: 15280047@sun.ac.za

The cardiac isoform of acetyl-CoA carboxylase (ACCβ) produces malonyl-CoA, a potent inhibitor of mitochondrial fatty acid (FA) uptake; thus increased ACCβ activity decreases FA utilisation thereby potentially leading to intracellular myocardial lipid accumulation and insulin resistance (IR). Previous studies show that greater flux through the hexosamine biosynthetic pathway (HBP) contributes to the development of IR. In light of this, we hypothesise that increased HBP flux induces ACCβ gene expression thereby contributing to the onset of IR. Our initial work focused on ACCβ gene promoter regulation and suggests that the HBP modulates upstream stimulatory factor 2 (USF2) thereby inducing ACCβ gene expression. Here, we further investigated HBP-mediated regulation of ACCβ gene expression by transiently transfecting cardiac-derived H9c2 cells with an expression vector encoding the rate-limiting HBP enzyme (GFAT) ± the full length ACCβ and 4 truncated promoter-luciferase constructs, respectively. GFAT overexpression increased ACCβ gene promoter activity for the full length and 3 larger deletion constructs ($p < 0.001$ vs controls). However, GFAT-mediated ACCβ promoter induction was blunted when co-transfected with the -38/+65 deletion construct suggesting that USF2 binds to the proximal promoter region (near start codon). Our study demonstrates that increased HBP flux induces ACCβ gene promoter activity via HBP modulation of USF2. We propose that ACCβ induction reduces FA oxidation, thereby leading to intracellular lipid accumulation (FA uptake>>FA oxidation) and the onset of cardiac IR.

9 Increased hexosamine biosynthetic pathway flux leads to PKB/AKT post-translational modification: Implications for onset of insulin resistance

Joseph D, Essop MF
Department of Physiological Sciences, Stellenbosch University, South Africa
Correspondence to: Danzil Joseph, e-mail: 14353830@sun.ac.za

With onset of insulin resistance (IR), hyperglycaemia-mediated oxidative stress may increase flux through the hexosamine biosynthetic pathway (HBP) resulting in greater *O*-GlcNAc (HBP end-product) modification of nuclear and cytosolic proteins. Although excess HBP flux is associated with IR onset, underlying mechanisms driving this process in the heart remain unclear. Here we hypothesised that higher HBP flux increases PKB/Akt *O*-GlcNAcylation, thereby attenuating its activity and diminishing insulin-mediated myocardial glucose uptake. Rat cardiac-derived H9c2 myoblasts were cultured in 5 mM glucose (controls) for 24 h and compared to cells treated with 25 mM glucose (hyperglycaemia). To assess the role of the HBP, we employed two pharmacologic modulators, i.e. 40 µM DON (HBP inhibitor) and 50 µM PUGNAc (increases HBP end-product). We also evaluated the role of oxidative stress by administering 250 µM 4-OHCA (antioxidant). We employed immunofluorescence microscopy to determine the degree of PKB/Akt (FITC-green) and *O*-GlcNAc (Texas Red) co-localisation. Our data show increased PKB/Akt *O*-GlcNAcylation after 24 h of hyperglycaemia. Interestingly, HBP inhibition (DON) blunted this effect while PUGNAc administration further increased it. Antioxidant treatment also reversed hyperglycaemia-mediated PKB/Akt *O*-GlcNAcylation. Our data show that hyperglycaemia-mediated oxidative stress triggers the HBP thereby increasing PKB/Akt *O*-GlcNAcylation. We suggest that activation of this detrimental signalling cascade may impair insulin-mediated myocardial glucose uptake thereby leading to IR.

10 The expression of the tumour suppressor Pdcd4 in 3t3-L1 pre-adipocytes and adipose-derived stromal/stem cells

[1] ***Van den Heever M,*** [2] ***Niesler CU,*** [1] ***Hough FS,*** [1] ***Ferris WF***
[1] Department of Medicine, Stellenbosch University, Tygerberg, South Africa
[2] Department of Biochemistry, University of KwaZulu-Natal, Petermaritzburg, South Africa
Correspondence to: Ms Martine van den Heever, e-mail: vdh@sun.ac.za

Stem cells are widely heralded for their therapeutic potential to repopulate diseased or damaged tissue. Adipose tissue is an abundant source of these pluripotent cells that may be differentiated into an adipocytic, osteoblastic, chondrocytic or myoblastic phenotype. However, for successful therapeutic use, the proliferation and differentiation of these stromal cells must be exquisitely regulated to avoid aberrant cell function. As Programmed Cell Death 4 (pdcd4)

has been associated with the control of proliferation in other cell types, the role for pdcd4 in the regulation of these cellular responses in stromal cells was assessed.

Aim: To examine the relationship between pdcd4 expression, proliferation and differentiation in 3T3-L1 pre-adipocytes and primary rat adipose-derived stromal/stem cells (ADSCs).

Methods: 3T3-L1s and ADSCs were differentiated into mature adipocytes and proliferation measured using tritiated thymidine incorporation after 0, 1, 2, 4, 6 and 8 days. Differentiation was assessed by Oil Red O staining of accumulated lipid and real-time quantitative PCR (QRT-PRC) analyses of the adipocyte markers PPARγ and aP2. QRT-PCR was used to measure pdcd4 expression at all time points.

Results and conclusion: Preliminary data indicate that the pdcd4 exhibits a more intimate association with differentiation than with the suppression of proliferation in both 3T3-L1s and ADSCs. The tumour suppressor may therefore play a more participative role in normal cellular function during differentiation and not be solely associated with the regulation of aberrant proliferative responses.

11 Efficacy of *Prunus africana* bark extract and metformin in the treatment of high fat diet induced fatty liver

***Tshidino SC*, Karachi J, Koekemoer T, Roux S**
Department of Biochemistry and Microbiology, Nelson Mandela Metropolitan University, Port Elizabeth, South Africa
Correspondence to: Shonisani C Tshidino, e-mail: Saartjie.roux@nmmu.ac.za

The aim of this study was to investigate the antioxidant potential of *Prunus africana* bark extract and metformin oral treatments, on high fat diet (HFD) induced fatty liver and peroxisomal oxidative stress. The rats were divided into four groups of 6 to 7 per group; the negative control group was fed 6.9% low fat diet (LFD) while the HFD group fed a 40% HFD. The experimental groups were gavaged with *P. africana* extract or metformin. The hepatic total lipids (TL) were extracted and used to determine triglyceride (TG) and total cholesterol (TC) levels. Malonaldehyde (MDA) was used as a marker for oxidative stress. The proxisomal H_2O_2 and superoxide producing and degrading enzymes, and nonenzymatic antioxidant were assayed including xanthine oxidases (XOx), suproxide dismutase (SODx), glutathione peroxidase (GPx), glutathione reductase (GRx), catalase (CTx) and glutathione (GSH) levels. The hepatic TL, TG, TC and MDA levels were significantly increased, while SOD activity was significantly inhibited in the HFD group compared to the LFD group. Treatment with *P. africana* extract significantly attenuated the TG and MDA levels, while significantly induced an increased in the GPx and GRx activities and GSH level compared to the HFD group, but not significant different from that of the LFD group. Metformin supplementation had no effect on the hepatic lipid parameters, while it increased the activity of CTx and GPx and GSH levels. These results suggest that compounds in *P. africana* bark extract may have potential in treating fatty liver disease and its associated oxidative stress.

12 *In vitro* safety and efficacy assessement of the anti-diabetic medicinal plant: *Sutherlandia frutescens*

***Koekemoer T*, van de Venter M, Roux S**
Department of Biochemistry and Microbiology, Nelson Mandela Metropolitan University, Port Elizabeth, South Africa
Correspondence to: Trevor Koekemoer, e-mail: Saartjie.roux@nmmu.ac.za

The identification of adverse effects such as poor efficacy, toxicity and herb-drug interaction, is an important requisite if traditional medicine is to be integrated into public health programmes. Plant extracts contain multiple components which can potentially mimic, increase, or decrease the effects of co-administered drugs through simultaneous interaction on the same therapeutic target or by affecting the metabolic stability of these drugs. In the present study various *in vitro* models designed to simulate specific anti-diabetic targets were used to screen an aqueous and ethanol extract prepared from *Sutherlandia frutescens*, a popular herbal remedy for the treatment of diabetes. The *in vitro* effects of *S. frutescens* extracts on carbohydrate digestion, protein glycation, hepatic glucose utilisation, insulin secretion, β-cell proliferation, muscle glucose metabolism, DPPIV activity, adipogenesis and adipocyte glucose utilisation were assessed in the presence and absence of the target specific conventional drugs: acarbose, metformin, glibenclamide, insulin and rosiglitazone. In addition, the potential for hepatotoxicity and CYP3A4 interaction was also evaluated. While the results confirm that *S. frutescens* has anti-diabetic properties at multiple therapeutic targets and with varying degrees of efficacy, it also raises the potential for drug interaction. Furthermore, differences in the efficacy between the aqueous and ethanol extracts suggest that *S. frutescens* contains multiple anti-diabetic components. The results from this study provide a much needed platform for further clinical studies aimed at evaluating the safety of the traditional medicinal plant *S. frutescens.*

13 *In vitro* assessment of the interaction between African medicinal plants and anti-diabetic prescription medicines

***Fang C*, Koekemoer T, Roux S**
Department of Biochemistry and Microbiology, Nelson Mandela Metropolitan University, Port Elizabeth, South Africa
Correspondence to: Cindy Fang, e-mail: Saartjie.roux@nmmu.ac.za

In South Africa, the use of medicinal plants as supplements is increasing, especially among the elderly and people with chronic diseases such as diabetes, and these are often combined with prescribed medicines. Previous *in vitro* studies have shown that many herbal remedies inhibit human hepatic Cytochrome P450 iso-enzymes (CYP450) and P-glycoprotein (P-gp). This is cause for concern since prescribed medicines are often used simultaneously with herbal remedies which may result in treatment failure. This study was undertaken to screen the effect of a few popular African anti-diabetic medicinal plants on CYP450 3A4 and 2C9 activity. These isoforms were selected since not all the CYP 450 iso-enzymes are inducible, and for diabetes, the only clinically relevant CYP450 iso-

enzymes are CYP3A4 and CYP2C9. The analysis used microsome-based *in vitro* models for assessing CYP450 enzyme activities. The results indicate that *Sutherlandia frutescens* extracts moderately inhibited CYP3A4 activity using resorufin benzyl ether (BzRes) as substrate.

For P-gp, the study assessed the effect of *S. frutescens* extracts on P-gp in both wild-type Caco2 cells and Vinblastine resistant Caco2cells. The study found that short term treatment (2 hrs) with *S. frutescence* did not significantly inhibit the efflux of the P-gp substrate Rhodamine-123 in both wild type and vinblastine resistant Caco2cells relative to the positive control verapamil. The results from this study provide useful information on the potential drug-herb interactions between some popular medicinal herbs and prescribed anti-diabetic drugs.

14 The regenerative capacity of pancreatic tissue following an 80–90% partial pancreatectomy in a Wistar rat

[1] ***Ghoor S***, [1] ***Muller C***, [1] ***Pheiffer C***, [2] ***Du Toit D***, [2] ***Page BJ***
[1] Medical Research Council, South Africa
[2] Stellenbosch University, South Africa
Correspondence to: Samira Ghoor, e-mail: samira.ghoor@mrc.ac.za

The rat partial pancreatectomy (Px) model has been widely used to study pancreas endocrine regeneration. Post-Px remnant pancreas undergoes regeneration to compensate for the deficit in endocrine mass. The aim of this pilot study was to determine the regenerative capacity of pancreatic endocrine tissue, following an 80–90% partial Px.

Experimental groups consisted of the Px group (n = 3) and the sham (Sx) control group (n = 3). Body weight and blood glucose levels were monitored. On day seven, 5-bromo-2'-deoxy-uridine (BrDU) was injected intraperitoneally. At termination, insulin levels were measured, and tissue was harvested for immunolabelling, and mRNA analysis. Sections were stained for BrDU, insulin and TCF7L2. Genes for mRNA expression analysis (using RT-PCR) included *islet-1, glut2, irs1* and *irs2.*

After 30 days the Px rats were hypoinsulinaemic, however they maintained normoglycaemic levels. BrDU labelling revealed increased proliferation of acinar tissue and intra-islet β-cells. Expression of *islet-1* was upregulated. No difference was noted in *glut2, irs1* and *irs2* expression.

The transcription factor *islet-1,* normally detected in the developing pancreas and later restricted to adult islets, suggests active endocrine regeneration. However, β-cell mass had not recovered to normal physiological functionality. Results showed that intra-islet β-cell proliferation contributes significantly to β-cell recovery.