**1. Body composition measures as predictors of hypertension in urban black South African woman**

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**Objectives:** Hypertension is becoming increasingly prevalent in South Africa (SA), especially amongst the black population. Since body composition is a major risk factor for hypertension, it is important to investigate which parameter of body composition best predicts this disease. The aim of this study was to evaluate whether baseline and/or change in body composition, could predict the development of hypertension over a 10 year period in a sample of black SA woman.

**Research design and methods:** This longitudinal study included 563 black SA women, aged 29-53 years, residing in Soweto, Johannesburg. Data on systolic and diastolic blood pressure, anthropometric, dual energy x-ray absorptiometry (DXA)-derived body composition and simple anthropometric measures were collected at baseline (2003) and at 10-year follow-up (2013). Results: Over the 10-year follow-up period, all anthropometric [eg. body mass index (BMI): 30.3±6.1 to 32.6±6.8, p<0.0001], DXA-derived body composition measures (p<0.001) and blood pressure (BP, systolic: 19.8±19.8 mmHg, diastolic: 12.6±12.0 mmHg; both p<0.001) increased significantly. A total of 321 participants (cumulative incidence 57.02%) developed hypertension [systolic (and/or diastolic) BP ≥140 (90) mmHg] during follow-up. Baseline BMI and waist circumference were associated with the development of hypertension at follow-up [adjusted odds ratio (95% CI) per unit change: 1.04 (1.01–1.08), p=0.012 and 1.03 (1.01–1.04), p=0.001, respectively]. Of the DXA-derived measures of body composition and body fat distribution, fat mass [adjusted odd ratio (95% CI) per unit change: 1.03 (1.00–1.05), p=0.049], fat mass [%] (1.05 (1.01–1.1), p=0.008), trunk fat mass (1.05 (1.00–1.1), p=0.046), arm fat mass (% total fat mass) (1.19 (1.04–1.37), p=0.013) and leg fat mass (% total fat mass) (0.96 (0.92–0.99), p=0.025) at baseline were also associated with increased risk for hypertension risk at follow-up. The changes in anthropometric and DXA-derived measures of body composition were not associated with hypertension at follow-up. Conclusion: Our study has shown that baseline body composition, rather than the change in body composition, is the better predictor of future risk of developing hypertension.

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**2. Adipose depot-specific differences in cyclic nucleotide signalling during adipogenesis**

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**Background:** IBMX (3-isobutyl-1-methylxanthine) is a standard constituent of the cocktail used to induce adipogenesis in vitro in cultured mammalian pre-adipocytes. IBMX is a non-selective phosphodiesterase (PDE) inhibitor, and can therefore elevate intracellular cyclic GMP and/or cyclic AMP, although it is often (erroneously) assumed to exclusively elevate cAMP. This study aimed to delineate the respective contributions of IBMX, cAMP and cGMP during adipogenesis in primary rat adipose-derived stromal cells (ADSCs) derived from subcutaneous and visceral adipose tissue, and to assess whether these compounds have depot-specific adipogenic effects.

**Methods:** Primary (non-immortalised) ADSCs were isolated from rat subcutaneous and perirenal visceral adipose tissue (scADSCs and pvADSCs, respectively) by collagenase digestion and subcultured until passage 2. Adipogenesis was induced in post-confluent ADSC cultures using adipogenic induction media [AM: standard growth media supplemented with IBMX, insulin, indomethacin and dexamethasone]. Alternatively, IBMX was omitted from AM and replaced with 8-Br-cAMP and/or 8-Br-cGMP. Control cultures were maintained in standard growth media. Intracellular lipid accumulation was stained and quantified with Oil Red O staining, and gene expression analysis was performed by means of semi-quantitative real-time PCR.

**Results:** AM induced the accumulation of intracellular lipid droplets in both scADSCs and pvADSCs, but no spontaneous lipid accumulation was observed in control cultures. Omission of IBMX from AM strongly inhibited adipogenesis in both cell-types by inhibiting the adipogenic gene expression program. Within the context of AM, cyclic GMP partially restored adipogenesis in scADSCs, but not in pvADSCs. Cyclic AMP had no adipogenic effects, but inhibited the adipogenic effects of cGMP.

**Conclusions:** Within the context of AM, IBMX has adipogenic effects on cultured ADSCs that cannot be accounted for by the actions of cyclic nucleotides. Our results indicate that cyclic nucleotides may have adipose depot-specific effects during adipogenesis, and that cAMP does not stimulate adipogenesis in ADSCs.

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**SEMDSA ORAL PRESENTATIONS**

1. **Body composition measures as predictors of hypertension in urban black South African woman**

2. **Adipose depot-specific differences in cyclic nucleotide signalling during adipogenesis**
3. The prevalence of type 2 diabetes among older people in Africa 2000-2013: a systematic review & meta-analysis

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Aim/hypothesis: There is little information on the prevalence and management patterns of diabetes in older person (>55 years) from the African continent. We performed a systematic review to determine the prevalence of type 2 diabetes in Africa in individuals aged >55 years, reported in studies from 2000-2013 to provide accurate data for monitoring further trends.

Methods: A comprehensive literature search of databases was undertaken using an African search filter to identify type 2 diabetes prevalence studies published from 2000-2013. Data was extracted and synthesized from full copies of articles that met the inclusion criteria. Data analyses used the statistical software R.

Results: Of total of 1448 citations, 39 studies in 14350 individuals met the inclusion criteria. The overall prevalence was 14.1% (95% CI: 11.4-17.1), and was higher in the studies based on oral glucose tolerance test [OGTT] [24.0% (95% CI: 17.7-30.7), 12 studies, n=3,413] than fasting blood glucose (FBG) criteria [10.5% (95% CI: 8.3-12.9), 27 studies, n=10,935], p=0.001. In non-STEPs [16.4% (12.7-20.4)], STEPS studies [10.4% (95% CI: 6.7-14.9)], p=0.042: in urban [21.0% (95% CI: 15.2-27.5)] vs. rural settings [10.5% (7.1-14.5)], p=0.003, but did not differ significantly across age groups, gender, sample size, year of publication region and population coverage. There was substantial heterogeneity.

Conclusions/interpretation: Regardless of the difference in type 2 diabetes prevalence by diagnostic criteria and study design among older populations in Africa, these data highlight the need to reduce diabetes risk factors and implement adequate management strategies.

4. Changes in body composition in menopausal, black urban African women in the Study of Women Entering and in Endocrine Transition (SWEET)

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Background & Aims: Data predominantly from studies performed in populations of European ancestry show that the menopausal transition (MT) is characterized by changes in body composition. However, no such investigations have been undertaken in women from sub-Saharan Africa. Such studies would be important because the prevalence of obesity in Africa is highest in middle aged, urban African females and the possible contribution of the MT to this statistic has not been analysed. The current study therefore aimed to determine whether there are differences in body adiposity, lean muscle mass, and bone mineral density (BMD) across MT groups of urban African women, and if any changes are related to an altered hormonal milieu.

Methods: Participants were 702 black urban women in the cross-sectional Study of Women Entering and in Endocrine Transition (SWEET). Menopause stage was defined using the Stages of Reproductive Aging Workshop+10 (STRAW+10) criteria, which are internationally accepted guidelines that have been previously validated in this population. Levels of follicle stimulating hormone (FSH), estradiol (E2), dehydroepiandrostosterone (DHEA), dehydroepiandrostosterone sulphate (DHEAS), testosterone (T) and sex hormone binding globulin (SHBG) were measured. Dual-energy X-ray absorptiometry and ultrasound scans were used to measure body composition.

Results: Whole body lean mass (p=0.002) and BMD (p<0.0005) were significantly lower in the post-menopausal compared to the pre-menopausal groups. No differences in total fat mass were noted. Serum levels of E2 (p=0.0005), SHBG (p<0.0005) and DHEAS (p=0.0007) were significantly lower in the groups at the end compared to those at the beginning of the MT, whilst FSH was higher (p=0.0005). FSH (log: pmol/L) correlated negatively (unstandardized β=-2.06, p<0.0005) with total lean mass (kg) whilst E2 (log: IU/L) correlated positively (unstandardized β=2.06, p=0.002) with BMD (mg/cm²).

Conclusions: The MT in this population is characterized by lower whole body lean mass and BMD in post- compared to premenopausal subjects but by minimal differences in fat mass. Lower lean mass and BMD were associated with higher FSH and lower E2 serum levels, respectively. Future research is needed to determine whether the MT-related sarcopenia is associated with changes in cardiometabolic disease risk factors.

5. Glycaemic Control in 8-25-year-old patients with Type 1 Diabetes Mellitus in the TEENs Study: South African Subset Analysis.

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Background: Type 1 Diabetes Mellitus (T1DM) is a common and demanding disease of childhood and early adulthood. Glycosylated haemoglobin (HbA1c) is the main objective measure of glycaemic control and assesses risk factor for complications.
**Aim:** To describe the treatment characteristics and determine the proportion of patients achieving HbA1c targets as defined by ISPAD and ADA recommendations (<7.5% in ≤18-years-olds; <7.0% in ≥19-years-olds).

**Methods:** The TEENS study is an international, observational cross-sectional study that investigated 5960 young persons with T1DM in the 3 age categories: 8-12-year-olds, 13-18-year-olds, and 19-25-year-olds. TEENS encompassed 20 countries including South Africa (SA). A total of 508 patients were assessed in 5 sites in the private and public health sector by paediatric endocrinologists in SA. Data were collected by interview, survey and from medical records. 507 (99.8%) patients were eligible for analysis. HbA1c was measured uniformly using point of care testing (A1cNow™) (reference range 4-6%).

**Results:** Among the 507 T1DM patients, mean age was 14.9 ±4.4 years and mean duration of T1DM was 7.1 years. Females comprised 53%, Caucasians 51%, and 30% and 9% were overweight and obese, respectively. The majority (67%) used injections/pensets. The most frequent treatment was basal bolus regimen in 320 (94%); of these 166 (52%) used analogue based insulin therapy. 32% patients were on insulin pump therapy and 1% used both injections and pump therapy. Mean number of blood glucose checks was 4.0±2.0/day with the highest (4.9±1.9) in 8-12-year-olds. The use of real time continuous glucose monitoring was uncommon (4.0% of youth). Mean HbA1c was 9.8 ±2.2% and increased with age: 8-12-year-olds: 9.4 ±2.0%; 13-18-year-olds: 9.9 ±2.1%; 19-25-year-olds: 10.1± 2.4%. Target HbA1c was achieved in only 52 patients (10.3%), with decreasing target attainment as age increased: 8-12-year-olds: 14%; 13-18-year-olds: 9%; 19-25-year-olds: 7%. Additionally, 39.6% of patients had very poor glycaemic control with an HbA1c ≥ 10%.

**Conclusion:** This sample of young patients with T1DM cared for in specialty centres in SA demonstrated overall suboptimal glycaemic control. There is urgent need to identify approaches to improving control; examining demographic, treatment and psychosocial factors associated with achieving target HbA1c may provide direction.

This study was supported by Sanofi

### 6. Chronic GSK-3 inhibition may be good for diabetes, but bad for the heart!

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Glycogen synthase kinase-3 (GSK-3) is a serine-threonine kinase regulating glycogen synthesis and known as a role-player in cardioprotection. Active GSK-3 is also recognized as the strongest anti-hypertrophic protein in the heart. However, myocardial GSK-3 is known to regulate expression of SERCA-2a, affecting contractility; II) phosphorylate and inhibit IRS-1 disrupting insulin signalling and (III) regulate growth via interaction with hypertrophic signalling pathways. Small, specific GSK-3 inhibitors are currently being developed for clinical use, treating e.g. affective disorders but are also considered as treatment for type 2 diabetes.

We aimed to study the effects of chronic GSK-3 inhibition on the heart, using pre-diabetic rats (DIO).

Wistar rats (obesity induced by supplementing their diet with sugar and fat for 16 weeks) were compared to age matched controls. Half of each group was treated with the GSK-3 inhibitor (CHIR118637 - 30mg/kg/day) from weeks 12 to 16 of the diet period. Glucose tolerance was established, biometric and biochemical parameters determined and protein expression ascertained in snap-frozen hearts using Western blotting and specific antibodies. Ca2+ATPase activity was determined spectrophotometrically and cardiomyocytes used to determine insulin sensitivity, cell size and localization of NFATc3 and GATA4, the latter by means of fluorescence microscopy.

GSK-3 inhibition: (i) did not affect body weight or intraperitoneal fat, both of which were increased by DIO; (ii) could increase SERCA-2a expression and phospholamban phosphorylation which were both downregulated by DIO; (iii) upregulated IRS-2, but not IRS-1 expression, IRS-1 expression was severely curtailed in DIO; (iv) positively modulated insulin signalling reflected in enhanced cardiomyocyte insulin sensitivity as measured by accumulation of [3H]2-deoxyglucose. Glucose uptake was attenuated in cells from DIO animals; (v) increased left ventricular weight and cardiomyocyte size in control animals; (vi) increased LV end-diastolic diameter in control animals; (vii) translocated both NFATc3 and GATA4 to the nucleus in cardiomyocytes from both control and DIO animals.

We conclude that chronic, specific GSK-3 inhibition improved glucose tolerance and insulin sensitivity of the heart, but showed clear signs of inducing cardiac hypertrophy, especially in normal animals.

### 7. The cardio-protective properties of PPAG against diabetic induced cardiomyopathy: an in vitro study

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**Introduction:** Myocardial infarction is the leading cause of death in diabetic patients. Hyperglycemia, in a diabetic state, is responsible for augmented production of free radical species as well as myocardial apoptosis leading to cardiomyopathy. While there is an increasing interest in investigating the efficacy of Aspalathus linearis against metabolic complications, less is known about the cardiac protective properties of some of its major phenolic compounds. This study examines the cardio-protective effect of phenylpyruvic acid-2-O-β-D-glucoside (PPAG) against hyperglycemic-induced cardiovascular complications using an in vitro cardiomyocyte model.
Methods: A cardiac myoblast cell line (H9c2) was exposed to either normal (5.5mM) or high (33mM) glucose for 48hrs. Cells exposed to high glucose were treated with metformin (1µM) or PPAG (1µM) as well as a combination of metformin and PPAG for 12hrs. The efficacy of PPAG to reverse altered cardiac energy metabolism was investigated by measuring the uptake and oxidation of fatty acids. Furthermore, mitochondrial membrane depolarization was assessed by measuring JC-1 fluorescence while apoptotic cell death was determined by annexin V/propidium iodide staining. Correspondingly, Bcl-2/Bax ratio and caspase-3 activation was detected by Western blot analysis.

Results: High glucose exposure resulted in a significant disruption in cardiac fatty acid metabolism, mitochondrial membrane potential as well increased myocardial apoptosis. Treatment with PPAG abolished this effect. This was evident as detected by decreased uptake of fatty acids and their oxidation, occurring concurrently with decreased apoptosis. Interestingly, the effect of PPAG was comparable to that of known cardio-protective agent, metformin.

Conclusion: These results suggest that PPAG has a strong potential in ameliorating hyperglycemic-induced cardiac complications that can lead to myocardial apoptosis.

8. Vanadate impedes lipid accumulation during adipogenesis of bone marrow stem cells

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Background: Glucocorticoids are commonly used to treat a variety of diseases, but chronic use of glucocorticoids may cause a reduction in bone mineral density (BMD), which leads to the development of osteoporosis. Glucocorticoid-induced osteoporosis (GIO) has also been associated with an increase in bone marrow adiposity. These adipocytes are thought to stem from the same progenitor/mesenchymal stromal cells (MSCs) as the bone forming osteoblasts. It has therefore been suggested that glucocorticoids may promote the adipocytic differentiation of MSCs at the expense of osteoblastic differentiation, thereby reducing osteoblast numbers and BMD. Our group has previously demonstrated that sodium orthovanadate, a protein tyrosine phosphatase inhibitor, prevents GIO in vivo in rats and that vanadate reduces adipocytic differentiation in adipose-derived MSCs (ADSCs). We have therefore hypothesized that glucocorticoids may promote the adipocytic differentiation of MSCs at the expense of osteoblastic differentiation, thereby reducing osteoblast numbers and BMD. Our group has previously demonstrated that sodium orthovanadate, a protein tyrosine phosphatase inhibitor, prevents GIO in vivo in rats and that vanadate reduces adipocytic differentiation in adipose-derived MSCs (ADSCs). We have therefore hypothesized that glucocorticoids may promote the adipocytic differentiation of MSCs at the expense of osteoblastic differentiation, thereby reducing osteoblast numbers and BMD. Our group has previously demonstrated that sodium orthovanadate, a protein tyrosine phosphatase inhibitor, prevents GIO in vivo in rats and that vanadate reduces adipocytic differentiation in adipose-derived MSCs (ADSCs). We have therefore hypothesized that glucocorticoids may promote the adipocytic differentiation of MSCs at the expense of osteoblastic differentiation, thereby reducing osteoblast numbers and BMD. Our group has previously demonstrated that sodium orthovanadate, a protein tyrosine phosphatase inhibitor, prevents GIO in vivo in rats and that vanadate reduces adipocytic differentiation in adipose-derived MSCs (ADSCs). We have therefore hypothesized that glucocorticoids may promote the adipocytic differentiation of MSCs at the expense of osteoblastic differentiation, thereby reducing osteoblast numbers and BMD. Our group has previously demonstrated that sodium orthovanadate, a protein tyrosine phosphatase inhibitor, prevents GIO in vivo in rats and that vanadate reduces adipocytic differentiation in adipose-derived MSCs (ADSCs). We have therefore hypothesized that glucocorticoids may promote the adipocytic differentiation of MSCs at the expense of osteoblastic differentiation, thereby reducing osteoblast numbers and BMD. Our group has previously demonstrated that sodium orthovanadate, a protein tyrosine phosphatase inhibitor, prevents GIO in vivo in rats and that vanadate reduces adipocytic differentiation in adipose-derived MSCs (ADSCs). We have therefore hypothesized that glucocorticoids may promote the adipocytic differentiation of MSCs at the expense of osteoblastic differentiation, thereby reducing osteoblast numbers and BMD. Our group has previously demonstrated that sodium orthovanadate, a protein tyrosine phosphatase inhibitor, prevents GIO in vivo in rats and that vanadate reduces adipocytic differentiation in adipose-derived MSCs (ADSCs). We have therefore hypothesized that glucocorticoids may promote the adipocytic differentiation of MSCs at the expense of osteoblastic differentiation, thereby reducing osteoblast numbers and BMD. Our group has previously demonstrated that sodium orthovanadate, a protein tyrosine phosphatase inhibitor, prevents GIO in vivo in rats and that vanadate reduces adipocytic differentiation in adipose-derived MSCs (ADSCs). We have therefore hypothesized that glucocorticoids may promote the adipocytic differentiation of MSCs at the expense of osteoblastic differentiation, thereby reducing osteoblast numbers and BMD. Our group has previously demonstrated that sodium orthovanadate, a protein tyrosine phosphatase inhibitor, prevents GIO in vivo in rats and that vanadate reduces adipocytic differentiation in adipose-derived MSCs (ADSCs). We have therefore hypothesized that glucocorticoids may promote the adipocytic differentiation of MSCs at the expense of osteoblastic differentiation, thereby reducing osteoblast numbers and BMD. Our group has previously demonstrated that sodium orthovanadate, a protein tyrosine phosphatase inhibitor, prevents GIO in vivo in rats and that vanadate reduces adipocytic differentiation in adipose-derived MSCs (ADSCs). We have therefore hypothesized that glucocorticoids may promote the adipocytic differentiation of MSCs at the expense of osteoblastic differentiation, thereby reducing osteoblast numbers and BMD. 

Results: Vanadate significantly reduced the quantity of lipid accumulated during adipocytic differentiation of bone marrow MSCs.

Conclusion: Vanadate causes a reduction in lipid accumulation during adipocytic differentiation of bone marrow MSCs, similar to our previous observations in adipose-derived MSCs. Future work will be focused on investigating adipocyte gene markers, to determine if vanadate reduces adipocytic differentiation or the lipid accumulation process. This work may contribute to our understanding of GIO and the development of new therapies for GIO.

9. The influence of obesity-associated type 2 diabetes on the migration capacity of bone marrow-derived mesenchymal stem cells
(bmMSCs)

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Chronic wounds continue to be a major cause of morbidity for type 2 diabetic patients and therapeutic approaches to improve wound healing need to address cellular changes induced by the pathological micro-environment associated with this metabolic disorder. The multi-functional properties of progenitor/mesenchymal stem cells (MSCs) play a key role in wound healing and tissue regeneration. However, the implications of long term overarching inflammation on the capacity of bmMSC to migrate towards injured areas that is required for healing is less well defined, especially in the context of type 2 diabetes.

In-depth micro-array analysis of bone marrow aspirates containing bmMSCs derived from obese diabetic (ob/ob) and lean healthy (control) mice was performed to improve our understanding of the underlying molecular mechanisms involved in the diminished regenerative capacity of MSCs and impaired wound healing associated with type 2 diabetes mellitus. Molecular signalling pathways of particular interest were the Signal Transducer and Activator of Transcription (STAT3) signalling pathway and its counterpart the Suppressor of cytokine signalling (SOCS3) pathway.

PCR Profiler micro-array analysis demonstrated that out of the 84 genes assessed, 32% were overexpressed (cc112, cci3, cci4, cd40, cebpda, csf2, csf3, cxcl12, il12a, il18r1, il1a, il2, il21, ll23a, ll24, il5, il6, il9, jak3, lif, lta, nfkb1, socs1, socs3, tnf) whereas 8% of genes were underexpressed (cadc25a, csf1, cxc4r, ikbkb, il10, il4, il6ra) in bmMSCs derived from ob/ob compared to those derived from control animals. In vitro assessment of the rate of wound closure (scratch assay) furthermore demonstrated impaired healing in ob/ob-derived bmMSCs. Taken together, our data suggest that stem cell impairment is at least in part due to IL-6/STAT3 dysregulation during obesity.

Ongoing work is examining whether anti-inflammatory substances such as IL-6 and the diabetes drug pioglitazone are able to rescue the in vitro migration abilities of bmMSCs by targeting the IL-6 / STAT3 molecular signalling pathway.
LY2963016 (LY IGlar) and Lantus® (IGlar) are both insulin glargine products with identical amino acid sequences. Even with identical primary structure, protein-based therapeutics manufactured by distinct processes must be shown to be clinically similar. Thus, we performed a 24-week, Phase 3, randomized, double-blind, parallel study to compare the safety and efficacy of LY IGlar and IGlar. The primary objective was to test noninferiority (0.3% margin) of LY IGlar to IGlar as measured by change in HbA1c from baseline to 24 weeks in T2DM on ≥2 OAMs. Patients were insulin-naïve (HbA1c ≥7.0% to ≤11.0%) or previously on IGlar (HbA1c 11.0%). Both groups had within-group similarly significant (p<.001) decreases in mean HbA1c (~ -1.3% [Endpoint: LY IGlar ≤11.0%]). Both groups had within-group similarly significant (p=.31) AE frequencies (LY IGlar, 52%; IGlar, 48%; p=.31) were similar in population and in the insulin-naïve/prestudy IGlar subgroups. In clinical efficacy between LY IGlar and IGlar were met. Noninferiority of IGlar to LY IGlar was non-inferior to IGlar. Noninferiority of IGlar to LY IGlar was also demonstrated; thus, criteria for equivalence in clinical efficacy between LY IGlar and IGlar were met. There were no treatment differences in secondary efficacy or safety outcomes, including hypoglycemia, in the total population and in the insulin-naïve/prestudy IGlar subgroups. AE frequencies (LY IGlar, 52%; IGlar, 48%; p=.31) were similar. In conclusion, LY IGlar compared with IGlar in combination with OAMS provided equivalent efficacy and similar safety profile in patients with T2DM.

11. Prevalence of coeliac disease among patients with Type 1 diabetes in Kwa-Zulu Natal: results from the Durban Diabetes and Coeliac Disease (DDCD) study.

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Objective: To assess the prevalence of coeliac disease among patients with type 1 diabetes mellitus (T1DM) attending the outpatient tertiary diabetes clinic at Inkosi Albert Luthuli hospital (IALCH) in Durban, Kwa-Zulu Natal.

Methods: This was a cross-sectional observational study on all subjects with T1DM attending the adult diabetes clinic at IALCH. Information collected from all patients included history, clinical examination and laboratory tests. Blood tests included glutamic acid decarboxylase (GAD) antibody, anti-TPO antibody, anti-parietal antibody, coeliac antibodies, B12, folate, ferritin, TSH, fT4, creatinine, serum lipids, fructosamine and HbA1c. Patients with a positive coeliac antibody were invited to have upper endoscopy and duodenal biopsy. Diagnosis of coeliac disease was based on histological criteria using the modified Marsh classification.

Results: A total of 202 (M:F=90:112) patients were recruited; of these 114 (56.6%) were African, 64 (31.7%) Indian, 9 (4.5%) white, 15 (7.4%) Coloured. The mean age and mean duration of diabetes were 26.4±11.4 and 10.7±9.1 years respectively; the mean BMI was 21.6±6.3 kg/m2. GAD antibody was found in 63.4% (n=128). One or more positive coeliac antibodies were found in 65 (32.2%) patients: endomysial antibody, 7.4% (n=15); tissue transglutaminase antibody, 8.4% (n=17) and antigliadin antibody, 30.2% (n=61). Upper endoscopy and duodenal biopsy was performed in 51 (25.3%) patients. Duodenal biopsy specimens were classified according to the modified Marsh classification and 5 patients (2.5%) met the criteria for a diagnosis of coeliac disease.

Conclusion: Patients with T1DM frequently have co-existent positive coeliac antibodies, however a smaller proportion have histologically proven coeliac disease. Further research is required in South Africa to verify the findings of this study and aid in the development of guidelines for screening of coeliac disease among patients with T1DM.

12. The impact of a diabetes management team on the metabolic control and prevalence of complications in paediatric patients with type 1 diabetes mellitus

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Background: In various centres a diabetes management team (DMT) including a diabetes nurse educator (DNE), was found to have an impact on glycaemic control.

Hypothesis: A DMT improves HbA1c, DKA and recurrent DKA (rDKA) rate, and decreases hospital stay and prevalence of complications of type 1 diabetic (TIDM) patients.

Study design: Before-after study.

Methods: 190 TIDM patients attending the paediatric diabetic clinic at Tygerberg Children’s Hospital between August 2004 and July 2011 were reviewed. The following data were extracted: HbA1c, DKA admissions, length of hospital stay, clinic attendances, insulin regimen and dose, and complications. Four time periods were compared: P1 (paediatric endocrinologist only), P2 (introduction of DMT), P3 (introduction of DNE), and P4 (substitution of DNE).

Results: HbA1c increased from 9 (7.85-10.15)% in P1 to 10.9 (9.6-12.2)% in P2, and decreased to 9.25 (8.75-9.75)% in P4 (p=0.01818). DKA rate improved from 32.5 (P1) to 23.5/100 patient years in P4, rDKA rate improved from 18.8% (P1) to 9.6% (P4). Admissions decreased from 0.79 (0.46-1.12) in P1 TO 0.18 (0.02-0.34) in P4 (P=0.00127). Patients staying longer than 30 days decreased from 30% (P2) to 15.1% (P4). Number of insulin injections increased from 2.97 (2.91-3.03) in P1, to 3.06 (2.97-3.14) in P2 (p=0.0015). Few complications were documented in P1. Prevalence of microalbuminuria was similar (26.9-46.2%)
in all periods, as was retinopathy (10.3-13.3%). Detection of limited joint mobility increased from 0% (P1) to 42.9% (P4). Levels of triglycerides were similar in all periods, LDL cholesterol decreased to 2.6 (2.38-2.81) mmol/l in P3 and HDL cholesterol decreased to 1.38 (1.27-1.49) mmol/l in P4.

**Conclusions:** After introduction of the full DMT (including the DNE), HbA1c decreased and showed less variation, DKA and rDKA rate decreased, hospital stay shortened, number of insulin injections/day increased and complications were more readily identified. This suggests that a multi-disciplinary approach to the management of diabetes plays a big role in the improved outcomes of paediatric diabetic patients. It is therefore recommended that inexperienced personnel should not be entrusted with the care of diabetes patients. For optimal care, the full DMT should continue to perform its vital function. In particular, the service of the DNE is invaluable and every effort should be made to retain her position.

13. **Cyclopia maculata and pancreatic beta-cell protection in type 2 diabetes**

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Protecting the vulnerable β-cell in type 2 diabetes (T2D) is becoming increasingly important, making it a high priority therapeutic target. Preservation of β-cell viability, as well as function in limiting the progression of T2D may be achieved by ameliorating chronic β-cell stress (e.g., insulin hyper-secretory demand, hyperglycemia, and hypertriglyceridemia), which has been shown to cause β-cell dysfunction and eventual failure. Polyphenol-rich extracts of *Cyclopia* spp, containing mangiferin, may provide novel adjuvant therapies for β-cell preservation, due to their numerous health-promoting properties, including antioxidant and anti-inflammatory properties. An aqueous extract of unfermented *Cyclopia maculata* (honeybush), containing more than 6% mangiferin, was assessed for its protective effect on pancreatic β-cells in vitro, in RIN-5F cells, and in vivo, in streptozotocin (STZ)-induced diabetic Wistar rats. A reduction in fasting glucose levels and the area under the curve in the oral glucose tolerance test in rats pretreated with *C. maculata* extract indicated an amelioration of STZ-induced diabetes. Pre-treatment with extract also improved serum triglyceride levels and the glucose-to-insulin ratio, with increased β-cell area in islets and with a concomitant increase in β-cell proliferation at the higher extract dose. An in vitro triitated thymidine incorporation assay showed that the extract was not mitogenic in RIN-5F cells. Extract pre-treatment also reduced the STZ-induced elevation of plasma nitrite levels, with no changes observed in serum catalase, serum glutathione, liver lipid peroxidation and liver nitrotyrosine levels. Pre-treatment with the aqueous, unfermented *C. maculata* extract used in this study improved glucose metabolism in diabetic Wistar rats, with evidence of pancreatic β-cell protection both in vitro and in vivo.

14. **Investigating microRNAs for the early detection of type 2 diabetes**

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**Introduction:** MicroRNAs (miRNAs) are small non-coding RNAs that regulate many biological pathways, thus play a fundamental role in cellular function. Alterations in miRNA expression are implicated in metabolic dysregulation, with several studies reporting the involvement of miRNAs in the pathophysiology of type 2 diabetes (T2D). Moreover, accumulating miRNAs have recently attracted considerable interest as biomarkers to identify individuals at risk for developing T2D, or as prognostic biomarkers to assess therapeutic response.

**Aim:** In this study, miRNA regulation during T2D disease progression was investigated by miRNA expression profiling of type 2 diabetic, pre-diabetic and non-diabetic controls using next generation sequencing and quantitative real-time PCR (qRT-PCR).

**Methods:** miRNAs were extracted from the blood of age and gender matched diabetic, pre-diabetic and non-diabetic individuals of mixed ancestry origin. After confirmation of good quality RNA using nanodrop spectrophotometry and an Agilent bioanalyser, samples were sent to ArrayStar (Rockville, USA) for next generation sequencing. In addition, the expression of three miRNAs (miR-33a, miR-126 and miR-9) that had previously been reported to be associated with T2D was quantified in these samples using qRT-PCR.

**Results:** Biochemical characteristics of study participants were as follows: Fasting Plasma Glucose concentrations (FPG) were 7.5 ± 0.9 vs. 5.4 ± 0.2 vs. 5.0 ± 0.1 mmol/L, glycated haemoglobin A1c (HbA1c) concentrations were 7.2 ± 0.8 vs. 5.3 ± 0.2 vs. 5.3 ± 0.1 and the area under the curve for the oral glucose tolerance test (OGTT) values were 15.0 ± 1.6 vs. 9.1 ± 0.2 vs. 5.0 ± 0.6 for diabetics, pre-diabetics and controls, respectively. Of the three miRNAs investigated, only miR-9 was differentially expressed. Levels of miR-9 were significantly higher in pre-diabetic compared to diabetic individuals (1.84-fold; p < 0.05). miRNA-9 is reported to induce the expression of inflammatory genes, suggesting that pre-diabetes is associated with increased inflammation compared to diabetes, a theory that has previously been suggested. MiRNA sequencing is currently being conducted and the results will be presented.

**Conclusion:** We optimistically expect that miRNA expression profiles will differ between type 2 diabetic, pre-diabetic and controls, and that these differences could be used as potential, minimally invasive biomarkers for the prediction of T2D risk.

15. **Pulmonary hypertension in patients with hyperthyroidism in a tertiary South African Hospital**

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**Objective:** Pulmonary hypertension is a complex disorder with multiple aetiologies. A few case reports and observational studies have documented hyperthyroidism as a cause for...
pulmonary hypertension, as well as the improvement of pulmonary pressures once the patients were treated for the thyroid disease. The objectives of the study were to i) document the incidence of pulmonary hypertension in patients with newly diagnosed hyperthyroidism at Steve Biko Academic hospital, ii) to document the change in pulmonary pressures in patients with both hyperthyroidism and pulmonary hypertension from hyperthyroid to euthyroid state, iii) to compare South African data with international literature.

**Method:** A prospective, observational study was performed in patients with newly diagnosed hyperthyroidism that presented to Steve Biko Academic hospital between September 2012 to March 2014. A transthoracic echocardiogram was performed on all patients to document their pulmonary arterial pressure (PAP). Patients were diagnosed with pulmonary hypertension if they had a PAP of more than 25mmHg. Patients with documented pulmonary hypertension underwent standard investigations to rule out other causes of pulmonary hypertension, and the transthoracic echo was repeated once their TSH value normalised.

**Results:** Of the 41 patients (8 males and 33 females) enrolled in the study, 25 (61%) had echocardiographic evidence of pulmonary hypertension. Seventy percent of females compared to 8% of males had pulmonary hypertension. Sixty eight percent of black females were diagnosed with pulmonary hypertension, compared to 77% of white patients enrolled. Both males with pulmonary hypertension were white. A reduction in pulmonary pressures was documented in 24(96%) of 25 patients once a euthyroid state was documented by a normal TSH value.

**Conclusion:** The incidence of pulmonary hypertension in patients with newly diagnosed hyperthyroidism was 61%. The female predominance and incidence of pulmonary hypertension correlates with international literature, although a gender disparity was noted. Hyperthyroidism is a reversible cause of pulmonary hypertension in our South African cohort.

**16. Reduced salivary cortisone, but similar cortisol day curves in Addison’s disease in patients on hydrocortisone replacement**

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**Background:** Salivary cortisol, as measured using electrochemiluminescence has been used to monitor patients with Addison’s disease (AD) on hydrocortisone replacement. Salivary cortisone has been suggested as an alternative to salivary cortisol, as it may accurately reflect plasma free cortisol. We wished to examine the pharmacokinetics of these analytes in patients and controls.

**Methods:** We measured salivary cortisol and salivary cortisone by liquid chromatography-tandem mass spectrometry, using a day curve with 16 time points in patients with AD on hydrocortisone and in healthy controls with endogenous cortisol secretion.

**Results:** There were 25 patients and 26 healthy controls. The median (interquartile range) area under the curve (AUC) for cortisol was not different for patients, 55.63 (32.91-151.07), compared with controls 37.49 (27.41-52.00) nmol*min*L\(^{-1}\); \(p=0.098\), whereas the peak cortisol (C\(_{\text{peak}}\)) was higher 32.61 (5.75-146.19) versus 8.96 (6.96-12.23) nmol/L; \(p=0.013\) and time to peak (T\(_{\text{peak}}\)) 1.5 (0.5-2.0), compared with controls 0.0 (0.0-0.5) hours; \(p<0.001\). The AUC for cortisone was reduced for patients, 23.65 (6.10-54.76), compared with controls, 227.73 (200.10-280.52) nmol*min*L\(^{-1}\); \(p=<0.001\), C\(_{\text{peak}}\) for cortisone was lower 11.11 (2.91-35.85) versus 33.12 (25.97-39.95) nmol/L; \(p=0.002\) and longer T\(_{\text{max}}\) 2.5 (2.0-9.5), compared with 0.25 (0.00-0.50) hours, respectively; \(p<0.001\).

**Conclusions:** Although there was no difference in cortisol exposure, there was less cortisone exposure in patients than controls, possibly due to the pharmacological effect of hydrocortisone on the 11-beta-hydroxysteroid dehydrogenase enzymes. Lack of cortisone exposure and less metabolism of cortisol in patients with AD may predispose to metabolic consequences.

**17. Obesity-related plasma metabolite profiles of black women spanning the epidemiologic transition**

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**Objective:** In developed countries, specific metabolites have been associated with obesity and metabolic diseases, e.g. type 2 diabetes. It is unknown whether a similar profile persists across populations of African-origin, at increased risk for obesity and related diseases.

**Methods:** In a cross-sectional study of normal-weight and obese black women (33.3±6.3yr) from the United States of America (US, N=69, 65% obese), South Africa (SA, N=97, 49% obese) and Ghana (N=82, 33% obese) serum metabolite profiles were characterized via gas chromatography-time of flight/mass spectrometry.

**Results:** In US and SA women, BMI correlated with branched-chain and aromatic amino acids, as well as dopamine and aminoadipic acid. The relationship between BMI and lipid metabolites differed by site; BMI correlated positively with palmitoleic acid (16:1) in the US; negatively with stearic acid (18:0) in SA, and positively with arachidonic acid (20:4) in Ghana. BMI was also positively associated with sugar-related metabolites in the US; i.e. uric acid, and mannitol, and with glucosamine, glucoronic acid and mannitol in SA.

**Conclusion:** While we identified a common amino acid metabolite profile associated with obesity in black women from the US and SA, we also found site-specific obesity-related metabolite suggesting that the local environment is a key moderator of obesity.
18. Expression of genes involved in melanin synthesis and oxidative stress in adipose tissue of black and white South African women and its association with insulin

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Introduction: We have previously shown that for a given BMI, black women are more insulin resistant than their white counterparts. However, the mechanisms are unclear. It has been proposed that increased oxidative stress may be one of the mechanisms involved. Melanin has been recently observed in visceral adipose tissue of obese subjects and is hypothesised to serve as an antioxidant in adipose tissue. Thus the aim of this study is to determine whether oxidative stress and antioxidant genes are expressed in adipose tissue, and whether this is associated with insulin sensitivity in black and white, normal-weight and obese South African (SA) women.

Methods: Sixty normal-weight and obese, black and white SA women, were measured for body composition (DXA) and insulin sensitivity (SI, FSIGT). Total RNA was isolated from the gluteal (GLUT), superficial and deep abdominal subcutaneous adipose tissue (SSAT, DSAT) depots. The expression of melanin synthesis-related genes: Microphthalmia-associated transcription factor (MITF), Tyrosinase related protein 1 (TYRP1), Dopachrome tautomerase (DCT); MITF and DCT was positively associated with S, in white women only, independent of fat mass (kg) (r=0.76, p=0.003 and r=0.64, p=0.018). GLUT expression of TYRP1 was negatively associated with insulin sensitivity (S) in black women only (r=0.44, p=0.035). DSAT expression of eNOS was positively associated with DCT and MITF in both ethnic groups (r=0.55, p=0.033 ; r=0.58, p=0.02 and r=0.72 p=0.001 ; r=0.82 p<0.001, respectively).

Conclusion: We demonstrate, for the first time that melanin synthesis genes are expressed in the SAT of black and white SA women, and that this expression differed by BMI and ethnicity. The results may also suggest that ethnic differences in S are associated with ethnic differences in melanin and antioxidant gene expression.

19. Audit of standard of care measures and complications in a tertiary type 1 diabetic (DM1) clinic.

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Background: Practice guidelines for the care of diabetics assist clinicians to deliver appropriate standards of care. Regular clinic audits are recommended to review usage of preventive services and assess severity of complications.

Aims: (1) Conduct an audit of the quality of care delivered to adult diabetics who attend our DM1 clinic. (2) Characterize their burden of illness.

Methods: A retrospective chart review of all patients attending the DM1 clinic in 2014 was performed.

Results: 174 patients (54% female), mean age 29.5 years attended the clinic during the 12 month period; totaling 455 patient visits. Of these, 131 (75%) had a lipogram, 74 (43%) a serum creatinine, and 47 (27%) a serum TSH performed. One patient was offered an HIV test. 159 (91%) patients received diabetic education. Three patients had dental health assessed. No patients were offered an influenza vaccine. Contraceptive use was addressed at 107 (45%) visits and foot care was assessed at 261 (61%) visits. Home glucose monitoring differed significantly between the genders: 53% of females performed monitoring compared to 36.8% of males (p=0.001). Metabolic control indices revealed 67% of HbA1Cs > 9% and only 7.8% of HbA1Cs ≤ 7%. 92% of systolic blood pressures ≤ 140 mmHg, and 80% of diastolic blood pressures ≤ 80 mmHg. 86% of triglycerides were ≤1.7 mmol/l, and 49% of LDLs were <2.5 mmol/l. Twenty eight patients who were asked about smoking, admitted to this habit. Target organ damage was present as follows: 18 (5%) of patients had proliferative retinopathy, 83 (20%) had evidence of renal impairment, 69 (28%) had peripheral neuropathy. Five patients had some form of macrovascular disease.

Conclusion: At our DM1 clinic, compliance with routine quality indicators e.g. measuring annual creatinine and TSH, as well as addressing associated health issues e.g. dental health, tobacco use, vaccinations, and contraception needs improvement. In addition, the majority of our patients have poor glycaemic control with less than 10% at A1C target. As a result of this audit, quality improvement interventions have been initiated.

20. Deoxyribonucleic acid (DNA) methylation and dysglyceamia in the Cape Town Bellville South population

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Introduction: DNA methylation is an epigenetic modification with potential implications in the pathophysiology of type 2 diabetes mellitus (T2DM). These epigenetic changes reflect genetic predisposition and environmental influences, and have potential applications for disease risk stratification or to monitor therapeutic response.
**21. Diabetes and hypertension screening in Zandspruit, Johannesburg 2012-2014.**

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**Background:** The Lilly Non Communicable Diseases (NCD) Partnership Project includes Project Hope (NGO) with the University of Pretoria as partner aiming to improve health care for people with NCD in the Zandspruit area, Johannesburg. As part of a NCD awareness campaign blood pressure and glucose screening was done between 2012 and 2014.

**Methods:** From June 2012 until June 2013, Project HOPE conducted mobile screening days, including door-to-door week-long campaigns (one five-day campaign per month from January to June 2013). Door-to-door campaigns and screening gazebos covered all major entry and exit points of the community to ensure visibility in Zandspruit. In 2014 Screenings were conducted by setting up gazebos in busy areas within the community instead of door-to-door.

**Results:** A total of 1099 and 1161 regions were hypermethylated in diabetics compared to prediabetics and controls respectively, while 223 and 255 regions were hypomethylated in diabetics compared to prediabetics and controls, respectively. Prediabetics had a total of 287 differentially methylated regions (DMRs) compared to controls, of which 124 were hypermethylated and 163 were hypomethylated. Many of these DMRs were identified in T2D causal genes such as Glucokinase (GCK), hepatocyte nuclear factor (HNF1A) and pancreatic and duodenal homeobox 1 (PDX-1), while other DMRs were identified in regulatory and biologically relevant pathways such as Wnt signaling, carbohydrate and glucose transport, and pancreatic development. Moreover, a few DMRs were progressively methylated across worsening stages of dysglycemia, or were specific to the prediabetic or diabetic group.

**Conclusion:** Functionally significant DMRs could be incorporated into algorithms to facilitate risk stratification, especially since traditional risk factors for T2DM have low predictive power in this population. Identifying individuals who will benefit from disease modifying intervention strategies could assist in decreasing the disease burden. Moreover, recent reports of DNA methylation changes in urine support the use of DNA methylation as a novel non-invasive marker to identify those individuals with a high risk of developing T2D.

**Source of funding:** The South African Medical Research Council and National Research Foundation
Conclusion: South Africa has several populations with founder effects for FH and familial chylomicronaemia. FH accounted for about 75% of all presentations to our paediatric lipid clinic. Homozygous FH accounted for 9% of all paediatric FH presentations reflecting earlier diagnosis due to its characteristic phenotype with cutaneous xanthomata. Appropriate dietary and drug therapy of paediatric lipid disorders may be lifesaving, but requires an accurate diagnosis and specialized therapeutic knowledge. It is important to preserve the necessary clinical and laboratory expertise in South Africa so that paediatric lipid disorders can continue to be managed appropriately.

23. Peroxidation status of edible oils in South Africa

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PUFA of the n-3 and n-6 series play important roles in the brain, skin and cardiovascular system. Ingestion of these fatty acids is important. However, unsaturated fats are susceptible to oxidation that leads to a range of products: conjugated dienes (CD), lipid hydroperoxides (LOOH) and thiobarbituric acid reactive substances (TBARS). These products may harm protein and DNA. Aim: To determine the concentration of oxidation products in edible oils, before and after heating, and to measure their extractable antioxidant capacity.

Study design: 31 oils (from olives, sunflower, canola, grapeseed, peanut, sesame, salmon, flax, coconut and palm) were purchased from retail outlets. Oils were classified by their predominant content as saturated (SFA, n=3), monounsaturated (MUFA, n=19), and polyunsaturated (PUFA, n=9) fatty acids.

Methods: The analyses were undertaken before and after heating the oils. CD and TBARS concentrations were calculated by molar extinction coefficient. LOOH were determined by the xylenol orange assay with butylhydroperoxide as standard. The Oxygen Radical Absorbance Capacity (ORAC) of a methanolic extract of each oil was determined using trolox as reference.

Results: The mean ± SD of oxidation markers before and after heating in the SFA group for CD, LOOH, TBARS and ORAC were 5.54 ± 0.95 [µmol/g and 10.93 ± 3.95 [µmol/g], 2.57 ± 1.41 [µmol/g] and 36.40 ± 22.07 [µmol/g], 3.85 ± 0.08 [nmol/g] and 3.30 ± 0.01 [nmol/g], 10.40 ± 2.45 [µmol/g] and 9.52 ± 1.80 [µmol/g] respectively. MUFA oils were 11.47 ± 1.56 [µmol/g] and 26.15 ± 7.60 [µmol/g], 35.35 ± 3.69 [µmol/g] and 75.21 ± 11.92 [µmol/g], 8.89 ± 1.19 [nmol/g] and 22.61 ± 5.38 [nmol/g], 79.98 ± 22.18 [µmol/g] and 86.61 ± 19.30 [µmol/g], and PUFA oils were 20.09 ± 3.75 [µmol/g] and 44.85 ± 16.69 [µmol/g], 7.31 ±9.62 [µmol/g] and 84.25 ± 13.25 [µmol/g], 26.81 ± 20.31 [nmol/g] and 35.66 ± 21.95 [nmol/g], 2.57 ± 8.07 [µmol/g] and 28.63 ± 9.88 [µmol/g] respectively.

Conclusion: SFA oils had low oxidation markers but heating generated LOOH. MUFA oils had lower CD but similar other markers to PUFA oils at baseline. Heating MUFA and PUFA oils increased all markers significantly but to similar concentrations for LOOH and TBARS. Salmon oil had high TBARS that heating increased similarly to other PUFA. Extractable ORAC values varied considerably but were not different between MUFA and PUFA. Further studies are required to define the harmful oxidation product(s) and their mechanism of action so that production, storage and consumption of oils can be optimised for health.

24. Modulation of lipid metabolism as a possible tool to attenuate the effects of the mycotoxin fumonisin B1

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Fumonisin B1 (FB1) was classified as a group 2B carcinogen based on toxicity and carcinogenicity studies in rats and mice and its carcinogenic effects were linked to changes in phospholipids, sphingolipids, fatty acids (FA) and cholesterol. Similarities and differences of lipid parameters were investigated using the mycotoxin FB1, and the 2-acetylaminofluorene/partial hepatectomy (AAF/PH) treatments as cancer promoters in rat liver. A typical lipid phenotype was observed, including increased membranal phosphatidylethanolamine (PE) and cholesterol content, increased levels of C16:0 and monounsaturated fatty acids in PE and phosphatidylcholine (PC), as well as a decrease in C18:0 and long-chained polyunsaturated fatty acids (PUFA) in the PC fraction. Insight into complex lipid profiles induced by two different cancer promoting treatments and their potential role in the development of hepatocyte nodules was used to identify targets for the development of chemopreventive strategies against cancer promotion in rat liver. Different dietary ω6/ω3 FA ratios were tested for their potential to reduce FB1-induced pre-neoplastic liver lesions in male Fischer 344 rats. Sunflower oil (ω6/ω3 ratio 700:1) or sunflower oil supplemented with C20:5ω3 and C18:3ω6 (SEG diet; ω6/ω3 ratio 6:1) served as dietary fat sources during short-term initiation/promotion protocols, utilising diethylnitrosamine (200mg/kg body weight) as cancer initiator and FB1 as promoter (250 mg/kg diet). The SEG diet feeding replaced long chain ω6 PUFA with ω3 counterparts, resulting in an increase in total long chain PUFA content, especially in the phospholipid PE. A biphasic effect of the SEG diet towards FB1-induced hepatocarcinogenesis was observed depending on the specific phase of carcinogenesis: i.e. the SEG diet may decrease toxicity required for FB1 induced initiation, but also provides stimuli that act in synergism with FB1, during cancer promotion. The interactions between FB1 and dietary fatty acids provide challenges and opportunities to elucidate mechanisms involved in cancer development in humans.

25. Heterozygous Familial Hypercholesterolaemia (FH): the Groote Schuur Hospital Lipid Clinic experience.

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Familial hypercholesterolaemia is a common autosomal dominantly inherited clinical phenotype with severe low
density lipoprotein (LDL) hypercholesterolaemia that is associated with tendon xanthomata and typically results in premature coronary artery disease. While the cause was identified to be mutations in the LDL receptor and founders for these mutations were documented in South Africa, two further genes were identified to cause the same clinical phenotype: familial binding defective apoB100 and proprotein convertase subtilisin/kexin type 9 (PCSK9). The clinical phenotype being identified, investigations were done on consenting patients’ DNA to identify mutations in the LDL receptor, apoB and PCSK9 genes. Previously identified common mutations were sought before an exon-by-exon approach was undertaken to identify mutations by sequencing heteroduplex formation and single strand conformational polymorphism and later by high resolution melting. Mutations are confirmed by restriction enzyme digests or sequencing. Large insertions and deletions have not been investigated fully. In all 1182 patients have had confirmation of a genetic cause for FH. Pathogenic mutations have been identified in all 3 genes: 87 in the LDL receptor, 4 in apoB, and 4 known gain of function mutations in PCSK9. An additional 8 mutations in PCSK9 are under investigation to confirm their pathogenic role. The LDL receptor mutations involve all exons except for 15 and 18; most mutations (16) are in exon 4, the binding domain, while exons 7,8 and 9 contain 8, 8 and 9 mutations respectively. The 10 commonest LDL receptor mutations account for 1012 cases. In descending order and using the previous designation of the sequence they are: D200E, V408M, D154N, D200G, del 197, G361V, C356Y, R329X, F382S and E207K. The apoB mutations are in exon 26: R3500Q, R3500W, R3531C, H3543Y. The PCSK9 mutations are S127R, R469W, R496W, H553R. Only a few mutations appear restricted to specific population groups but regional enrichments of specific mutations have been noted. Whilst genotyping is not essential for the diagnosis of the phenotype and decision about management, an accurate genetic diagnosis is preferable and does improve cascade screening where LDL concentrations are ambiguous and is especially useful in considering risk of having homozygous offspring.

26. An analysis of gradient gel electrophoresis (GGE) patterns in dysbetalipoproteinemia

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Dysbetalipoproteinemia is an uncommon but severe dyslipoproteinemia resulting from a predisposing genotype but can only be diagnosed by ultracentrifugation or electrophoresis of lipoproteins. It was previously shown that a non-denaturing gradient (polyacrylamide) electrophoresis had excellent sensitivity and specificity for this disorder. The aim of this study was to review the electrophoresis patterns typical of and confirmed to be due to dysbetalipoproteinemia on record in patients attending a referral lipid clinic.

Altogether 101 subjects (60 women) had 177 studies, all at first presentation and 44 had additional studies. The causal mutations were apoE2/2 (87), apoE R145C (18) and apoE K146Q (1). The 3-8% acrylamide gel was run overnight with fasted plasma pre-stained with Sudan Black. The retention factors (RF) had been calibrated previously with ultracentrifugally prepared lipoproteins in which small dense LDL was assigned the value of 1.0. Intermediate density lipoproteins had 0.70 < RF < 0.85. The RF was calculated for the lipoproteins from the large through a peak and to the small end of the range.

The pattern was diagnostic of dysbetalipoproteinemia. LDL particles were absent in 75% and the remainder had small amounts (17% large, 32% intermediate and 52% small LDL). In 114 with complete lipid investigations, the plasma triglyceride and cholesterol concentrations were 4.6 ± 3.2 and 8.2 ± 4.1 mmol/L respectively (mean, standard deviation). There was no significant difference in the lipid profile and electrophoresis patterns in the different genotypes. The RF of the dominant lipoproteins ranged from 0.08 to 0.73, indicating a range of lipoproteins from chylomicron to LDL (RF >0.85); the peak being 0.64 ± 0.10 [mean, standard deviation]. The triglyceride concentration correlated inversely with the RF (r=0.001, r² =0.20 for peaks) indicating larger lipoproteins exist with hypertriglyceridaemia.

GGE is an excellent screening test for dysbetalipoproteinemia that has replaced ultracentrifugation and is appropriate before embarking on genotyping to confirm dysbetalipoproteinemia.

27. Prevalence and pattern of dyslipidaemia in type 2 Diabetes Mellitus patients

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Diabetes mellitus (DM) is a common secondary cause of dyslipidaemia, particularly, if glycaemic control is poor, which in-turn is an important risk factor for atherosclerosis and coronary artery disease. The spectrum of dyslipidaemia in DM can include any and all the various types of dyslipidaemia.

Objectives: 1) To study the prevalence and pattern of dyslipidaemia in type 2 DM patients. 2) To determine the relationship (if any) between HbA1C and the lipid profile in type 2 DM.

Methods: This was a cross sectional study, done on 200 type 2 diabetic patients attending the Diabetic Clinic at the Helen Joseph Hospital. Patients suffering from other known causes of secondary dyslipidaemia were excluded. Each patient’s HbA1c, and lipidogram results were noted from their clinic files. The lipid profile included: Total cholesterol (TC), Triglyceride (TAG), High density lipoprotein cholesterol (HDL-C) and calculated low density lipoprotein cholesterol (LDL-C).

Patients having one or more of the above parameters outside the targets recommended by the, South African Lipid Guidelines were considered to have dyslipidaemia.

Results: A total of 200 type 2 DM patients (86 males and 114 females) were studied. Prevalence of dyslipidaemia amongst diabetics was 93.5% (187 out of the 200 patients). Amongst males it was 43%, and in females it was 57%, despite the use of lipid-lowering (statin therapy, with a mean dose of 20mg) in 187 patients.
The most common pattern of dyslipidaemia was a combined dyslipidaemia [two or more lipid abnormalities] affecting a total of 82 patients (43.8%). Of this, 47.5% were males and 52.4% were females. The most common pattern of combined dyslipidaemia in males were low HDL (≤1mmol/l) and elevated LDL levels (≥1.8mmol/l) (24.4%) and in females was an elevated TAG (≥1.7mmol/l) in combination with an elevated LDL level (28.8%).

**Conclusion:** The majority of the type 2 diabetic patients studied, had dyslipidaemia despite the use of lipid lowering therapy in all patients. The most common pattern of dyslipidaemia among males was combined dyslipidaemia with high LDL and low HDL and in females was high LDL and TAG levels. The most prevalent lipid abnormality was a high LDL (≥1.8mmol/l) followed by a low HDL (≤1.0mmol/l for males and ≤1.2mmol/l for females). No significant relationship was found between HbA1c and lipid levels.

### 28. Telomere Length of Circulating Leukocytes in Patients with Homozygous Familial Hypercholesterolemia

**Objective:** Homozygous familial hypercholesterolemia (HoFH) is characterized by severe elevations in serum total and LDL cholesterol levels, with increased morbidity and mortality consequent to premature and extensive atherosclerotic cardiovascular disease. Oxidative stress and inflammation are important role players in the pathogenesis of atherosclerosis. These factors in turn, have been identified as key contributors to accelerated telomere attrition. This study addresses the hypothesis that patients with HoFH have shorter leucocyte telomere lengths compared to healthy, age and gender matched individuals, in view of its common association with atherosclerosis and cardiovascular disease.

**Methods:** Individuals attending the Charlotte Maxeke Johannesburg Academic Hospital Lipid Clinic, with genetically and/or clinically confirmed HoFH were invited to participate in this cross-sectional study. Each patient's clinical and biochemical variables were compared to those of two healthy ethnic, age and gender matched participants from the community. Leukocyte telomere lengths of patients and participants were estimated by quantitative polymerase chain reaction.

**Results:** Thirty five patients (19 males and 16 females) with HoFH and 67 healthy subjects were included in this study. The HoFH subjects all met clinical criteria for HoFH which was confirmed genetically in 91% (32 out of 35). All patients were treated with high potency statins (atorvastatin 80mg) at the time of the study, with the addition of ezetimibe in 91% of patients. The average duration of statin use was 11 years, with no incident detection of type 2 diabetes mellitus during this period. The mean serum total cholesterol and LDL-cholesterol levels were 12mmol/l and 10.5mmol/l respectively. There was no significant difference in leucocyte telomere length between the HoFH patients and the healthy controls.

**Conclusion:** This is the first reported study comparing leucocyte telomere lengths in patients with HoFH to that of healthy ethnic, age and gender matched control subjects. In contrast to other related telomere studies, we report no significant reduction in telomere length, secondary to hypercholesterolemia and atherosclerosis. In view of these findings, the pleiotropic effect of long term statin use on telomere biology requires further investigation.