

## 52<sup>nd</sup> SEMDSA Congress - Abstracts Oral Presentations

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### KP1. New technology in diabetes

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### OP1. Comparing screening strategies for gestational diabetes in a South African population

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**Background:** Gestational diabetes (GDM) is carbohydrate intolerance first recognised in pregnancy. Globally there is an alarming increase in type 2 diabetes mellitus and obesity. The incidence of GDM parallels this increase. Women who develop GDM are at risk of adverse pregnancy outcomes as well as the long-term risk of developing type 2 diabetes. Likewise, the foetus and neonate is at risk of short- and long-term adverse outcomes.

The diagnosis of GDM has historically been shrouded in controversy. In late 2015 the WHO adopted the IADPSG criteria for the diagnosis of GDM. They recommended universal screening of all pregnant women with diagnosis made on the following thresholds: any one abnormal value on the 75 g-2 hour OGTT - 0hour  $\geq 5.1$  mmol/l, 1 hour  $\geq 10$  mmol/l, 2 hour  $\geq 8.5$  mmol/l.

We conducted a prospective cohort observational study at the Eyethu Yarona Clinic (Lion Park Clinic). We recruited pregnant patients less than 26 weeks pregnant. Demographic data, random glucose and HbA<sub>1c</sub> was collected at recruitment. OGTT were conducted at 24-28 weeks of gestation and GDM was diagnosed according to WHO criteria.

**Aims and Objectives:** Our aim was to determine an effective, cost-efficient screening strategy for GDM.

Establish the prevalence of GDM based on the current WHO criteria and compare this to the prevalence if alternate criteria were applied

Identify the role of risk factors in screening for GDM and to develop a scoring system based on baseline characteristics to identify patients at high risk of GDM

Evaluate the role of HbA<sub>1c</sub> in screening for GDM

Evaluate the role of point of care testing in the diagnosis of GDM/OGTT

To examine the association between biomarkers (CRP, insulin, adiponectin) and microRNAs, and GDM.

**Results:** We recruited 1000 patients. We had complete data for 554 patients available for analysis. The prevalence of GDM was 25.8% if universal screening and the WHO criteria was applied. If selective screening was applied the prevalence only 254 women would have had an OGTT and the prevalence was 15.2%. The commonly used NICE criteria had a prevalence of 17% with universal screening and 3.6% with selective screening.

The presence of risk factors performed poorly as a screening tool. The presence of one or more risk factors (maternal age > 35 years, BMI > 30 kg/m<sup>2</sup>, previous stillbirth, previous baby > 4 kg, family history of diabetes mellitus) performed poorly as a screening tool with a sensitivity of 58.7% and specificity of 58.6%. The application of published risk factor-based scoring systems also performed poorly on our low risk obstetric population. We propose a scoring system based on maternal BMI, birth of previous baby > 4kg, and random glucose. This will require prospective validation.

HbA<sub>1c</sub> performed poorly as a screening or diagnostic tool for GDM. However, the majority of patients were diagnosed as GDM based on the fasting glucose and thus the fasting glucose may be considered as an alternative diagnostic test.

The glucometer is a commonly used device for managing patients with diabetes mellitus. The Roche Accucheck Active glucometer performed poorly when used for the diagnosis of GDM with a sensitivity of 27% and specificity of 89.4%.

For the evaluation of biomarkers only HIV negative patients were considered. Three hundred and eighty-nine women were included in a nested case control trial. There was a significant difference between the GDM and non-GDM groups for fasting glucose ( $p=0.0000$ ), fasting insulin ( $p=0.0003$ ), and adiponectin ( $p=0.0460$ ).

**Conclusion:** The prevalence of GDM in South Africa is higher than anticipated. There is a need to develop a standardised national screening protocol that is effective and cost efficient. The use of fasting glucose needs to be considered as a diagnostic test to avoid the cumbersome OGTT. Other glucometers need to be evaluated as a point of care device would make the screening of GDM more timeous, efficient, and cost-effective. Biomarkers hold promise as a screening tool and their potential needs to be investigated further, possibly as a screening tool for GDM in early pregnancy.

### OP2. The prevalence of gestational diabetes amongst women living in Soweto, South Africa

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**Background:** South Africa has an emerging obesity epidemic with 31% of urban and 21% of rural women being classified as obese. Along with obesity, type 2 diabetes is on the rise and, most likely, so is gestational diabetes mellitus (GDM). GDM is defined as diabetes diagnosed for the first time in a woman during pregnancy. GDM can have adverse effects on the mother and the developing foetus and adds to the already heavily-burdened healthcare system in our country. Little is known about the prevalence of GDM in South Africa with only four studies having ever been published, only one of which involved black South African women and the use of the oral glucose tolerance test (OGTT) - the golden standard for diagnosing GDM. That study produced a GDM prevalence of 8.8% in rural Limpopo. Universal screening for GDM is not performed in the state hospital sector in Johannesburg; women are selectively screened for GDM only if they have certain risk factors. The aim of our study was to determine the prevalence of GDM amongst women living in urban Soweto, Johannesburg.

**Methods:** Black South African women, 18 years of age or older and less than 24 weeks pregnant with singleton pregnancies, were recruited from the antenatal clinic at Chris Hani Baragwanath Academic Hospital and invited to participate in the study. Women with pre-existing diabetes were excluded from the study. A total of 1805 women were recruited into the study. At 24-28 weeks gestation they underwent a two-hour 75 g OGTT. The International Association of the Diabetes and Pregnancy Study Groups (IADPSG) criteria for diagnosing GDM were used. One or more of the following glucose thresholds had to be equalled or exceeded for GDM to be diagnosed: fasting glucose of 5.1 mmol/l; 1-hour post glucose load of 10 mmol/l; 2-hour post-glucose load of 8.5 mmol/l. Women diagnosed with GDM were referred for further management and treatment to the Diabetic Clinic at Chris Hani Baragwanath Academic Hospital.

**Results:** A total of 175 women were diagnosed as having GDM. The prevalence of GDM amongst black women living in Soweto was calculated as 9.7%. The majority of GDM cases had one abnormal OGTT reading, usually a fasting glucose of > 5.1 mmol/l. Management of the women involved dietary modifications and some required metformin.

**Conclusions:** The prevalence of GDM amongst black South African women in urban Soweto is of concern. In addition, the majority of these women were not flagged through their regular antenatal check-ups for GDM screening and would therefore have missed being diagnosed. In a country where diabetes is becoming a serious problem, it might be worth considering universal screening for GDM, as opposed to selective screening, in order to diagnose and manage more women optimally.

### OP3. The co-existence of HIV infection and obesity with risk of gestational diabetes mellitus in black Africans in Sub Saharan Africa

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**Background:** Whilst coping with HIV, Africa is facing increasing prevalence of obesity and Type-2 diabetes mellitus (T2D). Obesity coupled with HIV infection and combination anti-retroviral therapy (cART) might, through heightened insulin resistance, enhance the risk of gestational diabetes mellitus (GDM).

**Methods:** We recruited 1070 black African pregnant women living in Johannesburg, South Africa < 14 weeks' gestation and assessed HIV, cART status and body mass index (BMI). Between 24-28 weeks' gestation we screened for GDM (oral glucose tolerance test; International Association of the Diabetes and Pregnancy Study Groups diagnostic criteria).

**Results:** HIV-negative, obese women (BMI  $\geq$  30 kg/m<sup>2</sup>) had greater risk of developing GDM (OR 2.5; 95% CI 1.2 - 5.4) than HIV-negative, normal weight women. Diagnosis of HIV in early pregnancy and initiation of cART treatment was associated with more than double the risk of GDM in obese women (OR 5.9; 95% CI 2.3 - 15.3). This risk was mitigated if HIV was diagnosed, and cART prescribed, before pregnancy (OR 1.9; 95% CI 0.6 - 6.4). Adjustment for age, socio-economic status and parity, attenuated GDM risk in obese HIV-positive women with antenatal cART therapy, but the odds

of developing the disorder remained twice that (OR 4.8; 95% CI 1.8 - 12.7) of HIV-negative obese women.

**Conclusions:** Despite early diagnosis of HIV and the initiation of cART in obese subjects during pregnancy, these women may be at significant risk for GDM, which could contribute to an accelerated risk of T2D in the mothers and potentially the next generation.

### OP4. Gestational Diabetes: Prevalence and predictive factors for sustained dysglycaemia at 6-12 weeks follow up.

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**Background:** The prevalence of gestational diabetes mellitus (GDM), like the prevalence of diabetes (DM) and pre-diabetes, is on the rise. GDM is a well-recognised risk factor for developing future DM. In resource-limited settings, pregnancy is often the first formal exposure to healthcare. This may lead to the identification of glucose abnormalities that may have, in fact, been present before pregnancy. Dysglycaemia of first onset in pregnancy usually subsides post-partum but is usually sustained in the undiagnosed diabetic patient. The identification of risk factors for sustained and/or recurrent dysglycaemia after GDM will assist in more accurate risk stratification of patients during pregnancy. This may lead to more appropriate and cost-effective surveillance of this high risk group post-partum.

**Aims:** To determine the prevalence of dysglycaemia in the cohort of patients diagnosed with diabetes in pregnancy, by means of oral glucose tolerance testing at 6-12 weeks postpartum.

To identify historical, clinical and biochemical parameters that may predict DM after pregnancy in patients diagnosed with diabetes in pregnancy.

**Methods:** The study was conducted prospectively at the post-partum diabetic clinic at Tygerberg Academic Hospital over a period of 12 months. Patients diagnosed with diabetes in pregnancy were evaluated consecutively by means of a standard OGTT (oral glucose tolerance test) and HbA<sub>1c</sub> at 6-12 week follow up. Patients known with pre-gestational DM were excluded.

**Results:** The average duration from delivery to postpartum evaluation was 7.5 weeks (SD $\pm$ 2.3). Forty six percent were found to be dysglycaemic at follow-up. DM was diagnosed in 21 patients (27%) and pre-diabetes in 15 (19%). Thirty seven percent had at least one plasma glucose value in keeping with overt DM at diagnosis in pregnancy. Of these half were diagnosed with DM postpartum and 14% with pre-diabetes. Fasting plasma glucose (FPG) identified the majority with DM postpartum. The concomitant HbA<sub>1c</sub> correctly identified 20 of the 21 patients with DM. The factors significantly associated with DM were ( $p < 0.05$ ): Gestation < 24 weeks at diagnosis (OR 11.75), FPG > 5.1 (OR 5.72) and HbA<sub>1c</sub>  $\geq$  6.2% (OR 3.97) at diagnosis; any insulin use HbA<sub>1c</sub>  $\geq$  6.2% at delivery (OR 5.07), age > 36 years (OR 3.1), family history of DM (OR 2.98) as well as pre-term labour (OR 3.14). HbA<sub>1c</sub> at diagnosis  $\geq$  6.2% (OR 14.8), any insulin use (OR 4.7), gestation at diagnosis < 24 weeks (OR 3.85) and elevated FPG > 5.1 at diagnosis (OR 1.5) retained the significance with multivariate analysis.

**Conclusion:** Half of the women with glucose abnormalities in pregnancy had dysglycaemia postpartum. HbA<sub>1c</sub> at diagnosis, any insulin use, gestation at diagnosis < 24 weeks and elevated FPG were associated with the highest risk of DM postpartum.

## KP2. Signatures of healthy adipose tissue

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## OP5. Visceral adipose tissue (VAT) and not subcutaneous adipose tissue (SAT) is associated with type 2 diabetes (T2D) and cardiometabolic risk in middle-aged black South African women: a longitudinal analysis

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**Background:** Central body fat distribution mainly increased visceral adipose tissue (VAT), rather than a general increase in adiposity has been strongly associated with insulin resistance (IR), type 2 diabetes (T2D) and increased cardiovascular risk in various populations. However, several studies in SA, mostly cross-sectional, have shown that black SA women, despite being more insulin resistant, have less VAT and more abdominal and gluteal subcutaneous adipose tissue (SAT) than their white counterparts. Therefore, this study aimed to investigate if body fat and its distribution can predict T2D risk and other markers of cardiometabolic risk in middle-aged black SA women.

**Methods:** A convenience sub-sample (n=178) of black SA women from the Birth to Twenty (Bt20) caregiver cohort was recruited for this study. Participants were eligible for inclusion if they fulfilled the criteria of being an African woman and resident in the urban township of Soweto, less than 65 years of age, HIV negative and willing to be tested for HIV. Whole body fat and body fat distribution, including VAT and SAT, were measured using dual-energy x-ray absorptiometry at baseline (age: 42 (38-47 years)) and 11 (11-13) years later. At follow-up fasting lipids, HbA<sub>1c</sub>, glucose and insulin concentrations were measured and participants completed an oral glucose tolerance test (OGTT), from which insulin resistance (HOMA-IR), insulin secretion (insulinogenic index) and insulin sensitivity (Matsuda index) were determined.

**Results:** Body weight and fat mass (FM) increased by 6.4 (1.2-13.3) kg and 5.7 (0.5-13.7) kg, respectively, over the follow-up period (both  $P < 0.001$ ). In addition, VAT and abdominal SAT areas increased by 41% and 18%, respectively (both  $P < 0.001$ ). Independent of age and FM, VAT at baseline was positively associated with fasting insulin levels ( $\beta=0.1$   $P=0.01$ ), HbA<sub>1c</sub> ( $\beta=0.09$   $P=0.021$ ), HOMA-IR ( $\beta=0.45$   $P=0.009$ ) and triglycerides ( $\beta=0.31$ ,  $P=0.004$ ), and inversely associated with insulin sensitivity (Matsuda Index;  $\beta=-0.62$ ,  $P < 0.001$ ) and high-density lipoprotein (HDL) ( $\beta=-0.24$ ,  $P < 0.001$ ) at follow-up. In contrast, baseline abdominal SAT did not predict T2D risk and measures of cardiometabolic risk. Gynoid FM at baseline was positively associated with insulin sensitivity ( $\beta=0.21$ ,  $P=0.014$ ) and HDL concentration ( $\beta=0.07$ ,  $P=0.016$ ), and inversely associated

with fasting glucose ( $\beta=-0.10$ ,  $P=0.002$ ), HbA<sub>1c</sub> ( $\beta=-0.06$ ,  $P < 0.001$ ) and triglycerides ( $\beta=-0.18$ ,  $P < 0.001$ ). Total cholesterol and low-density lipoprotein (LDL) levels were not associated with any of the body fat distribution measures.

**Conclusions:** Despite the fact that black SA women have less VAT than their white counterparts, VAT remains a stronger predictor than SAT of T2D risk and other markers of cardiometabolic risk. In line with this, gynoid FM was protective against T2D risk. Notably, body composition, body fat and its distribution did not predict LDL and total cholesterol levels in black SA women. Future research is needed to validate these findings by controlling for other factors that may contribute to body fat and its distribution, such as diet, physical activity and socio-economic status.

## OP6. The effect of non-surgical weight management on weight and glycaemic control in people with type 2 diabetes: Three-year outcomes from a real-life programme

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**Background:** Non-surgical weight management interventions are cornerstones of clinical guidelines for obese patients with type 2 diabetes, but there is a lack of systematic evidence regarding their effectiveness in the longer term.

**Methods:** We used electronic health records to prospectively follow 23,208 patients with type 2 diabetes and obesity in a NHS Greater Glasgow and Clyde area in Scotland for up to 3 years between 2005 and 2014. Patients were stratified according to whether they were referred to and attended a lifestyle-based weight management service intervention, and by attainment of a target weight loss of at least 5kg over 7-9 sessions ("successful completers"). Main outcomes included change in weight, HbA<sub>1c</sub>, and diabetes medications.

**Results:** Only 3471 potentially eligible patients were referred to the service, and less than half of those attended (n=1537). Of those who attended 7-9 sessions, > 40% achieved a 5 kg weight loss. Successful completers (n=344) maintained greater weight loss (change at 3 years -8.03 kg; 95%CI -9.44;-6.62) than the phase 1 non-completers (n=729; -3.26 kg  $p < 0.001$ ) and those not referred to the service (n=19,737; -1.00 kg  $p < 0.001$ ). Successful completers were the only patient group who did not increase their use of diabetic medications and insulin over 3 years. In adjusted models, successful completers had a clinically significant reduction in HbA<sub>1c</sub> (6.6 mmol/mol after 1 year, and 3.7 mmol/mol after 3 years;  $p \leq 0.001$  for both) compared to the group of non-completers and unsuccessful completers.

**Conclusions:** A real-life structured weight management programme can reduce weight and attain an improved glycaemic control which may prove to be a more effective treatment approach than many pharmacological alternatives. Challenges include getting a higher proportion of patients referred to and engaged with intervention services.

### OP7. The effects of a 12 week aerobic and resistance exercise intervention on body composition and body fat distribution in obese black South African women.

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**Background:** Black South African (SA) women have a high prevalence of overweight and obesity. Even though there is evidence that exercise interventions reduce body weight, there is a paucity of data on changes in body composition and body fat distribution in response to an exercise intervention in obese black SA women. This study examined the effect of a 12 week exercise intervention on changes in body composition and body fat distribution in a sample of obese black SA women. We hypothesise that 12 weeks of aerobic and resistance exercise training will improve body composition and reduce central adiposity.

**Methods:** Black women (n=45) aged 23.5 ±3.5 years with a body mass index (BMI) of 33.7±2.7 kg/m<sup>2</sup>, were recruited from an urban area in Cape Town. Forty-five participants were randomly assigned to either an exercise (n=23) or control (n=22) group. Data was collected before and after a 12-week aerobic and resistance training intervention and all participants were encouraged to maintain their dietary habits. The control group was instructed not to engage in any new exercise training activities. Dual energy x-ray absorptiometry (DXA) was used to measure whole body composition (BMI; fat-free soft tissue and fat-mass) and body fat distribution (visceral adipose tissue, subcutaneous adipose tissue; central and appendicular fat mass; android and gynoid fat mass). Exercise dose was calculated by multiplying the total number of exercise sessions attended with the mean (%) HR max obtained during the exercise sessions, over the 12 week intervention.

**Results:** The total of 34 (exercise, n=20; control, n=14) participants completed the intervention. There was no difference between groups in any of the baseline variables (p > 0.05). In response to the 12 week intervention there was significant group by time interaction in weight (p=0.010), BMI (p=0.009) and % gynoid fat mass (p=0.002), such that BMI and % gynoid fat decreased significantly in the exercise group (p < 0.05), but did not change in the control group (p > 0.05). In the control group, exercise dose (Spearman's test) was inversely correlated with change in body mass index (r=-0.446, p < 0.05) and appendicular fat mass (r=-0.492, p < 0.05).

**Conclusion:** These findings suggest that 12 weeks of exercise training was effective at reducing BMI and gynoid fat. Furthermore, the significant correlations demonstrate that there is an exercise dose effect on body composition and distribution. Collectively, exercise is a suitable approach for reducing metabolic risk factors in obese black SA women, and exercise dose must be considered when investigating adaptations in body composition.

### OP8. Mitochondrial function and H<sub>2</sub>O<sub>2</sub> production in skeletal muscle: Adaptations to exercise training in obese black South African women

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**Background:** Obesity and physical inactivity have shown to cause reduced mitochondrial function, and increased oxidative stress. Accordingly, exercise training has been utilised to stimulate mitochondrial biogenesis and reduce the risk of metabolic syndrome in obese populations. Black South African women have a high prevalence of obesity and associated morbidities, yet no study has previously examined skeletal muscle adaptations to an exercise intervention. This study aimed to determine the effect of a 12-week exercise intervention on mitochondrial function and H<sub>2</sub>O<sub>2</sub> production in skeletal muscle of obese black women.

**Methods:** Forty-five obese sedentary black South African women were randomised to a control (n=22) or exercise (n=23) condition. The exercise group completed 12 weeks of supervised aerobic and resistance training (40-60 min/day, 4 days/week), while the control group continued their normal physical activity and dietary patterns. Pre-and post intervention testing included, maximal oxygen consumption (VO<sub>2max</sub>) and needle muscle biopsy (*m. Vastus Lateralis*) for the simultaneous analyses of mitochondrial respiration and H<sub>2</sub>O<sub>2</sub> production using high-resolution respirometry and Fluorometry (Oroboros Oxygraph-2k), respectively. Oxygen flux is corrected to mitochondrial content (TMPD) and H<sub>2</sub>O<sub>2</sub> production is reported relative to mitochondrial content and oxygen flux (H<sub>2</sub>O<sub>2</sub>/O<sub>2</sub> %).

**Results:** The exercise condition (n=18) showed increases in VO<sub>2max</sub> compared to the control condition (p < 0.05). Skeletal muscle in the exercise condition showed increased flux during LEAK (Complex I + II contribution; p=0.01), and increased electron transfer system (ETS) coupling efficiency (≈E/E), compared to the control condition (p=0.02). Further, the exercise condition showed reduced LEAK control ratio (L/E; p=0.02) and oxidative phosphorylation (OXPHOS) control ratio (P/E; p=0.01), compared to the control condition. Mitochondrial H<sub>2</sub>O<sub>2</sub> production (H<sub>2</sub>O<sub>2</sub>/O<sub>2</sub> %) reduced in complex II specific respiratory state in the exercise condition, compared to no change in the control (p=0.05).

**Conclusions:** These results show that 12 weeks of exercise training in obese black South African women stimulated skeletal muscle mitochondrial adaptations specific to function and H<sub>2</sub>O<sub>2</sub> production. Indeed, exercise training improved ETS coupling efficiency and reduced LEAK and OXPHOS control ratios, suggesting increased efficiency in generating ATP during OXPHOS and ETS capacity. Further, exercise training reduced mitochondrial specific oxidative stress, as reflected by the reduction in complex II H<sub>2</sub>O<sub>2</sub> production.

### OP9. Rat proximal femur MSCs differentiate into adipocytes with "brown fat" characteristics that are modulated by glucocorticoids and vanadate

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**Background:** The expansion of marrow adiposity is frequently associated with a decline in bone mass and occurs during a number

of pathological conditions such as type-1 diabetes, radiotherapy, aging and osteoporosis. Previously it has been shown that vanadate, a broad-spectrum protein tyrosine phosphatase inhibitor, prevents glucocorticoid (GC)-induced osteoporosis (GIO) in rats, but the mechanism by which vanadate achieves this is not fully understood. It has been suggested that GC-induced marrow adiposity may be due to the skewing of progenitor cell differentiation from osteogenesis towards adipogenesis, thereby compromising bone formation. Recently we have shown that bone marrow and proximal femur each contain distinct populations of mesenchymal stem/progenitor cells (MSCs) but treatment of these cells with GCs alone does not initiate the adipogenesis programme. Vanadate however caused a reduction in adipogenesis of both these populations *in vitro*, suggesting that vanadate might prevent GIO by abrogating the increase in marrow adiposity that is associated with the decline in bone formation seen during chronic glucocorticoid use. Since proximal femur MSCs (pfMSCs) rapidly differentiate into adipocytes, are derived from a region of the femur that is most affected during GIO and are relatively understudied, we investigated, by means of PCR array, the effects of vanadate and glucocorticoids on the expression of adipogenesis-related genes in pfMSCs.

**Methods:** Adult male Wistar rats (n=3) were sacrificed and the femora were removed. The proximal ends of the femora were fragmented and denuded of surface cells using type-1 collagenase solution before being seeded in a cell culture dish with standard growth media (SGM: DMEM plus 10% foetal bovine serum). After a week, pfMSCs were observed to migrate from the bone fragments, and these cells were sub-cultured until post-confluence in passage 3. PfMSCs from each animal were subsequently treated with SGM only (control), glucocorticoids (1 µM dexamethasone), adipogenic induction media (AM) (SGM supplemented with dexamethasone, insulin, IBMX, ascorbic acid and indomethacin), or AM with 10 µM vanadate. After 7 days of treatment, RNA was isolated using an RNeasy Qiagen kit and 84 adipogenesis related genes were measured using the Qiagen RT2 adipogenesis array.

**Results:** Many adipogenesis-related genes were up-regulated in pfMSCs following AM treatment, including AdipoQ, Retn, Fabp4, PPARγ, C/EBPα, Cfd, Fasn, KLF15, Acacβ, Agt, Lipe, Slc2a4 and Lpl. Unexpectedly, genes associated with brown (thermogenic) adipocyte characteristics were also found to be up-regulated, including UCP-1, Dio2, Adrb2, PPARGc1α, PPARGc1β and PPARα. Vanadate supplemented AM caused a significant increase in Adrb2 and Klf4 with a significant decrease in Ddit3. Dexamethasone treatment resulted in an increase in the expression of some adipogenesis-related genes, including FABP4, PPARα, PPARGc1α, KLF15 and C/EBPδ.

**Conclusions:** As pfMSC-derived adipocytes express markers of both mature adipocytes and brown adipocytes, pfMSCs may play a role in thermogenesis as well as lipid storage within bone. Although glucocorticoid treatment was insufficient to initiate the complete adipogenesis programme *in vitro*, GCs increased pro-adipogenic transcription factors which may accentuate the sensitivity of the cells towards subsequent adipogenic signals, thereby augmenting adipogenesis.

### KP3: Update on acromegaly

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### OP10. An investigation into the effects of type 2 diabetes associated systemic inflammation on mesenchymal stem cell function in black South African Women

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**Background:** Obesity has become a global epidemic, affecting over 500 million people and is a major risk factor for the development of insulin resistant Type 2 Diabetes Mellitus (T2DM). In Sub-Saharan Africa, South Africa has a particularly high prevalence of obesity, particularly in black South African women. A large body mass index (BMI  $\geq 30$  kg/m<sup>2</sup>), excess abdominal fat mass, low levels of physical activity and elevated circulating levels of inflammatory cytokines are all associated with the development of T2DM and its co-morbidities such as impaired tissue regeneration. This study hypothesized that during disease progression, pro-inflammatory changes in the systemic environment contribute to a decline in the functional capacity of mesenchymal stem cells (MSCs).

**Methods:** A total of 47 (n=10 healthy lean control; n=37 obese) reproductive-aged (18-45 years) black Xhosa women volunteered to participate in the study. After completing lifestyle and dietary questionnaires, anthropometric measurements (body weight, height, waist/hip circumferences) and a dual-energy x-ray absorptiometry (DXA) scan were performed. Blood samples were collected at a single time point from each participant. Whole blood and/or serum were analysed for fasting blood glucose levels, total cholesterol, HDL, LDL, triglycerides and inflammatory biomarkers (CRP, IFN-γ, IL-2, IL-4, IL-6, IL-8, IL-10, TNF-α) and SDF-1α. Participants with metabolic syndrome were classified as having > 3 out of 6 metabolic risk factors. These risk factors included: hypertension, fasting blood glucose (> 5.6 mmol/L), HDL (< 1.04 mmol/L), LDL (> 4.13 mmol/L), triglycerides (> 5.64 mmol/L), waist-to-hip ratio (> 0.85). For the *in vitro* experiments, MSCs (Poietics cell line) were exposed to patient-derived serum and changes in cellular viability, proliferation and migration capacity assessed using standard tissue culture techniques.

**Results:** The obese participants were divided into three groups based on the outcome of metabolic measures: 1) healthy obese (n=11), 2) metabolic syndrome (n=19) and 3) T2DM (previously diagnosed) (n=7). Systemic inflammation was evident in the healthy obese (CRP 29.8±8 pg/mL) and metabolic syndrome (CRP 50.8±24 pg/mL) participants, whereas T2DM patients had elevated blood glucose (8.8±3.8 mmol/L) and triglyceride levels (1.6±1.8 mmol/L). Preliminary data from *in vitro* experiments suggest a possible association between the inflammatory serum cytokine profile and the functional capacity of MSCs.

**Conclusion:** Characterisation of the patient population confirmed that participants were in different stages of disease progression and therefore justified the use of our model for further *in vitro* experimentation to assess the effect of a pro-inflammatory systemic environment on the pro-regenerative capacity of MSCs. The

impairment of endogenous MSCs due to long-term exposure to a pathological systemic and localised micro-environment may be a contributing factor to disease progression and the development of co-morbidities.

#### **OP11. The low renin phenotype in Africans: Implications for all-cause and cardiovascular mortality**

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**Background:** Excessive activation of the renin-angiotensin-aldosterone system (RAAS) is linked to hypertension development and cardiac abnormalities associated with cardiovascular and renal diseases. Currently, cardiovascular complications related to hypertension are among the leading causes of death in black populations, who usually exhibit low renin levels. Since the prognostic significance of renin in cardiovascular-related mortality in blacks is unknown, we determined whether renin and its interactions with blood pressure are predictive of all-cause and cardiovascular mortality in a black population with low vs. high plasma renin.

**Methods:** We measured active plasma renin in 1502 black South African men and women from both rural and urban areas (age  $\geq 35$  years). All-cause and cardiovascular mortality was assessed over five years. We divided the population into low (N=1002) and high renin (N=500) groups based on the cut-off of the Renin III CISBIO kit (Cedex, France).

**Results:** In multivariable-adjusted Cox-regression analyses performed in the low renin group, systolic blood pressure (SBP)

and the interaction between renin and SBP, but not renin alone, predicted both all-cause [(HR, 1.42; 95% CI, 1.08-1.87;  $P=0.012$ ), (HR, 1.74, 95% CI, 1.06-2.83,  $P=0.027$ )] and cardiovascular mortality [(HR, 1.83; 95% CI, 1.14-2.93;  $P=0.012$ ), (HR, 2.38; 95% CI, 1.07-5.26;  $P=0.033$ )]. In the total group, renin and its interaction with SBP predicted all-cause, but not cardiovascular mortality [(HR, 1.32; 95% CI, 1.06-1.64;  $P=0.013$ ), (HR, 1.29; 95% CI, 1.05-1.58;  $P=0.015$ )]. No associations existed in the high renin group.

**Conclusions:** The interaction of renin with SBP and not renin *per se* is predictive of all-cause and cardiovascular mortality only in blacks with low renin levels, whereas in the total group renin and the SBP\*renin interaction predicted only all-cause mortality. Volume-overload potentially due to excessive salt intake, inadequate diuretic therapy and renal damage, is indicated as one of the causes of resistant hypertension in the South African Hypertension Practice guidelines. This study lends further support to the use of diuretics and calcium channel blockers for lowering high blood pressures observed in the low renin phenotype, thereby reducing the vulnerability to heart failures, strokes and hypertensive kidney disease.

#### **KP4. Exciting new potentials for hypoglycaemic agents in cardiovascular disease: more than meets the eye**

**R Chilton**

University of Texas Health Science Center, San Antonio, TX, USA

## SATURDAY 6<sup>th</sup> MAY 2017

### OP12. Branched-chain and aromatic amino acids and cardio-metabolic risk in black African and Asian Indian populations

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**Background:** A number of studies have shown that serum levels of branched chain amino acids (BCAAs: valine, leucine, isoleucine) and aromatic amino acids (AAAs: tyrosine, phenylalanine) are elevated in a variety of cardio-metabolic diseases in population groups resident in high-income countries. In this study, we sought to describe the association of BCAAs and AAAs with the metabolic syndrome and its individual components, i.e. HDL-cholesterol, triglyceride, blood pressure and plasma glucose concentrations in black African (BA) and Asian Indian (AI) populations in South Africa.

**Methods:** We used serum samples collected from AI (n=349) and BA (n=369) subjects recruited through the Birth to Twenty Study to measure blood levels of BCAAs and AAAs. Existing data on fasting blood glucose, lipid profile, blood pressure, waist circumference and body mass index (BMI) as well as measurements of visceral and subcutaneous abdominal fat were obtained for all individuals. The BCAAs and AAAs were quantified using liquid chromatography mass spectrometry (LC-MS). Multivariable regression models were created to test for the association of amino acid levels (included as independent variables) with metabolic syndrome and its components (included as dependent variables) with adjustment for possible confounders. Furthermore, the determinants of amino acid levels were assessed using multivariable regression models with the amino acids included as the dependent variable. Amino acid levels were compared across tertiles of visceral fat thickness using ANOVA.

**Results:** The serum total amino acid levels (sum of BCAAs and AAAs) were higher in AIs compared to BAs ( $p=0.004$ ). The BCAAs and AAAs were significantly and positively associated with metabolic syndrome and its individual components, particularly in AIs, in unadjusted regression models but most of these associations were lost after adjustment for age, gender, BMI, smoking, education, visceral fat and subcutaneous fat. The loss of significance was largely due to visceral fat. However, triglyceride concentration was still significantly associated with total amino acids, valine and leucine levels in BAs even after adjustment for the above co-variables ( $p < 0.01$  for all associations). In multivariable linear regression models, visceral fat was the principal determinant of total amino acid levels in both BAs ( $p=0.002$ ) and AIs ( $p < 0.001$ ). Individuals with visceral fat thickness in the top tertile ( $\geq 6$ cm) had significantly higher total amino acid levels compared to those with a visceral fat thickness in the bottom tertile ( $\leq 2.9$  cm) in BAs ( $p=0.012$ ) and AIs ( $p < 0.001$ ).

**Conclusion:** Visceral fat, through an unknown mechanism, is positively related to total serum amino acid levels in AIs and BAs whilst valine and leucine are strong indicators of hypertriglyceridaemia in BAs. Longitudinal studies are required to investigate the ability of these amino acids to predict hypertriglyceridaemia in the BA population.

### OP13. The Cardiometabolic Profile of Patients with Psoriasis- a Case-Control Study

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**Background:** Psoriasis (PsO) is an immune-mediated inflammatory disorder with a global prevalence that ranges between 0.91 and 8.5%. Cardiometabolic comorbidities are increasingly recognised in PsO but it is not known whether cardiometabolic disease (CMD) is a consequence of PsO or alternatively that PsO and CMD share a common aetiology, possibly through abdominal obesity and inflammation. Therefore, the aims of this study were to measure the prevalence of cardiometabolic diseases (CMD) in patients with PsO and to determine whether PsO is a risk factor for CMD.

**Methods:** Adult HIV-negative PsO patients (n=103) and controls (n=98) were recruited from the Dermatology and Rheumatology clinics at hospitals of the University of the Witwatersrand Academic Complex. Controls were matched for gender, ethnicity and body mass index (BMI). Clinical and demographic data was recorded. The metabolic syndrome (MS) and its components were defined according to the harmonised guidelines. Fasting blood samples were obtained for the measurement of insulin, glucose, lipids, and high-sensitivity C-reactive protein (hsCRP). Insulin resistance was measured using the HOMA method. A non-contrast CT scan of the abdomen was taken at the level of L4 to measure visceral and subcutaneous fat.

**Results:** Amongst the 103 patients (48 male, 55 female) recruited, 28 patients (27.2%) had psoriatic arthritis (PsA). The mean ( $\pm$ SD) age and disease duration were  $53.3 \pm 14.5$  and  $18.9 \pm 13.3$  years, respectively. The median [IQR] BMI in patients and controls were  $30.1$  [25.3, 36.1] and  $28.2$  [24.9, 32.6] ( $p=0.06$ ). The prevalence [n (%)] of MS was higher in subjects with PsO [54 (52.4%)] compared to controls [33 (33.7%);  $p < 0.001$ ]. Type 2 diabetes (T2DM) was more prevalent in subjects with PsO (25.2%) compared to controls (4.08%;  $p < 0.001$ ). The median [IQR] for hsCRP levels (mg/L) in patients with PsO was  $5.20$  [2.00, 11.0] compared to  $2.15$  [1.10, 5.20] in controls ( $p < 0.001$ ). Although there were no differences in total cholesterol levels between cases and controls, patients with PsO had a lower median [IQR] HDL cholesterol (mmol/L) than controls ( $1.21$  [1.05, 1.56] vs  $1.37$  [1.09, 1.76];  $p=0.04$ ). Multiple logistic regression analysis demonstrated that the major risk factors for PsO were visceral fat with an odds ratio (95%CI) of  $1.03$  [1.00, 1.06] ( $p=0.047$ ), hsCRP ( $1.06$  [1.00, 1.11];  $p=0.039$ ) and smoking ( $2.17$  [1.30, 3.60];  $p=0.003$ ). The risk factors for T2DM were PsO ( $6.49$  [1.74, 24.2];  $p=0.005$ ) and visceral fat ( $1.04$  [1.01, 1.08];  $p=0.02$ ), whilst the risk factors for MS were visceral fat ( $1.10$  [1.04, 1.20];  $p < 0.001$ ) and insulin resistance ( $2.00$  [1.24, 3.22];  $p=0.004$ ).

**Conclusions:** Psoriasis is characterised by obesity and high prevalence of T2DM and MS. Visceral fat is a risk factor for T2DM and MS and also increases the risk for PsO, but acts independently of inflammation. The risk of PsO is also increased by smoking. Psoriasis is a further risk factor for T2DM. These results suggest that CMD

screening should form part of routine care for PsO patients and that lifestyle modifications are necessary to reduce smoking and abdominal obesity.

### KP5. Genetic control of human puberty: From Mendel to populations

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Sexual maturation at puberty marks a highly significant biological and psychological milestone in every human life. However, the precise biological and genetic control of this developmental transition from childhood to adulthood remains one of the 125 critical yet "Unanswered Scientific Questions" according to Science Magazine. Patients with congenital idiopathic hypogonadotropic hypogonadism [IHH] present with a rare form of pubertal failure and infertility. IHH is caused by deficient secretion or action of gonadotropin-releasing hormone (GnRH) and presents with either anosmia (referred to as Kallmann Syndrome) or with normosmia (referred to as normosmic IHH). Genetic and physiologic studies in IHH patients have provided unprecedented insights into the regulation of human puberty and hormonal control of spermatogenesis. Rare genetic variants in > 25 genes result in Mendelian forms of KS and nIHH. Recent genome-wide association studies also show that low frequency and common variants in several genes, including some of the genes causing mendelian forms of IHH, can also determine timing of puberty in the general population. The identification of genetic abnormalities has begun to shed clinical insights into natural history of IHH, and allow clinicians to devise optimal management strategies for induction of puberty and treating their infertility.

### KP6. Update on Cushings

**BMK Biller**

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### OP14. The prevalence and associations of hypogonadism in coloured males with type 2 diabetes

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**Background:** A recent study reported a high prevalence of 28% for type 2 diabetes (T2D) amongst the coloured population drained by Tygerberg Academic Hospital (TAH). The association between T2D and hypogonadism has been previously well-documented with some studies reporting a prevalence of hypogonadism in excess of 50% in males with T2D. Hypogonadism has also been shown to independently and significantly impact quality of life and sexual function in patients suffering from T2D. Currently, the prevalence and impact of hypogonadism in the coloured male T2D population is not known.

**Methods:** Fifty consecutive coloured male patients (30-70 years old), attending TAH outpatients for T2D were included. Patients with hypogonadism due to causes other than T2D were excluded, as well as those on testosterone therapy. Demographic, biometric and other data pertaining to the duration, treatment, co-morbidities and complications of T2D were collected. Each participant completed

an Androgen Deficiency of the Aging Male questionnaire (ADAM). Fasting morning blood samples were obtained for HbA<sub>1c</sub>, lipogram, total testosterone (TT), sex hormone binding globulin (SHBG) and creatinine. Free testosterone (FT) was calculated using the Vermeulen formula. Luteinising hormone (LH) and prolactin levels were determined on specimens with low TT. Repeat morning blood samples were taken in all subjects with low TT to confirm hypogonadism. Urine was collected for microalbumin and a protein-creatinine ratio. All participants underwent DXA for determination of body fat and bone mineral density (BMD).

**Results:** The mean age of the population and duration of T2D was 53 and 13 years respectively with a mean HbA<sub>1c</sub> of 10.2%, mean BMI of 31kg/m<sup>2</sup> and waist circumference of 109cm. Retinopathy, nephropathy, neuropathy and coronary artery disease were respectively documented in 40, 68, 22 and 22% of subjects. Lack of libido was volunteered in 72% of subjects with 78% of subjects reporting erectile dysfunction.

SHBG was variable with a mean of 30 and SD of 17.7 nmol/l. Mean TT was 10.3 nmol/l (lower limit of normal 8.6 nmol/l) and mean FT 0.223 nmol/l (lower limit of normal 0.198 nmol/l). Seventeen subjects (34%) had unequivocally low TT whilst 22 subjects (44%) had TT values in the intermediate range (8.6-13 nmol/l). Eighteen (36%) subjects had low FT, however 5 subjects with low total testosterone had normal FT, whilst 6 subjects with normal TT had low FT. Three subjects in the intermediate range TT had low FT. BMD was normal in all subjects.

Low TT correlated significantly with duration of diabetes and HDL. There was no association of TT or FT with target organ damage, HbA<sub>1c</sub>, biometric data, BMD or body fat measurements and distribution (android versus gynoid). There was poor correlation between the ADAM score and TT or FT.

**Conclusions:** Symptoms of sexual dysfunction occurred frequently as did low levels of testosterone in our population. However, there was a poor correlation between clinical parameters and laboratory hypogonadism.

### KP7. An appraisal of the cardiovascular safety of drugs used to treat Type 2 diabetes

**R Chilton**

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### OP15. Age-dependent development of left ventricular wall thickness in type 2 diabetic (db/db) mice is associated with elevated low-density lipoprotein and triglyceride serum levels

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**Background:** Diabetic cardiomyopathy (DCM) is a disease of heart muscle that remains one of the leading causes of death in diabetic individuals. Shifts in substrate preference resulting in aberrant serum lipid content and enlarged left ventricular wall thickness are well-established characteristics associated with the development of DCM. As underlying mechanisms driving the onset of the DCM remain relatively unclear, this study sought to characterise age-dependent development of left ventricular (LV) wall thickness in leptin-receptor-deficient diabetic (*db/db*) mice. Such data were compared with low-density lipoprotein (LDL) and triglyceride serum levels to assess whether any correlation exists between the parameters here investigated.

**Methods:** Male homozygous *db/db* mice together with their heterozygous non-diabetic lean littermate controls (*db/+*) (n=six per group) were monitored from the age of six to sixteen weeks. Mice were terminated each week to measure body weights, heart weights, liver weights, tibia length and fasting plasma glucose levels. Heart tissues were stained with haematoxylin and eosin stain to measure LV wall and interventricular septum thickness (represented in  $\mu\text{m}$ ) together with an assessment of myocardial remodeling. Serum was collected weekly and used to measure LDL and triglyceride levels (represented in mmol/L).

**Results:** *Db/db* mice presented significantly increased body weights, liver/body weight and fasting plasma glucose levels from the age of six to sixteen weeks when compared to the *db/+* controls. While they displayed a marked enlargement of LV wall and interventricular septum thickness from the age of eleven weeks ( $1603 \pm 88$ ,  $p \leq 0.001$  and  $1420 \pm 90$ ,  $p \leq 0.05$ , respectively), increased percent heart weight/tibia length was recorded only from week sixteen ( $0.01 \pm 0$ ,  $p \leq 0.001$ ). From week eleven, the LV wall and interventricular septum thickness results corresponded with cardiac remodeling and raised LDL and triglyceride serum levels ( $1.5 \pm 0.1$ ,  $p \leq 0.001$  and  $1.9 \pm 0.2$ ,  $p \leq 0.001$ , respectively).

**Conclusion:** Age-dependent development of LV wall thickness in *db/db* mice is partially associated with increased LDL and triglyceride levels, elucidating a potential pathophysiological mechanism. Thus, monitoring serum LDL and triglyceride levels remain important in identifying diabetic patients at risk of developing LV wall thickness and subsequent cardiac dysfunction.

#### OP16. Hypoglycaemia among insulin-treated patients with diabetes: South African cohort of International Operations Hypoglycaemia Assessment Tool (IO HAT) study

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On behalf of the South African IO Hat study group

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**Background:** The non-interventional IO HAT study assessed the incidence of hypoglycaemia in patients with insulin-treated diabetes in Bangladesh, Colombia, Egypt, Indonesia, Philippines, Singapore, South Africa, Turkey and the UAE. This study evaluated the results of the South African cohort.

**Methods:** Two-part self-assessment questionnaire was used: Part-1 assessing baseline demographic and treatment information,

hypoglycaemia unawareness, perceptions of hypoglycaemia, history of severe hypoglycaemia (previous six months), and symptomatic hypoglycaemia (previous four weeks); and a four-week prospective evaluation (Part-2) assessing history of both severe and symptomatic hypoglycaemia after baseline. Patient diaries were provided to aid recall during the prospective period.

**Results:** 7289 patients enrolled in IO HAT, 915 were South African (type 1 diabetes [T1D] 173; type 2 diabetes [T2D] 742). Retrospectively, 76.2% (T1D) and 52.2% (T2D) of patients reported hypoglycaemia in the Part-1 SAQ. Almost all patients reported hypoglycaemia prospectively (Part-2 SAQ; T1D 98.2%; T2D 90.1%). Most (T1D 92.5%; T2D 87.9%) patients completed diaries in the prospective period, with  $\geq 1$  confirmed hypoglycaemic event recorded in the diary by 53.8% (T1D)/19.6% (T2D) of patients. Severe hypoglycaemia was reported by 40% of patients with T1D during the retrospective and prospective periods (6.0/8.8 events per patient-year [PPY], respectively), and by 37.7% (retrospective) and 47.8% (prospective) of patients with T2D (2.2/8.9 events PPY, respectively).

**Conclusion:** This is the first patient-reported dataset on hypoglycaemia in insulin-treated patients with diabetes in South Africa. The high incidence of any, and severe hypoglycaemia, in the prospective period shows hypoglycaemia is under-reported and therefore underestimated, and highlights a clear need for initiatives to reduce hypoglycaemia in insulin-treated patients.

#### KP8. Management of hypoglycaemia unawareness - How Neuroimaging informs therapy

P Choudhary

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#### OP17. Characteristics of subjects with diabetes mellitus diagnosed before 35 years of age presenting to Inkosi Albert Luthuli Central Hospital from 2003 to 2016.

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University of KwaZulu-Natal

**Background:** Most patients diagnosed with diabetes mellitus under 35 years of age will have type 1 diabetes (T1D). However, with the rising prevalence of obesity, type 2 diabetes (T2D) of youth is increasingly common and ketosis-prone type 2 diabetes (KPD) may also occur in this age group.

**Aim:** The study aimed to evaluate the clinical, biochemical and immunologic characteristics of patients diagnosed with diabetes under 35 years of age and presenting for management at the Diabetes Clinic in Inkosi Albert Luthuli Central Hospital (IALCH) in Durban over a 13 year period, to determine patterns of diabetes presenting in this age group.

**Methods:** The study was a retrospective chart review of all patients diagnosed with diabetes before age 35 years. Data for analysis included age at diagnosis, mode of presentation, anthropometry, blood pressure, presence of diabetes autoantibodies, c-peptide levels, most recent HbA<sub>1c</sub>, serum lipids and creatinine, urine albumin-creatinine ratio and details of treatment; in addition, information on diabetes complications, mortality and attendance status was collected. Diabetes was classified according to clinical and laboratory criteria.

**Results:** A total of 517 patients (57% female) were included in the analysis. Of these, 445 (86.1%) were diagnosed with T1D, 27 (5.2%) with T2D, 27 with KPD (5.2%) and 18 (3.5%) with other forms of diabetes. The mean age of the total group was  $28 \pm 10$  years. At diagnosis, mean age (yr.) was lower in T1D ( $15 \pm 7.0$ ) than in T2D ( $17.2 \pm 5.7$ ) or KPD ( $23.6 \pm 6.6$ ) ( $p < 0.001$  for T1D vs. KPD); mean BMI (kg/m<sup>2</sup>) was also lower in T1D ( $24.4 \pm 5.1$ ) compared to T2D ( $28.4 \pm 4.5$ ;  $p < 0.001$ ) and KPD ( $32.4 \pm 6.8$ ;  $p < 0.001$ ). Antibodies to glutamic acid decarboxylase (GAD) were found in 264/364 (72.5%) subjects with T1D vs. 0 (0%) in T2D and 2/25 (8.0%) subjects with KPD. Fasting and glucagon-stimulated c-peptide levels were lower in T1D ( $0.36 \pm 0.46$  ng/ml at 0 minutes and  $0.49 \pm 0.64$  ng/ml at 6 min) compared to T2D ( $1.84 \pm 1.31$  ng/ml at 0 minutes and  $3.66 \pm 2.97$  ng/ml at 6 minutes) ( $p < 0.001$ ) and KPD ( $1.43 \pm 0.89$  ng/ml at 0 minutes and  $2.99 \pm 1.91$  at 6 minutes) ( $p < 0.001$ ). Mean HbA<sub>1c</sub> (%) was higher in T1D ( $11.1 \pm 3.0$ ) than T2D ( $9.4 \pm 3.1$ ) ( $p < 0.05$ ) and KPD ( $8.6 \pm 3.3$ ) ( $p < 0.001$ ), as were insulin doses used (units/kg) ( $1.2 \pm 0.5$  vs.  $0.9 \pm 0.8$  vs.  $0.6 \pm 0.8$ ) ( $p < 0.01$ ). There were 18 (3.5%) known deaths. Default rate was high in all groups: T1D: 237(53.5%); T2D: 13(48.1%); KPD: 11(40.7%).

**Conclusion:** The majority of patients diagnosed with diabetes before 35 years of age in KwaZulu-Natal have T1D, with small numbers of other types. Glucose control is sub-optimal and there is a high rate of loss to follow up.

#### OP18. A meta-analysis of genome-wide association studies (GWAS) for type 2 diabetes in subjects from South Africa, Nigeria, Ghana and Kenya.

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**Aims:** GWAS for type 2 diabetes (T2D) have uncovered over 100 risk loci primarily in populations of European and Asian ancestry. Studies in populations of African ancestry provide the opportunity

to discover novel T2D loci (including African-specific variants) and fine map variants at known loci.

**Methods:** A meta-analysis of two GWAS was conducted in 4344 Africans: (i) 1602 cases and 976 controls of Zulu ethnicity (Durban Diabetes Study); (ii) 1031 cases and 738 controls from Nigeria, Ghana and Kenya (Africa America Diabetes Mellitus Study - AADM). Association between T2D and each variant was tested using linear mixed models to account for cryptic relatedness and population structure.

**Results:** The most significant variant was the widely-replicated rs7903146 at *TCF7L2* ( $p = 3.2 \times 10^{-12}$ ). One novel locus (rs73284431 near *AGMO* and *DGKB*) reached genome-wide significance ( $p = 4.9 \times 10^{-9}$ , MAF = 9.5%; MAF=0 in Europeans and Asians). A second African-specific variant (rs12277475, MAF = 21.9%) near the index variant *INS-IGF2* rs3842770 reached moderate significance ( $p = 2.2 \times 10^{-7}$ ). SNP rs73987668 (MAF = 1.2% in AADM) near *CA10* (locus associated with metabolic syndrome in Africans) showed genome-wide significance in the AADM study ( $p = 4.1 \times 10^{-9}$ ). Using direct and local replication approaches, we replicated ( $p < 0.05$ ) with directionally consistent effects, over 50% of 87 previously established T2D loci. Within replicated loci, variants other than the reported index SNPs often showed smaller P-values highlighting the fine-mapping capability of the reduced linkage disequilibrium present in African genomes.

**Conclusions:** We found two (rs73284431 and rs73987668) novel susceptibility loci for T2D in Africans and replicated over half of loci found in other populations. Results demonstrate the importance of performing GWAS in Africans for better understanding of the genetic architecture of T2D and provide a resource to larger consortia for further discovery, replication and fine-mapping.

#### KP9. An update on the diagnosis and management of the diabetic triopathy

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## SUNDAY 7<sup>th</sup> MAY 2017

### KP10. Challenges and solutions for the diabetic foot

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### OP19. Factors associated with 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) most commonly starts in childhood and early adulthood and requires a life-long insulin therapy. Despite improvements in care, many patients with T1DM fail to reach adequate glycaemic control, and a number of factors that influence glycaemic control have been identified in previous studies. There is a paucity of data from South Africa relating to factors associated with glycaemic control.

**Aim:** The aim of the study is to determine the proportion of South African patients with T1DM achieving glycated haemoglobin (A<sub>1c</sub>) targets as defined by ISPAD (< 7.5% for < 18 y/o) and ADA (< 7% for ≥ 18 y/o) recommendations and to further explore factors related to glycaemic control in T1DM South African youth.

**Methods:** The TEENs study is an international, observational cross sectional study undertaken in 2012 and investigated 5960 individuals in three age categories (8-12; 13-18; and 19-25) with T1DM from 20 countries including South Africa. The study determined the main predictive factors of glycaemic control and quality of life in order to generate recommendations to improve diabetes management. Data was collected by interviews, surveys and medical records during one study visit. Glycated haemoglobin (A<sub>1c</sub>) was measured uniformly using a point of care test. Subjects recruited from South African sites are described in this report.

**Results:** 500 South African patients were included in the study (mean age 14.8 years, mean duration of diabetes 7.2 years and mean age at diagnosis 8.2 years). The mean A<sub>1c</sub> in this cohort was 9.8% (SD=2.1%). The target A<sub>1c</sub> was only achieved in 50 (10%) patients, with the proportion of A<sub>1c</sub> attainment decreasing with age (13.3%, 9.3% and 6.3% in the 8-12, 13-18 and 19-25 age groups respectively). The majority of patients in the three age categories use insulin pens (66.8%) while a basal bolus regimen is the most frequently prescribed treatment regimen (94.0%). Almost all patients over the age of 13 years are on a basal bolus regimen {13-18 years (97.7%) and 19-25 years (99.8%)} in comparison to 86.2% in the 8-12 age group. The overall mean number of blood glucose checks per day is 4.1, with the highest in the 8-12 age group (4.9). The 19-25 age group

checks their blood glucose levels less often per day (3.1). In addition, the use of real-time glucose monitoring devices is uncommon in all age groups, with an overall use of 4.4%. Better glycaemic control was significantly associated with a number of modifiable factors including the frequency of glucose monitoring, availability of glucagon at home and exercising for at least 30 minutes per week. The overall proportions of diabetic ketoacidosis and severe hypoglycaemia were 6.0% and 8.8% respectively.

**Conclusion:** The overall glycaemic control is poor amongst all the age groups studied, the data reinforces the need to improve the modifiable factors that have been identified as key in achieving A<sub>1c</sub> target.

### OP20. Investigating the mechanisms of pharmacological chaperone rescue of mutant G protein-coupled receptors

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**Background:** Dysregulation of G protein-coupled receptors (GPCRs) is commonly associated with a vast variety of diseases, including reproductive disorders associated with mutations found in the luteinizing hormone receptor (LHR), which has an important role in the endocrine control of reproduction. Many GPCR mutations cause protein misfolding and subsequent detection and retention by the endoplasmic reticulum (ER) quality control systems, preventing their expression at the cell surface. The ER quality control system includes an array of molecular chaperones and has several levels of response to expression of misfolded proteins including the unfolded protein response (UPR), ER associated degradation (ERAD) and in extreme cases, apoptosis.

Pharmacological chaperones have been shown to "rescue" the cell surface expression of retained mutant receptors, including LHR-CHAP, which we have previously shown to rescue retained mutant LHRs. It is hypothesised that pharmacological chaperones, such as LHR-CHAP enable escape from the strict ER quality control system. However, the mechanisms behind this remain unclear. We examined the degree of retention of mutant receptors and aimed to determine whether the ability of LHR-CHAP to "rescue" correlated with cellular localisation of the retained receptors.

**Methods:** An enzyme-linked immunosorbent assay (ELISA), was employed to determine cell surface and total expression of extracellularly epitope tagged receptors in non-permeabilised and permeabilised cells, respectively. Cellular localisation of wild type or mutant LHRs was addressed through confocal microscopy by co-transfecting cells with fluorescently tagged wild type/mutant receptors and markers of different cellular compartments such as the ER, Golgi and plasma membrane. In each case, following transfection cells were treated for 24h in the presence/absence of 1 μM LHR-CHAP prior to analysis.

**Results:** It was found that different mutant LHRs are retained and rescued to different degrees and that LHR-CHAP treatment does not show similar rescue effects on all the mutations included in this study, with some displaying large increases in cell surface expression and others showing little/no increase.

Four mutant LHRs were selected for further examination by confocal microscopy. These receptors were selected based on their level of

retention and cell surface rescue after LHR-CHAP treatment. Mutants I374T and S616Y are partially retained and partially rescued, whereas T461I and C543R are fully retained and display complete rescue or no rescue, respectively.

Localisation studies demonstrated that wild type LHR is expressed on the cell membrane (as expected), whereas mutant LHRs co-localize with different cellular compartments involved in the folding and maturation of secretory receptors. LHR-CHAP treatment altered localisation of the retained receptors and resulted in cell membrane expression for only some of the mutant receptors, correlating with the ELISA results.

**Conclusions:** Different mutant LHRs are differentially processed by the cell and are retained and rescued to different degrees. Pharmacological chaperones, such as LHR-CHAP, show potential as drug therapies to rescue mutant LHRs associated with diseases such as infertility and we hope these studies will help further our understanding of the mechanisms of action of these compounds.

### OP21. Use of a novel cell-permeant molecule to rescue the activity of mutant LH receptors implicated in reproductive dysfunction

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**Background:** G protein-coupled receptors (GPCRs) are a large family of cell surface receptors that convey 80% of signal transduction across cell membranes. As a result, GPCR signaling is responsible for regulating most biological processes in humans, and GPCRs present very effective drug targets. Genetic mutations in GPCRs have been implicated in many diseases. Inactivating mutations can impede interactions with up-/down-stream signaling components, but the majority cause receptor mis-folding and consequent intracellular retention and/or degradation by the cellular quality control machinery preventing translocation to their site of action at the cell membrane. Some cell-permeant ligands (pharmacological chaperones) have been shown to 'rescue' the cell surface expression of such mutant GPCRs, presumably by stabilising the correct folding of the nascent protein. Here we describe a pharmacological chaperone (LHR-CHAP) that can restore function of mutant luteinizing hormone receptors (LHRs).

**Methods:** A number of mutant LHRs, reported as causing reproductive dysfunction in humans were identified. Receptor expression/localisation, hormone binding affinity and signaling competence of these receptors was determined, using a cell-based exogenous expression system. Radioligand hormone binding assays, cell signaling assays measuring second messenger generation and receptor ELISA assays were utilised in order to establish the cause of mutant receptor non-functionality as loss of ligand binding, loss of signal transduction and/or loss of cell surface receptor expression. The ability of the small molecule LHR agonist (LHR-CHAP) to rescue the localisation and/or function of these mutant receptors was then examined.

**Results:** 70% of the mutant receptors were found to be poorly expressed at the cell surface (retained), and LHR-CHAP was able to 'rescue' cell surface expression of two thirds of retained receptors with mutations in the receptor endo-domain, to some extent, although variable degrees of rescue were measured. Hormone

stimulation of LHR-CHAP-rescued mutant receptors was measured, confirming their functionality. Furthermore, mutant receptors with normal cell surface expression, but no response to hormone stimulation due to impaired hormone-binding or hormone-induced signaling ability, also elicited a robust response to allosteric stimulation by LHR-CHAP.

**Conclusion:** We have identified a small-molecule LHR agonist that functionally rescues a number of intra-cellularly retained, hormone binding-deficient or signaling-deficient mutant human LHRs. This approach has potential application for treatment of infertile patients bearing such mutations, for whom there is no current therapy. In addition, elucidation of the characteristics of mutations which cannot be rescued in this way may aid in the development of similar interventions for other disease-causing mutant GPCRs.

### KP11. Endocrine evaluation and management of anovulatory infertility in females

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Polycystic ovarian syndrome (PCOS) and functional hypothalamic amenorrhea (FHA) represent two common conditions characterized by anovulatory infertility in women. PCOS is a polygenic disorder characterized by oligo/anovulation, hyperandrogenism and polycystic ovarian morphology. FHA results from functional suppression of the hypothalamo-pituitary-ovarian (HPO) axis. In women presenting with anovulatory infertility, detailed personal history should be obtained with focus on menstrual pattern, symptoms and signs of hyperandrogenism (acne, hirsutism), galactorrhea, dietary history, caloric intake, history of eating disorders, exercise and athletic training, weight fluctuations, sleep patterns, stressors; mood, drug intake, substance abuse and history of fractures. Clinicians should also obtain a thorough family history with attention to eating disorders and reproductive disorders. Initial endocrine evaluation should include exclusion of pregnancy, thyroid function tests, prolactin, estradiol, luteinizing hormone (LH), follicle-stimulating hormone (FSH), anti-mullerian hormone (AMH), total testosterone and dehydroepiandrosterone sulfate (DHEA-S) and if suspected, 17-OH progesterone to exclude late onset congenital adrenal hyperplasia. While PCOS can be diagnosed with specific clinical and biochemical criteria, FHA is a diagnosis of exclusion. Medical management of PCOS includes lifestyle modifications, weight management, management of symptoms (acne/hirsutism) and ovulation induction for those desiring fertility. For women with FHA, a multidisciplinary treatment approach is necessary, including medical, dietary, and mental health support, aiming to reverse those inciting factors contributing to the functional HPO suppression and if unsuccessful, use transdermal E2 therapy with cyclic progestin in those not requiring fertility. In FHA patients seeking fertility, pulsatile gonadotropin-releasing hormone (GnRH) is ideal physiologic therapeutic option and if unavailable, gonadotropin therapy can be used for induction of ovulation.

## KP12. Does metabolically healthy obesity exist?

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## OP22. Genetic associations of common variants at CYP17A1 and SERPINA6/SERPINA1 loci with blood pressure and measures of central and whole body adiposity in black South African women

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**Background:** Common genetic variants that influence morning corticosterone and cortisol levels have been identified at *CYP17A1* and *SERPINA6/SERPINA1* loci, respectively, in Caucasians. *CYP17A1* encodes 17- $\alpha$ -hydroxylase, an enzyme catalysing the conversion of precursors for corticosterone synthesis to precursors for cortisol synthesis. *SERPINA6* encodes corticosteroid binding globulin (CBG, the major glucocorticoid binding plasma protein); and *SERPINA1* encodes  $\alpha$  1-antitrypsin (which inhibits cleavage of the reactive centre loop that releases glucocorticoids from CBG). We examined the associations of variants that represent African haplotype blocks, in addition to previously identified risk variants, at *CYP17A1* and *SERPINA6/SERPINA1* loci; with BMI, waist circumference (WC) and blood pressure in black South African women.

**Methods:** A sub-sample (n=853) of adult black female caregivers from the Birth to Twenty cohort, between the ages of 25 and 84 years, were selected on the basis of the availability of both DNA and matching serum, and excluded if they were pregnant or breastfeeding. Basic anthropometric measures (BMI, WC) were taken and blood pressure was measured using an Omron 6 automated machine. Single nucleotide polymorphism (SNP) selection was based on previously identified genetic variants at both *CYP17A1* and *SERPINA6/SERPINA1* loci. To select African-specific tagSNPs, we identified European haplotype blocks from which each previously identified European SNP resides. A total number of 12 SNPs were selected and genotyped using the Open Array Taqman assay in the QuantStudio 12K Flex platform.

**Results:** The women (48 $\pm$ 7.6 years) had a mean BMI of 32.7  $\pm$  7.5 kg/m<sup>2</sup>, WC of 96.2 $\pm$ 15.2 cm, systolic blood pressure (SBP) of 130.7 $\pm$ 22.7 mmHg and diastolic blood pressure (DBP) of 86.1 $\pm$ 13.5 mmHg. Age and smoking were included as covariates. There was an association between rs1004467 at *CYP17A1* and lower BMI ( $\beta$ =-2.18, p=0.030), and between rs1004467 and rs17115104 and lower WC ( $\beta$ =-2.21, p=0.027 and  $\beta$ =-2.32, p=0.021, respectively) and lower DBP ( $\beta$ =-2.18, p=0.030 and  $\beta$ =-2.07, p=0.039, respectively). However, when adjusting for BMI, the associations with DBP were no longer

significant (p=0.071 and p=0.078, respectively). Further, a SNP at *SERPINA6/SERPINA1* (rs2749529) was associated with lower BMI ( $\beta$ =-2.42; p=0.016) and WC ( $\beta$ =-3.12; p=0.007).

**Conclusions:** These data show that genetic variants which alter glucocorticoid secretion and plasma protein binding in Caucasians are also associated with obesity and blood pressure in black South African women. Although we have still to confirm associations of these SNPs with steroid and CBG phenotypes in this cohort, our findings are consistent with a causative contribution of variation in glucocorticoid signaling to the prevalence of obesity and hypertension in black South Africans.

## OP23. The effects of two high-fat diet formulations and a Green Rooibos Tea extract on *in vivo* adiposity and *ex vivo* function of cultured adipose-derived stromal cells

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**Background:** Naïve progenitor cells isolated from adipose tissue (adipose-derived stromal cells: ADSCs) can be differentiated into mature adipocytes in culture in order to study adipocyte biology *ex vivo*. We investigated the effects of two different high-fat (HF) diet formulations on *in vivo* visceral adiposity and on the *ex vivo* differentiation of ADSCs from subcutaneous and visceral fat (scADSCs and pvADSCs, respectively), and whether these effects could be counteracted by a patented Green Rooibos Tea (GRT) extract.

**Methods:** Male Wistar rats were randomised into treatment groups at 4 weeks after weaning and remained in these groups for 16 weeks: control (standard chow); high fat diet (HFD: chow with cooking fat, condensed milk and sucrose) and high fat/high fructose/cholesterol diet (HF-FCD: chow with cooking fat, condensed milk, cholesterol, fructose and casein). Within each diet group, a subgroup of animals received GRT at 60mg/kg body weight from weeks 10 to 16. The animals were subsequently sacrificed and inguinal subcutaneous (SC) and perirenal visceral (PV) adipose tissue biopsies were harvested. Total body weight (BW) and PV fat pad weight were recorded (n=10 for each group). ADSCs were isolated from adipose tissue (n=5 for each group, except for n=8 for the HFD group) through collagenase digestion and centrifugation, and were maintained in culture in standard growth media (DMEM plus 10% fetal bovine serum). ADSCs in passage 2 were differentiated into mature adipocytes through treatment with adipocytic media (AM: standard growth media supplemented with dexamethasone, indomethacin, isobutylmethylxanthine and insulin) for 12 days. Intracellular lipid droplets were stained with Oil Red O (ORO), quantified with image analysis software and expressed as percentage area stained.

**Results:** The BW of the HF-FCD group was significantly increased (P < 0.01) compared to all other groups. The *in vivo* visceral adiposity index (inVAI: PV weight / total body weight x 100%) increased in the HFD and HF-FCD groups and was normalised in the HFD-GRT group. The *ex vivo* visceral adiposity index (exVAI: % ORO staining

in pvADSCs / % ORO staining in scADSCs from the same animal) was increased in the HF-FCD group and in some individuals in the HFD group, which was subsequently stratified into responders (HFD-r: exVAI > 1; n=4) and non-responders (HFD-nr: exVAI < 1; n=4). Unexpectedly, the exVAI was also increased in all GRT groups. In terms of % area stained with ORO, AM-induced lipid accumulation was decreased in scADSCs harvested from HF-FCD and HFD-r animals as well as from all GRT groups, compared to control, whereas differences in lipid accumulation in cultured pvADSCs from the different groups were not statistically significant.

**Conclusions:** Our results confirm that isolated primary ADSCs retain a memory of their physiological milieu before isolation and culture, and can therefore be utilised as a model system to study diet-induced obesity *ex vivo*. Both HF diet formulations increased visceral adiposity *in vivo* and suppressed the lipid accumulation of cultured scADSCs, demonstrating that the adipogenic response of ADSCs can be permanently re-programmed by a high-fat diet. GRT did not normalise lipid accumulation in scADSCs from HFD or HF-FCD animals, but rather suppressed lipid accumulation in scADSCs independent of diet. Consequently, the dietary use of GRT may be associated with suppressed subcutaneous fat accumulation *in vivo*, possibly resulting in lipids being shunted into visceral depots or ectopic sites.

#### OP24. Longitudinal association of PCSK9 variants with LDL-C levels and biomarkers of type 2 diabetes in a black South African population

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**Background:** The strategy for PCSK9 inhibition is more effective in lowering levels of LDL-C than the standard use of conventional statin drugs. Longitudinal studies of carriers of the loss-of-function (LOF) PCSK9 variants provide a proxy for the implications of lowering LDL-C using PCSK9 inhibitor drugs. Recently, mounting evidence has been documented of the associations of the PCSK9 variants with increased type 2 diabetes (T2D) risk among Asians and Europeans, thereby raising safety concerns of the PCSK9 inhibition drugs. However, different PCSK9 variants are associated with LDL-C lowering among people of African ancestry and little is known of their association with type 2 diabetes risk. Amazingly, the PCSK9 variants found among people of African ancestry have a greater LDL-C lowering effect compared to those common among Europeans and Asians. This study was conducted to determine the longitudinal association of the PCSK9 variants with LDL-C and biomarkers of T2D among black South Africans.

**Method:** We conducted a longitudinal study, nested within the Prospective Urban and Rural Epidemiology study using data collected during 2005 and 2010 among 737 apparently healthy, male and female black South Africans of Setswana descent. Genotyping of the C679X and A443T PCSK9 variants was achieved using Taqman assays from Applied Biosystems. Generalised estimating equations were used to determine longitudinal association of the A443T and C679X PCSK9 variants with LDL-C, fasting glucose and glycated haemoglobin while correcting for age, sex, BMI and urbanisation, baseline data.

**Results:** Significantly lower LDL-C levels per risk allele ( $p < 0.001$ ) were noted for compound heterozygotes of C679X and A443T variants ( $\beta = -1.61$  mmol/L (95%CI -2.23,-0.99)), carriers of C679X variants ( $\beta = -0.91$  mmol/L (-1.20, -0.61)) and A443T variant carriers ( $\beta = -0.438$  mmol/L (-0.63, -0.61)) compare to non-carriers. Consequently, the compound heterozygotes of C679X and A443T and C679X risk allele only carriers were significantly associated with reductions in fasting glucose ( $\beta = -0.8$ mmol/L (-1.57, -0.03);  $p = 0.041$  and  $\beta -0.40$  mmol/L (-0.79, -0.02);  $p = 0.042$  per risk allele respectively). However, a reduction in glycated haemoglobin was noted to be associated with the evaluated PCSK9 variants, though it was not significant.

**Conclusion:** Our results indicated that the apparently healthy carriers of A443T and C679X variants exhibit sustained low LDL-C levels and fasting glucose levels compared to non-carriers. The discrepancy in the association patterns of the A443T and C679X variants with fasting glucose and those of other PCSK9 variants common among Europeans is suggestive of ethnic specific effects of these variants. However, more investigation is required to further explore this phenomenon.

#### OP25. Dried spot cards of biologic fluids for diagnostic investigation of metabolic disorders

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**Background:** Collection of biologic fluid for laboratory analysis requires sampling in several fragile tubes, usually with additives. These analytes or matrices may be unstable so testing needs to be carried out quickly. Collection on filter paper can overcome instability and lower the cost of transporting the sample to laboratory.

**Aim:** This project aimed to develop an inexpensive, convenient, comprehensive and reproducible patient sample collection system, which ensures integrity and ease of transport of small scale samples at room temperature as well as ensuring convenient long-term storage for subsequent analysis.

**Methods:** Samples (blood, buffy coat, serum, plasma and urine) were collected into various tubes and spotted onto filter paper cards. Concentrations of triglyceride, cholesterol and phospholipid were measured in the original sample and dried plasma spots (DPS). Determination of oxidation of lipids by measurement of conjugated dienes (CD) and thiobarbituric acid reactive substances (TBARS) on the filter paper card was carried out. In addition, DNA extracted from a dried buffy coat spot (DBCS) from a familial hypercholesterolaemia patient was analysed. Values obtained from serum and dried spots were compared.

**Results:** The cholesterol, phospholipid and triglyceride values of 14 samples analysed for comparison of sample directly and dried spots stored at different temperatures were highly correlated after one week, and three months. Plasma cholesterol for DPS at one week was not significantly different to that at both the three and seven month analyses ( $p > 0.05$ ). The means of plasma triglyceride and phospholipid concentration were significantly different ( $p < 0.0001$ )

between one week and seven months. PCR analysis was successful for DNA extracted DBCS stored at room temperature. CD and TBARS in dried serum spot (DSS) on the filter paper without BHT/ethanol were significantly increased after one week of storage ( $p < 0.05$ ), with significant reduction of TBARS after three months ( $p < 0.05$ ). BHT prevented the increase in CD from baseline to three months. Sealed DSS with BHT increased the stability of CD and TBARS for three months.

**Conclusion:** Dried filter paper spots may be used to transport and store biologic fluid samples for analyses of a number of water-soluble and water-insoluble analytes. To protect lipids from being oxidised, the filter paper has to be pre-treated in BHT/ethanol.

### KP13. Triglycerides and remnant cholesterol in diabetes and cardiovascular disease

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### KP14. Lipoprotein(a) as a cause of cardiovascular disease

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### OP26. Is Lipoprotein(a) involved in keloid formation?

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**Background:** Lipoprotein(a) [Lp(a)] is a cholesterol ester-rich LDL-like particle, with a single apolipoprotein B100 (apoB) disulphide linked to apo(a). Lp(a) is the main carrier of oxidized phospholipid-apoB (OxPL-apoB). Both genetic and epidemiologic studies have shown that an elevated Lp(a) level is an independent causal risk factor for both atherosclerotic cardiovascular disease and aortic stenosis. However the physiological role of Lp(a) remains undefined. It has been postulated to play a role in tissue healing and innate immunity. Lp(a) may have offered evolutionary advantage to humans by promoting accelerated wound healing and the repair of tissue injuries and vascular lesions. In a physiological setting without the presence of cardiovascular risk factors these properties may have been beneficial. However in the setting of cardiovascular risk factors such as hypercholesterolaemia and hypertension, and increased oxidative and inflammatory stress present in modern societies, Lp(a) is clearly pro-atherogenic when Lp(a) is elevated.

Keloids are benign growths of dense fibrous tissue which result from an abnormal healing response to a cutaneous injury, extending beyond the original borders of the wound or inflammatory process. Keloids are common among black Africans. A previous study has shown that keloid scarring is associated with carotid atherosclerosis not explained by traditional cardiovascular risk factors.

We postulated whether there could be a causal link between Lp(a) and the higher prevalence of keloids in this population group. The aim of this study was therefore to measure Lp(a) in black African patients with keloids and to determine whether there is any relationship between Lp(a) and keloid formation.

**Methods:** Two hundred black African subjects were recruited, one hundred with obvious keloid scarring and one hundred controls

without scarring. After informed consent 20mL blood was drawn from a cubital vein. Lipid profiles (total-cholesterol, triglyceride, HDL-cholesterol and calculated LDL-cholesterol), sensitive-CRP and serum creatinine were measured using standard assays. Lp(a) concentrations were measured by using a turbidimetric isoform insensitive assay. Oxidised phospholipid-apoB levels were measured in both the cases and controls. Keloid tissue was also stained for Lp(a) and oxPL-apoB.

**Results:** There were no significant differences in mean age (27 vs 29 years), gender (40% vs 44% females) or weight (70.5 vs 74 kg) between the cases and controls. There were also no significant differences in the lipid profiles. The mean Lp(a) level was significantly higher in the cases compared to the controls (57.8 vs 44.2 mg/dL;  $p=0.01$ ) as were the oxPL-apoB levels (17.4 vs 15.7 nmol/L;  $p=0.009$ ). There was a large overlap in the range of Lp(a) levels between the two groups however. Keloid tissue stained for oxPL-apoB but not for Lp(a).

**Conclusions:** Lp(a) levels are increased in black African subjects with keloids but it remains uncertain whether Lp(a) is involved in scar-tissue (keloid) formation in humans.

### OP27. International Cholesterol Management Practice Study (ICPLS): South African Results

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**Background:** Lowering low-density lipoprotein cholesterol (LDL-C) with lifestyle interventions and statin-based therapy is core to the prevention and treatment of atherosclerotic cardiovascular disease. Lower LDL-C concentrations are associated with greater benefit and current guidelines recommend that LDL-C be lowered to 1.8 mmol/L, 2.5 mmol/L and 3.0 mmol/L in very high, high and moderate risk patients, respectively.

**Aim:** To determine the percentage of patients reaching LDL-C targets, as defined by the 2011 ESC/EAS guidelines for the management of dyslipidaemia, in patients at very high, high and moderate CV risk in South Africa.

**Methods:** ICPLS is a multinational, multicentre, non-interventional, single visit, cross-sectional study documenting real life management of dyslipidaemic subjects.

Patients over 18 were eligible if they had been receiving stable lipid-modifying therapy for at least 3 months when LDL-C was measured. LDL-C could be measured up to 12 months prior to the study visit provided lipid-lowering medication was unchanged from the time of measurement to the study visit. Patients receiving PCSK9 inhibitors or participating in clinical trials were excluded. Here we present the results of the South African study arm.

**Results:** Nineteen investigators (11 general practitioners) enrolled 396 eligible patients. Three study sites were located at public hospitals. The mean (SD) age of patients was 60.0 (10.2) years. The majority (64%) of patients were male, 47% were current or former smokers, 51% were hypertensive, 24% were diabetic and 34% of all patients had documented coronary artery disease. Heterozygous FH was confidently identified in 1% of patients. Statins were the most commonly prescribed lipid-modifying therapy (99%), but 75% of participants were not receiving high-intensity statin therapy.

Fibrates and ezetimibe were used in 4% and 3% of patients, respectively. The average duration of lipid-modifying therapy was 7 years and the statin prescribed had remained unchanged in 72% of patients. Untreated LDL-C levels were available for 33% of patients and were a mean (SD) of 3.9 (1.4) mmol/L. Mean (SD) on treatment LDL-C was 2.6 (1.0) mmol/L with a range of 0.5-8.3 mmol/L. The mean % LDL-C reduction from untreated baseline was 31.3%, with 77% of patients achieving < 50% reduction. Overall 60% of patients did not reach their LDL-C target. The failure to reach target rate was 70%, 48% and 26% for very high, high and moderate risk patients, respectively. Investigators reported statin intolerance (any degree) in 23% of patients and only 34% of these patients reached LDL-C target.

**Conclusions:** The majority of patients receiving lipid-lowering therapy in South Africa do not reach their recommended LDL-C target. Target achievement was lowest in very high risk patients where the LDL-C target is 1.8 mmol/L. Factors contributing to the failure to reach target include frequent failure to use high intensity statins, low use of statin plus ezetimibe combinations, failure to move to higher potency statins and a high rate of investigator reported statin intolerance.

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**Research Area :** Research Area , Lipids / LASSA

### OP28. LDL-cholesterol target achievement in patients with heterozygous familial hypercholesterolaemia at Groote Schuur Hospital: Minority at target despite large reductions in LDLc

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**Background:** Familial hypercholesterolaemia (FH) is characterised by markedly increased LDL-cholesterol(LDLc) and premature cardiovascular disease (CVD). LDLc lowering is the cornerstone of therapy and South African guidelines set an LDLc target < 3.0 mmol/L in January 2013.

**Aim:** To evaluate LDLc target achievement and explore reasons for not reaching target in FH patients attending a public sector lipid clinic at Groote Schuur Hospital (GSH).

**Method:** Clinical records of patients with genetically confirmed FH were reviewed for the following information: untreated, presentation and best lipid levels, current medication in patients who had attended the clinic after January 2013 and the reason for not up-titrating lipid lowering therapy (LLT) in patients who had not

reached target of LDLc ≤ 3 mmol/L and who were not using powerful statin therapy (defined as atorvastatin 80 mg or rosuvastatin 40 mg) or a combination of a powerful statin with other LLT.

**Results:** Of 1232 genetically confirmed FH patients, a representative random sample of 454 FH patients (37%) was analysed. The mean age (± standard deviation (SD)) of the cohort was 37 ± 19 years, 220 (49%) were male. 186 (41%) of those younger than 50 years had CVD at presentation and 273 (60%) had tendon xanthomata. The mean (± SD) untreated and presentation LDLc values were 7.4 ± 1.9 and 6.7 ± 1.9 mmol/L, respectively. At presentation 151 (33%) patients were taking LLT: 112 statins, 31 resins, 20 fibrates, 1 ezetimibe. The mean (± SD) best LDLc of the whole cohort was 4.4 ± 1.5 mmol/L. A total of 64 patients were seen after January 2013. Amongst these patients, 59 (92%) were taking statins and 22 (34%) were taking ezetimibe in addition to a statin. Despite a mean LDLc reduction of 50% (7.8 ± 2.1 to 3.9 ± 1.2 mmol/L) in the group of 64 patients with follow up after 2013, 48 (75%) of these 64 patients did not reach an LDLc < 3 mmol/L. Among the 20 patients (42%) who did not reach target and did not use the highest statin dose, the reason for not using powerful statin treatment was attributed to ongoing dose titration in 7 (35%) and statin side effects in 5 (25%) patients. The 21 patients who used statin (any dose) in combination with ezetimibe achieved a reduction from highest to best LDLc of 8.2 ± 2.0 to 3.7 ± 1.0 mmol/L.

**Conclusion:** The GSH FH population is at extraordinary high risk when compared to many other FH cohorts. CVD is highly prevalent. Untreated LDLc levels are so high, that 75% of patients did not reach LDLc target despite the use of powerful statins achieving a mean LDLc reduction of 50%. These findings emphasize the importance of early identification and aggressive treatment of FH including the use of combination LLT, with ezetimibe and other LLT.

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### OP29. Familial hypercholesterolaemia: classification of mutation severity according to observed LDL-cholesterol is useful for predicting cardiovascular disease risk

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**Background:** The phenotypic variability of familial hypercholesterolaemia (FH) is largely related to the severity of the underlying mutation. International guidelines advise grouping FH mutations according to receptor negative or defective status, but do not incorporate data on observed severity in terms of LDL-cholesterol (LDL-c) levels, surrogate markers of atherosclerotic

burden or cardiovascular disease (CVD) event rate. Recently, an analysis of the large Dutch FH registry showed good correlation between mutation stratification based on LDL-c percentile per mutation and coronary artery disease risk, but these results require external validation.

**Aim:** To establish CVD risk in South African patients with confirmed pathogenic *LDLR* mutations stratified according to mean LDL-c per mutation.

**Methods:** We studied patients seen at the lipid clinic of Groote Schuur Hospital between 1980 and January 2016 in whom the clinical diagnosis of FH has been confirmed by identifying a pathogenic *LDLR* mutation. The mean untreated LDL-c of each FH mutation was used to stratify mutations into quartiles: below 7.5, at 7.5, between 7.5 and 8.5, and above 8.5 mmol/L. We compared the mean carotid intima media thickness (cIMT) by mutation quartile adjusting for traditional cardiovascular risk factors, except lipid levels. We used Kaplan Meier with log rank test and Cox proportional hazard models to estimate and compare survival free from symptomatic CVD from birth until first non-fatal CVD event or first presentation at lipid clinic, whichever came first. Symptomatic CVD was defined as angina, myocardial infarction, percutaneous coronary intervention, coronary artery bypass graft, cerebrovascular accident or peripheral vascular disease requiring intervention.

**Results:** The median date of entry was December 1998. We identified 72 different pathogenic *LDLR* mutations in 1154 FH heterozygotes. The mean age ( $\pm$  standard deviation (SD)) at diagnosis was  $37 \pm 16$  years and 545 (47%) were male. Mean untreated LDL-c ( $\pm$  SD) were  $6,7 \pm 1,1$ ,  $7,5 \pm 1,8$ ,  $8,1 \pm 1,8$  to  $9,1 \pm 2,2$  mmol/L in ascending sequence of quartiles. Carotid IMT was performed in 317 individuals and mean cIMT ( $\pm$  standard error) increased per quartile from  $0,665 \pm 0,026$ ,  $0,681 \pm 0,013$ ,  $0,700 \pm 0,013$ ,  $0,724 \pm 0,024$ ,  $p$  for trend  $< 0,001$ . Event free survival (95% confidence interval) decreased from 58 (54-62), 55 (52-57), 55 (52-58), to 48 (44-51) years in quartiles 1, 2, 3, and 4 respectively, log rank  $p=0,038$ . The adjusted hazard ratio (95% confidence interval) associated with one step change in quartile was 1,20 (1,04-1,39),  $p=0,013$ .

**Conclusions:** Classifying mutation severity in heterozygous FH using observed mean LDL-c per mutation allows for more granular and, therefore, better risk stratification than the current approach of dichotomising *LDLR* mutations into receptor defective and receptor negative. The proposed approach will likely be of clinical use, when counseling patients on their expected cardiovascular risk, based on their identified FH mutation.

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**Research Area:** Research Area, Lipids / LASSA

### OP30. Proprotein Convertase Subtilisin Kexin type 9 (PCSK9) mutations: High prevalence likely explaining severe familial hypercholesterolaemia in Western Cape region

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**Background:** Familial hypercholesterolaemia (FH) is a prevalent genetic disorder characterised by autosomal dominant inheritance of hypercholesterolaemia and a markedly increased risk of cardiovascular disease (CVD). In FH a causal mutation is mostly identified in the low-density-lipoprotein cholesterol receptor (*LDLR*) gene or in the apolipoprotein B (*APOB*) gene, with a small minority ( $< 1\%$ ) having proprotein convertase subtilisin kexin type 9 (*PCSK9*) gain of function (gof) mutations. Complete genetic work up of the 3 FH genes in a cohort of FH patients selected using strict clinical criteria in Cape Town, found a remarkably high prevalence of *PCSK9* mutations where no other causal mutation had been identified in *LDLR* or *APOB*.

**Aim:** To describe the phenotype of patients with *PCSK9* mutations, stratified by known pathogenic and uncertain significance, and compare the phenotype to that of FH patients with *LDLR* or *APOB* mutations.

**Methods:** Individuals referred to the lipid clinic of Groote Schuur Hospital between 1980 and 2016 with clinically diagnosed FH underwent genetic analysis of the *LDLR*, and if applicable the *APOB* and *PCSK9* genes. Dichotomous variables were compared with chi-square tests and normally distributed continuous variables with the independent student t-test. We compared survival free from symptomatic CVD from birth until first non-fatal CVD event or first presentation at lipid clinic, whichever came first, with Kaplan Meier (log rank test) and Cox proportional hazard models.

**Results:** We identified 1234 persons with one mutated FH allele, of which 1154 were in the *LDLR* and 43 in *APOB*. Thirteen different non-synonymous *PCSK9* mutations were identified in 37 patients. Two mutations were found to be non-pathogenic (loss of function) in subsequent cascade screening. Of the remaining 11 *PCSK9* mutations 4 (7 individuals) were classified pathogenic based on published reports. One patient carried 2 pathogenic variants (R496W and E669K), 3 patients carried the S127R mutation and 3 the R469W mutation. The 7 patients with known pathogenic *PCSK9* mutations had non-significantly higher untreated LDL-c levels (mean  $\pm$  standard deviation) of  $8,84 \pm 2,84$  mmol/L than carriers of *LDLR* or *APOB* mutations with levels of  $7,88 \pm 1,93$  mmol/L and  $6,66 \pm 1,75$  mmol/L, respectively. The event free survival (95% confidence interval) was not significantly different between carriers of *PCSK9*, *LDLR* and *APOB* mutations at 52 (39-65), 55 (53-56) and 61 (56-66) years, respectively. The 24 FH patients with one of the 7 *PCSK9* mutations of uncertain significance had an intermediate phenotype

in the range between *LDLR* and *APOB* mutation carriers.

**Conclusions:** FH was due to *PCSK9* mutations in at least 0.6%, and possibly up to 2.8%, of patients in a large cohort of patients with clinical FH. Known pathogenic mutations give rise to a particularly severe FH phenotype. In South African FH patients without *LDLR* or *APOB* mutations analyzing *PCSK9* is likely to contribute to molecular diagnosis. Genetic cascade screening is necessary to establish pathogenicity of the 7 *PCSK9* variants of unknown significance, which have thus far not been found.

**Conflict Declaration:** An unconditional grant from Regeneron was made available for evaluating these *PCSK9* variants of unknown significance. RH acknowledges that his post doc research project in Cape Town was kindly supported by the Fritz Thyssen, the Atheros, and AmSTOL foundations.

### OP31. An investigation of the *PCSK9* gene polymorphism E670G and the risk of coronary artery disease in the South African black population.

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**Introduction:** The serum concentrations of LDL are determined primarily by the activity of LDL receptors (*LDLR*) in the liver. Recently, *PCSK9* the ninth member of the proprotein convertase family that promotes *LDLR* degradation through an endosomal / lysosomal pathway was identified. Variants in the *PCSK9* gene that affect *PCSK9* levels and activity result in either hypercholesterolaemia or hypocholesterolaemia. Thus, gain-of-function mutations in *PCSK9* reduce *LDLR* expression on the cell surface, which decreases cellular uptake of LDL resulting in elevated serum LDL concentrations. Conversely, a loss of function mutation promotes cellular uptake of LDL and lowers serum LDL concentrations thereby conferring protection against coronary artery disease (CAD). Recent studies have shown that the *PCSK9* polymorphism E670G plays a key role in determining plasma LDL concentrations, thus modulating the severity of atherosclerosis. Epidemiological studies suggest that the incidence of CAD is increasing in the South African black population and there is as yet no data on the prevalence of the *PCSK9* E670G polymorphism in this population. Therefore, the aim of this study was to determine the prevalence of the E670G polymorphism and its association with CAD in a South African black population group.

**Methods:** A group of 58 African subjects with CAD were recruited from the Charlotte Maxeke Johannesburg Academic Hospital cardiac clinic as well as a convenience sample of 60 African subjects with no history of CAD. Anthropometric measurements were taken and fasting serum lipid and plasma glucose concentrations were measured using routine laboratory methods. Whole blood was taken for DNA isolation and the *PCSK9* (E670G) polymorphism identified using a standard RFLP-based PCR method. The alleles at this polymorphic locus were A and G, with G being the risk allele.

**Results:** Subjects with CAD had higher glucose ( $p=0.003$ ), total cholesterol ( $p < 0.0001$ ), LDL ( $p < 0.0001$ ) and triglyceride ( $p < 0.0001$ ) but lower HDL levels ( $p < 0.0001$ ) than subjects without CAD. The BMI was similar across groups ( $p=0.75$ ) but the CAD group were older ( $p < 0.0001$ ) and included a higher number of male subjects ( $p < 0.0001$ ) than the non-CAD group. Subjects carrying the G allele i.e. GG or AG were more prevalent in the CAD group (72.4%) than

the non-CAD group (52.3%;  $p=0.02$ ). When comparing subjects with the GG or AG genotype to those with the AA genotype, there were no differences in LDL ( $p=0.98$ ), total cholesterol ( $p=0.39$ ) or HDL ( $p=0.76$ ) levels between the groups but triglyceride levels did tend to be higher in the subjects carrying the G allele ( $p=0.05$ ). Logistic regression analyses demonstrated that subjects carrying the G allele had an odds ratio (with 95% CIs) for CAD of 2.39 (1.12, 5.13) ( $p=0.02$ ) compared to subjects with the AA genotype.

**Conclusions:** The G allele of the E670G polymorphism in the *PCSK9* gene increases the risk of CAD in black African subjects by over 2-fold. It is possible that this effect is mediated by the modulation of lipid levels by this polymorphism however this hypothesis can only be confirmed in subjects with recent coronary events who have not been treated with lipid-lowering agents.

### OP32. Comparing the *in vitro* level of foam cell formation in young and older adults

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**Background:** In 2012, an estimated 17.5 million people died from CVDs, representing 31% of all global deaths. An estimated 7.4 million of these deaths were due to coronary heart disease and 6.7 million were due to stroke. The major cause of heart disease, myocardial infarction and stroke in western society is atherosclerosis, a chronic inflammatory disease characterized by lipid and cholesterol accumulation within the walls of arteries. A critical event in atherogenesis is the focal accumulation of oxidised-LDL (OxLDL)-laden foam cells (FC) derived from macrophages. Atherosclerotic disease is more prevalent in older subjects; however there is no data comparing FC formation across different age groups. Therefore the aim of this study was to compare *in vitro* FC formation between young and older adults.

**Methods:** Whole blood (45ml/person) was obtained from healthy young (20-25 years,  $n=11$ , 6 females) and older ( $\geq 40$  years,  $n=7$ , 6 females) adult volunteers. Weight, height, hip and waist circumference were measured in all subjects. Monocytes were extracted from buffy coats by gradient centrifugation using Histopaque. The monocytes were cultured overnight with RPMI medium. After 24 hours of culture the monocytes adhered to the culture plate and non-adherent lymphocytes were removed through washing with RPMI. Monocyte purity was determined using an antibody that binds to the monocyte/macrophage-specific antigen, calprotectin. Monocytes were differentiated into macrophages by incubation over 5 days in standard media supplemented with 100 ng/ml of macrophage colony-stimulating factor (MCSF). Formation of FCs was induced through exposure of macrophages to copper-oxidized human low density lipoprotein (OxLDL) at a concentration of 3.0  $\mu\text{g/ml}$  for 48 hours. The level of FC formation was determined by measuring intra-cellular lipid accumulation using the lipid-specific dye Oil red O. The level of lipid accumulation in the cells treated with OxLDL was expressed as a percentage of that observed in untreated cells.

**Results:** The young ( $23.3 \pm 1.35$  years) and older ( $57.1 \pm 10.4$  years;  $p < 0.001$ ) groups did not differ significantly in terms of BMI ( $23.4 \pm 3.29$  vs  $27.6 \pm 6.73$ ;  $p=0.11$ ). Lipid accumulation, used as a measure of foam cell formation, was significantly higher in the older ( $134 \pm 15.6\%$ ) than the younger ( $99.9 \pm 22.1\%$ ;  $p=0.003$ ) group. When only females were analysed across the age groups, the same significant trend ( $131 \pm 15.6\%$  versus  $98.2 \pm 25.3\%$ ;  $p=0.02$ ) was observed. If females ( $98.2 \pm 25.3\%$ ) were compared with males ( $102 \pm 20.3\%$ ;  $p=0.79$ ) in the younger age group, no significant differences in foam cell formation were observed.

**Conclusion:** This study shows that older female subjects have a higher level of *in vitro* foam cell formation than younger subjects. This may partially explain the increased prevalence of atherosclerotic disease observed with aging. Our data also shows that foam cell formation does not differ between genders in the younger age group. Due to the small number of older male subjects we were not able to analyse foam cell formation across age groups for this gender. Further studies are therefore required to confirm age-related differences in foam cell formation in male subjects.

### OP33. Erroneous low density lipoprotein cholesterol concentration with homogenous assay due to LpX in a patient with jaundice.

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**Background:** Hypercholesterolaemia is linked to atherosclerosis and risk is refined by determining low density lipoprotein cholesterol (LDLC) and high density lipoprotein cholesterol (HDLC) concentrations. Initially LDLC and HDLC were measured by ultracentrifugation; later concentrations of plasma triglyceride (TG), total cholesterol (TC) and HDLC were measured in routine chemistry laboratories and LDLC was calculated by the Friedewald formula provided that TG concentration is  $< 4.5$  mmol/L, and abnormal lipoproteins (dysbetalipoproteinaemia, lipoprotein X (LpX)) are absent. LpX is associated with cholestatic liver disease. Homogenous assays were developed for high throughput in automated machines; they rely on physical chemical properties to differentially measure cholesterol in lipoproteins. Unusual lipoproteins may react differently in these assays.

**Aim:** To explain severe hypercholesterolaemia in a jaundiced patient who had an unaccountable cholesterol fraction after determination of HDLC and LDLC by homogenous assays.

**Method:** A 54 year old hypertensive, non-insulin dependent diabetic woman with a history of single vessel coronary artery disease, transmural inferior myocardial infarction and severe congestive heart failure (LVEF 20%), on losartan, bisoprolol, hydrochlorothiazide, low-dose aspirin, metformin, long-acting insulin, and low-dose statin, developed weight gain and jaundice. She had overt congestive heart failure, jaundice, and mild hepatomegaly. Sonography revealed liver congestion, ascites, a normal sized spleen, and a normal biliary tree.

Echocardiographic studies confirmed severe congestive heart failure, ascribed to diabetic cardiomyopathy and a previous transmural inferior infarct. Angiography revealed a chronic total occlusion of the RCA, with unobstructed LCA. The hyperbilirubinaemia of 260 micromol/L was predominantly conjugated (55%); alkaline phosphatase and gamma-glutamyl transpeptidase activities were 1026 and 1194 U/L respectively whilst transaminases were normal. Serum albumin was 14 g/L. The TG, TC, HDLC and LDLC were 3.7, 18.6, 0.9 and 8.0 mmol/L respectively.

Apolipoproteins B (1.09 g/L) and A1 (0.51 g/L) concentrations were low. Markers for viral and auto-immune disease were negative. Non-denaturing gradient gel electrophoresis was performed after staining lipoproteins with sudan black (predominantly neutral lipid stain) and separating apolipoprotein B-containing lipoproteins. This was compared to lanes with variable dyslipidaemias including selected samples with large and small LDL.

**Result:** The patient had low intensity staining of LDL compared with other patients and its size was large. Compared with very low density lipoproteins, there was a narrower band of staining in this size range that was previously shown to be due to LpX. Utilising the area-under-the curve of densitometric analyses in the LDL range, the patient's LDLC was estimated to be  $< 2.6$  mmol/L.

**Conclusion:** Severe hypercholesterolaemia in this patient with intrahepatic cholestatic jaundice and congestive heart failure, is attributed to LpX. LpX not only accounts for the hypercholesterolaemia determined by the difference between TC and LDLC, but also for much of the apparent LDL hypercholesterolaemia. The concentration of apolipoprotein B is in support of a lower LDL concentration. The homogenous LDLC result is compatible with a monogenic disorder such as familial hypercholesterolaemia that could cause premature ischaemic heart disease but as demonstrated by electrophoresis and a lipid stain, is erroneously high. Homogenous assays for LDLC are not reliable in severe hypercholesterolaemia in the presence of jaundice.