ABSTRACTS

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MANAGEMENT OF GRAVES’ ORBITOPATHY: PRESENT AND FUTURE
Luigi Bartalena, Maria Laura Tanda, Adriana Lai
University of Insubria, Varese, Italy

Graves’ orbitopathy (GO) is an autoimmune disorder representing the main extrathyroidal expression of Graves’ disease. GO is present in about 50% of Graves’ patients, often in mild and self-limiting forms, but it is sight-threatening in 3-5%. Mild GO usually does not need specific treatments but local measures, such as artificial tears, ointments, dark glasses, prisms. Patients with sight-threatening GO, generally due to dysthyroid optic neuropathy, should receive immediate attention and be treated with high-dose IV glucocorticoids (GC), or, if not promptly responsive to the latter, be submitted to orbital decompression. Management of moderate-to-severe GO (20-30% of cases) depends on disease activity. If GO is active (florid inflammation) treatment relies on the use of IV GC with or without associated orbital radiotherapy. If GO is inactive (burnt-out disease), immunosuppression is ineffective, and treatment should be surgical (orbital decompression for disfiguring proptosis, squint surgery for extracocular muscle dysfunction, or eyelid surgery for lid retraction). Rehabilitative surgery is eventually required in at least one third of patients. Other medical treatments have been attempted in active GO. Currently available somatostatin analogs (octreotide or lanreotide) provided disappointing results in randomized clinical trials, but novel analogs (e.g. SOM230) might be more effective. The CD20+ B cell depleting agent, rituximab, was effective in an open study. Other immunomodulating agents, such as IL-1 or TNF-alpha antagonists, might be employed as in rheumatoid arthritis, but randomized clinical trials are warranted to support their use.

TREATMENT OF GRAVES’ HYPERTHYROIDISM
Luigi Bartalena, Maria Laura Tanda

University of Insubria, Varese, Italy

Graves’ hyperthyroidism is due to autoantibodies reacting with the TSH receptor on thyroid cells (TSH-receptor antibody (TRAb)). The ideal treatment should eliminate the cause, but this is presently unfeasible. Thus, management of hyperthyroidism is aimed at reducing excessive thyroid hormone synthesis by antithyroid drugs (ATDs) or ablating the thyroid by radioiodine (RAI) or thyroideectomy (Tx). Both RAI and Tx have the deliberate goal of inducing hypothyroidism. Selection of treatment depends on several factors, including age, goiter size, presence of the orbitopathy (GO), availability of an expert surgeon, concern with radioiodine therapy, compliance with ATDs. Indications for ATDs are: young age (including the neonatal period), pregnancy and lactation, small goiter, preparation to Tx or RAI. ATDs are usually safe (side effects in <5% of cases). The most serious adverse effect is agranulocytosis (rare, 0.1-0.3%). The major limitation of ATDs is the high recurrence rate (40-70%), almost inevitable if TRAb is present at the end of ATD course. If hyperthyroidism relapses (and goiter is small) RAI is the treatment of choice. RAI is the most used treatment in the elderly and generally is not used in children. Pregnancy and lactation are contraindications to its use. GO may progress after RAI, but this undue effect can be prevented by concomitant glucocorticoid treatment. Tx is the preferred treatment if goiter is large and hyperthyroidism is poorly controlled, in childhood, or if there is suspicion of associated malignancy. The surgeon must be expert to minimize complications, such as larveal nerve paralysis and hypoparathyroidism.

OSTEOPHGENESIS IMPERFECTA AND OSTEOPOROSIS, A POTENTIAL DIAGNOSTIC DILEMMA
Peter Beighton
Division of Human Genetics, Faculty of Health Sciences, University of Cape Town

Osteogenesis imperfecta (OI) is a heterogeneous genetic disorder in which bone fragility and radiolucency of the skeleton are major features. The overall population prevalence of OI is about 1:20,000, and in the Cape, more than 500 affected persons have been documented during the past 35 years. Osteoporosis (OP) is a common and major cause of disability which presents with reduced bone density and a propensity to pathological fractures. In these respects, OI and OP are very similar; at the mild end of the OP spectrum of involvement diagnostic differentiation at the clinical and radiological levels can be difficult. As both disorders are comparatively common, this issue can arise in routine clinical practice. Accurate diagnosis facilitates appropriate prognostication, management and medicinal therapy. With the advent of targeted drugs which may be specific for OI and not OP, and vice versa, diagnostic precision will be crucial for effective treatment.

ASSOCIATION BETWEEN THE PROINSULIN GENE POLYMORPHISM IVS-69 AND ADIPOSITY IN BLACK WOMEN
J Bermas, M Collins, H Baumgarten, C Seelig, O Jennings, Y Joffe, E Lambert, N E Levitt, J Dave, S West, M Faulenbach, S E Kahn, J H Goodecke

1UCT, 2MRC and 3University of Washington, USA

Objective: To examine the association between a 4bp proinsulin gene insertion polymorphism that affects pre-mRNA splicing (IVS-69) and a) fasting plasma insulin concentration, b) insulin secretion in relation to insulin sensitivity, and c) body composition, in black South African women.

Methods: 265 normal-weight (BMI<25 kg/m², n=115) and obese (BMI>30 kg/m², n=153) pre-menopausal black South African women aged 18-45 y were under went measurement of body composition (DXA), body fat distribution (ICT), fasting glucose, insulin and lipid profile, and proinsulin genotype. Insulin sensitivity (S_air) and acute phase insulin response (AIR) were assessed by a frequently sampled intravenous glucose tolerance test with Minimal Model analysis in a sub-sample of 14 normal-weight and 14 obese subjects.

Results: The frequency of the proinsulin VS-69 insertion allele was higher in the class 2 obese group (BMI>35kg/m²) compared to the normal-weight group (OR=2.0, 95%CI: 1.1-3.5, p=0.029). Subjects with the insertion allele had greater fat mass (32.0±15.4 vs 27.4±12.4 kg, p=0.039), greater fat-free soft tissue mass (43.1±6.8 vs 40.9±5.7 kg, p=0.028), and more subcutaneous adipose tissue (462±252 vs 360±211 cm², p=0.004) but similar visceral fat (p=0.118), than homoyzogotes for the wild type allele.

In the sub-sample of subjects carrying the 4bp insertion tended to have higher fasting insulin levels (12.1±8.7 vs 10.2±6.9 mU/l, p=0.078), be more insulin resistant (p=0.077), and, when adjusted for body fat, have improved β-cell function (disposition index, DI, (~3 x AIR); 432±259 vs 301±194×10⁻⁶ μU/ml, p=0.094), compared to subjects homozygous for the wild type allele.
Conclusion: The proinsulin gene variant (IVS-69) is associated with extreme adiposity in black South African women. Further research is required to examine the association between this insertion allele and altered β-cell function in these women.

A DESCRIPTIVE ANALYSIS OF TREATMENT IN TYPE 2 DIABETIC PATIENTS AT CHRIS HANI BARAGWANATH HOSPITAL, IN THE YEARS 2005, 2006 AND 2007

S Bhana, B Pauly
Division of Endocrinology, Chris Hani Baragwanath Hospital and University of the Witwatersrand, Johannesburg

Methods: An audit was undertaken to assess the standard of care delivered by registrars at the Diabetic Clinic. The type 2 clinic is managed by registrars, and they are expected to follow SEMDSA guidelines. From 2005 patients were regularly referred from the registrar managed diabetic clinic to the endocrine department. An inpatient file was created with the aim of capturing data and a SPSS-13 statistical programme was used to analyse the data. The following parameters were recorded: duration of illness; sex; age; presence of co-morbid illnesses (hypertension, stroke and/or obesity), family history of cardiovascular disease, recreational habits, use of aspirin and other treatments, clinical markers of vascular disease. BMI, waist measurements, MAC, %KE, HbA1C, lipogram, dyna-map reading of blood pressure and ECG.

Results: A total of 952 files were made from the period 2005 to 2007. 47 files were excluded for this evaluation for the following reasons. 24 were classified as type 1 DM, 6 had diabetes secondary to pancreatitis and 17 files had too little information for evaluation. Out of the remaining 905 patients 66.2% were females and 33.8% male the mean age is 57.7 years (range: 25 to 91). Mean duration is 10.27 years (range: 3 months to 42 years). 90% of patients required insulin of which 72.5% are on a biphasic insulin, 16.8% on once daily long acting insulin and a minority were on other insulin regimens. 49.2% reported a positive family history of type 2 diabetes. 22% had a history of cardiovascular disease. 2.8% had a history of ischaemic heart disease. 3.8% had a documented stroke. 3.8% have peripheral vascular disease. 87.9% were known to have hypertension, of which only 30.5% of the patients had a systolic blood pressure of less than 130mmHg, and 47.5% had a diastolic blood pressure of less than 80mmHg. The average systolic blood pressure was 143mmHg, and diastolic blood pressure 79.5mmHg. Erectile dysfunction was assessed in 61.5% of the male patients, of which 56.5% reported ED. The average Hba1c was 10.28% over the three year period. 35.3% of males and 71.3% of females were judged as overweight or obese without objective measurements. BMI was not measured in 27.7% of patients. The remaining 72.3% were found to have a BMI above 27 (SEMDSA guideline for action). Males had an average BMI of 28.76, and females had an average BMI of 34.7. Mean weight 85.04 Kg. Waist was measured in 72% of the females, of these 56.7% had waist circumference < 82 (SEMDSA). 85% of males had a waist measurement, of these 32% had waist circumference < 94 (SEMDSA).

83% had microalbumin/creatinine ratios assessed. The mean was 23.5 (range 0-579). 9.8% had macro and 27.1% had microalbuminuria. GFR levels were obtained in 87%: 3.1% had a GFR of less than 30, 16.8% had a GFR of between 30-60, and 67.8% of the patients greater than 60. 66.4% of patients had records of fundoscopy, of which 60.3% were reported as abnormal. 12.9% of the patients were not assessed for neuropathy, of the remainder, 41.9% had abnormalities. Aspirin therapy rose from 53.2% in 2005 to 68.5% in 2006 and 70.3% in 2007. In 2005 only 35% of patient files had a recorded full lipid screen prior to assessment. This percentage rose to 49% in 2006 and 67% in 2007. 8.4% admitted to smoking and 9% admitted to using alcohol.

A MATERNAL HIGH FAT DIET THROUGHOUT GESTATION AND LACTATION OR LACTATION ONLY INDUCES GLUCOSE INTOLERANCE IN WEANLING OFFSPRING

M E Cerf, C S Chapman, C J F Muller, J Louw
Diabetes Discovery Platform, Medical Research Council, PO Box 19070, Tygerberg, 7505, Cape Town, South Africa

We sought to determine the effects of high saturated fat programming during pregnancy and/or lactation on glucose tolerance in weanling Wistar rats. Dams were fed a high fat diet (HFD; 40% energy as fat) during specified periods of gestation and/or lactation. Their food intakes, body weights and glucose concentrations were monitored. Weanling offspring were produced from dams maintained on HFD throughout gestation (HFDG), throughout lactation (HFL) or throughout both gestation and lactation (HFG). Control weanlings were produced from dams maintained and a standard laboratory diet (10% energy as fat) throughout gestation and lactation. For an OGTT, weanlings were fasted overnight, glucose (50% solution at 2 g/kg) was administered orally, and blood glucose concentrations measured at 0, 15, 30 and 60 min.

At day 7 of lactation HFG dams had reduced food intakes in contrast to the greater food intakes of HFL dams. The increased food intakes of HFL dams persisted until day 14 of lactation. There were no changes in either the body weights or blood glucose concentrations in any of the dams during lactation. The OGTT revealed that HFL and HPGL weanlings had greater glucose concentrations at 10, 15, 30 and 60 min.

A lactational HFD induces a greater feeding response in dams. Maternal high fat feeding induces adverse programming effects in maternal high fat feeding induces adverse programming effects in...
weaning progeny. The high glucose concentrations during an OGTT, in both HFL and HFGL weanlings, suggest that these offspring are glucose intolerant.

**BONE GEOMETRY IN BLACK AND WHITE SOUTH AFRICAN FEMALES**

M Conradie, M M Conradie, F S Hough

*Division of Endocrinology and Metabolism, Tygerberg Academic Hospital and Stellenbosch University*

Numerous studies have confirmed the relationship between hip axis length (HAL) and risk of hip fracture, independent of bone mineral density status. A near doubling of hip fracture risk has been documented with every SD increase in HAL. Vertebral strength is also influenced by the geometry of the vertebrae and vertebral size has been shown to be reduced in women predisposed to vertebral fractures. Most studies of hip geometry have documented a shorter HAL in African-Americans compared with whites. A shorter HAL was also previously documented in SA black female nurses compared with whites. To assess previous observations of racial differences in skeletal geometry, geometric assessments were performed on our study cohort of 187 black and 186 white females. The HAL, femoral neck width and the vertebral cross-sectional area were compared in the total and mean area of vertebrae L1-L4. The DIEXA measurements were all performed using a Hologic QDR-1000 scan.

Adult black females in our study had a shorter HAL than whites. The approximately one standard deviation difference in HAL between our black and white cohorts, suggests a significantly lower hip fracture rate previously reported in SA black females compared with whites. Average vertebral size was smaller in black females and thus fails to explain the apparent lower vertebral fracture risk previously reported in this population.

**A COMPARATIVE STUDY OF KNOWN DETERMINANTS OF BONE STRENGTH IN BLACK AND WHITE SOUTH AFRICAN FEMALES**

M Conradie, M M Conradie, F S Hough

*Division of Endocrinology and Metabolism, Tygerberg Academic Hospital and Stellenbosch University*

Skeletal fracture rates are reportedly low in SA black females. Compared with whites, hip bone density (BMD) is higher in blacks, although previous studies conducted in black populations from the African continent, including SA, in fact suggest similar vertebral BMDs compared with whites. This comparative study aimed to assess fracture risk by evaluating known determinants of bone strength in a study cohort of 187 black women and 186 white SA women. Vertebral and femoral BMD was measured employing DEXA, quantitative ultrasound (QUS) variables were measured using the SAHARA sonometer and bio-chemical markers of bone turnover were determined. Vertebral BMD was similar in blacks and whites, whereas significantly higher femoral BMD was documented in blacks. An apparent slower decline in vertebral and femoral BMD was noted in blacks with ageing. Higher QUS measurements were documented in elderly blacks compared with whites. These variables showed no decline with ageing in blacks, in contrast to a significant deterioration in whites. Bone turnover was similar in the total pre- and postmenopausal black and white cohorts, but differed with ageing – a slower bone turnover rate was observed in blacks at the time of the menopausal transition. Bone turnover increased with ageing in postmenopausal blacks only and this can be partially ascribed to the more pronounced secondary hyperparathyroidism noted in blacks. Vertebral fractures occurred in a similar percentage of blacks (11.5%) and whites (8.1%) in our study, disputing the perception that blacks suffer more vertebral fractures. Most studies of hip geometry have documented a shorter HAL in African-Americans compared with whites. A shorter HAL was also previously documented in SA black female nurses compared with whites. To assess previous observations of racial differences in skeletal geometry, geometric assessments were performed on our study cohort of 187 black and 186 white females. The HAL, femoral neck width and the vertebral cross-sectional area were compared in the total and mean area of vertebrae L1-L4. The DIEXA measurements were all performed using a Hologic QDR-1000 scan.

Adult black females in our study had a shorter HAL than whites. The approximately one standard deviation difference in HAL between our black and white cohorts, suggests a significantly lower hip fracture rate previously reported in SA black females compared with whites. Average vertebral size was smaller in black females and thus fails to explain the apparent lower vertebral fracture risk previously reported in this population.

**BMD CHANGES IN OSTEOPOROTIC PATIENTS TREATED FOR AT LEAST 3 YEARS WITH INTRAVENOUS PAMIDRONATE DISODIUM**

M M Conradie, B H Ascott-Evans, F S Hough, M Conradie

*Division of Endocrinology, Stellenbosch University and Tygerberg Academic Hospital*

Bisphosphonates (BPs) are potent inhibitors of bone resorption and are widely used for the treatment of all forms of osteoporosis. Intravenous infusions (IVI) of the amino-BP pamidronate (PAM) disodium, with relatively long dosing intervals, overcome the dual problems of poor absorption and upper gastrointestinal side-effects associated with oral bisphosphonates. From our database of over 140 osteoporotics (treated with IVI PAM for at least twelve months), we conducted a retrospective study of forty-eight patients treated with PAM for three to six years. Twenty-seven of the study population received 30 mg of PAM every three months and 21 received 60 mg PAM every six months. The aims of the study were to investigate (1) whether there is a difference in BMD response between the two treatment groups and (2) to determine the increase from baseline in BMD (using dual-energy X-ray absorptiometry – DXA) after a minimum of 36 months of treatment. Statistically, when comparing the two treatment groups, results showed no difference in BMD response (Statistics 8; repeated measures ANOVA; L1-L4, p = 0.43; Total Hip, p = 0.44). Therefore, for further analysis, results of both groups were combined. The length of treatment and total dose of PAM assessed were 36 months and 360mg respectively. Mean baseline T-score at lumbar (L1-L4) spine, total hip and femoral neck were -3.07 ± 0.87, -2.32 ± 0.79 and -2.43 ± 0.71, respectively. After 36 months of PAM treatment, there was a significant increase in L1-L4 BMD (0.786 g/cm² vs. 0.850 g/cm², p < 0.005), but not in the total hip and femoral neck areas. Further analysis of the group up to 72 months of treatment confirmed that this increase from baseline was maintained (L1-L4: 0.793 g/cm² vs. 0.849 g/cm², p < 0.02). In conclusion (1) no significant difference in BMD between the two treatment regimes was noted, and (2) intravenous PAM treatment significantly increased BMD of the lumbar spine, but not total hip or femoral neck. No further change occurred from 3 to 6 years of treatment.
following time-spaced tetracycline labelling, confirmed a significant GC-induced reduction in osteoblast appositional rate and bone formation rate. However, similar results were observed in both the wild type and MKP-1 knockout mice, without any significant difference in the magnitude of the effect. We conclude that, although MKP-1 mediates the effects of GCs in immortalised MDA15.4 cells, it does not exclusively do so in vivo. Since vanadate can rescue the effects of GCs in rats, it is likely that other tyrosine phosphatases may mediate some of the effects of GCs in bone.

**HOME Glucose Monitoring and SCHOOLING as Determinant of Knowledge of Diabetes Control Parameters**

**J M A Cronje, D G van Zyl**

Department of Internal Medicine, Kalafong Hospital, University of Pretoria

**Objective:** To assess patient understanding and recall of diabetes control parameters (blood glucose, HbA1c level and blood pressure) after consultations in patients with different levels of schooling. To determine if patients using home monitoring of blood glucose, have an improved understanding of control parameters.

**Methods:** A convenience sample of 139 consecutive diabetic patients was selected from the two adult diabetic clinics at Kalafong hospital. Patients were requested to complete a questionnaire on their clinic visit immediately after leaving the consultation room. Patients that were illiterate were assisted with completion of the questionnaire. The questionnaire included demographic information and what they could recall from their doctors consultation.

**Results:** The mean age of patients was 55.1 years (SD: 14.0). One-hundred-and-four patients (74.8%) had 5 or more years of schooling. Of the patients 46 (33.1%) were treated with insulin only, 50 (36.0%) with oral agents and 43 (30.9%) were on combination of insulin and oral agents. Only 43 (31.7%) patients were doing home glucose monitoring. One hundred and fifteen (82.7%) of patients stated that their blood glucose, and 92 (66.2%) that their blood pressure were monitored. Patients doing home monitoring did not show better recall of these parameters than those who do not do home monitoring. Doctors taking care of diabetic patients should make sure that the patients are aware of whether glucose and blood pressure are controlled at each clinic visit. Novel ways of transferring patient control information may mediate some of the effects of GCs in bone.

**Conclusion:** It seems that patients had better recall of information about their control parameters if they attended school for 5 or more years. Patients doing home monitoring did not show better recall of these parameters than those who do not do home monitoring. Doctors taking care of diabetic patients should make sure that the patients are aware of whether glucose and blood pressure are controlled at each clinic visit. Novel ways of transferring patient control information may be needed to enhance patient understanding.

**Influence of Catch-Up Growth on Insulin Action and Secretion in 7-Year-Old Children**

**N J Crowther, N Cameron, J Truster, M Toman, S A Norris, J P Gray**

Department of Chemical Pathology and Paediatrics, University of the Witwatersrand, Johannesburg, South Africa, and Department of Human Sciences, Loughborough University, England

The aim of this study was to investigate the effect of catch-up growth occurring at different stages of childhood on glucose levels and beta-cell function at seven years of age. Oral glucose tolerance tests (OGTT) were performed on 152 seven-year-old children. Anthropometric data were available from birth to seven years of age. Children were split into catch-up, catch-down and normal growth groups based on growth rates between birth and one, birth and five and birth and seven. Fasting, 30 and 120 minute blood samples collected during the OGTT were assayed for glucose, insulin, proinsulin and des-31, 32 proinsulin and area-under-the-curve (AUC) values calculated. Children with catch-up growth between birth and five or birth and seven years had greater AUC insulin levels than catch-down children. Children with catch-up growth only between birth and seven years exhibited higher proinsulin levels and a greater insulin secretory response to glucose than those who experienced catch-up growth between both birth and one and birth and seven years of age. Low birth weight children with no catch-up growth between birth and seven had the highest glucose and lowest insulinogenic index levels while children with high birth weight and catch-up growth had the highest insulin levels. Extremes of birth weight in conjunction with extremes of postnatal growth are all detrimental to childhood metabolism. The negative metabolic effects of catch-up growth between birth and seven may be attenuated if catch-up growth also occurs between birth and one year of age.

**Growth hormone treatment and the transition period**

**P R Czernichow**

Paediatric Endocrinology, Necker Enfants Maladies Hospital, 149 rue de Sevres, Paris, France

Improved adult height is a major goal in growth hormone (GH) treatment of children with GH deficiency. However it has been clearly shown that adults with severe GH deficiency have an increased risk of mortality for cardiovascular diseases. This is due to increased cardiovascular risk factors including elements of the metabolic syndrome. GH replacement in adults improves abdominal fat distribution and metabolic profile. Therefore, in GH deficient individuals, replacement throughout the life cycle is indicated.

In this respect, clinical programmes need to be developed for adolescent with GH deficiency for the transfer of care from paediatric to adult endocrine clinics.

Transition refers to a broad set of physical and psychological changes starting at the end of puberty and ending with full adult maturation. Reassessment of aetiology and anterior pituitary function is of crucial importance as GH deficiency may be transient, and because the level of GH secretion is mandatory to decide if GH treatment is necessary.

Transition care requires a dedicated service with contributions from paediatric and adult endocrinologists. For the patients continuing GH, a treatment regimen to optimise body composition and cardiovascular function needs to be delivered. A long-term surveillance programme is also recommended for those who will stop GH treatment.

**Neonatal diabetes mellitus: a disease linked to multiple mechanisms**

**P R Czernichow**

Paediatric Endocrinology, Necker Enfants Maladies Hospital, 149 rue de Sevres, Paris, France

Transient (TNDM) and permanent (PNDM) neonatal diabetes mellitus are rare conditions occurring in 1:350 000 live births. In TNDM growth retarded infants develop diabetes in the first few weeks of life only to go into remission in a few months with possible relapse to a permanent diabetes state usually around adolescence or as adults. We believe that pancreatic dysfunction in this condition is maintained throughout life with relapse initiated at times of metabolic stress such as puberty or pregnancy. In PNDM, insulin secretion failure occurs in the late fetal or early post-natal period. A number of conditions are associated with PNDM, some of which have been elucidated at the molecular levels. Among those, the very recently elucidated mutations in KCNJ11 and ABCC8 gene, encoding the Kir6.2 and SUR1 subunit of the pancreatic KATP channel involved in regulation of insulin secretion accounts for one third to a half of the PNDM cases. Patients with TNDM are more likely to have intrauterine growth retardation and less likely to develop ketoadidas than patients with PNDM. In TNDM, patients are younger at the diagnosis of diabetes and have lower initial insulin requirements. Considerable overlap occurs between the two groups, so that TNDM cannot be distinguished from PNDM based on clinicial features. Very early onset diabetes mellitus seems to be unrelated to autonomic maturity in most instances. Recurrent
Young rats are more sensitive to changes in the fat content of the diet. High-fat diets can induce insulin resistance and type 2 diabetes mellitus in women with thyroid disease were compared by quantitative RT-PCR. Male rats were fed from weaning (3 weeks of age) on a diet 3/19/08 2:57:32 PM

Diabetes mellitus is common in patients with “transient” neonatal diabetes mellitus and, consequently, prolonged follow-up is imperative. Molecular analysis of chromosome 6 anomalies, the KCNJ11 and ABCC8 genes encoding Kir6.2 and SUR1 provide a tool to identify transient from permanent neonatal diabetes mellitus in the neonatal period. This analysis also has potentially important therapeutic consequences leading to transfer some patients, those with mutations in KCNJ11 and ABCC8 from insulin therapy to sulfonylureas.

Results: PPAR gamma expression was normalised with 2 stably expressed housekeeping genes. Comparison of the different fat deposits indicated that PPAR gamma expression was constant in all 3 deposits, so data were pooled for subsequent analyses. The relative changes in PPAR gamma mRNA in 2, 4 and 9 month old rats on 10%, 20% and 40% fat diets were analysed using qBase software. Two month old rats showed a 3-fold increase in PPAR gamma mRNA when fed 20% fat, and a 4-fold increase when fed 40% fat, as compared to normal control rats. By 4 months PPAR gamma was expressed at a constant level (equivalent to that of 2 month old rats fed 40% fat) regardless of the fat content of the diet.

Conclusion: Young rats are more sensitive to changes in the fat content of their diets than older rats.

EFFICACY OF BAZEDOXIFENE FOR THE TREATMENT OF POSTMENOPAUSAL OSTEOPOROSIS

T de Villiers, S Silverman, C Christianся, H Genα, J Zanchettaα, I Valteβ, G Constantine, A Chinesα

1Panorama MediClinic and Stellenbosch University, Cape Town, South Africa; 2Cedars-Sinai Medical Center and UCLA, Los Angeles, CA; 3CCBR, Ballerup, Denmark; 4UCSF and Synarc Inc., San Francisco, CA; 5University of El Salvador, Metabolic Research Institute, Buenos Aires, Argentina; 6CCBR, Tallinn, Estonia; 7Wyeth Research, Collegeville, PA

This phase III study was designed to evaluate bazedoxifene (BZA), a novel selective estrogen receptor modulator (SERM), for the treatment of postmenopausal osteoporosis.

Generally healthy postmenopausal women with osteoporosis (N=7482: mean age, 66.4 y) were randomized to daily therapy with BZA 20 or 40 mg, raloxifene (RLX) 60 mg, or placebo for 3 years; all women were supplemented with up to 1200 mg calcium and 400-800 IU vitamin D. The primary endpoint was the incidence of new vertebral fractures after 3 years. Secondary endpoints included nonvertebral fracture (NVF) incidence, bone mineral density (BMD) response, and serum bone markers.

Relative to placebo, estimates of the reduction in new vertebral fracture risk were 42%, 37%, and 42% with BZA 20 and 40 mg and RLX 60 mg, respectively (p<0.001). The incidence of NVFs was similar among groups. In a subgroup of women at higher fracture risk (n=1772), BZA 20 mg showed a significant reduction in NVF risk relative to placebo or RLX (p<0.05); combined data for BZA 20 and 40 mg showed a significant reduction in NVF risk relative to placebo (p=0.03). BZA treatment was associated with significant increases in BMD at all skeletal sites evaluated (p<0.001) and significant reductions in serum marker levels compared with placebo (p<0.001). BZA was generally well tolerated over 3 years of therapy.

BZA treatment of postmenopausal women with osteoporosis effectively reduced the risk of new vertebral fractures and, in women at higher fracture risk, decreased the risk of NVF.

AN ANALYSIS OF PREGNANT THYROID PATIENTS PRESENTING TO CHRIS HANI BARAGWANATH HOSPITAL

C Druck, K R L Huddle, R Shires

Division of Endocrinology, Chris Hani Baragwanath Hospital and University of the Witwatersrand

Aim: An analysis of the causes of thyroid diseases and outcomes of pregnancy in patients presenting between 2004 and 2008.

Methods: Maternal characteristics, causes of thyroid disease and perinatal outcomes were reviewed. The subgroup of patients with Graves’ disease, diagnosed by the presence of Graves’ ophthalmopathy, positive thyroid antibodies, or both, was analysed separately.

Results: 85 pregnancies in mothers with thyroid disease were managed between 2004 and 2008. The mean age of these patients was 28.8 years and mean duration of pregnancy at presentation was 20 weeks. They were followed for an average of 18 weeks. 53 delivered at Chris Hani Baragwanath Hospital, 11 will deliver in the next 3 months; 3 had intrauterine fetal deaths; 2 aborted spontaneously;
there was 1 molar pregnancy in the group and 15 were lost to follow up or delivered elsewhere. Mean APOAR scores at 1 and 5 minutes were 8.7 and 9.8 respectively.

There were 67 hypothyroid pregnancies of which 47 were in 42 women with Graves’ disease. 66% of the previously diagnosed patients were hypothyroid at the time of conception.

The results demonstrate markedly impaired androgen binding. 34.5% of control, which was not thermolabile in our patient with Reifenstein’s syndrome.

Conclusion: We report Reifenstein’s disease in two South African black men, including an evaluation of androgen binding.

**HIGH FAT FEEDING DURING GESTATION DOES NOT ALTER LIPIDEMIA OR CHOLESTEROLEMIA IN WISTAR RATS**

U Gerber, K Williams, C J F Muller, J Louw, M E Cerf
Diabetes Discovery Platform, Medical Research Council, PO Box 19070, Tygerberg, 7505, Cape Town, South Africa

Our aim was to determine whether gestational high fat (HF) feeding alters the plasma free fatty acid (FFA), triglyceride and cholesterol concentrations. Rats were either fed a chow (control) or high fat diet (HFD) throughout gestation (HFG). After 3h fasting, blood was collected from the tail vein at e0 (pre-pregnancy) and at e7, e14 and e20 of gestation. Total plasma FFA, triglyceride and cholesterol concentrations were determined.

Total plasma FFA, triglyceride and cholesterol concentrations were measured in control dams at e20 vs. e0, e7 and e14. In HFG dams, FFA concentrations were elevated at e14 and e20 vs. e0. There were no differences between the FFA concentrations of HFG and control dams at any stage of gestation. Triglyceride concentrations were elevated at e20 vs. e0 in control dams. In HFG dams, triglyceride concentrations were elevated at e14 and e20 vs. e0. Total plasma cholesterol concentrations were reduced at e14 vs. e7, and elevated at e20 vs. e0 and e14. No differences were found in cholesterol concentrations in HFG dams vs. control dams. In control dams, cholesterol concentrations were reduced at e14 vs. e0, and elevated at e20 vs. e0 and e14. No differences were found in cholesterol concentrations in HFG dams at any stage of gestation. There were no differences in cholesterol concentrations when comparing the HFG and control dams at any stage of gestation.

Hypertriglyceridermia was induced by a HFD during early (e7) gestation and mid-gestation (e14). HF feeding at each stage of gestation parallels control feeding in relation to the total FFA and cholesterol profiles. Thus HF feeding during gestation does not appear to induce either hyperlipidemia or hypercholesterolemia.

**ETHNIC DIFFERENCES IN BODY COMPOSITION AND INSULIN SENSITIVITY: ROLE OF TISSUE-SPECIFIC ADIPOKINE EXPRESSION**

J H Goedecke, B Walker, S E Kahn, T Olsson, on behalf of the SA Women Obesity Research Group

1Medical Research Council and University of Cape Town, South Africa; 2University of Edinburgh, Scotland; 3University of Washington, USA; 4Umea University, Sweden

Objective: To examine ethnic differences in body composition, insulin sensitivity and tissue-specific adipokine expression in lean and obese black and white South African women.

Methods: Sixty lean and obese black and white premenopausal women with no known disease were recruited for the study. Body composition and distribution (DXA and CT) and insulin sensitivity in patient A

During repair of the hypospadias, a scrotal skin biopsy was performed and, for a control, a specimen of genital skin was obtained from a normal patient of similar age undergoing circumcision. Fibroblasts from these specimens were harvested and cultured. Androgen binding of [3H]-methyltrienolone, a non-aromatizable androgen, to fibroblast monolayers was measured at 37°C and 42°C.

**RESULTS**

| Binding of [3H]-methyltrienolone in fibroblast monolayers (fmol/mg protein) |
|---------------------------------|------------------|------------------|
| **Patient A** | **Control** | **Patient A** | **Control** |
| 37°C | 12.9 | 37.4 |  |
| 42°C | 10.4 | 38.2 |  |

**RESULTS**

The results demonstrate markedly impaired androgen binding.

**CONCLUSION**

We report Reifenstein’s disease in two South African black men, including an evaluation of androgen binding.
relation to insulin secretion (frequently sampled intravenous glucose tolerance test with minimal model analysis) were measured. Biopsies were taken from the deep and superficial subcutaneous adipose tissue (SAT) and gluteal depots for measurement of adipokine mRNA levels by quantitative real-time PCR.

Results: Lean and obese black and white women were matched for body fatness (30.5±1.3 vs. 29.7±1.3 % and 46.9±1.2 vs. 44.4±1.3 %, p=0.40). Although there were no ethnic differences in fat distribution between lean women, obese white women accumulated more VAT (144±11 vs. 102±11 cm², p<0.01), whereas obese black women accumulated more superficial SAT (321±13 vs. 244±14, p=0.01), with no ethnic differences in deep SAT (p=0.92). Lean and obese black women were less insulin sensitive than their white counterparts (S̄_glu: 5.7±5.5 and 1.8±5.5 vs. 3.6±5.5 x 10^{-6} mIU/l, p<0.01) but had a greater acute insulin response to glucose (AIRg: 171±43 vs 55±41 and 295±38 vs 76±41 mU/l). It correlated with % body fat and VAT in white (R=0.59, p=0.01 and R=0.51, p<0.01), but not black (R=0.24, p=0.21 and R=0.27, p=0.20) women. Glucocorticoid receptor-α mRNA levels were lower (p<0.001) and leptin, C/EBPβ and STAT5a mRNA levels were higher (p<0.001, p<0.05 and p<0.05, respectively) in black compared to white women.

Conclusion: The association between body composition and insulin sensitivity is ethnic specific, and is associated with differential adipokine expression.

INcorporation of the c9:11 and c10:12 cLA ISOMERS INTO HUMAN Skeletal Muscle and ADIPOSE TISSUE

Julia H Goedecke 1,2, Dale E Rae 1, Marius Smuts 3, Estelle V Lambert 1, Marianne O'Shea 3

1UCT/MRC Research Unit for Exercise Science and Sports Medicine, Department of Human Biology, Faculty of Health Sciences, University of Cape Town, South Africa; 2Medical Research Council of South Africa; 3Loders Croklaan Lipid Nutrition, The Netherlands

Background: A previous study from this laboratory showed that 12 weeks of CLA supplementation decreased mean insulin levels in response to an oral glucose tolerance test in normal weight exercising females compared to a control group.

Object: The aim of this study was to measure the incorporation of the c9:11 and c10:12 CLA isomers into skeletal muscle and adipose tissue in a sub-sample of this cohort with a view to understanding the observed alteration in insulin sensitivity.

Methods: In this randomised, double-blind, placebo-controlled trial, 25 individuals (10 males, 15 females) ingested either 3.9 g-d-1 CLA (50:50 c9:11:c10:12) (CLA group, n=14), BMI: 24.2±2.2 kg·m^{-2}) or 3.9 g·d-1 high oleic acid sunflower oil (placebo group, n=11, BMI: 24.5±2.4 kg·m^{-2}). Skeletal muscle phospholipids were measured.

Results: The c10:12 CLA isomer was significantly incorporated into adipose tissue triglyceride of the CLA group following the supplementation period (p<0.001). There was a trend for the c9:11 CLA isomer to be incorporated into the skeletal muscle phospholipids of the CLA group following the supplementation period (p=0.001). In addition, these changes were accompanied by a significant incorporation of oleic acid (p=0.019) into the adipose tissue triglyceride, and a trend towards an increase in oleic acid in the skeletal muscle phospholipids (p=0.056).

Conclusion: CLA is incorporated into adipose tissue and skeletal muscle in an isomer-specific manner in normal weight, exercising individuals following 12 weeks of CLA supplementation.

IMPACT survey of industrial action on diabetic PATIENTS AT A PUBLIC SECTOR hospital

T Gunther, D G van Zyl

Department of Internal Medicine, Kalafong Hospital, University of Pretoria

Objectives: To assess the impact and subjective perceptions of the public sector strike in June 2007.

Setting: Diabetic outpatients attending a diabetic clinic in Kalafong Hospital (a secondary hospital).

Methods: A survey was done in patients attending the clinic during the first month after the strike ended. All patients were requested to complete a questionnaire on the impact that the strike had on them as well as their perceptions surrounding the strike. HbA1c values before and after the strike were compared to assess the impact of the strike on glycaemic control.

Results: The survey forms were distributed to 500 diabetic patients, of which 410 were completed (response rate: 82%). Of these 403 were adequately completed for evaluation. A significant proportion of patients, 58.6%, stated that they were affected by the strike. The proportion of patients that reported that they were slightly, moderately and severely affected was 30.3%, 17.1% and 11.2% respectively. One hundred and forty-four (35.7%) of the surveyed patients missed an appointment with a diabetes clinic doctor and 165 (49.9%) could not collect prescribed medication from the hospital pharmacy. Patients reported being afraid to come to hospital or anticipated that they would not be helped was 192 (47.6%) and 133 (33%) respectively. With regards to medication 25.8% were without diabetic tablets for at least one day, 15.4% without insulin and 22.6% without blood-pressure medication. The HbA1c before and after the strike did not change significantly, the proportion of patients with well-controlled blood glucose (HbA1c < 7%) declined slightly 25.8% to 23.9% (p = 0.456).

Conclusion: The strike in public sector hospitals during June 2007 affected a significant proportion of diabetic patients attending the Kalafong Diabetes Clinic. This however did not reflect in the glycaemic control of patients.

Growth hormone: a method comparison study of the Automated chemiluminescent immunoassay IMMULITE 1000® VS the manual immunoradiometric CISbio® assay

C Haukansson, J King, T S Pillay

Chemical Pathology, C17 NHLS Laboratory, Groote Schuur Hospital and University of Cape Town, Cape Town, South Africa

Aim: Discontinuation of commercial assay kits is a common cause for diagnostic laboratory assay switching. Before such a change is undertaken, a method comparison study serves as an interassay bias study. The planned introduction of a new manual immunoradiometric CISbio® growth hormone assay during August 2007, performed in C17 lab, to replace an interim send-away assay performed on the automated chemiluminescence Immulite 1000® assay necessitated a method comparison study. Additionally, as a small subsidiary project, an easier modification of the label CISbio® separation technique was investigated.

Methods: 28 frozen samples, which had been assayed on the Immulite 1000® platform, were thawed and analyzed using the CISbio® kit according to the package insert. Linear regression, absolute and relative difference graphs were plotted.

Results: Plotting CISbio® on the y axis and Immulite 1000® on the x axis, a regression equation of y = 0.77x + 0.21 with an R value of 0.97 was obtained: the Immulite 1000® yield higher readings. Taking total analytical error for growth hormone as 20%, the absolute and relative difference plots showed that a clinically significant bias between the two assays exists. Although, the label separation study was hampered by low sample numbers, it yielded a regression equation of y = 0.63x + 1.24; R value = 0.99.

Conclusion: Immunoassay standardization is a universal problem in laboratory diagnosis. These two assays compare favorably but a significant bias exists that will influence result interpretation, especially if literature-derived “cut-offs” are used.
Maturity Onset Diabetes of the Young (MODY) in South Africa: Database on Incidence, Clinical Features, Biochemistry and Genetics

M Hoffman, B H Ascott-Evans, Y Yako, T Matsha, M Kotze, R T Erasmus

NHLS/Division of Endocrinology/Genetics, Stellenbosch University and Tygerberg Academic Hospital

MODY was described in the 1960s, before the present pandemic of obesity so that our clinical ability to categorise diabetes in the young. MODY occurs in children and young adults, usually with a strong family history of similar early onset diabetes. Characteristically, persistent hyperglycaemia is not associated with progression to ketoacidosis and insulin is not required ab initio. Many different subsets of MODY and the genes responsible have now been described, in all forms there is a dominantly inherited monogenic defect of insulin secretion, usually presenting before age 25. All known alleles are expressed in beta cells; insulin sensitivity is normal.

Although non-genetic factors play no major role in the pathogenesis of MODY, conditions which reduce insulin sensitivity (e.g. pregnancy) may bring out or aggravate the diabetes. There are no reported studies from RSA looking at the genetics or other aspects of MODY in our local populations. Our multi-disciplinary team (including molecular geneticists) will look at all aspects of MODY (including clinical and biochemical) and act as a national referral centre for genetic testing.

We aim to identify new cases of MODY and screen families for asymptomatic cases by developing a simple diagnostic tool with appropriate genetic testing. In particular, we aim to categorise the frequency of previously identified mutations in our MODY patients of different ethnicity, as well as to look for new mutations if indicated. Mutation analysis of the MODY gene will use state of the art molecular genetic techniques to not only identify sequence variants in MODY genes (bioinformatics tools, PCR, high resolution melt (HRM) analysis, etc), but to also categorise those sequence variants. We will then be able to analyse the DNA of family members. We will also genotype normal individuals using these mutations, using iRIM analysis, as part of our genotype-phenotype correlation studies.

Bone Disease in Diabetes Mellitus

Stephen Hough

Division of Endocrinology, Department of Medicine, Faculty of Health Sciences, Stellenbosch University and Tygerberg Academic Hospital

A variety of musculoskeletal disorders are associated with diabetes mellitus and include limited joint mobility, neuropathic arthropathy (e.g. Charcot joint), hand abnormalities (e.g. carpal tunnel), Dupuytren’s, diabetic scleodactyly, reflex sympathetic dystrophy etc), diabetic muscle infarction and diabetic bone disease. The earliest effect of diabetes on bone is seen in the increased prevalence of skeletal malformations in the fetuses of diabetic mothers. Bone abnormalities and fractures may also result from late complications of diabetes e.g. renal failure, neuropathy, visual impairment etc. It is, however, the role of diabetes as the cause of a metabolic bone disease resulting in a generalised decrease in bone mass and skeletal fragility (osteoporosis) that has attracted attention recently.

We and others have shown that Type 1 diabetes is associated with impaired bone formation, a low bone mass (BMD) and an increased risk (up to 12X) of fracture. Hyperglycaemia, insulinopaenia, malnutrition, acidosis and alterations in various other hormones (IGF, vitamin D, amylin, GLP) have been proposed as causes of the low bone formation osteoporosis associated with Type 1 diabetes mellitus.

Type 2 diabetes (T2DM) also predisposes to fracture. Although changes in insulin, leptin and adiponectin have been implicated, the cause of the skeletal fragility in T2DM remains unknown and BMD in this disease has been shown to be low, normal or even increased. Furthermore, drugs used to treat T2DM like the TZDs have been shown to stimulate adipogenesis, decrease osteoblastogenesis, decrease bone formation and BMD, and predispose to fractures. It is concluded that diabetes and its treatment are clinically important risk factors for osteoporotic fractures.

AngII Receptor Antagonism Improves Nitric Oxide Production, ENOS and PKB/Akt Expression in Hearts from a Rat Model of Insulin Resistance

B Huisamen, S Pérel, S O Friedrich, H Strijdom, A Lochner

Department of Biomedical Sciences, Division of Physiology, Faculty of Health Sciences, Tygerberg

Physiological concentrations of NO play an important role in maintaining normal vascular function. NO synthase (NOS) activity and NO production may be chronically impaired in insulin resistance because exogenous insulin therapy appears to improve endothelial function in such patients. Insulin stimulates the production of NO in endothelial cells and cardiomyocytes by activating a signalling pathway including insulin receptor substrate-1 (IRS1), phosphatidylinositol-3-kinase and protein kinase B (PKB/Akt).

AngII plays a pivotal role in the development of atherosclerosis and hypertension. AngII type 1 (AT1) receptor-evoked oxidative stress is implicated in the inactivation of NO, leading to impaired endothelium-dependent vasodilatation. Blocking the actions of AngII with an AT1 receptor antagonist (Losartan), has beneficial effects in patients with insulin resistance or type 2 diabetes mellitus.

In view of the above, we investigated whether (i) elevated AngII influenced myocardial insulin resistance, insulin signalling and NO production in a rat model of diet-induced obesity (DIO) and (ii) Losartan can alleviate this.

Hyperphagia-induced obese, insulin resistant rats (DIO) were compared to age-matched controls. Half the animals were treated with 10 mg/kg Losartan for 1 week. Afterwards, isolated hearts were perfused with or without 0.03 uIU/mL insulin. Fasting blood glucose, insulin (RIA), and body weight were recorded. Western Blotting and flow cytometric methods were utilized to determine protein expression, phosphorylation and NO production respectively. Stat: ANOVA followed by the Bonferroni correction (p<0,05 as significant).

Results: Hearts from DIO rats were insulin resistant as reflected by elevated SerP of IRS1, lower insulin stimulated phosphorylation of both PKB/Akt and eNOS and lower NO production. Losartan, restored the impaired NO production and PKB/Akt and eNOS expression in hearts from the DIO animals. Losartan also unmasked that AngII signaling modulates myocardial PKB/Akt and NO expression. We conclude that AngII signaling leads to inhibition of NO production in insulin resistance that can be restored by AT1 antagonism.

Increased Flux Through the Hexosamine Biosynthetic Pathway Induces Gene Promoter Activity of Acetyl-CoA Carboxylase

Jamie Imbriale, M Faadiel Essop

Department of Physiological Sciences, Stellenbosch University

The cardiac isoform of acetyl-CoA carboxylase (ACCo) produces malonyl-CoA, a potent inhibitor of mitochondrial fatty acid (FA) uptake. Increased flux through the hexosamine biosynthetic pathway (HBP) under hyperglycemic conditions may contribute to insulin resistance (IR). We hypothesized that increased HBP flux induces cardiac ACCO gene expression thereby diminishing mitochondrial FA uptake. This may lead to intracellular lipid accumulation and the onset of myocardial IR in the pre-diabetic context (hyperglycaemia).

We transiently transfected cardiac-derived rat H9c2 myoblasts with a 1317 bp human ACCO promoter-luciferase construct (pFL-P; 1317) and an expression construct encoding the rate-limiting step of the HBP P-gluotamine:fructose 6-phosphate amidotransferase (pGAT) ± HBP inhibitors/activators.

pGAT overexpression increased pFL-P; 1317 activity by 75 ± 23% vs. controls (p<0.001). Moreover, in the presence of varying concentrations of L-glutamine (0-8 mM), an HBP substrate, pFL-P;
1317 activity was dose-dependently increased. To corroborate this, we administered two GFAT inhibitors, i.e. 40 μM aza and 40 μM 6-diacyl-5-oxo-L-norleucine (DON) to transfected cells for 24 hrs. Both aza and DON attenuated pPII-1317 activity vs. controls (p<0.01). In agreement, co-transfections with 2 dominant negative GFAT constructs also diminished pPII-1317 activity (p<0.01). Furthermore, inhibition of an HBP enzymatic regulator acting downstream of GFAT, i.e. O-GlcNAc transferase, with 2 mM alloxan attenuated pPII-1317 activity by 35.6 ± 1.9% vs. controls (p<0.001). To gain further insight into transcriptional mechanisms, we investigated upstream transactivation factors (USF1, USF2) as candidates. USF2 induced pPII-1317 activity by 44 ± 23% vs. controls (p<0.001). Co-transfection of the GFAT construct with a reporter-promoter construct containing consensus USF binding elements resulted in a marked vs. controls. (p<0.001).

This study demonstrates that increased HBP flux induces ACCO gene promoter activity, implicating USF2 as a transcriptional modulator regulating this process. We further propose that such an induction would reduce cardiac FA oxidation, leading to intracellular lipid accumulation (sarcocremal FA uptake exceeding mitochondrial FA oxidation) triggering the onset of myocardial IR.

**EFFECT OF ⁹⁹mTc-MDP INJECTION ON BMD MEASUREMENTS USING A LUNAR DXA DENSITOMETER**

Sumaya Ismail

Department of Nuclear Medicine, Groote Schuur Hospital, Cape Town

Although manufacturers of dual X-ray absorbismetry (DEXA) machines recommend a delay of a bone density scan after the administration of radiocurcides, they do not provide any reason for it. Studies performed to investigate this phenomenon provide conflicting results. It has been demonstrated that different results are obtained using different machines.

The main objective of this study was to determine whether the presence of an intravenous dose of ⁹⁹mTc-methylene diphosphonate (⁹⁹mTc-MDP) had an effect on measured bone mineral density (BMD) and whether the magnitude of this effect was clinically significant. Secondary objectives were to determine potential associations between BMD, recorded weight and the measured dose of ⁹⁹mTc-MDP administered.

Twenty eight patients attending the clinic scheduled for a radionucleide bone scan had BMD measurements done prior to the administration of a diagnostic dose of ⁹⁹mTc-MDP and 2 hours after the injection using a Lunar DPX-1Q densitometer. BMD measurements were done at the lumbar spine (L1-L4) and both hips at the femoral neck, trochanter as well as the total hip. The mean changes in BMD measurements for the lumbar spine were 0.136 g/cm², the equivalent of a reduction of 12.36% (p=0.004). Although all measured sites for the hip showed a reduction in readings these did not exceed the L2C for these regions. Correlations with weight showed that bigger differences in readings correlated with increased weight. No likely correlations were found with ⁹⁹mTc-MDP dose.

Our study confirms the reports of a significant effect of ⁹⁹mTc-MDP on lumbar BMD using a Lunar densitometry. These effects were not seen at femoral sites of measurement. Lumbar BMD measurements following ⁹⁹mTc-MDP need to be interpreted with caution.

**INHIBITION OF THE INSULIN RECEPTOR KINASE BY ANTIRETROVIRAL PROTEASE INHIBITORS**

W. L. W. Ismail, T. S Pillay

Division of Chemical Pathology, Department of Clinical Laboratory Sciences, Faculty of Health Sciences, University of Cape Town

Human immunodeficiency virus protease inhibitors (PIs) are potent antiretroviral agents. Unfortunately, prolonged use of the drugs has caused many complications among patients, including insulin resistance, lipodystrophy, hyperglycaemia, and type 2 diabetes mellitus. The molecular basis for these drug-induced metabolic syndromes is still unknown. Few studies have addressed the molecular pathways involved. Thus, this study was designed to elucidate how PIs may affect the insulin signalling pathway in Chinese Hamster Ovary cells transfected with high levels of human insulin receptor (CHO-IR cells) and in differentiated 3T3-L1 murine adipocytes. We have also examined the effects of these drugs on lipoprotein lipase in the adipocytes. Cells were treated with a range of doses of PIs (saquinavir and indinavir) for 16 h and then stimulated with insulin. Insulin-stimulated tyrosine phosphorylation was analyzed by immunoblotting with anti-phosphotyrosine antibody. Saquinavir (30-40 μM) displayed potent inhibition of the tyrosine phosphorylation of the insulin receptor β-subunit and IRS proteins in both cell types. In contrast, indinavir (60 μM) had mild effects on insulin signalling. Thus both drugs display differential effects in their effects on insulin signalling and this may influence the propensity to cause the side effect of insulin resistance seen in patients on the drugs. We postulate that the inhibition of the insulin receptor kinase may occur because of direct effects on the kinase domain or because of changes in the level or activity of tyrosine phosphatases.

**THE TNFA GENE -308 G/A POLYMORPHISM MODULATION OF THE RELATIONSHIP BETWEEN DIETARY FAT INTAKE AND OBESITY RISK IN BLACK SOUTH AFRICAN WOMEN**

Yoel Jeff, Madelaine Carstens, Malcolm Collins, Courtney Jennings, Lize van der Merwe, Naomi Levin, Kellie Lambert, Julia H Goecks

1ESSM, Department of Human Biology, and the 2Diabetes and Endocrine Unit, Department of Medicine, University of Cape Town, and 3South African Medical Research Council, Cape Town

Objective: To explore diet-genre interactions between dietary fat intake and the TNFA -308 G/A polymorphism on obesity and its associated risk factors for cardiovascular disease in black South African women.

Methods: 105 normal weight and 118 obese urbanised black South African women were recruited. Body composition (DXA), body fat distribution (CT), and fasting serum glucose, insulin and lipid levels were measured and insulin resistance estimated (HOMA-IR). Dietary intakes was assessed using a validated food frequency questionnaire. Subjects were genotyped for the functional -308 G/A polymorphism within the TNFA gene.

Results: There were no significant differences in the genotype (p>0.345) or allele frequency (p>0.463) of the TNFA -308 G/A polymorphism between normal weight and obese groups. In addition, no significant genotype associations were found for body fatness or distribution, fasting [glucose], [insulin], HOMA-IR, or serum lipid levels. However, there were significant interaction effects between dietary fat intake and TNFA -308 G/A genotypes. An increase in dietary fat intake (% energy) was associated with increased obesity risk in subjects with the pro-inflammatory TNFA A allele, but not the G allele (OR=1.25, CI: 1.1-1.4, p=0.016). There was also a significant interaction effect between the omega 3 fatty acid, α-linolenic acid intake (ALA) (% energy), and TNFA -308 G/A polymorphism on the TC:HDL-C ratio (p=0.008). An increase in ALA intake was associated with an increased TC:HDL-C ratio in subjects with the TNFA -308 GG genotype, whereas the opposite was observed in subjects with the A allele.

Conclusion: The TNFA -308 G/A polymorphism modifies the relationship between dietary fat intake and obesity risk and serum lipid levels in black SA women. Therefore, a better understanding of diet-genre interactions is required when devising dietary recommendations for disease prevention.

**MULTIPLE ENDOCRINE NEOPLASIA TYPE 2A – A CASE REPORT**

Klisiewicz A M, Raal F J

Division of Endocrinology and Metabolism, Department of Medicine, Johannesburg Hospital

Introduction: MEN type 2A is an autosomal dominant genetic syndrome consisting of medullary thyroid carcinoma,
pheochromocytoma and hyperparathyroidism. Genetic testing for mutations in the RET proto-oncogene should now be part of the standard workup in all patients with suspected MEN type 2A.

Case report: A 34 year old black male originally from the Congo presented to the Johannesburg Hospital with a hypertensive emergency. A diagnosis of pheochromocytoma was made based on his urine metanephrine results and the patient underwent a laparoscopic left adrenalectomy following on tumour localization by means of CT, MRI and I-123 MIBG imaging. Pre-operative work up showed elevated calcium, PTH and calcium levels and a diagnosis of MEN 2A was made. Following on initial surgery for the pheochromocytoma the patient underwent total thYROidecToMy with central node dissection and parathyroidectomy. Histology confirmed the diagnosis of adenalf pheochromocytoma, medullary thyroid carcinoma and parathyroid hyperplasia. The patient underwent genetic testing which was positive for a mutation in exon 11 of the RET gene (G634R).

Conclusion: MEN 2A is still under diagnosed in many areas of the world, despite the fact that genetic testing and early management can result in dramatically lower mortality. DNA analysis is the gold standard for assessment of at risk individuals and identification of the RET mutation is an indication for early thyr oidectomy and close observation.

DETECTION OF LIPOPROTEIN X BY NON-DENATURED POLYACRYLAMIDE GRADIENT GEL ELECTROPHORESIS

M le Riche1, B Ratanes2, P Byrnes2, R T Erasmus2, A D Marais2

1Chemical Pathology Division, Department of Pathology, NHLS, Tygerberg Hospital, Stellenbosch University; 2Lipidology Division, Department of Medicine, Groote Schuur Hospital, MRC Cape Heart Group, University of Cape Town

Lipoprotein X (LpX) is an abnormal cholesterol-containing particle that may be present in the serum of subjects with cholestasis, lecithin:cholesterol acyltransferase (LCAT) deficiency and intravenous lipid infusions. This study investigated the utility of a non-denaturing polyacrylamide gradient gel electrophoresis (GGE) system in the detection of LpX.

Icteric samples received at the Chemical Pathology laboratory, Tygerberg Hospital, were analysed by GGE. Total bilirubin (TB) concentration was measured in all samples. Depending on sample volume, various parameters and ratios known to associate with LpX were also evaluated, including conjugated bilirubin (CR), alkaline phosphatase (ALP), gamma-glutamyltransferase (GGT), total cholesterol (TC), HDL-cholesterol (HDL-C) (including subtypes), triglycerides (TG), phospholipid (PL), bile acid (BA) and free cholesterol (FC) concentration, and agarose gel electrophoresis (AGE) was performed. Laboratory, demographic and clinical data was reviewed.

Serum concentration of CR, ALP, GGT, FC, PL, FC:TC and CR:TC all strongly associated with LpX-positive GGE results (p<0.0001). The best predictor of LpX was FC:TC > 0.6. LpX-positivity in GGB and AGE agreed in 84% of cases. In the setting of obstructive liver disease LpX was seen in 66% of patients if total cholesterol was >7.5 mmol/L.

The study showed that this GGE system is a useful test in demonstrating LpX in icteric plasma and has potential for a screening test in LCAT deficiency.

VITAMIN D DEFICIENCY IN GAUTENG

Stanley Lipschitz

Osteoporosis Clinic, Rosebank, Johannesburg

Vitamin D is essential for intestinal absorption of Calcium. Lack of Vitamin D results in secondary hyperparathyroidism, and increased bone resorption. Vitamin D deficiency is associated with low BMD and increased risk of hip fracture, as well as elevated risk of falls in the elderly.

UVB rays from sunlight on the skin converts Pro Vitamin D3 to Vitamin D3, which is then hydroxylated in the liver and kidney to the active metabolite 1-25 (OH) Vitamin D.

The main circulating metabolite of Vitamin D is 25(OH) Vitamin D3, which is the correct functional indicator of the body’s Vitamin D status.

In a cross sectional international study of 2588 women with osteoporosis from 18 countries, Lips et al. demonstrated that 30.8% had Vitamin D levels <20ng/ml, and 63.9% levels <30ng/ml. These findings were consistent through all countries studied (Northern and Southern Hemisphere).

No study of Vitamin D status has been conducted in Gauteng, South Africa. In this study we attempt to define the following:

1. The incidence of Vitamin D deficiency in a cohort of post-menopausal women referred to an osteoporosis clinic for assessment
2. The relationship of low Vitamin D levels to PTH levels
3. The relationship of Vitamin D levels to BMD T scores at spine and hip.

EARLY CHANGES FOLLOWING BARIATRIC SURGERY IN SOUTHERN AFRICA

M G Logan1,2, M S Pepper1, M T van der Merwe1,2,3, G K Fetter4

1Molecular and Metabolic Medicine Research Group, UP; 2NetCare Bariatric Centers of Excellence, 3Department of Endocrinology, UP; 4University of the Witwatersrand and Sunninghill Hospital

Aim: To provide a descriptive analysis of a retrospective study on a typical cohort of patients undergoing bariatric surgery in two hospitals in SA in Bariatric Centers of Excellence setting.

Materials and methods: 330 patients (2005-2007) mean BMI 45.87 ± 0.63 (mean ± SEM) characterized pre-op (clinical anthropometric & DEXA). 130 matched for same parameters post-op over 9-12 month observational period. Statistical analysis: Students paired t-test & Pearson’s correlation regression analysis.

Results:

Parameter Pre-op Post-op ≤ 3 months ≥ 3 months

| FG | 5.4 ± 0.1 | 5.14 ± 0.09 * | 4.85 ± 0.1 ** |
| Cholesterol | 5.18 ± 0.1 | 4.45 ± 0.1 *** | 4.51 ± 0.12 *** |
| Triglyceride | 1.73 ± 0.1 | 1.39 ± 0.06 ** | 1.36 ± 0.11 *** |
| Waist | 137 ± 2.65 | 118 ± 2.19 *** | 114 ± 3.29 *** |
| Hip | 148 ± 2.02 | 131 ± 2.2 *** | 126 ± 4.04 *** |
| Weight | 137 ± 3.4 | 118 ± 3.82 ** | 110 ± 5.2 ** |
| BMI | 46.2 ± 1.16 | 41.5 ± 1.4 *** | 39.1 ± 1.92 *** |

*p<0.05, **p<0.01, ***p<0.005, ****p<0.001

Conclusion: Average post-op weight loss – 20% of initial pre-op weight. Co-morbid diseases & anthropometric measurements illustrated significant changes post-op. Risk factor scoring is a valuable pre-op tool for assessing eligibility for surgery.

INVESTIGATION INTO THE DEVELOPMENT OF INSULIN RESISTANCE IN RATS

MacKenzie J, Roekemoer T, Deallty G, v d Venter M, Roux S

Department of Biochemistry and Microbiology, Nelson Mandela Metropolitan University, Port Elizabeth

The prevalence of diabetes mellitus (DM) type 2 in children is on the rise world wide. The aim of our study was to investigate the development of insulin resistance using a rat model. Weaned male Wistar rats were fed with high fat diet (HFD) (40% fat kcal) and a control group was fed with low fat diet (LFD) (9.5% fat kcal) for 12 weeks. The rats were sacrificed at different time points (day 0, day 7, day 14, week 8, and week 12) and blood plasma samples were analysed. Fasting plasma glucose levels were stable over a period of 12 weeks but corresponding insulin levels were significantly increased in the HFD group from day 14. Calculating the quantitative insulin sensitivity check index (Quicki) we were able to observe a...
significant decrease of the insulin sensitivity compared to the control diet. Analysis of the free fatty acids (FFA) in the blood plasma showed that already after 7 days of HFD a significant increase can be seen compared to the control diet. This difference in FFA can also be observed at all the later time points. Since it has been shown that increased free fatty acids in the plasma can lead to the development of DM type 2, this aspect will receive prominent attention in further research. Our findings confirm that our rat model did develop insulin resistance on the high fat diet. With this model we are now able to investigate detailed metabolic changes in different tissues.

ESTABLISHING A RAT MODEL FOR INSULIN RESISTANCE AND USING IT AS A SCREENING MODEL FOR POTENTIAL TREATMENTS
Kevin K, Koekemoer T, Siko S, Smith N, Dealtry G, van der Merwe M, Roux S
Department of Biochemistry and Microbiology, Nelson Mandela Metropolitan University, Port Elizabeth

Diabetes mellitus (DM) type 2 is on the increase worldwide. In South Africa various plant extracts are used by traditional healers to treat DM. For most of them no scientific data exist on how they effect the pathological changes in the body. The aims of our study was 1) to establish an insulin resistant rat model and 2) to use Metformin as treatment to test a standard for expected results. Male Wistar rats were fed with a high fat diet (HFD) (43% fat kcal) (not commercially available) for 3 months. Rats were sacrificed to confirm insulin resistance. A second group of rats continued with the HFD and was treated for 28 days with Metformin. Rats fed with low fat diet (LFD) were used as control. After 3 months on HFD a significant decrease in insulin sensitivity could be observed, taking fasting plasma glucose and insulin levels into account. Metformin treatment for 28 days increased the insulin sensitivity. The measurement of glucose uptake into different tissues showed a decrease in the HFD group. Treatment with Metformin increased the glucose uptake in liver and muscle. Analyzing free fatty acids (FFA) in plasma we can confirm the correlation between the onset of insulin resistance and increased FFA levels. Other plasma lipid parameters also confirm a pathological change. Triglycerides and total cholesterol levels are increased in the HFD group and are lowered with the Metformin treatment. Concluding our findings, we were able to establish an insulin resistant model by feeding rats with a self made diet high in fat. Treating those rats for four weeks with Metformin showed positive results and can now be used to compare indigenous plant extract in further studies.

MACROPROLACTINOMA IN PREGNANCY
A Magan, N Goolam Mahyoodeen, K Huddle
Division of Endocrinology, Department of Medicine, Chris Hani Baragwanath Hospital and University of the Witwatersrand, Johannesburg

Aim: To describe the progress and outcome of pregnancy in a 24 year old woman with a macroprolactinoma.

Methods: The patient was followed throughout pregnancy and the postpartum period. Relevant details were recorded and analysed.

Results: A macroprolactinoma (PRL 634 μg/L; 15x11mm on CT scan) was first diagnosed in June 2004. Bromocriptine was used initially but failed to achieve complete control and the side effects proved intolerable. Clinical and biochemical control was achieved after 17 months of cabergoline 1μg twice weekly. The patient conceived in November 2005 and stopped therapy shortly thereafter. Cabergoline 0.5μg twice-weekly was restarted at 14 weeks gestation because of the reappearance of severe headaches, although the MRI showed no change in tumour size. The course thereafter was uneventful. The patient now be used to compare indigenous plant extract in further studies.

Conclusion: We report the successful outcome of a macroprolactinoma in pregnancy treated with cabergoline. This is consistent with other reports in the literature. However, more data are still required with regards to this drug’s safety in pregnancy.
Conclusion: Screening with a fundal camera improved the quality of care for diabetic patients and is feasible in the South African public sector, primary care setting. A single technician should be able to photograph almost 10,000 patients a year. It is hoped that this project will be a model for other health districts.

A PROPOSED METHOD TO MEASURE BODY COMPOSITION IN OBSESE INDIVIDUALS USING DXA

Lisa Mckliefeld1, Sorrel Reid1, Linda Bewerunge2, Elaine Rush3, Julia Goodeck1,3

1UCT/MRC Research Unit for Exercise Science and Sports Medicine, Department of Human Biology, Faculty of Health Sciences, UCT, SA; 2Institute of Sport and Recreation Research New Zealand, Faculty of Health and Environmental Sciences, AUT University, Auckland, New Zealand; 3MRC, Parow, South Africa

Objective: Dual-energy x-ray absorptiometry (DXA) is a tool to measure body composition. However, its application is limited in individuals who do not fit in the scanning area. Therefore, the aim of this study was to i) validate a new method to measure body composition in obese individuals using DXA, and ii) compare to an existing method. Methods: Body composition was measured using DXA in 50 individuals between 17-68 years with varying body fatness (BMI: 19.9-39.3 kg/m²) and body weight (56.2-120.9 kg). Body composition (fat mass, fat-free soft tissue and bone mineral content) was compared using the arm-replaced (AR) vs. existing half-body (HB) method, compared to the reference whole-body scan (RS). Linear regression and Bland-Altman analyses assessed the accuracy and intra-tester reliability.

Results: There was no significant difference in fat mass, fat-free soft tissue or bone mineral content between the AR method and the HB method, and neither were significantly different to the RS. However, there were smaller differences for the AR compared to the HB method for fat mass (AR: r=1.000, limits of agreement [LOA]=-0.24-0.33 kg vs. HB: r=0.999, LOA=-0.93-0.90 kg), fat free soft tissue mass (AR: r=1.000, LOA=-0.62-0.26 vs. HB: r=0.997, LOA=-2.06-0.75 kg), and bone mineral content (AR: r=1.000, LOA=-0.03-0.01 vs. HB: r=0.991, LOA=-0.18-0.06). Intra-tester reliability was not significantly different between the two methods. Conclusion: The AR method is a valid and accurate measure of body composition, and is less variable than the HB method.

Reference: Mckliefeld LK, Reid S, Bewerunge L, Rush EC, Goodeck JH. Validation of a new method to measure body composition in very-obese individuals using dual-energy x-ray absorptiometry. International Journal of Body Composition Research 5 (4): 147-151, 2007.

SITE-SPECIFIC DIFFERENCES IN BONE MASS BETWEEN SA CHILDREN OF DIFFERENT ETHNIC GROUPS

Lisa Mckliefeld1, Shane Norris1, Estelle V Lambert1, Lize van der Merwe1, John M Pettifor2

1UCT/MRC Research Unit for Exercise Science and Sports Medicine, UCT, SA; 2Wits/MRC Mineral Metabolism Research Unit, University of Witwatersrand, Johannesburg, SA; 3Biostatistics Unit, MRC, Cape Town, SA

We have previously shown that although shorter and lighter, SA children of mixed ancestral origin have greater whole body bone mineral content compared to black and white IS and SA children. To more closely investigate site-specific differences between ethnic groups within South Africa, we compared lumbar spine (LSBMC), proximal femur (PBFMC) and femoral neck (PNBMC) bone mineral content in black (SAR, n=263) and white (SAW, n=73) children from Johannesburg, and in children of mixed ancestral origin (RAM; n=64) from Cape Town, South Africa, measured using Hologic DXA machines.

After adjustment (age, weight and height), the following pattern was significant for LSBMC between the girls: ISM>SMB>SAR. The same pattern was significant for adjusted FSMBMC in both girls and boys.

reosorption that is initiated when osteoclast precursors, derived from the circulation and adjacent marrow, most likely through capillary sinusoids in bone remodelling compartments (BRCs), are programmed to differentiate into osteoclasts. These resorb a certain amount of bone and die. The resorption cavity is prepared for the reversal phase, in which the resorbed bone is replaced when incoming osteoclast precursors differentiate and form new bone. Growth factors released from resorbed bone matrix can contribute to preosteoclast differentiation and bone formation. The preosteoclasts themselves, growing in the resorption space, can communicate through cell contact and paracrine signalling mechanisms to differentiate. Osteoclasts can sense the need for bone repair by detecting damage and pressure changes, and signalling to surface cells to respond appropriately. Now that it has been shown through mouse genetics thatPTHrP generated locally in bone is a crucial physiological regulator of bone formation, and probably also of resorption, we need to understand how local PTHrP release is controlled in bone in the remodelling process, so that it can both promote differentiation of osteoblasts and inhibit their apoptosis.

There is some evidence to support a view that osteoclasts in the BMU might also generate activity that contributes to bone formation, and could even complement the direct effect that PTH has in promoting differentiation of committed osteoclast precursors. First, both human and mouse genetics provide evidence supporting the view that osteoclasts, despite in some circumstances being unable to resorb bone, e.g. failure of acidification or of cathepsin K activity, can nevertheless be associated with normal, or even increased bone formation. An implication is that it may be possible to design reosorption inhibitors that do not block bone formation. Second, PTH administered intermittently in an anabolic regime results in transient activation of osteoclasts, and prevention of the latter in a number of experimental approaches has been associated with blunting of the PTH anabolic effect. It is possible that osteoclasts, transiently activated by PTH can contribute to the coupling of bone formation to reosorption by producing activity that influences preosteoblast participation in bone formation.

SCREENING FOR DIABETIC RETINOPATHY IN PRIMARY CARE WITH A MOBILE FUNDAL CAMERA: EVALUATION OF A SOUTH AFRICAN PILOT PROJECT

Bob Mash1, Di Powell2, Felicity du Plessis3, Unita van Vuuren2, Margaret Michalowska3, Naomi Levitt3

1. Division of Family Medicine and Primary Care, Department of Interdisciplinary Health Sciences, Stellenbosch University; 2. Cape Town, Metropolitan District Health Services and Programmes, Provincial Government of the Western Cape; 3. Division of Endocrinology, Department of Medicine, University of Cape Town

Background and aims: Diabetic retinopathy is a leading cause of adult blindness. An efficient screening can reduce the incidence. This project aimed to implement and evaluate a new service for retinal screening in the Cape Town metropolitan district. Screening in primary care was offered using a non-myrdriatic mobile fundal camera. This is the first time such a service has been evaluated in a South African primary care context.

Methods: The service was implemented as an operational research study at three community health centres and data were collected to evaluate the operational issues, screening, reporting and referral of patients.

Results: Out of 400 patients screened 84% had a significantly reduced visual acuity. 63% had retinopathy (22% severe non-proliferative, 6% proliferative and 15% maculopathy), 2% of eyes could not be screened and 14% of patients required dilatation. Referral was necessary in 27% of cases for cataracts, in 7% for laser treatment and in 4% for other specialist services. Repeat photography was needed in 8% and urgent follow-up in 12%. A SWOT analysis of the pilot project was completed and recommendations were made on how to integrate it into the district health system.
After adjustment, SAM boys and girls had significantly higher PFBMC than their SAB and SAW peers. In addition, PFBMC was significantly higher in SAB boys than SAW boys. There was no difference between SAW and SAB girls for adjusted PFBMC.

Sets of best predictors were selected for BMC at each of the 3 sites from linear models containing the following candidate variables: gender, current height, current weight, age, birth weight and ethnic group. Linear models containing the best predictors accounted for between 40 and 59% of the variance at these sites. Birth weight contributed significantly to the models for LSBMC and FNBM. Ethnicity, height and weight were significant contributors to BMC at all 3 sites.

It was well established that black South African adults and children have higher FNBMC than SA whites, but the finding of higher FNBM in children of mixed ancestral origin is of interest, as this ethnic group has not been studied previously.

**GOITRE, THYROID DYSFUNCTION AND CLINICAL ASPECTS OF THYROTOXICOSIS IN THE FREE STATE**

Molleertz W F, Oosthuizen G M, Joubert G

Departments of Internal Medicine and Biostatistics, University of the Free-State, Bloemfontein

Although the World Health Organization (WHO) regards South Africa as a country with optimal iodine intake, total goitre prevalence in Africa as a region increased from 15.6% in 1993 to 28.3% in 2003. Although a few thyroid-related hospital-based studies were published in South Africa over the last two decades few population-based studies focusing on goiter, biochemical thyroid dysfunction and the clinical spectrum of thyroid diseases in adults were conducted in South Africa up to now. A number of studies mostly in children were recently conducted in this region as part of a global WHO iodine nutrition supplementation programme. Similarly, very little is known about the long-term outcomes of the different treatment modalities, including the use of radio-active iodine, in the management of thyrotoxicosis in South African populations.

**THE PREVALENCE OF METABOLIC SYNDROME (MET S) IN A RURAL SOUTH AFRICAN COMMUNITY OF ZULU DESCENT USING 2005 INTERNATIONAL DIABETES FEDERATION (IDF) DEFINITION**

Mollet A A 1, Esterhuizen T M 1, Pirie F J 1, Gouws E 2, Omar M A 1,2

1University of KwaZulu-Natal; 2Medical Research Council, Durban, South Africa

The prevalence of metabolic syndrome (Met S) using 2005 International Diabetes Federation (IDF) definition was evaluated in a rural South African community of Zulu descent, selected by random cluster sampling of adults >15 yr. All subjects had demographic, anthropometric and biochemical measurements including a 75g OGTT. The definition of Met S using the third report of the National Cholesterol Education Program Adult Treatment Panel (NCEP-ATPIII) was also applied for comparison.

Of 987 subjects (M: P = 0.784) studied, the crude overall prevalence of Met S was 22.7% with a higher prevalence in women (26.6%) than in men (19.3%); the age-adjusted rates were 18.7%, 20.8% and 15.5%, respectively. The prevalence increased with age, both in women and men. The prevalence of clustering of components of Met S decreased with increasing number of “other” components plus a high waist circumference (WC).

In 971 subjects, the crude overall prevalence of Met S was lower with ATPIII (18.3%) than with 2005 IDF (23.1%) definition. 17.6% were identified by both sets, with “almost perfect” agreement between the two systems (κ=0.91 [95% CI 0.766 – 0.956]).

This study in a rural South African community has highlighted a high prevalence of Met S, especially in women, the prevalence is higher with IDF than ATP definition but with excellent agreement between them and suggests that this community unlike other sub-Saharan African communities, is well into the epidemic of Met S.

**RELATIONSHIP BETWEEN INSULIN RESISTANCE AND BETA CELL PROINSULIN PROCESSING**

N H Narar, N J Crowther

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

**Aims:** The aim of this study was to investigate the effects of insulin resistance on proinsulin-processing efficiency in non-diabetic subjects.

**Methods:** Fasting insulin, glucose, proinsulin (PI) and des-31, 32 proinsulin (des-PI) levels were measured in 425 non-diabetics subjects. Insulin resistance was calculated using HOMA. Insulin, des-PI and PI were expressed as a % of all insulin-related species e.g. PI/insulin+PI+des-PI. The study cohort was split into octiles based on HOMA levels and trends across the octiles for insulin, PI, des-PI and % levels were analysed via ANCOVA and post hoc analysis (Tukey HSD test).

**Results:** Des-PI levels (%M) from lowest to highest HOMA octile were (mean±SEM) 3.02±0.35, 3.97±0.45, 4.36±0.44, 5.05±0.54, 5.68±0.70, 6.05±0.78, 11.52±0.89, 20.91±1.97 (p<0.0001 for octiles 7 and 8 versus all other octiles). The trend of significantly higher values in octiles 7 and 8 than all other octiles was also observed for PI levels. Des-PI % levels from lowest to highest HOMA octile were: 11.66±1.72, 8.47±0.91, 7.66±0.69, 7.30±0.74, 6.93±0.71, 6.07±0.68, 8.92±0.56, 9.76±0.89. Thus, % levels fall from octile 1 to 6 and then increase from octile 6 to 10. Some trend was observed for PI % levels but the exact opposite trend was observed for insulin % levels.

**Conclusions:** In non-diabetic subjects, as insulin resistance increases proinsulin-processing efficiency rises until at a HOMA value of 3.1, proinsulin-processing efficiency declines. This suggests that one role played by insulin resistance in the aetiology of type 2 diabetes is to attenuate the ability of beta cells to convert proinsulin to insulin.

**CURRENT SOCIO-ECONOMIC MEASURES, AND NOT THOSE MEASURED DURING INFANCY, AFFECTS BONE MASS IN POOR URBAN SOUTH AFRICAN CHILDREN**

Shane A Norris 1, Zoë A Sheppard 2, Paula L Griffiths 1, Noël Cameron 1 2, John M Pettifor 1

1MRC Mineral Metabolism Research Unit, University of Witwatersrand; 2Department of Human Sciences, Loughborough University, Loughborough, UK

Understanding the impact of socio-economic status (SES) on physical development in children is important, especially in South Africa where considerable inequalities persist. This is the first study to examine the association between SES on bone development at the whole body, femoral neck, and lumbar spine, in Black children living in Soweto, South Africa. We postulated that SES would impact height, body composition, nutrition (especially calcium intake), and sports participation both at and out of school. Therefore, we modelled the SES variables together with potential mediators, such as growth (height), body composition (fat and lean tissue mass), dietary calcium intake and physical activity to discern the direct and indirect SES association with bone mineral content and area.

Linear regression models were used to investigate associations between SES during infancy, and current SES, anthropometric and DXA-derived bone mass in 9/10 year old children (n=309).

Findings suggest that current SES measures, rather than SES during infancy, are stronger predictors of current whole body bone area (BA) and whole body bone mineral content (BMC) after adjusting for body size, pubertal development, physical activity, habitual dietary calcium intake, and body composition. SES had no significant effect on body size, pubertal development, biochemical thyroid dysfunction and the clinical spectrum of thyroid diseases in adults were conducted in South Africa up to now. A number of studies mostly in children were recently conducted in this region as part of a global WHO iodine nutrition supplementation programme. Similarly, very little is known about the long-term outcomes of the different treatment modalities, including the use of radio-active iodine, in the management of thyrotoxicosis in South African populations.

**Relationship between insulin resistance and beta cell proinsulin processing**

N H Narar, N J Crowther

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

**Aims:** The aim of this study was to investigate the effects of insulin resistance on proinsulin-processing efficiency in non-diabetic subjects.

**Methods:** Fasting insulin, glucose, proinsulin (PI) and des-31, 32 proinsulin (des-PI) levels were measured in 425 non-diabetics subjects. Insulin resistance was calculated using HOMA. Insulin, des-PI and PI were expressed as a % of all insulin-related species e.g. PI/insulin+PI+des-PI. The study cohort was split into octiles based on HOMA levels and trends across the octiles for insulin, PI, des-PI and % levels were analysed via ANCOVA and post hoc analysis (Tukey HSD test).

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**Conclusions:** In non-diabetic subjects, as insulin resistance increases proinsulin-processing efficiency rises until at a HOMA value of 3.1, proinsulin-processing efficiency declines. This suggests that one role played by insulin resistance in the aetiology of type 2 diabetes is to attenuate the ability of beta cells to convert proinsulin to insulin.
SEB has a significant independent effect on whole body BMC through its impact on RA. This suggests that poverty alleviation policies in South Africa could have a positive effect on bone health.

**LIVER FUNCTION TEST ABNORMALITIES IN PATIENTS WITH TYPE 2 DIABETES MELLITUS**

**Paruk I, Kolowale B, Motala A A, Pirie F J, Omar M A K**

**Department of Endocrinology, University of KwaZulu-Natal, Durban**

**Aims:** To determine the prevalence of liver function test abnormalities in adult patients with type 2 diabetes mellitus attending a tertiary diabetes clinic at Inkosi Albert Luthuli Central Hospital in Durban.

**Study design:** The study was a retrospective chart review of patients with type 2 diabetes attending the diabetes clinic. Data that was obtained included a history of liver disease, alcohol abuse, drug therapy, duration of diabetes, height, weight, body mass index (BMI), lipid profile and liver function tests (LFT).

**Results:** The charts of 313 patients were reviewed. Abnormalities of LFTs were found in 118 patients (37.6%). Of these, 12 patients had a history of alcohol abuse and were excluded from further analysis. Elevation in serum gamma-glutamyl transferase, alkaline phosphatase and alanine transaminase was found in 23.9% (n=76), 18.2% (n=57) and 13.7% (n=43) respectively. Serum total cholesterol, triglycerides and LDL cholesterol were higher in the group with LFT abnormalities, when compared with subjects with normal LFTs; mean BMI was similar in the 2 groups (32.5 vs 33.2 kg/m²). Morbidly obese patients (n=42), demonstrated a higher frequency of liver enzyme derangements (69%).

**Conclusions:** There is a high prevalence of LFT abnormalities in this group of patients with Type 2 diabetes, particularly in the morbidly obese subjects, comparable with the reported prevalence in the western world. Lipid abnormalities were more frequent in the group with liver enzyme derangements.

**AN ANALYSIS OF LIPID MANAGEMENT IN PATIENTS WITH TYPE 2 DIABETES AT CHRIS HANI BARAGWANATH HOSPITAL, JOHANNESBURG**

**B Pauly, R Shires, S Bhana**

**Division of Endocrinology, Chris Hani Baragwanath Hospital and University of Witwatersrand, Johannesburg**

**Objective:** To assess the management of dyslipidaemia in patients with Type 2 diabetes at Chris Hani Baragwanath Hospital for the period 2005 - 2007.

**Method:** The results of 905 patients with T2DM attending the diabetes clinic at Chris Hani Baragwanath Hospital were evaluated between 2005 and 2007. The T2DM clinic is largely managed by registrars. An inpatient file was created and data captured on a computer data base. The following information was recorded for each patient and analysed using the SPSS-13 statistical programme: total cholesterol (TC), triglyceride (TG), HDL-C and LDL-C values, drug therapy and response to therapy. SEMDSA guidelines for target lipid values were used to assess impact of management, i.e. TC<5.0, TG<1.5, HDL>1.2, LDL<3.0. Registrars were familiarised with the SEMDSA guidelines for managing lipid disorders.

**Results:** As of 2005 only 41.3% of patient files had a documented full lipid screen prior to assessment. This percentage rose to 53.1% in 2006 and to 67.7% in 2007. Of all those with a prior lipid screen (2005 - 7), 86.9% had abnormal lipid profiles, but only 31.0% received simvastatin. In the group who did not have a previously documented lipid measurement 77.3% were found to have dyslipidaemia.

Ultimately 96.8% of our patients had repeat lipid measurements. Of the patients who had previously documented lipid profiles and who were already on treatment with simvastatin, only 15.6% had achieved the SEMDSA target values.

80.3% of patients fulfilled SEMDSA criteria for treatment of their dyslipidaemia, but had not received any therapy.

**Conclusion:** Lipid management is an integral part of diabetic care. Audit review help to analyse current clinical practice and can be used to measure success or failure. Although the number of lipid screens done by registrars at Chris Hani Baragwanath Hospital increased from 41.3% in 2005 to 67.7% in 2007, target levels were seldom achieved. The results of this audit will help to focus on more effective management strategies.

**THE PHOSPHATONINS AND HYPOPHOSPHATAEMIC RICKETS**

**John M Pettitfor**

**MRC Mineral Metabolism Research Unit, Department of Paediatrics, University of the Witwatersrand and Chris Hani Baragwanath Hospital, Johannesburg**

Although relatively uncommon individually, the various causes of hypophosphataemic rickets have provided an impetus for unravelling the mechanisms of phosphate homeostasis and bone mineralization. Over the past 10 years, considerable advances have been made in establishing the gene mutations responsible for a number of the inherited causes and in understanding the mechanisms responsible for tumour induced osteomalacia/rickets. The most exciting aspects of these discoveries have been the discovery of a whole new class of hormones or phosphatonin which are thought to control phosphate homeostasis and 1alpha-hydroxylase activity in the kidney, through a bone-kidney-intestinal tract axis. Although our understanding of the interrelationships is far from complete, it raises possibilities of improved therapeutic agents in the long term, and has resulted in improved diagnostic abilities in the short term.

**GENETIC POLYMORPHISMS IN INDIAN AND AFRICAN SUBJECTS WITH TYPE 2 DIABETES IN KWAZULU-NATAL**

**Fraser Pirie, Ayesha Motala, Rosemary Pegoraro, Imran Paruk, M A K Omar, Nash Ranjit, Lee Rom, Thirumalai Govender**

**Diabetes and Endocrine Unit, Nelson R Mandela School of Medicine, University of KwaZulu-Natal, Durban, South Africa**

**Aim:** The study aimed to evaluate the association between single nucleotide polymorphisms (SNP) in the PPAR gamma, KCNJ11, TCF7L2 and FTO genes in Indian and African subjects with type 2 diabetes attending a diabetes clinic in Durban, KwaZulu-Natal.

**Methods:** Indian (N=161) and African (N=104) subjects, with type 2 diabetes, attending the diabetes clinic at Inkosi Albert Luthuli Central Hospital were enrolled. Healthy controls (300 Indian subjects and 99 African subjects) were selected from two previous epidemiology studies. Five SNPs (rs1801282 in the PPAR gamma gene, rs5219 in the KCNJ11 gene, rs7903146 and rs12255372 in the TCF7L2 gene and rs9911650 in the FTO gene) were evaluated by pre-designed allelic discrimination assays using the Applied Biosystems Taqman system on a 7500 Thermal Cycler.

**Results:** In Indian subjects, the minor alleles in PPAR gamma rs1801282, KCNJ11 rs5219 and rs12255372 in the TCF7L2 gene were associated with type 2 diabetes: rs1801282 GG genotype, p=0.042; rs5219 AA genotype, p=0.044; rs12255372 TT genotype p=0.012. The FTO SNP was not associated with type 2 diabetes in Indian subjects. In African subjects the PPAR gamma and KCNJ11 polymorphic alleles were rare, and each was found only in one person with type 2 diabetes. Although the TCF7L2 and FTO loci were polymorphic in African subjects, no association with type 2 diabetes was found.

**Conclusion:** The polymorphic profile of the gene variants studied here, in association with type 2 diabetes in Indian subjects, is similar to that found in other population studies. This is not the case for the African population in KwaZulu-Natal in whom the nature of the genetic contribution to type 2 diabetes remains unknown.
GENDER AND ETHNIC DIFFERENCES IN VITAMIN D STATUS AND BIOCHEMICAL BONE MARKERS IN CHILDREN
Machenue A. Poopedi, Shane A. Norris, John M. Pettifor
MRC Mineral Metabolism Research Unit, Birth to Twenty Research Programme, University of the Witwatersrand, Johannesburg, South Africa

The aim of the study was to determine gender and ethnic differences in relation to vitamin D [(25(OH)D] levels and biochemical bone markers in healthy 10 year old urban South African children.

Data were collected on 476 children (159 Black girls, 64 White girls, 181 Black boys, and 71 White boys) with a mean age of 10.6 years, who formed part of the Birth-to-Twenty longitudinal cohort. The evaluation of subjects included DXA-derived bone mass, anthropometric measurements, nutrition, lifestyle factors, 25(OH)D status and biochemical bone markers.

White children had higher 25(OH)D than Black children (p=0.0001). There were significant ethnic and seasonal variations in 25(OH)D, in particular, during autumn (p=0.01) and summer (p=0.0001) months with White children having increased 25(OH)D. Black females and males had higher alkaline phosphatase (ALP) than their White peers (p=0.0002, p=0.0004 respectively). No significant differences in PTH were observed between the ethnic groups. 25(OH)D correlated negatively with ALP (r=−0.18, p=0.0002) in all subjects. There was no correlation between 25(OH)D and PTH in all subjects. In conclusion, hypovitaminosis D is uncommon with 16% of children having 25(OH)D levels of less than 20 ng/ml. The relationship between 25(OH)D and ALP was only found when both ethnic groups were combined. The relationship disappears when the subjects were stratified by gender and ethnicity. Higher ALP in Blacks than Whites could possibly reflect greater bone formation rates, unrelated to vitamin D status.

HYPERGLYCEMIA-INDUCED ACTIVATION OF THE HEXOSAMINE BIOSYNTHETIC PATHWAY CAUSES MYOCARDIAL CELL DEATH
Ubrha Rajamani, M Faadiel Essop
Department of Physiological Sciences, Stellenbosch University

Hyperglycemia-mediated myocardial cell death may contribute to contractile dysfunction in Type 2 diabetic patients. The diabetic heart exists within a hyperglycemic milieu and the accumulation of intracellular glucose metabolites results in greater flux through the hexosamine biosynthetic pathway (HBP) leading to O-GlcNAc modification of target proteins. Here we hypothesized that under hyperglycemic conditions there is increased O-GlcNAc modification of apoptotic proteins thereby resulting in higher rates of myocardial apoptosis.

To begin to investigate our hypothesis we established an in vitro model (cardiac-derived rat H9c2 myoblasts) of hyperglycemia-induced apoptotic cell death. H9c2 myoblasts were exposed to 5, 22 and 33 mM glucose, respectively, for 5 days and apoptotic cell death determined using Hoechst nuclear staining. We also administered 40 μM of 6-diazo-5-oxo-L-norleucine (DON), an inhibitor of the HBP rate-limiting enzyme, i.e. glutamine:fructose 6-phosphate amidotransferase (6PT) for 5 days. Mannitol experiments were conducted in parallel to rule out cell death by osmotic effects.

Hoechst nuclear staining counts demonstrated that the number of apoptotic nuclei was increased by 1.9- and 2.2-fold in response to 22 and 33 mM glucose, respectively (p<0.01 vs. 5 mM controls). No cell death was observed in response to increasing doses of mannitol (5, 22, 33 mM). Hoechst nuclear staining data also revealed that DON administration blunted hyperglycemia-induced apoptotic cell death usually observed in response to 22 and 33 mM glucose levels by 24.5 ± 7.9% and 53.3 ± 6.2% respectively (p<0.01 vs. 5 mM controls).

This study reports for the first time that hyperglycemia-induced myocardial apoptotic cell death is also mediated via the hexosamine biosynthetic pathway. Our findings may result in the development of novel therapeutic agents to limit the detrimental effects of hyperglycemia on the heart's function.

BONE AND NON-BONE EFFECTS OF CALCIUM
Ian R Reid
University of Auckland

The Auckland Calcium Study was a randomized controlled trial in 1471 normal postmenopausal women, assessing the effects of calcium on bone density over 5 years. The women received calcium 1g/day calcium or placebo.

Calcium reduced bone loss by two-thirds in both the hip and total body sites. Fracture data were inconclusive, but there were downward trends in hazard ratios for most fracture categories. Serum PNP levels were 22% lower in the calcium group at 5 years (p=0.03).

Calcium reduced LDL cholesterol by almost 10%, increased HDL comparably, thus increasing the HDL/LDL ratio by almost 20%. In the calcium group, there were small transient decreases in blood pressure at six months, but thereafter the two groups were indistinguishable. There was no evidence of any between-groups difference in weight loss, lean mass or fat mass.

There were no between-groups differences in falls, tooth loss, or iron status. Constipation and discontinuation of trial medication were more common in the calcium group. Myocardial infarction was more common in those allocated to calcium, though this effect was of marginal significance following event adjudication.

It is concluded that calcium produces sustained benefits on BMD but these are likely to be overwhelmed from any deleterious effect on heart health.

THE LINKS BETWEEN BODY COMPOSITION AND BONE
Ian R Reid
University of Auckland

Body weight impacts on both bone turnover and bone density, and is therefore an important risk factor for vertebral and hip fractures, ranking in importance alongside that of age. The effect of body weight is probably contributed to by both fat mass and lean mass, though in postmenopausal women, fat mass has been more consistently demonstrated to be important. A number of mechanisms for the fat-bone relationship exist and include the effect of soft tissue mass on skeletal loading, the association of fat mass with the secretion of bone active hormones from the pancreatic beta cell (including insulin, amylin, and preptin), and the secretion of bone active hormones (e.g. estrogen and leptin) from the adipocyte.

These factors alone probably do not fully explain the observed clinical associations, and further study of the actions on bone of novel hormones related to nutrition is an important area of further research. An understanding of this aspect of bone biology may open the way for new treatments of osteoporosis. More immediately, the role of weight maintenance in the prevention of osteoporosis is an important public health message that needs to be more widely appreciated.

CONTROL OF REGULATED SECRETION BY OSTEOCLASTS AND OSTEOBLASTS
F Patrick Ross
Department of Pathology and Immunology, Washington University School of Medicine, St Louis, MO, USA

Osteoclasts (OCs) and osteoblasts (OBs) are polarized, like all adherent cells. We focused on the molecular mechanisms of polarization for both cell types, as they may represent therapeutic targets. Cell polarity involves a series of events that require movement to the cell surface of specialized vesicles containing specific cargo. In the case of OCs and OBs, such cargo includes cathepsin K and matrix proteins such as collagen I, osteopontin and bone sialoprotein. Based on known models we hypothesized that one or more synaptotagmin (Syt) family regulates vesicle targeting and hence cell polarization. Since Syt VII modulates secretion by myeloid and mesenchymal cells, precursors of OCs and OBs respectively, we examined the cellular and whole animal phenotype of Syt VII null mice. These animals have a low turnover phenotype with suppressed OC and OB function consequent on decreased secretion. We purified secretory vesicles from OCs and

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performed proteomics, thereby identifying several hundred proteins. These studies represent important approaches to understanding the function of OCs and OBs. This work is supported by NIH funding.

A LOW PREVALENCE OF RAISED INTRAOCULAR PRESSURE IN A SOUTH AFRICAN COHORT OF ADDISON’S PATIENTS

I L Ross1, C Cook2, N S Levitt1, A Karlsson1, L van der Merwe1, N Cockburn3, D Schatz4

1Division of Endocrinology, University of Cape Town; 2Division of Ophthalmology, University of Cape Town; 3Division of Medicine, Uppsala, Sweden; 4Specialist Ophthalmologist, Pretoria; 5Department of Paediatrics, University of Florida, USA

Background: Both topical and systemic glucocorticoids may elevate the intraocular pressure, sometimes within pathological ranges of pressure elevation. Raised intraocular pressure is a risk factor for the development of glaucoma and consequent permanent visual disturbance.

Objectives: We examined whether patients with Addison’s disease on stable doses of hydrocortisone are at risk for raised intraocular pressure.

Methods: Patients from the South African Addison Study who were regular attendees at two major centres were approached to take part. The intraocular pressure was measured using a Goldman’s tonometer and bilateral pachymetry was performed to assess corneal thickness. Appropriate intraocular pressure adjustments for the thickness of the cornea were made. Bone mineral density was recorded, when available.

Results: Of the thirty patients approached, three refused to participate and 27 patients were encilitled in the study. There were 18 (67%) female patients and the mean ± standard deviation at age of enrolment was 48 ± 17.2 years. 13 years had elapsed between the diagnosis of Addison’s disease and enrolment. 70.4% had autoimmune Addison’s disease. The mean (SD) corrected intraocular pressure was 14.2 ± 3.32 and 13.7 ± 3.0 mmHg for the right and left eyes respectively. The median (IQR) hydrocortisone dose/kg was 0.30 (0.25–0.35) mg, median (IQR) hydrocortisone dose/m² was 12.1 (10.4–15.0) mg and median total daily dose of hydrocortisone (IQR) was 20.0 (20.0–30.0) mg. The median (IQR) total daily fluorocortisone dose was 0.10 (0.06–0.10) mg. Age, weight and hydrocortisone doses did not correlate with intraocular pressure but there was a negative correlation between daily fluorocortisone dose and intraocular pressure on the corrected intraocular pressure of the left eye (r = 0.46 (p = 0.016) and there was a negative trend for fluorocortisone and corrected intraocular pressure on the right eye. 26% and 7% of this cohort had WHO criteria for the diagnosis of osteoporosis and osteopenia respectively.

Conclusions: We have been unable to demonstrate a single case of raised intraocular pressure in this cohort, despite the higher than expected prevalence of osteoporosis supporting excess hydrocortisone doses. The significant negative correlation between intraocular pressure and daily fluorocortisone dose, may suggest that the fluorocortisone may be protective and negate the effect of hydrocortisone on the intraocular pressure.

HIGH-FAT DIET INDUCED BETA-CELL COMPENSATION IN ADULT OBESE RATS

C R Row1, C J F Muller, K Williams, M E Cerf, J Louw

Diabetes Discovery Platform, South African Medical Research Council, Tygerberg, 7505, South Africa

Rats fed a high-fat diet (HFD) develop obesity and symptoms of metabolic disease, i.e. a degree of insulin resistance in adulthood, but have been shown to be able to maintain normoglycemia. This study aims to determine how the islets of obese rats compensate to increased insulin demand after long-term high fat feeding. Wistar rats (n=10) were fed a HFD (40 % energy as fat, 15% protein, 46% carbohydrates) from 3 weeks of age for a 12 month period. Control rats (n=6) received standard rat chow.

After 12 months the body mass of the rats maintained on a HFD was significantly higher than the controls (808.6 ± 114.6 vs. 620.7 ± 36.12, p<0.0001). The mean blood glucose concentrations (6.05 ± 1.07 vs. 5.79 ± 0.9 mmol/l; p=NS) were slightly higher in the HFD rats. There was no significant difference in the mean serum insulin concentrations (5.24 ± 2.9 vs. 4.45 ± 2.95 nmol/ml). Image analysis of the islets from HFD rats revealed a slight increase in the islet/pancreas ratio (0.051 ± 0.021). The islets were larger (mean islet size 220643.59 ± 253934 µm² vs. 174659.16 ± 249697 µm²) and the β-cell/α-cell ratio was slightly increased (4.17 vs. 3.46).

These results showed that maintaining Wistar rats on a HFD from weaning for 12 months induced obesity without any overt signs of the development of type 2 diabetes. The islets adapt morphologically to the increased body mass by increasing islet size and increasing the β-cell/α-cell ratio.

CHARACTERIZATION OF MESENCHYMAL STEM CELLS AS A MODEL SYSTEM FOR STUDYING GLUCOCORTICOID-INDUCED OSTEOPOROSIS

H Sadie1, F S Hough1, W Smith3, E F du Toit2, W F Ferris1

1Division of Endocrinology and Metabolism, Department of Medicine, Stellenbosch University, Tygerberg; 2Department of Medical Physiology, Stellenbosch University

Physiological (nanomolar) concentrations of glucocorticoids (GCs) are required for normal osteoblast differentiation. In contrast, pharmacological (micromolar) GC concentrations can result in osteoporosis, characterized by a decrease in the number and function of osteoblasts and marrow adiposity, the latter indicating possible transdifferentiation of osteoblasts into adipocytes. Osteoblasts arise from the mesenchymal progenitor lineage, and therefore share a common origin with adipocytes. We have isolated mesenchymal stem cells (MSCs) from different adipose depots in lean and diet-induced obese male Wistar rats and have compared the response of these cells to well-established osteoblast differentiation media (ascorbic acid, βglycerol-2-phosphate, dexamethasone). In addition, we studied the effects of pharmacological doses of GCs on MSC and pre-osteoblast proliferation. Cells from lean subcutaneous (LS), lean visceral (LV), obese subcutaneous (FS) and obese visceral (PV) depots were compared.

Upon initiation of osteoblast differentiation, naïve MSCs exit the cell cycle, resulting in inhibition of proliferation. We observed that osteoblast differentiation media induced an almost complete inhibition of proliferation within 24 hours in cells from subcutaneous depots (LS, FS), while the effect in cells from visceral origin (LV, PV) was much less pronounced. Increased alkaline phosphatase activity, a marker of osteoblast function, was consistently observed in LS cells after 7 days in osteoblast media, while the response in other cell-types was inconsistent. Treatment with osteoblastic media also resulted in intracellular lipid accumulation in LV and PV cells, and this effect was shown to be mediated by the dexamethasone (GC) in the differentiation media. In contrast, this effect was not observed in cells from subcutaneous origin (LS, FS). Our results suggest that cultured MSCs isolated from subcutaneous adipose depots can differentiate into functional osteoblasts in response to osteoblast differentiation media. However, cells from visceral depots have an impaired ability to differentiate into osteoblasts. Overall, our results clearly demonstrate unique depot-specific responses of rat adipose-derived MSCs in culture, indicating in vivo preprogramming that persists in vitro.

GLUCOCORTICOID-INDUCED INHIBITION OF PROLIFERATION IN RAT ADIPOSE-DERIVED PLURIPOTENT CELLS

Micheline Sanderson, Hané Sadie, Stephen Hough, William Ferris

Division of Endocrinology and Metabolism, Department of Medicine, Faculty of Health, Stellenbosch University, Cape Town, South Africa

Exposure to prolonged pharmacological doses of glucocorticoids (GC) causes osteoporosis in 50% of patients. This is due to a reduction in the proliferative capacity of preosteoblasts, leading to retarded bone growth.
Four hundred and forty one (22%) children had sustained fractures. Rates in childhood are as high as those in the elderly. Recent research has been undertaken to understand the reasons for this, but there is little information available on ethnic differences in childhood fracture rates.

**Introduction.** Fracture rates in childhood are as high as those in the elderly. Recent research has been undertaken to understand the reasons for this, but there is little information available on ethnic differences in childhood fracture rates.

**Objective.** To determine the number of fractures, rates of fractures, the common sites of fractures, the causes of fractures and the types of trauma causing fractures in children from birth until 15 years of age living in Johannesburg.

**Methods.** Using the Birth to Twenty longitudinal cohort of children, we retrospectively obtained information on fractures and their sites from birth to 14.9 years of age on 2031 participants. The ethnic breakdown of the children was Black (B) 78%, White (W) 9%, Mixed Ancestry (MA) 10.5% and Indian (I) 1.5%.

**Results.** Four hundred and forty one (22%) children had sustained a fracture one or more times during their lifetime (males 27.5% and females 20.5%). The most common site of fracture was the upper limb (57%) followed by the lower limb (22%). In Black (B) children fractures were distributed as follows: upper limb (57.2%), lower limb (21.6%), skull (11.8%), facial (4.2%), trunk (2.6%) and other (0.6%). In White (W) children fractures were distributed as follows: upper limb (53.1%), lower limb (22.8%), skull (11.2%), facial (5.9%) and trunk (0.3%).

**Conclusions.** Greater osteoid thickness in B children may reflect greater osteoblast efficiency as it did in B adults. This may have contributed to greater Ec wall thickness and greater cortical thickness also in B children. Since bone strength depends mainly on cortical bone these differences may contribute to lower fragility fracture rates in B compared to W children.
IMPROVEMENTS IN THE HYPERINSULINAEMIC EUGLYCÆMIC CLAMP
Magdalena J Turysyniecka, C D Byrne
Endocrinology and Metabolism, DohSAD Division, University of Southampton, UK

The glucose clamp technique is regarded as a gold standard method to assess the whole body insulin. The modification of the standard clamp, the stepped hyperinsulinaemic clamp, consists of low and high doses of insulin to assess lipolytic and peripheral insulin sensitivity respectively. However there is little literature if any discussing the practical aspect of performing this complicated and laborious test. I have performed more than 80 stepped hyperinsulinaemic euglycæmic clamps over a period of about twelve months and gained a considerable experience with this technique. I have identified a number of critical steps in performing this technique. These include the following: analytical instrument performance (frequent instrument calibration [YSI 2300], monthly membrane change, importance of using plasma versus whole blood); retrograde cannulation (adequate hand pre-warming and positioning, large, straight versus small, curvy vein, wrist as opposed to hand, topical anaesthetics elevate discomfort in some, alcohol wipes for vein enlargement); patency maintenance (continues, slow rate 0.9%NaCl infusion); arterialized venous blood sampling (measurement of blood gasses for saturation); -insulin infusion; glucose infusion (small rate increments rather than large and frequent); unexpected glucose results (sample re-analysis, analyzer re-calibration or re-sampling versus instantaneous glucose rate change)

We conclude that the above mentioned steps will facilitate the successful completion of clamp.

COMPARISON OF DIETARY INTAKE IN TWO SOUTH AFRICAN ETHNIC GROUPS
Weisberg R, Palker J E, Crowther N J
Department of Chemical Pathology, NHLS, Johannesburg

Objective: One of the aims of this project was to compare dietary food intake in two South African ethnic groups namely Indian and African.

Methods: Nutrient status was assessed using a validated food frequency questionnaire. Fifty subjects in each of the ethnic groups from an urban population of women residing in the Greater Johannesburg area were included in the study. Low energy reporters (LERs) were defined as those having an energy intake / basal metabolic rate ratio of <1.14.

Results: Figure 1 shows a comparison of dietary intake for total energy, intake of fat and carbohydrate groups from an urban population of women residing in the Greater Johannesburg area. Total energy intake (kJ), 8691±373 and 6131±230; total fat (g), 86±3.3 and 80±3.2; total carbohydrate (g), 255.7±11.1 and 167.2±6.5; total dietary fibre (g), 20.5±1.1 and 14.3±0.6; calcium (mg), 860±61.1 and 586±40.2; lycopene (μg): 41.6±9.2 and 88.6±22.7. Data shown as mean values ± SEM; *p<0.05; **p<0.001. There were no significant differences between the energy intake of the two groups. However, there were significant differences in levels of fat, calcium and lycopene intake between the two groups. Levels of fat and calcium were higher in the Indian group while the levels of lycopene were higher in the African group.

Conclusions: Greater energy intake in African than Indian females may explain the higher prevalence of obesity in the African population. Greater proportion of fat in the Indian diet may predispose this population to cardiovascular disease. Higher dietary consumption of dairy products in black females explains the greater calcium intake whilst lower intake of tomato-based products explains their lower lycopene levels.

ASSESSMENT OF MYOCARDIAL GLUT4 TRANSLLOCATION BY IMMUNOFLOUORESCENCE MICROSCOPY
Gordon Williams, M Faadiel Essop
Department of Physiological Sciences, Stellenbosch University

Perturbations of myocardial signaling cascades result in decreased translocation of the insulin-responsive glucose transporter (GLUT4) from intracellular vesicles to the sarcolemma, leading to reduced glucose uptake and the onset of insulin resistance. Although considerable efforts are being put into unraveling molecular mechanisms underlying this process, less is known regarding the spatio-temporal regulation of GLUT4. In light of this, our aim was to establish a fluorescence microscopy-based method to visualize and quantify GLUT4 translocation in cardiac-derived myocytes.

Rat cardiac-derived H9c2 myocytes were cultured until ~80-90% confluent, thereafter sub-cultured in chamber slides (~15,000 cells per well) for 24 hrs. Cells were then serum-starved for 3 hrs followed by administration of 150 nM insulin for 0, 5 and 30 mins. respectively. These experiments we performed ± 200 nM wortmannin, an inhibitor of PI3-kinase (key regulator of myocardial GLUT4 translocation). Subsequently, myocytes were fixed and probed for 24 hrs with antibodies specific for intracellular and membrane-bound GLUT4. To assess GLUT4 translocation we employed the following secondary antibodies: a) FITC Green for intracellular GLUT4, and b) Texas Red for membrane-bound GLUT4. Cells were thereafter viewed by multi-dimension imaging using an inverted system microscope (Olympus IX81).

Our results show a peak response after 5 mins of insulin treatment, i.e. intracellular GLUT4 density was reduced while membrane-bound GLUT4 density was increased. Co-administration of wortmannin abolished GLUT4 translocation. However, after 30 mins membrane-bound GLUT4 returned to control levels.

We have successfully established a fluorescence microscopy-based method to visualize and quantitatively assess GLUT4 translocation. Here the insulin response peaked after 5 mins with a reversal to control levels after 30 mins. We are currently employing our model to investigate the effects of various stresses on myocardial GLUT4 translocation.

DEVELOPMENTAL EFFECTS OF INTRA-UTERINE PROGRAMMING ON THE NEONATAL RAT
K Williams*, U Gerber*, C Muller*, J Loun*†, J Estebayse*, J van Rooyen*, M Cerf*†
'Diabetes Discovery Platform, MRC; "Biomedical Sciences, CPITU

A stimulus or insult during critical developmental windows is believed to programme the physiology and metabolism of the animal with long-lasting consequences. One form of developmental programming is the manipulation of maternal nutrition during gestation and/or lactation. The aim of the study was to investigate the developmental effects of a maternal diet varying in fat content on the neonatal rat.

Pregnant Wistar rats were fed a diet varying in fat content (10%, 20%, 30% and 40%) throughout gestation. The 10% fat (as energy) diet was used as the control and the 40% fat diet as the high fat diet (HFD). At birth (postnatal day 1), neonates were weighed and body measurements recorded. Neonates were then killed and blood taken for the determination of blood glucose concentrations. The pancreas, heart, brain and liver was excised and weighed. Plasma glucose concentrations and head circumference was increased in the 20% group. Heart to body length increased in all the experimental groups. In both the 20% and 30% groups the body and pancreas weight was increased. Liver weight was increased in the 30% and HFD groups. Brain weight was reduced in the 20% and HFD groups. Heart weight was reduced in the HFD group.

A maternal gestational diet, varying in fat content, programmes the growth trajectory and organ development in progeny. This may compromise their physiology and metabolism predisposing these progeny to develop metabolic disease later in life.

GENETIC ANALYSIS OF INSULIN RESISTANCE IN A HUMAN HEPATOCYTE CELL LINE
S Williams, G Deatly, M van de Venter, S Roux
Department of Biochemistry and Microbiology, NMMU, Port Elizabeth, South Africa

Objective: To identify the genetic changes associated with the development and reversal of insulin resistance in the liver and to
compare the response of insulin resistant hepatocytes to ciglitazone and a South African medicinal plant extract.

**Methods:** The human Chang hepatocyte cell line was cultured in the presence of insulin and fructose to establish insulin resistance. Once resistant, the cells were treated with an extract of a South African medicinal plant, or with ciglitazone to reverse the insulin resistance. Insulin resistance was confirmed by glucose uptake and lipid accumulation assays. Samples of RNA from control, insulin resistant and treated cells were analysed using a SuperArray PCR based matrix for Diabetes associated genes. A panel of 10 genes showing the greatest changes in expression were selected and their expression at time points during the development and reversal of insulin resistance was analysed.

**Results:** The selected genes were PPAR gamma, PPAR alpha, ACLY, αPortal Hypothesis postulates 2 of insulin resistance invokes ectopic fat in liver and accumulation assays. Samples of RNA from control, insulin resistant and treated cells were analysed using a SuperArray PCR based matrix for Diabetes associated genes. A panel of 10 genes showing the greatest changes in expression were selected and their expression at time points during the development and reversal of insulin resistance was analysed.

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**Conclusions:** The above genes provide useful markers for insulin resistance and identification of molecular targets for the medicinal plant and ciglitazone.

**A GLUCOCENTRIC APPROACH TO TYPE 2 DIABETES – WHOSE AGENDA?**

**John S Yudkin**

**University College London**

The discovery of insulin and of antibiotics such as streptomycin and penicillin provided the medical profession with the proverbial ‘Magic Bullets.’ These drugs were able to save lives, with a Number Needed to Treat (NNT) of 1. The advent of the statins was pitched as the arrival of a new Magic Bullet, agents which could precisely target the underlying aetiology of the major global public health threat. There are, though, vital differences between drugs targeted at reducing quantitative risk factors (cholesterol, blood pressure, glucose) and those targeting disease. Even in patients at extremely high risk it is necessary to treat large numbers of people who will not benefit for every one who will. For example in a patient with a 5-year coronary heart disease event risk of 20%, 19 out of 20 will have an identical outcome with or without taking a statin for that period (NNT = 20). In consequence, individual clinicians are serving a predominantly public health function, although patients may retain traditional ‘Magic Bullet’ views of their treatments. Because of the organisational and financial costs of outcome studies, both registration and promotion of new agents depend on surrogate endpoints. And with the global pandemic of type 2 diabetes, with the consequent search for the latest ‘Blockbuster Drug,’ glucose lowering has become a surrogate endpoint of choice for pharmaceutical companies. Moreover, Guideline Committees and health care financing organisations incorporate strict glycemic targets for management and reimbursement. There are, though, both qualitative and quantitative differences between putting a high-risk patient, usually mature in years, on a statin and on insulin. Furthermore, in the UKPDS, serious macrovascular endpoints (myocardial infarction and stroke) were 5 times more frequent than microvascular ones (sight-threatening retinopathy and renal failure), but no study has shown cardiovascular benefits of glucose lowering. Indeed, there is recent evidence suggesting that both intensive insulin regimens (ACCORD) and thiazolidinediones (rosiglitazone meta-analysis) may increase event rates. It is suggested that the ‘Medical-Industrial Complex’ is responding to these public health challenges by pushing a Poly-Pill approach to prevention which may not benefit the majority of individual patients.

**HOW DOES YOUR BIG TOE KNOW THAT YOU’RE FAT? EXPLORING ADIPOSE TISSUE SIGNALS IN THE WHOLE ORGANISM**

**John S Yudkin**

**University College London**

Obesity is associated both with altered insulin action in many tissues, and with endothelial dysfunction and cardiovascular disease. Both of these consequences are more marked when fat is distributed centrally (truncal obesity). There is, then, a need to explain how adipose tissue signals to remote tissues and organs. Non-esterified fatty acids (NEFAs) delivered to the liver have been implicated as the main insulin resistance signal from fat (Portal Hypothesis), but much evidence suggests other signalling pathways. Inflammatory cascades are stimulated by NEFA, impairing insulin signalling, but additional fat-derived proinflammatory molecules also impair insulin action in target tissues. The Endocrine Hypothesis postulates that adiponectin, interleukin-6 and retinol binding protein 4 may play signalling roles but tumour necrosis factor-α is unlikely to have systemic effects other than in septic shock. An OverFlow Hypothesis of insulin resistance invokes ectopic fat in liver and muscle under situations of nutritional excess. Recent data suggest that low grade inflammation in fat, with recruitment and activation of macrophages through expression of monocyte chemotactant protein-1, induces hepatic steatosis, and that activated macrophages are integral to hepatic and skeletal muscle insulin resistance – suggesting a novel Cellular Signalling Hypothesis. Similarly, perivascular fat and its recruited macrophages may play an important role in inhibiting endothelial insulin action and in atheroecrosis through effects on downstream vascular endothelium and skeletal muscle tissue – a concept we have termed a Vasocrine Hypothesis. Understanding these mechanisms depends on careful in vivo studies in humans and animals, and a move away from reductionist cell and molecular approaches.

**SUCCESSFUL SHORT TERM TREATMENT OF OSTEOPOROSIS ASSOCIATED WITH A CASE OF STICKLER’S SYNDROME: A CASE REPORT**

**E W Zöllner, R Pitcher, A Spitaels**

**Stellenbosch University, Tygerberg Hospital, and University of Cape Town, Red Cross Children’s Hospital**

A 13-year-old boy with Stickler’s syndrome was investigated for short stature. Clinically there was no evidence of an additional endocrinopathy or another chronic disease. He was in early puberty (testicular volume 5 ml) with a short upper segment and mild scoliosis. XR spine revealed severe spondelectasia with 3 biconcave/ wedge compression fractures. The BMD of the total lumbar spine was 0.481 g/cm² (Z-score -3.87). Serum calcium, magnesium, phosphate was normal. The serum PTH was 4.8 nmol/l at a corrected serum Ca of 2.32 mmol/l. Serum alkaline phosphatase (AP) was 224 u/l and the bone specific AP 118 u/l. Urinary free deoxypyridinoline crosslink was 34.58 nmol/mmol urinary Cr. Serum testosterone was 2.4 nmol/l. Serum free cortisol and GH were normal. The bone formation markers osteocalcin and pro-collagen type I N-terminal propeptide were normal, but the bone formation marker osteocalcin was 446 nmol/l. The bone mineral density (BMD) of the total lumbar spine was 0.524 g/cm² (Z-score -2.8) and there were no further fractures. To the best of our knowledge this is the first documented case of osteoporosis in Stickler’s syndrome which was also successfully treated.