BS5-2

Incretin secretion is independent of glucokinase function

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Aims: Incretin hormones glucagon-like peptide-1 (GLP-1) and gastric inhibitory peptide (GIP) are released from intestinal cells in response to luminal but not systemic glucose, and act to potentiate glucose stimulated insulin release by the pancreatic beta cell. Incretin secretion and function are reduced in Type 2 diabetes and are the targets of novel treatments. The enzyme glucokinase (GCK) which is the glucose sensor in pancreatic beta cells has also been proposed as the main glucose sensor in the gut. Subjects with diabetes as a result of a mutation in the *GCK* gene are a good model for studying this proposition. We hypothesized that the secretion of incretins in response to a 75g oral glucose load would be lower in *GCK* mutation carriers compared to controls.

Methods: We studied 79 adult subjects, 28 with *GCK* mutations and 51 familial controls without diabetes. We measured glucose, insulin, c-peptide, GLP-1 and GIP at 5 time points during a 75g OGTT (0, 30, 60, 90 and 120 minutes) and also measured anthropometric data.

Results: *GCK* mutation carriers had higher mean plasma glucose concentrations compared with non-carriers (9.9 vs 6.5 mmol/L, $p<0.001$). Insulin and c-peptide profiles were similar between mutation carriers and non-carriers ($p=0.07$ and $p=0.20$). GIP and GLP-1 profiles during the OGTT were not different between *GCK* mutation carriers and non carriers ($p=0.70$ and $p=0.32$). Peak GIP or GLP-1 did not alter with age, sex, BMI or peak plasma glucose.

Conclusions: *GCK* mutation carriers have higher plasma glucose but equivalent insulin and c-peptide profiles during OGTT than non-mutation carriers, consistent with reduced plasma glucose sensing function of pancreatic beta cell *GCK*. *GCK* mutation carriers had similar incretin secretion in response to 75g oral glucose load compared with controls, suggesting that *GCK* is not the main luminal glucose sensor in the gut.

BS5-3

Dexamethasone inhibits insulin stimulated glucose uptake by reducing akt substrate of 160 kDa (AS160) phosphorylation in human adipocytes

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Background: Glucocorticoids are widely used in clinical therapy. However, they cause adverse effects, including insulin resistance and Type 2 diabetes, by as yet unclear mechanisms. Insulin stimulates glucose uptake via the insulin receptor substrate (IRS) 1/phosphoinostide-3-kinase (PI3K)/protein kinase B (Akt) pathway and promotes the redistribution of glucose transporter (GLUT) 4 from intracellular storage compartments to the plasma membrane. Akt was reported to play a role in the late step of GLUT4 trafficking. Akt substrate of 160 kDa (AS160) was recently identified as a Rab-GAP involved in GLUT4 trafficking. Insulin stimulated phosphorylation of AS160 is downstream of Akt and appears to be essential for exposure of GLUT4 at the plasma membrane (PM) and glucose uptake. This is mediated through the association of phosphorylated A160 with 14-3-3 in the cytosol. We hypothesise that dexamethasone inhibits insulin-stimulated glucose uptake at a level distal to Akt by dys-regulation of AS160.

Methods: Differentiated human SGBS adipocytes were treated –/+ 1 μmol/l dexamethasone for 24h –/+ co-treatment with 10 μmol/l RU486, the glucocorticoid receptor (GR) antagonist. Cells were incubated with 1 nmol/l insulin for 20 min at 37°C to stimulate glucose transport. Insulin-stimulated GLUT4 activity was measured by [^{3}H]-2-deoxyglucose uptake. GLUT4 translocation was assessed by immunoblotting of PM, high and low density membrane fractions. GLUT4 expression at the PM was further validated using the plasma membrane lawn assay in 3T3-L1 adipocytes. Akt activation and AS160 phosphorylation were examined by immunoblotting using phosphospecific antibodies. AS160 interaction with 14-3-3 was assessed by immunoblotting of AS160 immunoprecipitates.

Results: Dexamethasone significantly inhibited insulin-stimulated glucose uptake by ~50% ($p<0.001$) in SGBS adipocytes, but was without effect on expression or phosphorylation of proximal signalling molecules (IRS-1, PI3K, Akt) or GLUT4. Dexamethasone decreased insulin-stimulated translocation of GLUT4 to the PM as assessed by PM lawn assay and subcellular fractionation/immunoblotting. AS160 phosphorylation at T642 residue (a key Akt phosphorylation site) significantly decreased by ~50% ($p<0.01$). Consequently, insulin-stimulated AS160 association with 14-3-3 was dramatically decreased. This defect was completely restored by RU486 indicating the involvement of GR-mediated mechanisms. At 1 nmol/l insulin, AS160-T642 phosphorylation is maximal at sub-maximal glucose uptake i.e. its phosphorylation is not a limiting factor. RU486 did not completely rescue the dexamethasone-mediated inhibition on insulin-stimulated glucose uptake.

Conclusions: Collectively, these results suggest that defects at the level of AS160 phosphorylation contribute to dexamethasone-induced inhibition of glucose uptake. Our finding that the GR antagonist completely abrogates dexamethasone effect on AS160 phosphorylation, but only partially restores glucose uptake, are consistent with additional dexamethasone-induced defects. AS160 presents a novel target in the improvement of glucocorticoid-induced insulin resistance.

BS5-5

Black seed (Nigella sativa) regulates glucose, insulin level and lipid profile in patients with Type 2 diabetes

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Background: Black seed (*Nigella sativa (NS)*) has been tried as an anti diabetic agent in animal models of Type 2 diabetes with considerable efficacy, but no systematic research has been reported on humans. However NS in combination with various herbs have been studied on human patients for the treatment of diabetes This study investigated the effects of NS seed powder on the levels of blood glucose, insulin and lipids in patients with Type 2 diabetes in order to investigate possible side effects such as a fall in leukocyte and platelet counts. This was the first ever study of its type on human subjects that was initiated following approval from the Directorate of Advanced Studies and Research Board, University of Arid Agriculture, Rawalpindi, Pakistan. The study included 46 patients with Type 2 diabetes.

Methods: Selection criteria 1) Patients of either sex with known Type 2 diabetes. 2) Fasting blood glucose greater than normal values, even with their usual diabetes control medicine. 3) Age between 30-60 years. 4) Patients not on insulin therapy. 5) Patients not suffering from any other chronic disease. 6) Not taking any medication other than that for diabetes. 7) Not taking

black seed in any form or any other herbal treatment. 8) Pregnant women were not included in this study. All the registered patients signed a consent form before the start of study. *Nigella sativa* seeds were identified at "Herbarium Medicinal Botanic Centre; Pakistan Council of Scientific and Industrial Research (P.C.S.I.R.) Laboratories Complex, Peshawar, Pakistan". A voucher specimen No. (PES): 9747 was deposited there for future reference. All patients consumed NS seed powder for 40 days followed by a placebo for another 40 days. Fasting blood samples were collected from each subject on 0, 40th and 80th day of the study. Glucose, insulin, total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, total leukocyte count and platelet count were analysed using standard methods. Data collected from all the patients were analysed using SPSS (Statistical Package for Social Sciences) Version-12. The results are expressed as mean ± SEM. Where necessary, 95% confidence intervals on differences (95% CI) are given. For each parameter, mean values were compared by paired sample test. Correlations between different parameters were analysed by the Chi Square test.

Results: A highly significant decrease in fasting blood glucose ($p<0.001$), total cholesterol ($p<0.001$), LDL cholesterol ($p=0.001$), triglycerides ($p<0.001$) and increase in insulin ($p<0.001$) and HDL ($p=0.011$) was observed after treatment with NS seed powder. All values except HDL reversed significantly again at the end of the placebo phase, indicating that these changes were due to the treatment with the NS seed powder. No significant change was observed in total leukocyte and platelet count throughout the study.

Conclusion: Our results demonstrate that NS seed powder improves the levels of blood glucose and insulin and lipid profile in patients with Type 2 diabetes with reasonable safety. We also recommend further human studies on a pilot scale.

Diabetes in the Western Pacific region

DWP1-1

Surveillance of Type 2 diabetes in China: a subgroup analysis of diabetes duration in the DIABCARE 2006 study

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Background and aims: The DIABCARE 2006 project, with the intention of the Western Pacific Declaration on Diabetes, was a part of DIABCARE studies initiated from 1998. DIABCARE 2006 is important for understanding current diabetes control, diabetes management and diabetes complications status, thereby improving diabetes care in China.

Materials and methods: A subgroup of 2699 Chinese subjects with Type 2 diabetes, who registered for management of diabetes for more than 12 months at 60 hospitals in 18 cities were classified into three groups based on diabetes duration (years): ≤5 (n=917), 5-10 (n=801) and >10 group (n=981). Data were collected on a retrospective manner by reviewing medical records, interview and laboratory assessments. A centralised analysis for HbA_{1c} was carried out. All data were tabulated followed by descriptive statistical analysis.

Results: The mean ages of subjects were 57.8, 61.7 and 65.9 years in the diabetes duration groups of ≤5, 5-10 and >10 years, respectively. The onset age of diabetes was approximately 55 years in both groups of ≤5 years and 5-10 years, and 50 years in the group of >10 years. The waist-hip ratio (~0.90) and BMI (>24.5kg/m^2) were comparable in the three groups. The overall mean HbA_{1c} in 2006 was significantly lower than that in 1998 (8.7±2.0%). The mean HbA_{1c} was highest in the group of >10 years (7.8±1.5%) and lowest in the group of ≤5 years (7.3±1.6%). The percentages of subjects who achieved optimal HbA_{1c} target <6.5% decreased as duration of diabetes increased (≤5: 32.3%; 5-10: 22.9%; >10: 14.5%). There was also a significant improvement in overall mean fasting plasma glucose (FPG) from 1998 (9.0±3.4mmol/L) to 2006 (7.7±2.5mmol/L) in all subjects. An increase of more than 10% was observed in diabetes complications when comparing subjects in the group of >10 years with the group of ≤5 years. Our data showed more than 75% of subjects with less than 10 years of diabetes used Biguanides or Sulphonylureas as OAD therapy. Whereas, glucosidase inhibitors (~40%) were the most frequently used in the subjects who had Type 2 diabetes for more than 10 years. Subjects with a longer duration of diabetes appeared more likely to be treated with insulin (≤5: 30.4%; 5-10: 46.3%; >10: 68.5%) and to have a longer mean duration of insulin treatment (≤5: 1.03 years; 5-10: 1.72 years; >10: 3.05 years). The daily insulin units per kilogram also increased with increased diabetes duration (<5: 0.44U; 5-10: 0.49U; >10 group: 0.57U). Most subjects were treated with twice-daily injections, regardless of diabetes durations. The number of self-monitoring blood glucose and urine glucose increased with diabetes progression. With regards to answers about quality of life, a preponderance percentage of subjects rated their quality of life to be good or at least acceptable in all groups. Approximate half of the subjects expressed psychological insulin resistance to insulin initiation treatment.

Conclusions: An improvement in glycaemic control was observed in all subgroups of diabetes durations in patients with Type 2 diabetes. However, insulin therapy and diabetes care were not satisfactory. The gap between therapeutic guidelines and glycaemic control strongly indicates promoting awareness of treat-to-target treatment and continuing medical education in clinical practice is necessary.

DWP1-2

The individual and societal cost of Type 2 diabetes in Vanuatu

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Background: Three out of four deaths in Pacific Island countries (PICs) are due to non communicable diseases (NCDs) with Type 2 diabetes playing a critical role. The precise prevalence of diabetes in Vanuatu is unknown but a 2005 WHO STEPS survey indicated high rates of diabetes risk factors and diabetes prevalence is presumed similar to other PICs e.g. Samoa (22%), Tonga (15%) and Nauru (16%). The economic cost of NCDs in PICs is reported to consume US$1.95 million, almost 60% of the health budget of Tonga, and in Fiji absorbing 39% of the health budget in 2002, but little definitive information is available on the cost of diabetes.

Aims: This study aimed to determine the individual and societal cost of Type 2 diabetes in Vanuatu i.e. cost of treatment (health system cost), cost to people with diabetes (out-of-pocket expenses) and the impact of diabetes on quality of life.

Methods: This study was modelled on the Diabco$t Australia study [1]. The "Questionnaire for Persons with Diabetes in Vanuatu" was adapted locally to ensure relevance to the Vanuatu health system context and culture, and was administered to a convenience sample of 199 people with Type 2 diabetes by local staff who were trained to conduct the survey. A self reported survey questionnaire asked about respondents' demographics; health care access over the previous 3 months (such as prescription medications, health care encounters); cost to people with