DIABETIC RETINOPATHY IN NEWLY DIAGNOSED SUBJECTS WITH TYPE 2 DIABETES MELLITUS : CONTRIBUTION OF β-CELL FUNCTION

Authors:

Sharmistha Roy Chowdhury, MRCP(GIM), MRCP(D&E), Diabetes Research Unit, Centre for Endocrine and Diabetes Sciences, University Hospital of Wales, Cardiff, CF14 4XN, UK, Consultant Physician - Diabetes, Endocrinology and General Internal Medicine

Rebecca L Thomas, PhD, Diabetes Research Group, Swansea University, Singleton Park, Swansea, SA2 8PP, UK, Research Officer

Gareth J Dunseath, MPhil, Diabetes Research Group, Swansea University, Singleton Park, Swansea, SA2 8PP, UK, Research Assistant

Rajesh Peter, MD, Diabetes Research Group, Swansea University, Singleton Park, Swansea, SA2 8PP, UK, Consultant Diabetologist

D. Aled Rees, PhD, Centre for Endocrine and Diabetes Sciences, Institute of Molecular and Experimental Medicine, School of Medicine, Cardiff University, Cardiff CF14 4XN, UK, Senior Lecturer/Consultant Endocrinologist

Rachel V North, PhD, School of Optometry & Vision Sciences, Cardiff University, Maindy Rd, Cardiff, CF24 4LU, UK, Professor

Stephen D Luzio, PhD, Diabetes Research Group, Swansea University, Singleton Park, Swansea, SA2 8PP, UK, Professor

David R Owens, MD, Diabetes Research Group, Swansea University, Singleton Park, Swansea, SA2 8PP, UK, Professor

Corresponding Author: Dr. Sharmistha Roy Chowdhury e-mail sroyc14@hotmail.com Diabetes Research Unit, Centre for Endocrine and Diabetes Sciences, University Hospital of Wales, Heath Park, Cardiff, CF14 4XN, UK.

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ABSTRACT

Purpose: The association of hyperglycaemia and Diabetic Retinopathy (DR) in established type 2 Diabetes Mellitus (T2DM) subjects is well accepted. However the association between β-cell responsiveness and insulin sensitivity leading to fasting and postprandial hyperglycaemia with DR in newly diagnosed treatment naïve T2DM subjects remain unreported.

Methods: 544 newly diagnosed treatment naïve T2DM subjects were screened for DR (digital photography) and underwent a standardised 'Meal Tolerance Test'. Serial Plasma glucose and insulin levels were measured and fasting (M_0) and postprandial (M_1) β-cell responsiveness calculated {CPR (Calculating Pancreatic Response) Program} along with HOMA-B and HOMA-S. A subgroup of 201 subjects also underwent a 'Frequently Sampled Intravenous Glucose Tolerance Test' and the acute insulin response to glucose (AIR_G), insulin sensitivity (S_I) and glucose effectiveness (S_G) estimated (MinMod model).

Results: 16.5% (90) subjects had DR at diagnosis. Subjects with DR had significantly reduced M_0, HOMA-B and S_G leading to higher fasting and postprandial (2hour) glucose and significantly lower fasting and postprandial (2hour) insulin. Factors independently associated with DR in multivariate logistic regression analysis were M_0, HOMA-B and S_G with fasting and postprandial (2hour) glucose and insulin. There was no statistical difference in HbA_{1c}, systolic blood pressure, AIR_G and S_I between those with or without DR.

Principal conclusions: In this cohort of newly diagnosed T2DM subjects DR is associated with reduced β-cell responsiveness, resulting from β-cell failure rather than insulin resistance, leading to fasting and postprandial hyperglycaemia and hypoinsulinaemia.

INTRODUCTION:

Diabetes Mellitus (DM) is a worldwide epidemic and recent estimates (1) indicate that the number of people living with DM is expected to rise from 366 million in 2011 to 552 million by 2030, with 90% having Type 2 DM (T2DM). A recent analysis reported that of individuals with DM there are approximately 93 million people (~35%) with Diabetic Retinopathy (DR), and 28 million with vision-threatening DR (~10%) worldwide (2). In the United Kingdom, DR remains a leading cause of blindness in the working age population (3); thus early detection and treatment of modifiable risk factors known to influence its onset and progression is imperative. After 20 years of known DM duration approximately 40-60% of subjects with T2DM have some DR, with 10% of all T2DM subjects having developed sight threatening lesions related to proliferative DR and/or exudative maculopathy (4).

Various risk factors have been associated with the development and progression of DR including hyperglycaemia (5), duration of DM (5), hypertension and dyslipidaemia (6-8). The UKPDS and DCCT, along with their 10 year follow-up, have demonstrated the benefits of early and sustained improvement in glycaemic control with respect to DR (9, 10). Furthermore the UKPDS had shown that for every 1% decrease in HbA1c, there was a 37% risk reduction in microvascular complications in T2DM, predominantly DR (11). Recently the Accord Eye Study Group has shown that intensive glucose and lipid lowering, but not intensive blood-pressure control, reduce the rate of progression of DR (12).

Further analysis of the DCCT study (13) reported that the total glycemic exposure (HbA_{1C} and duration of diabetes) was able to explain 11% of the variation in retinopathy risk in the complete cohort, but other factors (e.g. environmental, genetic, glycemic variation and other measures of glycemia) could explain the remaining variation in risk on their own or through an intercorrelation with $HbA_{1C.}$

Investigating the possible association between hyperglycaemia and the presence of DR has over the years involved measurement of various metabolic indices, predominantly HbA_{1C} and/or fasting plasma glucose (11, 14). In 2005 Shiraiwa et. al. showed that postprandial hyperglycaemia and postprandial hypoinsulinemia were possible predictors for incident DR in Japanese T2DM subjects who were not on insulin treatment (15). The Diabetes Prevention Program (DPP), having studied subjects with impaired glucose tolerance and recent onset T2DM, found a higher baseline systolic blood pressure (SBP) and HbA_{1c} amongst those with retinopathy, but found no difference in insulin secretion as estimated by the Corrected Insulin Response (CIR) (16). In contrast a community based study in Taiwan demonstrated that both β-cell dysfunction and insulin resistance (IR) (both measured by the HOMA methodology) were associated with DR in established T2DM patients (17). Similarly, over the last decade, there have been other reports associating IR with DR (18-20). However the relationship between β-cell function, glucose effectiveness (S_G) and insulin sensitivity (S_I) contributing to the level of fasting and postprandial dysglycaemia, with the presence of DR in newly diagnosed subjects with T2DM remains unreported.

The aim of this study was to examine the association between β-cell function and insulin sensitivity (S_I) contributing to various fasting and postprandial glycaemic indices and the presence of DR in newly diagnosed and treatment naïve T2DM.

MATERIALS AND METHODS:

Subjects:

A total of 544 newly diagnosed, Caucasian subjects with T2DM were recruited into the study within 1-2 weeks after diagnosis of DM prior to any treatment between 1981 and 2007. The

subjects were referred by primary care on clinical presentation and were diagnosed by either fasting glucose or Oral Glucose Tolerance Test (OGTT) according to WHO criteria (21). No formal dietetic or lifestyle advice or anti-diabetic medication was given prior to study enrollment.

Ethical approval was obtained from South Glamorgan/Bro Taf Local Research Ethics Committee and all subjects gave informed consent.

Metabolic Tests:

All subjects were admitted at approximately 8am to an Investigation Unit following a 12-hour overnight fast and remained on bed rest throughout the morning of each of the study days.

Basic demographic data i.e. age, sex, height, weight and resting supine blood pressure were recorded and BMI calculated. Glycated haemoglobin and total cholesterol were measured. All subjects (n=544; Group A) underwent a standardized Meal Tolerance Test (MTT). This involved consuming a 500-kcal meal over a 10 minute period (58% carbohydrate, 23% fat, and 19% protein) commencing at time 0 min (22). Blood samples were taken from -30 to 240 minutes at 30 minute intervals, to determine plasma glucose, insulin, and C-peptide concentrations.

From 1991 onwards a subgroup of 201 subjects (Group B) additionally underwent a 'Frequently Sampled Intravenous Glucose Tolerance Test' (FSIVGTT), following a second sequential overnight fast.

The FSIVGTT consisted of baseline blood samples taken at -30, -15 and 0 minutes followed by a bolus of glucose (0.3g/kg body weight) given intravenously at 0 minute over a 2 minute

period. Thereafter, blood samples were taken at one minute intervals over 10 minutes. Following the intravenous bolus of insulin (Actrapid; Novo Nordisk, Bagsvaerd, Denmark) at a dose of 0.05 U/kg insulin given at 20 minutes (22), blood samples were taken at frequent intervals up to 180 minutes. At each time point, measurements of plasma glucose and insulin were made.

For both the procedures (MTT and FSIVGTT), an indwelling intravenous cannula was inserted into an antecubital fossa vein and connected via a three-way tap to a slow-running saline infusion, to maintain the patency of the canula allowing for repeated blood sampling. The technique was regularly checked to avoid any dilutional effect of the saline infusion.

Retinal Photography

Retinal images were obtained (Canon CR6-45NM) Non-Mydriatic Retinal Camera) through dilated pupils. Two 45° images were taken, one centred on the macula and one nasal field per eye. Classification of DR was based on the Diabetic Retinopathy Screening Service for Wales grading protocol, which is an enriched version of the UK National DR grading protocol (23). The highest grade for both eyes used for classification. All grading was carried out by a senior grader from the Diabetic Retinopathy Screening Service for Wales (DRSSW) and a diabetologist trained to grade fundus photographs with the DRSSW grading protocol and any differences were reconciled by reference to a second diabetologist who was also trained to grade fundus photographs.

Assay methods:

Duing the metabolic tests blood was withdrawn and placed into tubes containing different anti-coagulants; fluoride/oxalate for glucose and lithium-heparin for measurement of C-

peptide and specific insulin. Within approximately 10 minutes from collection, the blood tubes were spun in a refrigerated centrifuge for 5 minutes at approx 3000 rpm, and the plasma aliquoted into labelled tubes and stored frozen at -20°C until assay.

Glucose was measured by a glucose oxidase assay (YSI 2300, YSI, Hants, UK) and C-peptide and specific insulin by immunoassay (24). The within- and between-assay coefficients of variation were 1.8% and 1.9%. 5.4% and 8.8%, and 4.1% and 8.8%, respectively for the glucose, C-peptide and insulin assays.

Glycated haemoglobin measurements were performed in a routine Haematology Department. HbA_{1c} measurements were determined using a high-performance liquid chromatography assay (TOSOH HLC-723 G7; Tosoh Corporation, Tokyo, Japan) (25) which was Diabetes Control and Complications Trial (DCCT) aligned and the laboratory participated in an external quality assessment scheme. The general assay performance for the HPLC method used had a reported coefficients of variation within and between assay of <2.0% (25). HbA_1 measured in the early part of the study by column chromatography was converted to HbA_{1c} utilising the formula ($HbA_{1c} = 0.83HbA_1 - 0.54$) (26).

Data analysis:

Glucose and insulin levels: Fasting plasma glucose (FPG) and fasting plasma insulin (FPI) were measured. The postprandial plasma glucose (PPG) and plasma insulin (PPI) were represented by the 120 minute values and the areas under the curve $AUC_{(0\text{-}240min)}$ for plasma glucose and insulin over the 4 hour MTT period were calculated.

CPR program: The CPR (Calculating Pancreatic Response) program was used to quantify pancreatic ß-cell responsiveness during the MTT. M_0 (C-peptide response to fasting glucose)

representing fasting prehepatic insulin secretion and M_1 (C-peptide response to postprandial glucose) representing the increase in prehepatic insulin secretion in response to an increment in postprandial glucose were calculated (27).

Minimal model analysis: The minimal model analysis of FSIVGTT provided data on S_I (ability of insulin to enhance the net glucose disappearance from plasma) and S_G (ability of glucose to promote its own disposal and a marker of insulin-independent component of glucose tolerance) (28, 29). The acute insulin response to glucose (AIR_G) was the incremental area under the curve from 0-10 minutes during the FSIVGTT (30). The Disposition Index (DI), representing the composite measure of insulin sensitivity and pancreatic ß-cell responsiveness, was calculated as $DI = S_I \times AIR_G$ (30).

HOMA calculation: HOMA-B, HOMA-S and HOMA-IR were calculated using fasting plasma glucose and specific insulin levels using the Homeostasis Model Assessment (HOMA; version 2.2.2) (31), utilising fasting concentrations before the MTT.

Retinopathy Classification: Details of the method of retinal examination and classification of DR (23) have previously been described. For the purpose of this study, subjects were divided into 2 groups based on the absence (NDR) or presence of any diabetic retinopathy (DR) which included Background DR (BDR), Pre-proliferative DR (PPDR), Proliferative DR (PDR), possible Maculopathy (M1), and exudative Maculopathy (M2).

Statistical Analysis:

Descriptive analyses was conducted with Independent Sample t-test and Mann-Whitney U test for continuous variables and the Chi-square test for categorical variables. Normally

distributed variables were presented as the mean (±SD) and non-normally distributed variables expressed as median (interquartile range).

The designated putative risk factors were assessed using logistic regression methods with non-normally distributed variables [(FPG, FPI, PPG, PPI, $AUC_{Glucose(0-240min)}$, $AUC_{Insulin(0-240min)}$, HOMA B, M_0, M_1 and S_g)] log transformed. A non-correlated subset of clinical and metabolic variables were determined based on statistical and clinical relevance. All multivariate analyses were adjusted for age, gender, BMI and risk factors like systolic blood pressure and total cholesterol which have previously been reported to have an association (6-8) with DR with the final model additionally including parameters of ß-cell responsiveness/ ß-cell function and glycaemia. All analysis were conducted using SPSS 20 with $p<0.05$ taken as statistical significance (two-tailed).

RESULTS:

Of the 544 subjects (Group A), (393 male and 151 female, 2.6:1) with a mean age of 54 (SD±10) years, 83.5% (454) had no evidence of DR and 16.5% (90) had evidence of DR at presentation. Of those with DR, the majority 84.4% (76) had lesions of BDR (including M1) and 15.6% (14) had PPDR; none had either exudative maculopathy or PDR. In the subgroup of 201 subjects (Group B) undergoing FSIVGTT in addition to MTT, 85% (171) subjects had no evidence of DR at presentation while 15% (30) had DR comprising 12.5% (25) with BDR and 2.5% (5) PPDR; none had either exudative maculopathy or PDR.

Baseline characteristics including age, weight, BMI, systolic and diastolic blood pressure, total cholesterol and HbA_{1c} of the patients with DR and NDR in Groups A and B are detailed

in Table 1. At baseline, Group A subjects with DR had significantly lower body weight at diagnosis of DM (p=0.02) compared to those without DR. BMI was also lower in Group A and HbA1c was higher although both failed to reach statistical significance. There was no significant difference for the remaining baseline characteristics measured between those with or without DR. In Group B weight and BMI were lower in those with DR compared to those without DR however, this was not significant.

The metabolic variables measured during the MTT for Group A subjects with either DR or no DR are detailed in Table 2. Those with DR had a lower estimated ß-cell responsiveness i.e. M_0 (p=0.014) and ß-cell function (HOMA-B) (p=0.044), associated with higher fasting glucose (p=0.021) and lower fasting insulin concentrations (p=0.036). In the postprandial state, individuals presenting with DR had higher postprandial (2 hour) glucose (p=0.023) and lower postprandial insulin levels (p=0.001). Those with DR had numerically lower but non-significant (p=0.065) postprandial ß-cell responsiveness [M_1\{ 13.5 (7.9-23.8) vs 16.9 (9.1-30.0)*10^{-9} pmol/kg/min}].

Over the 4 hour MTT study period, subjects with DR had significantly higher $AUC_{Glucose\ (0-240min)}$ (p=0.023) and lower $AUC_{Insulin(0-240\ min)}$ (p = 0.001) in comparison to those without DR (Table 2). The glucose and insulin profiles and indices of ß-cell responsiveness (M_0 and M_1) during the MTT in subjects with DR and without DR are illustrated in Figures 1a and b.

The baseline characteristics and the metabolic responses in Group B subjects with either NDR or DR who underwent FSIVGTT are detailed in Table 3. Insulin sensitivity (S_I) was not significantly different between the two groups however, the S_G was significantly reduced in those with DR compared to those without DR (p=0.012). There was no difference in the AIR_G and DI between those with or without DR.

In univariate logistic regression analysis postprandial glucose, $AUC_{Glucose\ (0\text{-}240min)}$, postprandial insulin, $AUC_{Insulin\ (0\text{-}240min)}$, M_0, HOMA-B and S_G were significantly associated with the presence of DR (Table 4).

Factors associated with DR in multivariate logistic regression analyses are detailed in (Table 4). Measures of ß-cell function M_0 (OR 0.66 [95% CI 0. 0.484, 0.894] p=0.007) and HOMA-B (OR 0.74 [95% CI 0.570, 0.958] p=0.022) were associated with DR along with S_G (OR 0.20 [95% CI 0.066, 0.602] p=0.004).

The associtation of fasting glucose (OR 2.23 [95% CI 1.038, 4.791] p=0.04), postprandial glucose (OR 2.09 [95% CI 1.063, 4.123] p=0.033), $AUC_{Glucose\ (0\text{-}240min)}$ (OR 2.25 [95% CI 1.087, 4.664] p=0.029), fasting insulin (OR 0.76 [95% CI 0.585, 0.986] p=0.039), postprandial insulin (OR 0.66 [95% CI 0.511, 0.863] p=0.002) and $AUC_{Insulin\ (0\text{-}240min)}$ (OR 0.61 [95% CI 0.453, 0.828] p=0.001) with the presence of DR at diagnosis of type 2 diabetes shows the contribution of fasting, postprandial and overall hyperglycaemic/insulinopaenic exposure that leads to the development of DR. However, in this group of subjects there was no significant association with HbA_{1c} (OR 2.3 [95% CI 0.900, 5.859] p=0.082) with DR, when adjusted for the mentioned variables (age, gender, BMI, systolic blood pressure and total cholesterol) though the HbA_{1c} was higher in subjects with DR. Each 1 mmol/L increase in fasting and postprandial glucose was associated with a two-fold increase the risk of DR. Also each 1 pmol/L decrease in fasting and postptandial insulin was associated with increased risk of DR by 24% and 34% respectively.

In the multivariate logistic regression models once adjusted for glycaemia (using either HbA_{1c}/FPG/PPG) in addition to those mentioned above (age, gender, BMI, systolic blood

pressure and total cholesterol), ß-cell responsiveness or ß-cell function were the most significant risk factors for the presence of DR at diagnosis of diabetes. (Table 5).

DISCUSSION:

Our findings have shown that the presence of DR is associated with a reduced fasting β-cell responsiveness and function. This has resulted in hyperglycaemia in both the fasting and postprandial state, concurrent with fasting and postprandial insulinopaenia. In addition the insulin-independent component of glucose tolerance was reduced and independently associated with the presence of DR at diagnosis.

In this study, whilst employing both the CPR program (27) and the HOMA methodologies, we have established an independent association of M_0 and HOMA-B with the presence of DR by measuring β-cell function in response to a standardised meal challenge. This relationship of DR with β-cell function (HOMA-B) has preiously been analysed in a community-based study in Taiwan by Tung et. al. (17), involving patients with T2DM of varying duration, who were treated with lifestyle modifications and/or oral hypoglycaemic agents. They observed that those subjects with better preserved β-cell function were less likely to have DR. The UKPDS has reported that the severity of retinopathy at diagnosis of T2DM was related in both sexes to higher fasting plasma glucose levels, higher systolic and diastolic blood pressure, lower serum insulin levels, and reduced beta-cell function (32). The association between fasting β-cell dysfunction and DR in established T2DM patients as reported by Tung et al (17) is also present in our newly-diagnosed, treatment naïve, T2DM subjects. This contrasts with the DPP study involving newly diagnosed T2DM subjects, where no difference in insulin secretion estimated by the CIR was found (16).

We have also shown an independent association for the presence of DR with both fasting and postprandial hyperglycaemic and insulinopaenic responses to the MTT, as well as to the 4 hour ($AUC_{(0\text{-}240min)}$) response to the meal. Thus our study shows both fasting and postprandial glycaemic exposure exhibit an independent association with DR. Though HbA_{1c} was higher in the subjects with DR the difference narrowly failed to reach significance ($p = 0.06$). In 2005 Shiraiwa et.al. studied Japanese T2DM subjects known to have DM but not on insulin treatment (15) and established postprandial hyperglycaemia but not HbA_{1c} to independently correlate with the presence of DR (15) and stated postprandial hyperglycaemia as a possible predictor for incident DR in their subjects. Contrary to our findings two recent studies from UK identified an independent association for the presence of DR with HbA_{1c} and SBP in newly diagnosed T2DM within the first year of their diagnosis (33, 34). The DPP study involving newly diagnosed T2DM subjects has also reported a higher HbA_{1c} amongst those with DR (16).

Glucose effectiveness (S_G) represents the capacity of glucose, *per se*, to enhance glucose cellular uptake and to suppress endogenous glucose production and has been reported to be an important determinant of glucose metabolism (35). The glucose transporter protein GLUT-1 is widely distributed on the plasma membrane of various body tissues contributing an important role in insulin-independent glucose uptake (36, 37). Thus, in the presence of significant β-cell dysfunction and resultant insulinopaenia, a relatively poor S_G will further worsen glycaemia. This might explain our findings, where the newly diagnosed T2DM subjects with worse S_G are more likely to present with DR.

Our study therefore demonstrates the significant contributions of β-cell dysfunction, fasting and postprandial hyperglycaemia/insulinopaenia and reduced glucose effectiveness. Thus it adds to the evidence base of co-contributory factors towards development of diabetic

complications. Several epidemiological studies have confirmed the association between hyperglycaemia and the development of late diabetic complications (9, 38). However most of the previous studies have employed the time-averaged mean levels of glycaemia measurement of HbA_{1c} as a measure for glycaemic status. Over the last decade there has been increasing recognition that HbA_{1c} is not a complete expression of the degree of hyperglycaemia and that other aspects of dysglycaemia contribute to the increased risk of diabetic complications and HbA_{1c} was reported to account for 11% of the risk of retinopathy in the DCCT (13) . Recent research has also suggested that postprandial glucose levels and glucose variability, may confer additional risk for the development of micro- and macrovasular diabetic complications (39, 40).

In our study we measured insulin sensitivity both by the MINMOD program (following FSIVGTT) and HOMA (following MTT) and found no difference between T2DM subjects presenting with DR compared to those without DR at the time of diagnosis. Our study cohort differs from previous reports because it comprised of only newly diagnosed, treatment naïve participants with T2DM, and thus lacked the confounding effects of therapeutic interventions. By contrast, other cross-sectional studies have associated insulin sensitivity (assessed by euglycemic clamp) with the presence or severity of DR (18-20). The numbers of subjects and controls in those studies were modest and the subjects recruited had established T2DM that was being treated with variety of hypoglycaemic agents, both oral and insulin. It is therefore unclear whether the association that they found was entirely independent of the underlying confounders such as duration and treatment modalities of DM. Thus in our subjects presenting with DR there is no significant contribution from diminished insulin sensitivity/resistance at time of clinical diagnosis.

The DPP reported that more than 12% of subjects with T2DM had DR within approximately 3 years of diagnosis (16). 16.5% of our subjects with newly diagnosed T2DM, who presented with DR were studied within 1-2 weeks of diagnosis, possibly indicating a slightly longer pre-clinical period in our cohort. Whilst our study is limited by its cross sectional design that makes it difficult to confirm a cause and effect relationship, the strength of our study lies in the recruitment of subjects at clinical diagnosis. Thus, we were able to rule out confounding factors such as known duration of DM and treatment modalities, however we do acknowledge that duration prior to clinical diagnosis may have been substantial. It also presents a detailed analysis of the metabolic response of a T2DM subject emanating from a diminished fasting functional β-cell state, resulting in both fasting and postprandial dysglycaemia leading to DR but not being affected by an element of insulin resistance/sensitivity.

To summarise, in newly diagnosed treatment-naïve T2DM subjects, the presence of DR is associated with relatively worse functional status of both the insulin dependent (as manifested by lower β-cell responsiveness with resultant relative insulinopaenia) and insulin independent (as manifested by reduced S_G) components of glucose tolerance. Thus in this cohort of newly diagnosed T2DM subjects, DR is associated with reduced β-cell responsiveness resulting from β-cell failure rather than insulin resistance leading to a fasting and postprandial state of hyperglycaemia and hypoinsulinaemia.

Author Contributions

All authors contributed to the writing of this report. SRC processed, analysed and interpreted the data. DRO and SDL contributed to the conception, study design, interpretation of the data and writing of the report. SDL and GJD performed the laboratory analysis. RLT, GJD and RP contributed to processing and interpreting the data and RVN and DAR provided expert advice. All authors approved the final version of this manuscript. SRC and DRO had full access to all of the data in this study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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REFERENCES:

1. Whiting DR, Guariguata L, Weil C, Shaw J. IDF diabetes atlas: global estimates of the prevalence of diabetes for 2011 and 2030. Diabetes Res Clin Pract. 2011 Dec;94(3):311-21.
2. Yau JW, Rogers SL, Kawasaki R, Lamoureux EL, Kowalski JW, Bek T, et al. Global prevalence and major risk factors of diabetic retinopathy. [Review]. Diabetes Care. 2012;35(3):556-64.
3. Bunce C, Wormald R. Causes of blind certifications in England and Wales: April 1999-2000. Eye. 2008;22:905-11.
4. Stefansson E, Bek T, Porta M, Larsen M, Kristensen JK, Agardh E. Screening and prevention of diabetic blindness. Acta Ophthalmologica Scandinavica. 2000;78:374-85.
5. Zhang X, Saaddine JB, Chou CF, Cotch MF, Cheng YJ, Geiss LS, et al. Prevalence of diabetic retinopathy in the United States, 2005-2008. JAMA. 2010;304(6):649-56.
6. Chew EY, Klein ML, Ferris FL III, Remaley NA, Murphy RP, Chantry K, et al. Association of elevated serum lipid levels with retinal hard exudate in diabetic retinopathy. Early Treatment Diabetic Retinopathy Study (ETDRS) Report 22. Archives of Ophthalmology. 1996;114(9):1079-84.
7. Klein R, Klein BEK, Moss SE, Davis MD, DeMets DL. Is blood pressure a predictor of the incidence or progression of diabetic retinopathy? Archives of Internal Medicine. 1989;149(11):2427-32.
8. UK Prospective Diabetes Study Group. Risks of progression of retinopathy and vision loss related to tight blood pressure control in type 2 diabetes mellitus. UKPDS 69. Archieves of Ophthalmology. 2004;122:1631-40.
9. Holman RR, Paul SK, Bethel MA, Matthews DR, Neil HA. 10-Year follow-up of intensive glucose control in type 2 diabetes N Engl J Med. 2008;359:1577- 89.
10. The Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications Research Group. Retinopathy and nephropathy in patients with type 1 diabetes four years after a trial of intensive therapy. N Engl J Med. 2000 Feb; 10(342(6)):381-9.
11. Stratton IM, Cull CA, Adler AI, Matthews DR, Neil HA, Holman RR. Additive effects of glycaemia and blood pressure exposure on risk of complications in type 2 diabetes: a prospective observational study (UKPDS 75). Diabetologia. 2006;49(8):1761-9.
12. ACCORD study group, Accord Eye Study Group. Effects of Medical Therapies on Retinopathy Progression in Type 2 Diabetes. N Engl J of Medicine. 2010;15(363(3)):233-44.
13. Lachin JM, Genuth S, Nathan DM, Zinman B, BN R, DCCT/EDIC Research Group. Effect of glycemic exposure on the risk of microvascular complications in the Diabetes Control and Complications Trial—revisited. Diabetes. 2008;57(4):995-1001.
14. Miki E, Kikuchi M. Diabetic retinopathy and control of diabetes with special reference to blood glucose levels. Diabetes Research & Clinical Practice. 1994; 24 Suppl:S177-89.
15. Shiraiwa T, Kaneto H, Miyatsuka T, Kato K, Yamamoto K, Kawashima A, et al. Post-prandial hyperglycaemia is an important predictor of the incidence of diabetic microangiopathy in Japanese type 2 diabetic patients. Biochemical and Biophysical Research Communications 2005;336:339-45.
16. Diabetes Prevention Program Research Group. The prevalence of retinopathy in impaired glucose tolerance and recent-onset diabetes in the Diabetes Prevention Program. Diabet Medicine. 2007 24(2):137-44.

17. Tung TH, Shih HC, Tsai ST, Chou P, Chen SJ, Lee FL, et al. A community-based study of the relationship between insulin resistance/beta-cell dysfunction and diabetic retinopathy among type II diabetics in Kinmen, Taiwan. Ophthalmic Epidemiology. 2007;14(3):148-54.
18. Suzuki M, Kanazawa A, Shiba M, Kojima H, Harano Y. Insulin resistance in diabetic microangipopathies Journal of Diabetes and its Complications 2000;14(1): 40-5.
19. Nakano S , Kitazawa M, Ito T, Hatakeyama H, Nishizawa M, Nakagawa A, et al. Insulin resistant state in type 2 diabetes is related to advanced autonomic neuropathy. Clinical and Experimental Hypertension. 2003 25(3):155-67.
20. Katsumori K, Wasada T, Kuroki H, Arii H, Saeki H, Aoki K, et al. Prevalence of macro - and microvascular diseases in non-insulin-dependent diabetic and borderline glucose-intolerant subjects with insulin resistance syndrome. Diabetes Research and Clinical Practice 1995;29:195-201.
21. Report of a WHO/IDF consultation. Definition and diagnosis of diabetes mellitus and intermediate hyperglycaemia
http://wwwwhoint/diabetes/publications/diagnosis_diabetes2006/en/. 2005.
22. Owens DR, Luzio SD, Coates PA. Insulin secretion and sensitivity in newly diagnosed NIDDM Caucasians in the UK. Diabetic Medicine 1996;13(Suppl 6):S19–S24
23. Harding S, Greenwood R, Aldington S, Gibson J, Owens DR, Taylor R, et al. Grading and disease management in national screening for diabetic retinopathy in England and Wales. Diabetic Medicine. 2003;20:965-71.
24. Andersen L, Dinesen B, Jorgensen PN, Poulsen F, Roder ME. Enzyme immunoassay for intact human insulin in serum or plasma. Clin Chem. 1993;39:578–82.
25. Terreni A1, Paleari R, Caldini A, Ognibene A, Mosca A, G. M. Evaluation of the analytic performances of the new HPLC system HLC-723 G7 for the measurement of hemoglobin A1c. Clin Biochem. 2003. Nov;36(8):607-10.
26. UK Prospective Diabetes Study (UKPDS). VIII. Study design, progress and performance. Diabetologia. 1991;34(12):877-90.
27. Hovorka R, Chassin L, Luzio SD, Playle R, Owens DR. Pancreatic beta-cell responsiveness during Meal Tolerance Test: Model assessment in normal subjects and subjects with newly diagnosed non insulin dependant diabetes mellitus. The Journal of Clinical Endocrinology and Metabolism 1998;83(3):744-50.
28. Bergman RN, Finegood DT, Ader M. Assessment of insulin sensitivity in vivo Endocr Rev. 1985 6:45-86.
29. Bergman RN, Steil GM, Bradley DC, Watanabe RM. Modeling of insulin action in vivo. Annu Rev Physiol 1992;54:861-83.
30. Kahn SE, Prigeon RL, McCulloch DK, Boyko EJ, Bergman RN, Schwartz MW, et al. Quantification of the relationship between insulin sensitivity and beta-cell function in human subjects. Evidence for a hyperbolic function. Diabetes. 1993(42):1663–72.
31. Levy JC, Matthews DR, Hermans MP. Correct Homeostasis Model Assessment (HOMA) Evaluation uses the computer program. Diabetes Care. 1998;21:2191-2.
32. Kohner EM, Aldington SJ, Stratton IM, Manley SE, Holman RR, Matthews DR, et al. United Kingdom Prospective Diabetes Study, 30: diabetic retinopathy at diagnosis of non-insulin-dependent diabetes mellitus and associated risk factors. Archives of Ophthalmology. 1998;116(3):297-303.
33. Looker HC, Nyangoma SO, Cromie D, Olson JA, Leese GP, Black M, et al. Diabetic retinopathy at diagnosis of type 2 diabetes in Scotland. Diabetologia. 2012;55(9):2335-42.
34. Kostev K, Rathmann W. Diabetic retinopathy at diagnosis of type 2 diabetes in the UK: a database analysis. Diabetologia. 2012.

35. Best JD, Kahn SE, Ader M, Watanabe RM, Ni TC, Bergman RN. Role of glucose effectiveness in the determination of glucose tolerance. Diabetes Care. 1996;19:1018-30.
36. Henriksen JE, Alford F, Handberg A, et al. Increased glucose effectiveness in normoglycaemic but insulin resistant relatives of patients with non-insulin-dependant diabetes mellitus: a novel compensatory mechanism. J Clin Invest. 1994;94:1196-204.
37. Ebeling P, Koistinen HA, Koivisto VA. Insulin-independant glucose transport regulates insulin sensitivity. FEBS Lett 1988;436:301-3.
38. The Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complication (DCCT/EDIC) Study Research Group. Intensive diabetes treatment and cardiovascular disease in patients with type 1 diabetes. N Engl J Med. 2005;353:2643-53.
39. Brownlee M, Hirsch IB. Glycemic variability: a hemoglobin A1c-independent risk factor for diabetic complications. JAMA. 2006;295:1707–8.
40. Hanefeld M, Temelkova-Kurktschiev T. Control of postprandial hyperglycemia– an essential part of good diabetes treatment and prevention of cardiovascular complications. Nutr Metab Cardiovasc Dis. 2002;12:98–107.

Table 1: Baseline characteristics in subjects with No Diabetic Retinopathy (NDR) compared to those with Diabetic Retinopathy (DR) at diagnosis of T2DM. Group A: 544 subjects who underwent MTT, Group B: 201 subjects who underwent FSIVGTT

		All subjects	**NDR**	**DR**	**p value**
Group A	**Number**	**544**	**454**	**90**	
	Age at presentation (years)	54 (10)	54 (10)	56 (11)	0.28
	Male Sex (%)	393 (72)	324 (71)	69 (77)	0.31
	Weight (kg)	88 (17)	88 (17)	85 (19)	0.02
	BMI (kg.m^2)	30.2 (5.0)	30.4 (5.3)	29.6 (5.8)	0.06
	Systolic blood pressure (mmHg)	137 (19)	137 (20)	139 (18)	0.25
	Diastolic blood pressure (mmHg)	83 (11)	83 (11)	83 (11)	0.71
	Total Cholesterol (mmol/L)	5.4 (1.2)	5.5 (1.2)	5.2 (1.2)	0.08
	HbA_{1c} {%} [mmol/mol]	{7.7}(2.0) [61] (22)	{7.7}(2.0) [61] (22)	{8.0}(1.8) [64] (20)	0.06
Group B	**Number**	**201**	**171**	**30**	
	Age at presentation (years)	55 (10)	55 (10)	55 (11)	0.79
	Male Sex	145 (72)	125 (73)	20 (67)	0.47
	Weight (kg)	90 (17)	91 (16.7)	86 (16.5)	0.16
	BMI (kg.m^2)	31.2 (5.5)	31.3 (5.6)	30.6 (4.8)	0.54
	Systolic blood pressure (mmHg)	135 (19)	135 (18)	134 (19)	0.68
	Diastolic blood pressure (mmHg)	81 (10)	81 (10)	81 (10)	0.95
	Total Cholesterol (mmol/L)	5.4 (1.1)	5.4 (1.2)	5.4 (1.0)	0.77
	HbA_{1c} {%} [mmol/mol]	{7.6} (1.9) [61] (21)	{7.6}(2.0) [61] (22)	{7.7} (1.5) [61] (17)	0.80

Data expressed as Mean (± SD); Sex: Number (%); BMI = Body Mass Index

Table 2: Comparison of the metabolic variables during the Meal Tolerance Test in subjects with No Diabetic Retinopathy (NDR) and those with Diabetic Retinopathy (DR) at diagnosis of T2DM

Group A	**NDR (n=454)**	**DR (n=90)**	**p value**
Fasting Glucose (mmol/L)	9.6 (7.6 - 12.7)	10.6 (8.5 – 13.8)	0.021
Postprandial Glucose (mmol/L) (120 mins)	13.4 (9.8 - 17.3)	15.1 (11.1 - 18.1)	0.023
AUC $_{\text{Glucose (0-240min)}}$ (mmol/L)	11.8 (9.0 – 15.4)	13.6 (9.8 - 16.3)	0.023
Fasting Insulin (pmol/L)	61.8 (34.0 -99.0)	50.5 (33.9 – 86.36)	0.036
Postprandial Insulin (pmol/L) (120 mins)	278.5 (162.0 – 459.3)	189.0 (108.3 – 335.5)	0.001
AUC $_{\text{Insulin (0-240min)}}$ (pmol/L)	199.2 (117.7 - 317.2)	130.5 (83.8-225.7)	<0.001
M_0 (*10^{-9} pmol/kg/min)	5.3 (3.1-7.8)	3.7 (2.6-7.3)	0.014
M_1 (*10^{-9} pmol/kg/min)	16.9 (9.1-30.0)	13.5 (7.9-23.8)	0.065
HOMA-B (%)	34.9 (19.1-60.3)	26.1 (14.7-48.2)	0.044
HOMA-S (%)	59.7 (37.7-105.5)	78.7 (45.2-108.6)	0.094
HOMA-IR	1.7 (0.9-2.7)	1.3 (0.9-2.2)	0.094

Data expressed as median (1^{st} – 3^{rd} Inter Quartile Range)

AUC = Area Under the Curve

Table 3: Comparison of metabolic variables following Frequently Sampled Intravenous Glucose Tolerance Test in subjects with No Diabetic Retinopathy (NDR) and those with Diabetic Retinopathy (DR) at diagnosis

Group B	**NDR (n=171)**	**DR (n=30)**	**p value**
S_I x 10^{-4} [(microU/ml)$^{-1}$.min^{-1}]	0.8 (0.4-1.4)	0.9 (0.6-1.3)	0.610
S_G x 10^{-2} (min^{-1})	1.4 (1.2-1.7)	1.2 (0.8-1.6)	0.012
$AIR_{G\ (0\text{-}10min)}$ (microU/ml. min)	111.4 (65.4-177.7)	94.8 (62.2-191.0)	0.703
DI x 10^{-2}	0.89 (0.39-1.53)	0.82 (0.51-1.70)	0.744

Data expressed as median (1^{st} – 3^{rd} Inter Quartile Range)

S_I = Insulin Sensitivity; S_G = Glucose effectiveness; $AIR_{G\ (0\text{-}10min)}$ = Acute Insulin Response to glucose; DI = Disposition Index

Table 4. Univariate and multivariate logistic regression depicting variables independently associated with the

	Number	Crude OR (95% CI)	p	Adjusted OR (95% CI)
			(<0.05)	(for age and sex)
HbA1c (%)	506	2.329 (0.931, 5.823)	0.071	2.515 (0.997, 6.346)
Fasting Glucose (mmol/L)	544	2.078 (0.982, 4.400)	0.056	2.238 (1.051, 4.765)
Postprandial Glucose (mmol/L) (120 mins)	543	1.944 (1.004, 3.763)	0.049	2.054 (1.058, 3.987)
AUC $_{\text{Glucose (0-240min)}}$ (mmol/L)	544	2.081 (1.021, 4.242)	0.044	2.196 (1.075, 4.487)
Fasting Insulin (pmol/L)	494	0.782 (0.607, 1.007)	0.057	0.784 (0.607, 1.012)
Postprandial Insulin (pmol/L) (120 mins)	534	0.681 (0.526, 0.882)	0.004	0.686 (0.529, 0.890)
AUC $_{\text{Insulin (0-240min)}}$ (pmol/L)	534	0.625 (0.465, 0.840)	0.002	0.631 (0.468, 0.850)
M_0 (*10^{-9} pmol/kg/min)	540	0.693 (0.514, 0.934)	0.016	0.697 (0.517, 0.940)
HOMA-B (%)	494	0.750 (0.582, 0.968)	0.027	0.745 (0.577, 0.963)
S_G x 10^{-2} (min^{-1})	201	0.206 (0.069, 0.618)	0.005	0.211 (0.070, 0.642)

** for age, sex, BMI, SBP, TCh

BMI = Body Mass Index, SBP = Systolic Blood Pressure; TCh = Total Cholesterol

AUC = Area Under the Curve, S_G = Glucose effectiveness

Table 5. Univariate and Multivariate logistic regression depicting variables independently associated with the

a)	Number	Crude OR (95% CI)	p (<0.05)	Adjusted OR (95% CI)	p (<0.05)	A
				(fully adjusted *)		(a
M_0 (*10^{-9} pmol/kg/min)	540	0.693 (0.514, 0.934)	0.016	0.658 (0.484, 0.894)	0.007	0.
HOMA-B (%)	494	0.750 (0.582, 0.968)	0.027	0.739 (0.570, 0.958)	0.022	0.
b)				(fully adjusted *)		(a
M_0 (*10^{-9} pmol/kg/min)	540	0.693 (0.514, 0.934)	0.016	0.658 (0.484, 0.894)	0.007	0.
HOMA-B (%)	494	0.750 (0.582, 0.968)	0.027	0.739 (0.570, 0.958)	0.022	0.
c)				(fully adjusted *)		(a
M_0 (*10^{-9} pmol/kg/min)	540	0.693 (0.514, 0.934)	0.016	0.658 (0.484, 0.894)	0.007	0.
HOMA-B (%)	494	0.750 (0.582, 0.968)	0.027	0.739 (0.570, 0.958)	0.022	0.

* for age, sex, BMI, SBP, TCh

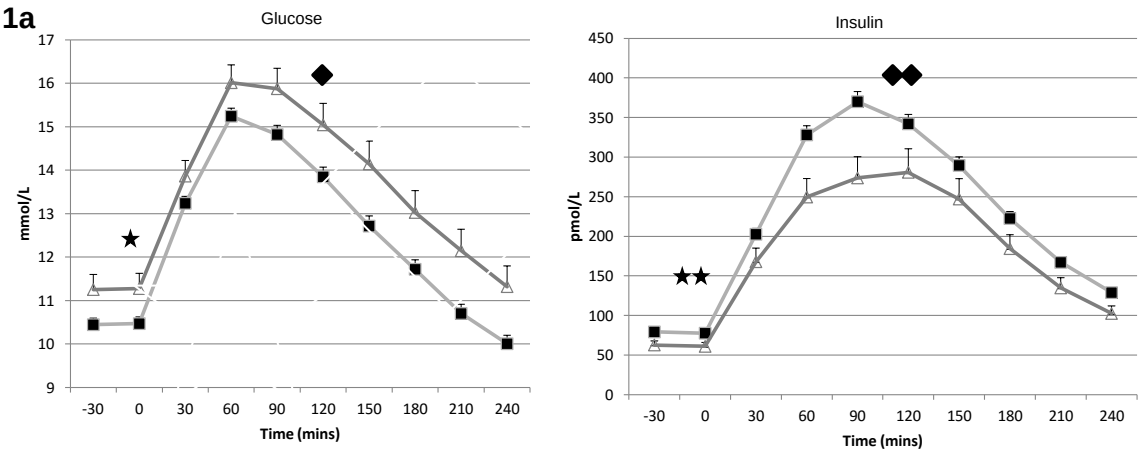
Θ for age, sex, BMI, SBP, TCh, HbA_{1c}

© for age, sex, BMI, SBP, TCh, FPG

® for age, sex, BMI, SBP, TCh, PPG

BMI = Body Mass Index, SBP = Systolic Blood Pressure; TCh = Total Cholesterol, FPG = Fasting Plasma Glucose

1a



1b

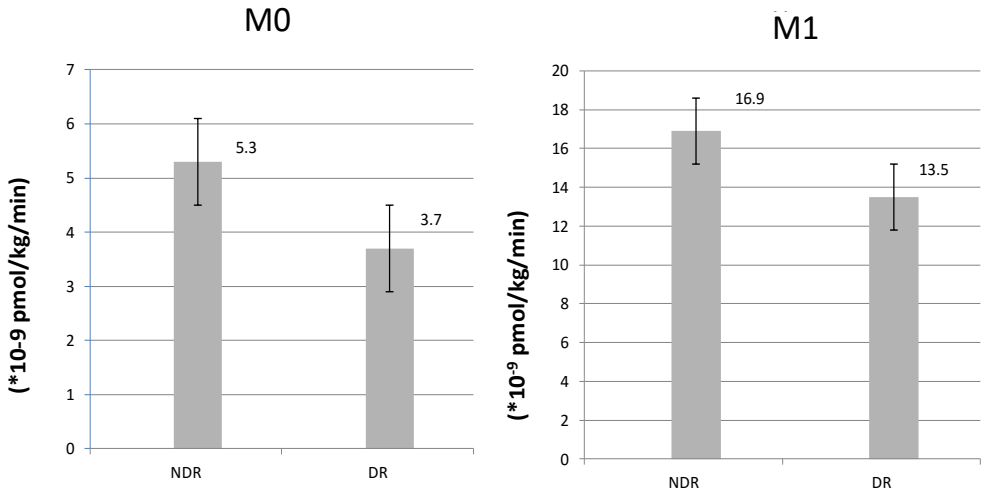


Figure: 1 Glucose and Insulin Profiles with β-cell responsiveness in patients with and without diabetic retinopathy.

1a) Plasma glucose and insulin profile (mean+SEM) during MTT in subjects with NDR (Filled square) (n=454) and those with DR (Open triangle) (n=90) at diagnosis of T2DM. Significant difference between NDR and DR : ★ Fasting Glucose (p = 0.021), ♦ Postprandial Glucose (p = 0.023), ★★ Fasting Insulin (p = 0.036) and ♦♦ Postprandial Insulin (p = 0.001).

1b) Fasting (M_0) and Post-prandial (M_1) β-cell responsiveness during MTT in subjects with NDR and those with DR at diagnosis of T2DM