INCIDENCE OF DIABETIC RETINOPATHY IN NEWLY DIAGNOSED SUBJECTS WITH TYPE 2 DIABETES MELLITUS OVER 5 YEARS: CONTRIBUTION OF β-CELL FUNCTION

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Abstract

Aims:

Identifying and modulating risk factors is essential to prevent visual impairment due to diabetic retinopathy (DR). This study examines incident DR with metabolic and hormonal factors in newly-diagnosed, treatment naïve, individuals with Type2 Diabetes Mellitus (T2DM), over a 5 year period from diagnosis.

Methods:

233 T2DM subjects underwent serial DR screening using digital photography and standardised Meal Tolerance Tests at diagnosis and after 1, 2 and 5 years. Subjects (179) with no DR throughout the 5-year study period were compared with those who developed DR (54).

Results:

Of 233 subjects, 54(23.2%) developed DR by 5 years, background DR in 50(93%) and exudative maculopathy in 4(7%) individuals. Of these subjects, 12(22%) developed DR after 1 year, 15(28%) after 2 years and 27(50%) after 5 years.

At baseline, those with DR at 5 years had higher HbA_{1c} (p=0.017), higher fasting plasma glucose (PG) (p=0.031) and postprandial PG (p=0.009). They were associated with reduced basal β-cell secretory function (M_0) (p=0.025), lower (p=0.000) postprandial β-cell responsiveness (M_1) and β-cell function (HOMA-B) (p=0.044).

Conclusions

There is an independent association between glycaemic control and β-cell dysfunction at the time of diagnosis of T2DM, with incident DR over a follow-up period of 5 years.

Introduction

The incidence of Diabetic Retinopathy (DR) in individuals with Type 2 Diabetes Mellitus (T2DM) ranges from 22.0% over 6 years in the UK to 66.9% in USA over 10 years (1). In a recent systematic review by Sabanayagam (2), the annual incidence of DR based on studies in Asia, Africa and North America, ranged from 2.2% to 12.7%. Therefore, identifying risk factors involved in the development and progression of DR is essential to define strategies to prevent visual impairment and blindness resulting from DR.

Exposure to prolonged hyperglycaemia is acknowledged to be the predominant risk factor for development and progression of DR in both type 1 and type 2 diabetes mellitus (3-6). Most studies report glycaemic control based on glycosylated haemoglobin in the form of HbA1c (6-9) and/or fasting plasma glucose (FPG) (4, 8, 10, 11), with fewer reporting on postprandial plasma glucose (PPG) (8, 12).

However, the relationship of DR with indices of β-cell function and insulin sensitivity remains inadequately addressed. The aim of this study was to examine these pathophysiological variables with progression to DR over a period of 5 years in newly-diagnosed, treatment naïve individuals with T2DM who had no evidence of DR at diagnosis.

Research Design and Methods

Subject recruitment, experimental protocol (including retinal images), data analysis and basic statistical analysis have been detailed in a previous publication (13). A total of 544 newly diagnosed Caucasian subjects with T2DM were recruited into the study within 1–2 weeks after

diagnosis of DM before any treatment between 1981 and 2007. Subjects were referred by primary care on clinical presentation and diagnosed by either fasting glucose or oral glucose tolerance test according to World Health Organization criteria - 1985. No formal dietetic or lifestyle advice or antidiabetic medication was given before study enrolment. Ethical approval was obtained from South Glamorgan/Bro Taf Local Research Ethics Committee, and all subjects gave informed consent.

Of 314 subjects with data at 5 years post diagnosis, data was available both at diagnosis and during the 5-year study period for 293 participants. Of these 293 subjects recruited, 60 subjects were excluded from the analysis, as DR was evident at diagnosis (Figure 1). In this analysis we have compared those with no DR (NDR) throughout (n=179) with those who developed DR during the course of the 5-year study (n=54).

All subjects were admitted at approximately 8 AM to an Investigation Unit following a 12-hour overnight fast and remained on bed rest throughout the morning of the study days. All subjects underwent a standardized Meal Tolerance Test (MTT). This involved consuming a 500-kcal meal over a 10-minute period (15 gm Weetabix, 100 gm skimmed milk, 250 ml pineapple juice, 50 gm white meat chicken, 60 gm wholemeal bread, 10 gm polyunsaturated margarine) (58% carbohydrate, 23% fat, and 19% protein) commencing at time 0 minutes (14). Blood samples were taken from 30 to 240 minutes at 30-minute intervals, to determine plasma glucose, insulin, and C-peptide concentrations.

Retinal images were obtained (Canon CR6-45NM) non-mydriatic retinal camera) through dilated pupils. Two 45° images were taken, one centered 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 (15). The highest grade for both eyes was used for classification.

Indices of β-cell function (HOMA-B) and insulin sensitivity/resistance (HOMA-S and HOMA-IR) were calculated, employing only the FPG and specific insulin levels obtained at baseline (time 0 minutes) during the MTT, using the Homeostasis Model Assessment (HOMA; version 2.2.2) (16). The CPR (Calculating Pancreatic Response) program (17) was used to further quantify pancreatic β-cell responsiveness during the MTT. Fasting β-cell responsiveness (M_0) is the ability of fasting glucose to stimulate insulin secretion, based on the C-peptide representing fasting pre-hepatic insulin secretory response to fasting glucose. Postprandial β-cell responsiveness (M_1) is the ability of postprandial glucose to increase insulin secretion as represented by the C-peptide response equating to pre-hepatic insulin secretion (17).

Following initial descriptive analysis and comparison between the two groups, a non-correlated subset of clinical and metabolic variables were defined. These designated putative risk factors [FPG, fasting plasma insulin (FPI), PPG, postprandial plasma insulin (PPI) $AUC_{Glucose\ (0\text{-}240min)}$, $AUC_{Insulin\ (0\text{-}240min)}$, HOMA B, M_0, M_1 and S_g)] were assessed using logistic regression methods, with log transformation of non-normally distributed variables. (The postprandial levels were represented by the 120-minute values and the areas under the curve ($AUC_{0\text{-}240mins}$) estimated half-hourly up to 4 hours during the MTT.

All multivariate analyses were adjusted for age, gender, BMI and risk factors that included systolic blood pressure and total cholesterol, which have previously been variously reported to have an association with DR. All analyses were conducted using SPSS 20, with $p<0.05$ taken as statistically significant (two-tailed).

We further calculated the mean of all the measured metabolic variables at each time point, as indicators of diabetes control.

Results

Baseline characteristics of the study population with type 2 diabetes:

The 233 subjects who underwent meal tolerance tests included 179 (76.8%) subjects who remained without DR and 54 (23.2%) who developed DR during the 5-year observation period (Supplementary Table 1). Of those who developed DR during the 5-year study from diagnosis, background DR (non-proliferative DR) was evident in 12 (22%) after 1 year, a further 15 (28%) after 2 years and 27 (50%) after 5 years (Supplementary Figure 1). Of those with DR at 5 years, the majority 93% (50) had BDR, with only 1.7% (4) who developed mild non-centrally placed exudative maculopathy but none progressed to pre-proliferative or proliferative DR.

Our study has shown that individuals with DR at Year 5 presented with a significantly higher $HbA_{1c,}$ ($p=0.017$) at diagnosis (Table 2). Although there was a greater percentage of males in the NDR group, the difference was not statistically significant. Other baseline characteristics were not significantly different between those with or without DR at Year 5 (Supplementary Table 1).

Comparison of baseline metabolic variables and indices of β-cell function at diagnosis for subjects with T2DM, who did or did not develop DR at 5 years

The metabolic variables observed at diagnosis of T2DM during the MTT for subjects, with or without DR, during the 5-year period, are detailed in Table 1 and the glucose and insulin profiles and indices of β-cell secretory function (M_0 and M_1) during the MTT are illustrated in Figure 1. Over the 4-hour MTT study period, subjects with DR had significantly higher FPG (p=0.031), 2-hour PPG levels (p=0.009) and AUC $_{\text{Glucose (0-240min)}}$ (p=0.007) with lower AUC $_{\text{Insulin (0-240 min)}}$ (p=0.042), in comparison to those without DR at Year 5. At diagnosis there was reduced fasting basal β-cell secretory function, as defined by M_0 (p=0.025) and HOMA-B (p=0.044), in those who developed DR, although the lower fasting insulin concentrations were not significantly different (p=0.177). In addition, in the postprandial state, there was highly significantly poorer estimated postprandial β-cell responsiveness to the test meal M_1 (p=0.000) and lower postprandial insulin levels (p=0.044) in those who developed DR

Univariate and Multivariate regression analysis

Based on the inter-group differences (Table 1), univariate logistic regression analysis demonstrated that HbA_{1c}, FPG, PPG and AUC $_{\text{Glucose(0-240min),}}$ at diagnosis were all positively related to the development of DR by Year 5. In addition, the β-cell function/secretory capacity represented by $M_{0,}$ $M_{1,}$ HOMA-B, postprandial insulin and AUC $_{\text{Insulin (0-240min),}}$ were negatively related to the appearance of DR within the 5-year observation period (Table 2a). Factors identified as associated with DR in the univariate logistic regression analyses were adjusted for age and sex, BMI, total cholesterol and systolic blood pressure and are detailed in (Table 2a), with the p value calculated using the likelihood ratio test.

We demonstrated that the risk of developing DR within 5 years of diagnosis of T2DM was related to baseline variables of HbA_{1c} (p=0.021), FPG (p=0.045), PPG (p=0.011) and AUC $_{Glucose\ (0\text{-}240min)}$ (p=0.011). Incident DR was also associated at baseline with fasting insulin (p=0.027), postprandial insulin (p=0.031) and AUC $_{Insulin\ (0\text{-}240min)}$ (p=0.006). The greater deficiency in β-cell function in those who developed DR was further supported by additional measures of β-cell secretory function fasting i.e. M_0 (p=0.015), HOMA-B (p=0.007) and postprandial M_1 (p=0.000), both independently associated with the development of DR (Table 2b). The M_0 however lost its significance when adjusted for insulin sensitivity (HOMA-S) and HbA1c but M_1 retained its significance when adjusted for insulin sensitivity (HOMA-S) (OR 0.96 [95% CI 0.93, 0.99] p=0.006) and HbA1c (OR 0.95 [95% CI 0.92, 0.99] p=0.006) thus portraying the effect of postprandial glycaemic exposure in our subjects developing DR (Table 2a).

In summary, each 1 mmol/L increase in fasting and postprandial glucose at diagnosis was associated with a two to three fold increase in the risk of DR by 5 years after diagnosis. In addition, each 1 pmol/L decrease in fasting and postprandial insulin was associated with increased risk of DR by 41% and 34% respectively.

Comparative analysis of subjects with T2DM with and without DR at 5 years with metabolic parameters at years 1, 2 after diagnosis

At end of Year 1, HbA_{1c} (p=0.045), FPG (p=0.006), PPG (p=0.007), and AUC $_{Glucose\ (0\text{-}240min)}$ (p=0.012) were associated with DR at 5 years. Similarly at end of Year 2 HbA_{1c} (p=0.032), PPG (p=0.005), and AUC $_{Glucose(0\text{-}240min)}$ (p=0.020) were also associated with DR at 5 years (Supplementary Tables 2 and 3). These findings further confirm the contribution of continuing

fasting, postprandial and overall hyperglycaemic exposure at Years 1 and 2, leading to DR by 5 years. In addition, an independent association with DR for measures of β-cell secretory capacity were seen at both Year 1 i.e. HOMA-B (p=0.016) and at Year 2 M_1 (p=0.006).

Comparative analysis of subjects with T2DM, with and without DR at 5 years, in relation to the mean averaged metabolic variables over the 5-year study period from diagnosis of T2DM (Years 0, 1, 2 and 5)

We have shown that individuals with DR at Year 5, compared to those without, had a significantly higher mean averaged HbA_{1c} (p=0.003) and a higher mean averaged PPG (2-hour) (p=0.007) and AUC $_{Glucose\ (0\text{-}240min)}$ (p=0.0015) over the 5 years from diagnosis (Table 3). However, there was no significant difference in the mean averaged insulin levels between the two groups, although those with DR by Year 5 had a lower mean averaged estimated postprandial β-cell responsiveness i.e. M_1 (p=0.001) compared to those without DR at 5 years.

Based on the inter-group differences (Table 3), univariate logistic regression was conducted, which demonstrated that the cumulative HbA_{1c} (p=0.004), PPG (p=0.022) and AUC $_{Glucose(0\text{-}240min)}$ (p=0.023) over 5 years post-diagnosis of T2DM were significantly related to the development of DR by Year 5 (Table 2b). A reduced estimated postprandial β-cell responsiveness i.e. M_1 (p=0.001) over the 5 years was also significantly related to the development of DR

Factors that were independently associated with DR in univariate logistic regression analyses when adjusted for age and sex, BMI, total cholesterol and systolic blood pressure are detailed in (Table 2b). Measures of β-cell function, M_0 (p=0.014) and M_1 (p=0.001) show independent

association with DR (being adjusted for insulin sensitivity {HOMA-S} as well) (Table 2b). HbA_{1c} (p=0.008), PPG (p=0.023), and $AUC_{Glucose(0\text{-}240min)}$ (p=0.027), show the contribution of postprandial and overall hyperglycaemic exposure over 5 years in subjects leading to DR by 5 years, when adjusted for the above mentioned variables.

There was a significant increase in the PPG levels and AUC in the individuals who developed DR, compared to those that did not, over the 5-year study period (Years 0, 1, 2, and 5), as shown in Figure 3. Furthermore, there was also a significant decrease in the postprandial β-cell responsiveness in those who developed DR. However, the other measurements including fasting glucose, insulin profiles and the mean HOMA-B and HOMA-S over the 5 years, were not significantly different between those with and without DR, by 5 years post-diagnosis of T2DM.

Subjects requiring oral hypoglycaemic medications at Year 1 [NDR-69 (38.5%), DR-30 (55.6%), p=0.027], Year 2 [NDR-81 (45.3%), DR-38 (70.3%), p=0.001] and Year 5 [NDR-114 (63.7%), DR-44 (81.5%), p=0.014] had a significantly greater chance of developing DR by Year 5, with no difference in the outcome between the use of metformin or sulphonylurea. There was no effect of insulin use or antihypertensive over the 5 years on development of DR by Year 5.

Discussion

In this study, we demonstrate a strong and independent association between incident DR after 5 years of Type 2 diabetes and glycaemic control at the time of diagnosis, as represented by $HbA_{1c,}$ fasting and 2-hour postprandial hyperglycaemia, as well as the overall 4-hour glucose

($AUC_{(0\text{-}240min)}$) response to a standardised test meal. We have further shown that calculated measures of β-cell function fasting (M_0) and HOMA-B and postprandial (M1), conducted at diagnosis were also significantly associated with DR. Our findings demonstrate that in our study population of individuals with T2DM, at diagnosis, substantial loss in β-cell function had occurred. This resulted in excess glycaemia, which presented a greater risk of developing DR within the post-diagnosis 5-year period. The data emphasise the contribution of both postprandial and overall glycaemic exposure over the 5-year follow-up period to development of DR. The degree of dysglycaemia seen in the individuals at diagnosis reflects the reduced fasting and postprandial β-cell responsiveness to a meal challenge at diagnosis.

The significantly higher baseline HbA_{1c} (8.6%) in newly-diagnosed T2DM subjects, who went on to develop DR by 5 years, compared to those who did not have DR (HbA1c 7.4%), remained significant on multivariate logistic regression. Thus, for every 1% rise in HbA_{1c} at diagnosis, there was a four-fold greater likelihood of developing DR in the following 5-year period. There was also a significant independent association between the mean HbA_{1c} level, measured across Years 1, 2 and 5, with the development of DR during the study period. This emphasises the importance of the early impact of preceding dysglycaemia and β-cell dysfunction evident at the time of diagnosis and was not seen 5 years after treatment had been implemented.

Our findings of 23.2% of subjects developing DR over 5 years, are similar to the UKPDS, which demonstrated that newly-diagnosed subjects with T2DM had a DR incidence of 22% over the same period of time (9). They further demonstrated that glycaemic control at diagnosis and overall glycaemic exposure were significant contributory factors. The Wisconsin Epidemiologic Study of Diabetic Retinopathy (WESDR) also reported that in subjects with T2DM, glycaemic control over 6 years represented by HbA_{1c} was associated with increased

risk of DR development (7). However, in contrast to our findings, they did not find a relationship between DR incidence and β-cell function, as defined in their study by plasma C-peptide measurement. Our study is also consonant with that of Yoshida et al. who followed T2DM subjects with no DR at the initial visit, and found significant contribution of baseline overall glycaemic exposure (HbA_{1c}) and duration of DM (6) to DR development. The Verona Diabetes Study, conducted over 5 years, further showed by multivariable logistic regression analysis that development of DR was independently predicted by average glycaemia over time (HbA_{1c}) or mean FPG (18). The analyses in our study thus support the association of both baseline and overall glycaemic exposure with development of DR.

Similar to our findings, an epidemiological study from Mauritius showed baseline FPG independently contributed to 6-year incidence of DR (4). In their newly-diagnosed DM subjects at baseline their 6-year incidence of DR at 23.8% was very much in line with our 5-year incidence of 23.2%. The Atherosclerosis Risk in Communities (ARIC) study from USA with a 3 year DR incidence of 10.1% (19) and the Blue Mountains Eye Study in an Australian population-based cohort over 5 year) (20) both showed the association of baseline FPG with incident DR.

A Japanese study of T2DM subjects demonstrated, PDR development was significantly associated with HbA_{1c} more than 5 years earlier and with mean FPG more than 10 years earlier (21). This support a legacy effect and are consistent with results of the DCCT/EDIC (22) and UKPDS 80 (23). Our study demonstrating the independent association of FPG both at diagnosis and Year 1 with development of DR by 5 years post diagnosis also provides support to the legacy effect.

Our study established that 2-hour PPG following standardised meal at diagnosis, 1 and 2 years post diagnosis and mean PPG over 5 years all have an independent association with development of DR by 5 years from diagnosis. The PPI (2hour post meal) at baseline indicated a significant contribution to DR development by 5 years. The importance of post-prandial hyperglycaemia that we observed in our study has not been extensively documented before, although Shiraiwa et al. has shown an independent correlation of PPG and insulin with the progression of DR in T2DM Japanese subjects, surveyed over a 5-year period. PPG was shown to be a stronger predictor than HbA_{1c} in their subjects (12). Of note, our cohort were treatment naïve at baseline, with subjects majorly on oral hypoglycaemics at years 1, 2 and 5.

As in our study Voutilainen-Kaunisto et al. examined newly-diagnosed T2DM subjects over 10 years prospectively in Finland, at diagnosis and 5 and 10 years of follow-up (8). In these subjects, FPG, 2-hour PPG and HbA_{1c} at 5 years but not at diagnosis, predicted development of DR at the 10-year follow-up. In contrast to our study, their fasting insulin and C-peptide levels failed to show any association with DR development. Our findings noting an association between the incidence of DR and FPG, 2-hour PPG and $HbA_{1c,}$ 5 years prior to its development reflects a similar legacy effect ("metabolic memory"). They demonstrated that risk of developing DR was 7.7 times greater with elevated PPG levels compared with 4.2 times for elevated FPG. Our study demonstrating a greater contribution of postprandial and overall glycaemic exposure to the development of DR than FPG, is therefore concordant with the Finnish study.

In our study we observed that the $AUC_{Glucose(0\text{-}240min)}$ in response to a meal challenge at time of diagnosis, 1 and 2 years' post-diagnosis and the mean $AUC_{Glucose(0\text{-}240min)}$ over the 5-year follow-up period have an independent association with the DR development over this period

of time. These findings indicate that excess glycaemic exposure, measured over 4 hours during a MTT at diagnosis is significantly related to the development of DR over a 5 year observation period.

The AUC $_{\text{Insulin (0-240min)}}$ at diagnosis showed a significant negative contribution to the development of DR by 5 years. Those newly-diagnosed T2DM subjects who developed DR within 5 years had a significantly lower fasting and postprandial β-cell responsiveness and function at diagnosis and also a significantly lower mean fasting and postprandial β-cell responsiveness over these 5-years. Interestingly, there was no noted association with any parameters of insulin resistance/sensitivity at any time during the study period.

Our Mean M_0 and M_1 retained its significance even after being adjusted for insulin sensitivity. Our analysis established an independent association of M_0, HOMA-B and M_1 with incidence of DR by measuring β-cell function in response to a standardised meal challenge, employing both the CPR program (17) and the HOMA methodologies. This analysis of newly-diagnosed T2DM subjects indicates that lower fasting and postprandial β-cell responsiveness at diagnosis and over the 5-year study period are a basis for increased fasting, postprandial and overall glycaemic exposure, and all contribute significantly to the development of DR. The fasting and postprandial insulinopaenia at diagnosis in subjects who go on to develop DR is a reflection of a failing β-cell function at diagnosis and despite the introduction of therapeutic intervention subsequently, did not have an effect on the incidence of DR. This consolidates the importance of early and continuing glycaemic status and β-cell function on the future incidence of DR in individuals with Type 2 diabetes.

Suzuki et al studied retrospectively the role of pancreatic β-cell insulin secretory capacity (24-hour urinary C-peptide) in the development of PDR in T2DM subjects with a known duration of DM of greater than 10 years (24). The incidence of PDR during the follow-up period (~10 years) was highest in the group with the lowest 24-hour urinary C-peptide concentration. These data are consistent with the view that a low pancreatic β-cell insulin secretory capacity may be a risk factor for the development of PDR via the resulting hyperglycaemia (24). Ahlquist et al. have recently identified that a cluster of patients who were insulin deficient had the highest risk of DR compared to the cluster most resistant to insulin (25). Similarly, in our study there was no difference in the insulin sensitivity between those with or without DR by Year 5. This is very much in synchrony with a recently proposed β-cell centric classification of DM suggests that pancreatic β-cell deficiency is a basic requirement for the development of DM (26).

Our data is of a Caucasian population from South Wales between 1981and 2007 (13). However, the level of hyperglycaemia at presentation is similar to other population groups who present elsewhere in UK (9). The sustained contribution of the postprandial component of glucose level in our study (mean PPG) is stronger than the mean FPG on the development of DR. Of note, most parameters of glycaemic control and β-cell secretory status at diagnosis, and soon after (within 1 to 2 years after diagnosis), appear to be the main contributors to development of DR, with no significant relationship to HbA_{1c} or other glycaemic parameters, when determined at year 5. This is compatible with the early history of glycaemic exposure leading to the legacy effect described in the UKPDS (23) and "metabolic memory" described in the DCCT (22) being an important component in the development of DR. In addition, the insulin-independent component of glucose tolerance at diagnosis was reduced and independently associated with the incidence of DR by 5 years in newly-diagnosed T2DM subjects.

In conclusion, our study has shown an independent association between glycaemic control and β-cell dysfunction at the time of diagnosis of T2DM, with incident DR over a follow-up period of 5 years. The pre-diagnostic duration of DM in our cohort of persons with Type 2 DM may be slightly longer than in other studies, which is possibly reflected in the slightly greater percentage of people presenting with DR and higher HbA1c at baseline. Therefore, there is an imperative to diagnose T2DM as early as possible, in order to intervene and lower the glycaemic exposure to prevent/delay DR.

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Author contributions: S.R.C. and D.R.O. 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. All authors contributed to the writing of this report. S.R.C. processed, analyzed, and interpreted the data. D.R.O. and S.D.L. contributed to the conception, study design, and interpretation of the data. S.D.L. and G.J.D. performed the laboratory analysis. R.L.T., G.J.D., contributed to processing and interpreting the data. FSW and DRO revised the manuscript. All authors approved the final version of this manuscript.

Data Availability

The datasets generated during and/or analysed during the current study are not publicly available but are available from the corresponding author on reasonable request.

Disclosure Summary: D.R.O. has received honoraria for participation in advisory boards and/or lectures for Sanofi, NovoNordisk, Boehringer Ingelheim, Eli Lilly and Roche Diagnostics. The other authors have nothing to disclose.

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Legends for Figures and Tables:

Table 1: Comparison of metabolic variables at diagnosis in subjects who underwent a Meal Tolerance Test with No Diabetic Retinopathy throughout the 5 years from diagnosis, compared to those who developed Diabetic Retinopathy by 5 years from diagnosis of T2DM.

Table 2 a): Univariate and Multivariate logistic regression depicting variables independently associated with development of Diabetic Retinopathy by 5 years from diagnosis of T2DM.
Table 2 b): Univariate and Multivariate logistic regression depicting variables independently associated with development of Diabetic Retinopathy by 5 years from diagnosis of T2DM. All parameters are mean average values measured over diagnosis, Year 1, 2 and 5 years post diagnosis.

Table 3: Comparison of the mean averaged metabolic variables over a 5 year period (Years 0, 1, 2 and 5) during the Meal Tolerance Test in subjects with No Diabetic Retinopathy and those with Diabetic Retinopathy by 5 years post diagnosis of T2DM.

Figure 1: Baseline metabolic parameters. A) Glucose B) Insulin Profiles C) Fasting (M_0) and D) postprandial (M_1) β-cell responsiveness (mean ± SEM) in subjects at diagnosis with (DR) and without (NDR) diabetic retinopathy at 5 years post diagnosis

Figure 2: Mean metabolic parameters over 5 years. A) Mean Fasting (FPG), Postprandial (PPG), AUC Glucose $_{(0\text{-}240\ min),}$ B) Mean Fasting Insulin (FPI), Postprandial (PPI) and AUC Insulin $_{(0\text{-}240\ min)}$ Profiles C) Mean HOMA-B and S D) Mean fasting (M_0) and postprandial (M_1) β-cell responsiveness (mean ± SEM) over 5 years in subjects with (DR) and without diabetic retinopathy (NDR) by 5 years post diagnosis of T2DM.

Table 1: Comparison of metabolic variables at diagnosis in subjects who underwent a Meal Tolerance Test with No Diabetic Retinopathy throughout the 5 years from diagnosis, compared to those who developed Diabetic Retinopathy by 5 years from diagnosis of T2DM.

	No Diabetic Retinopathy (n=179)	**Diabetic Retinopathy (n=54)**	**p value**
Fasting Glucose (mmol/L)	10.1 (7.8-13.3)	11.6 (9.6-13.6)	**0.031**
Postprandial Glucose (mmol/L) (120 mins)	13.9 (10.2-17.7)	16.0 (13.3-18.1)	**0.009**
AUC $_{\text{Glucose (0-240min)}}$ (mmol/L)	11.6 (8.6-14.6)	13.7 (11.2-15.6)	**0.007**
HbA1c (%) [mmol/mol]	7.4 (6.4-9.6), [57 (46 -81)]	8.6 (7.8-10.0), [70 (62 - 86)]	**0.017**
Fasting Insulin (pmol/L)	61.2 (40.0-97.0)	56 (28-92)	0.177
Postprandial Insulin (pmol/L) (at 2 hour)	270 (145-428)	185 (94-391)	**0.044**
AUC $_{\text{Insulin (0-240min)}}$ (pmol/L)	192 (106-303)	155 (68-270)	**0.042**
M_0 ($*10^{-9}$ pmol/kg/min)	5.2 (2.7-7.8)	3.9 (1.9-7.0)	**0.025**
M_1 ($*10^{-9}$ pmol/kg/min)	17.2 (10.4-28.5)	9.8 (6.9-15.5)	**0.000**
HOMA-B (%)	34 (16-60)	25 (11-43)	**0.044**
HOMA-S (%)	59 (39-89)	72 (43-115)	0.191
HOMA-IR	1.7 (1.1-2.6)	1.4 (0.9-2.4)	0.191

Table 2 a): Univariate and Multivariate logistic regression depicting variables independently associated with development of Diabetic Retinopathy by 5 years from diagnosis of T2DM.
Table 2 b): Univariate and Multivariate logistic regression depicting variables independently associated with development of Diabetic Retinopathy by 5 years from diagnosis of T2DM. All parameters are mean average values measured over diagnosis, Year 1, 2 and 5 years post diagnosis.

		Univariate		Multivariate	
	Number	Crude OR (95% CI)	p	OR (95% CI)	p
a)				(fully adjusted **)	
HbA1c (%)	233	4.27 (1.21, 15.13)	**0.024**	4.48 (1.26, 15.96)	**0.021**
Fasting Glucose (mmol/L)	230	2.77 (1.01, 7.59)	**0.047**	2.78 (1.02, 7.64)	**0.045**
Postprandial Glucose (mmol/L) (120 mins)	230	3.44 (1.34, 8.85)	**0.011**	3.44 (1.34, 8.86)	**0.011**
AUC $_{\text{Glucose (0-240min)}}$ (mmol/L)	230	3.60 (1.34, 9.72)	**0.011**	3.62 (1.34, 9.76)	**0.011**
Fasting Insulin (pmol/L)	224	0.77 (0.52, 1.13)	0.184	0.59 (0.36, 0.94)	**0.027**
Postprandial Insulin (pmol/L) (120 mins)	225	0.66 (0.45, 0.95)	**0.026**	0.66 (0.46, 0.96)	**0.031**
AUC $_{\text{Insulin (0-240min)}}$ (pmol/L)	229	0.64 (0.43, 0.95)	**0.028**	0.53 (0.34, 0.83)	**0.006**
M_0 (*10^{-9} pmol/kg/min)	227	0.62 (0.41, 0.93)	**0.022**	0.59 (0.39, 0.91)	**0.015**
	227	-	**-**	0.92 (0.83, 1.02)	0.131 ***
M_1 (*10^{-9} pmol/kg/min)	224	0.48 (0.33, 0.70)	**0.000**	0.46 (0.32, 0.68)	**0.000**
	224	-	**-**	0.96 (0.93, 0.99)	**0.006 *** **
HOMA-B (%)	224	0.70 (0.50, 0.98)	**0.040**	0.60 (0.41, 0.87)	**0.007**
b)					
Mean HbA1c (%)	233	1.51 (1.14 – 2.01)	**0.004**	1.48 (1.12 – 1.97)	**0.008**
Mean Fasting Glucose (mmol/L)	230	1.15 (0.96 – 1.38)	**0.143**	1.13 (0.93 – 1.36)	**0.227**
Mean Postprandial Glucose (mmol/L) (120 mins)	230	1.15 (1.02 – 1.30)	**0.022**	1.15 (1.02 – 1.30)	**0.023**
Mean AUC Glucose (0-240min) (mmol/L)	230	1.17 (1.02 – 1.35)	**0.023**	1.18 (1.02 – 1.36)	**0.027**

Mean M0 (*10-9 pmol/kg/min)	227	0.89 (0.78 – 1.02)	**0.100**	0.81 (0.68 – 0.96)	**0.014**
	227	-	**-**	0.80 (0.66 - 0.98)	**0.030 *** **
Mean M1 (*10-9 pmol/kg/min)	224	0.93 (0.89 – 0.97)	**0.001**	0.93 (0.86 – 0.97)	**0.001**
	224	-	**-**	0.91 (0.86 - 0.96)	**0.001 *** **
Mean HOMA-B (%)	224	0.10 (0.98 – 1.01)	**0.505**	0.99 (0.97 – 1.01)	**0.207**
Mean HOMA-S (%)	224	1.00 (0.10 – 1.01)	**0.779**	1.01 (1.00 – 1.01)	**0.124**

** adjusted for age, sex, BMI, SBP, TCh: SBP = Systolic Blood Pressure; TCh = Total Cholesterol, BMI = Body Mass Index FPG = Fasting Plasma Glucose; PPG = Post Prandial Glucose; AUC = Area Under the Curve.

*** adjusted for age, sex, BMI, SBP, TCh, HOMA-S

Table 3: Comparison of the mean averaged metabolic variables over a 5 year period (Years 0, 1, 2 and 5) during the Meal Tolerance Test in subjects with No Diabetic Retinopathy and those with Diabetic Retinopathy by 5 years post diagnosis of T2DM.

	No Diabetic Retinopathy	**Diabetic Retinopathy**	**p value**
Number	**179**	**54**	**-**
Fasting Glucose (mmol/L)	9.1 (7.6 – 10.5)	9.5 (8.5 – 10.5)	0.116
Postprandial Glucose (mmol/L) (120 mins)	11.8 (9.5 – 13.4)	13.0 (11.4 – 14.4)	**0.007**
AUC $_{\text{Glucose (0-240min)}}$ (mmol/L)	9.6 (8.1 – 11.4)	10.5 (9.5 – 11.7)	**0.015**
HbA_{1c} (%) [mmol/mol]	7.0 (6.3 – 8.0), [53 (45 - 64)]	7.6 (6.9 – 8.2), [60 (52 - 66)]	**0.003**
Fasting Insulin (pmol/L)	63.5 (45.3 - 95.3)	67.1 (37.3 – 94.8)	0.957
Postprandial Insulin (pmol/L) (120 mins)	297.7 (202.1 – 468.3)	298.5 (156.0 – 426.8)	0.414
AUC $_{\text{Insulin (0-240min)}}$ (pmol/L)	205.1 (146.7 – 315.3)	171.3 (98.4 – 318.1)	0.212
M_0 (*10^{-9} pmol/kg/min)	6.2 (4.4 – 8.2)	4.8 (3.5 - 7.1)	0.083
M_1 (*10^{-9} pmol/kg/min)	22.6 (15.8 – 31.5)	16.1 (10.6 – 22.6)	**0.001**
HOMA-B (%)	44.4 (33.2 – 65.0)	42.1 (28.0 - 58.1)	0.257
HOMA-S (%)	70.0 (45.2 – 99.2)	62.8 (46.8 -118.0)	0.873
HOMA-IR	1.6 (1.2 - 2.5)	1.7 (1.0 - 2.5)	0.873

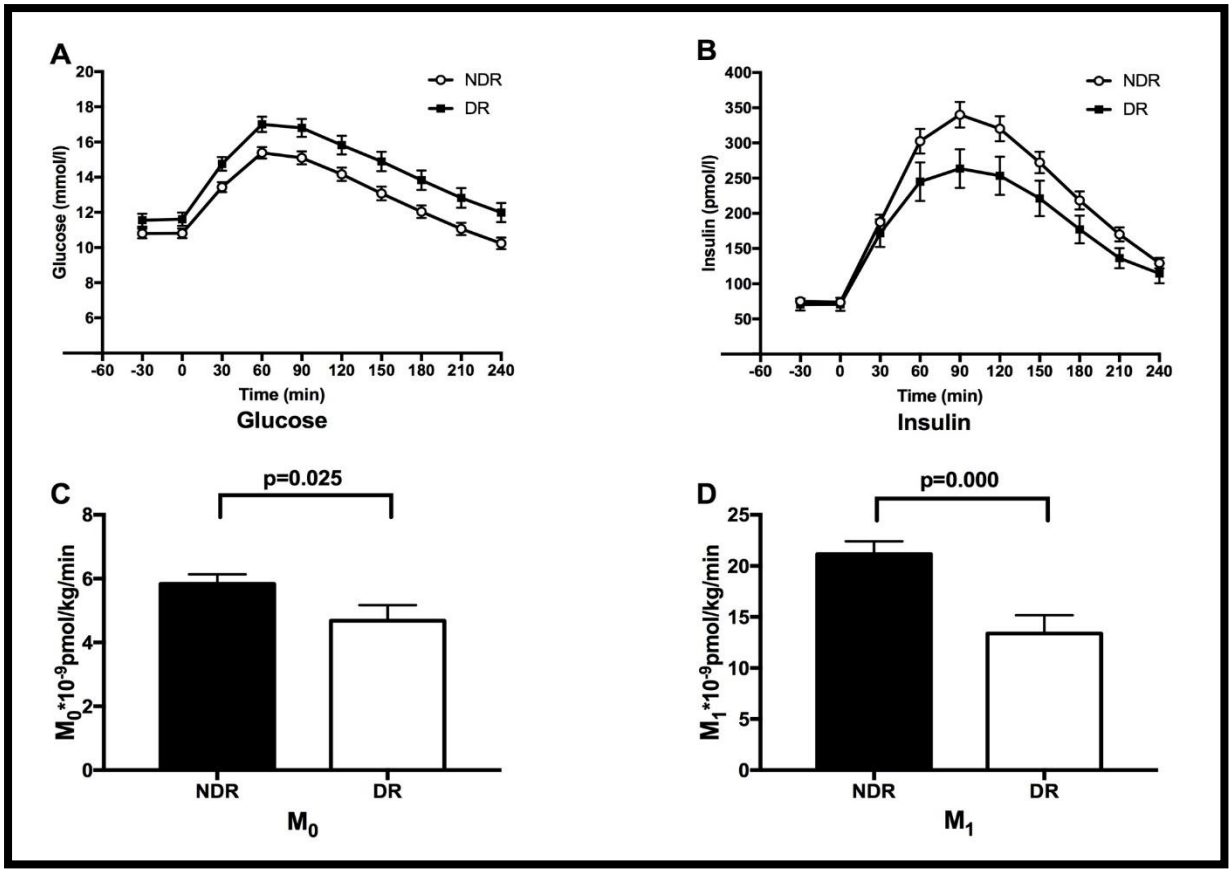

Figure 1: Baseline metabolic parameters. A) Glucose B) Insulin Profiles C) Fasting (M_0) and D) postprandial (M_1) β-cell responsiveness (mean ± SEM) in subjects at diagnosis with (DR) and without (NDR) diabetic retinopathy at 5 years post diagnosis

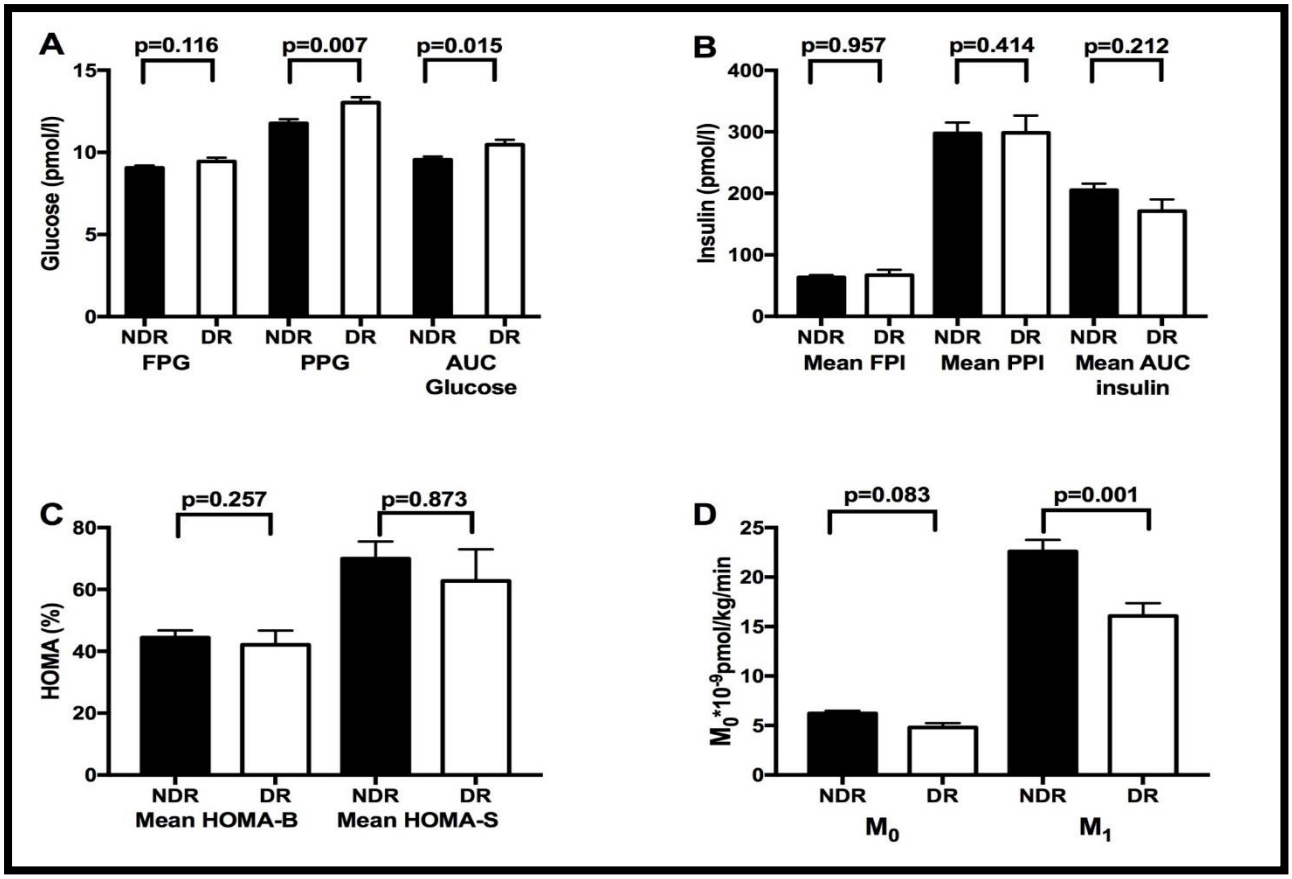

Figure 2: Mean metabolic parameters over 5 years. A) Mean Fasting (FPG), Postprandial (PPG), AUC Glucose (0-240 min), B) Mean Fasting Insulin (FPI), Postprandial (PPI) and AUC Insulin (0-240 min) Profiles C) Mean HOMA-B and S D) Mean fasting (M_0) and postprandial (M_1) β-cell responsiveness (mean ± SEM) over 5 years in subjects with (DR) and without diabetic retinopathy (NDR) by 5 years post diagnosis of T2DM.

Supplementary Table 1: Baseline characteristics in subjects with No Diabetic Retinopathy (NDR) throughout 5 years since diagnosis compared to those who developed Diabetic Retinopathy (DR) by 5 years from diagnosis of T2DM.

Subjects	**All subjects**	**No Diabetic Retinopathy (NDR)**	**Diabetic Retinopathy (DR)**	**Comparison of NDR and DR (p value)**
Number	**233**	**179**	**54**	**-**
Age at presentation (years)	54 (9)	54 (10)	55 (8)	0.53
Male Sex (%)	75	78	67	0.10
Weight (kg)	88 (16)	88 (16)	88 (16)	0.78
BMI (kg/m^2)	30 (5)	30 (5)	30 (6)	0.48
Systolic blood pressure (mmHg)	134 (18)	134 (17)	134 (18)	0.74
Diastolic blood pressure (mmHg)	83 (10)	83 (10)	82 (10)	0.85
Total Cholesterol (mmol/L)	5.5 (1.3)	5.5 (1.3)	5.7 (1.4)	0.20
HbA1c (%) [mmol/mol]	**8.2 (2.1) [66 (23)]**	**8.1 (2.1) [65 (23)]**	**8.8 (1.8) [73 (20)]**	**0.017**

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

Supplementary Table 2: Comparison of the metabolic variables at **1,2 and 5 years** post diagnosis of T2DM during the Meal Tolerance Test in subjects with No Diabetic Retinopathy (NDR) over 5 years from diagnosis of T2DM to those with Diabetic Retinopathy (DR) by 5 years from diagnosis of T2DM

	NDR	**DR**	**p value**
Number	**179**	**54**	
Year 1			
Fasting Glucose (mmol/L)	7.9 (6.9 – 8.9)	8.8 (7.6 – 10.7)	**0.003**
Postprandial Glucose (mmol/L) (120 mins)	9.7 (7.8 – 12.1)	11.1 (9.5 – 13.7)	**0.005**
Fasting Insulin (pmol/L)	64.6 (41.0 – 96.1)	63.0 (43.8 – 96.3)	0.965
Postprandial Insulin (pmol/L) (120 mins)	289.5 (177.8 – 443.3)	244.0 (155.0 – 396.5)	0.215
M_0 (*10^{-9} pmol/kg/min)	5.8 (3.8 – 9.5)	5.2 (3.9 – 7.2)	0.180
M_1 (*10^{-9} pmol/kg/min)	21.7 (12.7 – 34.7)	15.5 (11.5 – 29.9)	0.123
HOMA-B (%)	51.2 (34.6 – 71.5)	39 (25.3 – 60.2)	0.051
HOMA-S (%)	60.8 (41.4 – 97.0)	63.8 (42.9 – 90.4)	0.870
Year 2			
Fasting Glucose (mmol/L)	8.0 (7.1 – 9.4)	8.7 (7.6 – 10.7)	0.113
Postprandial Glucose (mmol/L) (120 mins)	10.0 (8.2 – 13.0)	11.7 (10.0 – 14.2)	**0.005**
Fasting Insulin (pmol/L)	64.5 (40.0 – 91.8)	61.0 (33.0 – 106.5)	0.595
Postprandial Insulin (pmol/L) (120 mins)	281.0 (171.0 – 493.0)	259.0 (134.3 – 426.3)	0.234
M_0 (*10^{-9} pmol/kg/min)	6.4 (4.0 – 9.0)	5.7 (3.8 – 8.0)	0.444
M_1 (*10^{-9} pmol/kg/min)	23.2 (12.0 – 37.7)	17.5 (8.8 – 27.0)	**0.009**
HOMA-B (%)	46.7 (31.4 – 74.1)	44.2 (27.2 – 71.2)	0.349
HOMA-S (%)	60.7 (40.2 – 95.0)	65.3 (36.9 – 110.7)	0.617
Year 5			
Fasting Glucose (mmol/L)	8.7 (7.5 – 11.1)	9.2 (7.9 – 11.6)	0.366
Postprandial Glucose (mmol/L) (120 mins)	12.7 (9.8 – 15.3)	13.3 (10.7 – 16.3)	0.155
Fasting Insulin (pmol/L)	61.4 (38.8 – 109.0)	65.6 (43.1 – 100.3)	0.849
Postprandial Insulin (pmol/L) (120 mins)	227.5 (173.7 – 426.0)	249.0 (119.8 – 458.0)	0.219
M_0 (*10^{-9} pmol/kg/min)	6.5 (4.0 – 8.7)	5.6 (2.6 – 8.3)	0.993
M_1 (*10^{-9} pmol/kg/min)	19.8 (12.1 – 31.4)	13.4 (7.3 – 19.0)	0.568
HOMA-B (%)	42.3 (26.4 – 65.3)	41.8 (20.0 – 62.1)	0.623
HOMA-S (%)	61.3 (34.6 – 99.4)	59.3 (39.0 – 90.3)	0.991

Data expressed as median (1^{st} – 3^{rd} Inter Quartile Range), AUC = Area Under the Curve

Supplementary Table 3: Univariate and Multivariate logistic regression depicting variables (Year 1 to 5) independently associated with development of Diabetic Retinopathy by 5 years after diagnosis of T2DM.

		Univariate		Multivariate	
	Number	Crude OR (95% CI)	p	OR (95% CI)	p
			(<0.05)	(fully adjusted **)	(<0.05)
Year 1 HbA1c (%)	228	7.29 (1.24 – 42.86)	**0.028**	6.20 (1.04 – 36.82)	**0.045**
Year 1 Fasting Glucose (mmol/L)	217	7.85 (1.82 – 33.92)	**0.006**	7.71 (1.78 – 33.29)	**0.006**
Year 1 Postprandial Glucose (mmol/L) (120 mins)	217	4.66 (1.56 – 13.93)	**0.006**	4.57 (1.52 – 13.69)	**0.007**
Year 1 AUC $_{\text{Glucose (0-240min)}}$ (mmol/L)	218	5.12 (1.46 – 17.85)	**0.011**	5.00 (1.43 – 17.50)	**0.012**
Year 1 HOMA-B (%)	191	0.60 (0.34 – 1.04)	0.069	0.49 (0.26 – 0.87)	**0.016**
Year 2 HbA1c (%)	226	7.64 (1.61 – 36.31)	**0.011**	5.69 (s 1.16- 27.94)	**0.032**
Year 2 Postprandial Glucose (mmol/L) (120 mins)	209	4.54 (1.52 – 13.56)	**0.007**	4.83 (1.60 – 14.64)	**0.005**
Year 2 AUC $_{\text{Glucose (0-240min)}}$ (mmol/L)	210	3.90 (1.20 – 12.87)	**0.024**	4.15 (1.25 – 13.76)	**0.020**
Year 2 M_1 (*10^{-9} pmol/kg/min)	195	0.62 (0.43 – 0.90)	**0.011**	0.59 (0.41 – 0.86)	**0.006**
Year 5 HbA1c (%)	120	1.93 (0.294 – 12.73)	0.493	1.81 (0.25 – 13.19)	0.560

** adjusted for age, sex, BMI, SBP, TCh: SBP = Systolic Blood Pressure; TCh = Total Cholesterol, BMI = Body Mass Index
FPG = Fasting Plasma Glucose; PPG = Post Prandial Glucose; AUC = Area Under the curve

Supplementary Figure 1: Consort Flow Diagram depicting development of Diabetic Retinopathy (DR) during the 5 year study from diagnosis