Assessment of putative risk factors in the development of Diabetic Retinopathy in Wales

Dr. Sharmistha Roy Chowdhury

A thesis submitted for the fulfilment of the requirement for the degree of Doctor of Medicine

Diabetes Research Unit

School of Medicine

Cardiff University

2016

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This work has not been submitted in substance for any other degree or award at this or any other university or place of learning and not being submitted concurrently in candidature for any degree or other award.

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Acknowledgements

I would like to thank my supervisory team Professor David R Owens and Professor Susan Wong for the help, support, expert guidance and patience they provided during this MD. It has been a long journey throughout which they have been a constant source of knowledge and encouragement. I would also like to thank Professor Stephen Luzio, Dr Rebecca Thomas and Dr Gareth Dunseath for their support and encouragement during the MD process. I would also like to thank my other colleagues within the diabetes research unit at University Hospital of Llandough, Cardiff and University Hospital of Wales, Cardiff who have helped me immensely in this journey including Nadia Worlock, Kate Eyre and Annie Hutchings.

I would like to thank all the diabetes research fellows, diabetes research nurses, research technicians at the Diabetes Research Unit: University Hospital of Llandough, Cardiff who have tirelessly worked over two and a half decades in gradually building up this database. I appreciate the opportunity to be involved in this longitudinal study, in conducting the metabolic challenge tests and metabolic data modeling during my research tenure and also to analyse the data for presentation in this Thesis. It has also been most encouraging to have had a manuscript accepted for publication during the course of this Thesis. I would also like to thank all the subjects involved in this study. I would like to thank Professor D R Owens, who was involved in the initial grading of the retinal images and Dr. R.L. Thomas for re-grading them and finalising them for the database.

I can't thank enough my parents Dr. A.K. Roy Chowdhury and Dr. Siti Roy Chowdhury for their constant encouragement and support in these long years of constant juggling of work, examinations, home, children and research commitments. I am so grateful to them for everything they have done to help and support me through this exciting and demanding period of my professional career and for never giving up on me. I would like to thank my husband Anirban and my darling boys Anish and Ashmit for making me laugh and being my light at the end of some very long days. The sacrifice of their time has proved invaluable for me in this very special journey. Special thanks also to my 11 year old, Anish who has helped with generating some of my diagrams in the Introduction.

Summary

The aim of this thesis was to assess the relationships between glycaemic exposure and β-cell function with prevalence, incidence and progression of diabetic retinopathy (DR) over 5 years in newly-diagnosed treatment-naïve subjects with type2 diabetes mellitus (T2DM).

At diagnosis, we studied 544 subjects and demonstrated DR was independently associated with fasting and postprandial hyperglycaemia and reduced fasting β-cell function during standardized meal and intravenous glucose challenge. The insulin-independent component of glucose tolerance (S_G) was also impaired and independently associated with presence of DR at diagnosis.

We followed up 233 subjects over 5 years and established independent association between chronic glycaemic exposure (HbA_{1c},/fasting/ postprandial hyperglycaemia) at diagnosis and incident DR during this period. We also demonstrated that fasting and postprandial β-cell responsiveness to nutrient challenge along with S_G at baseline was independently associated with development of DR over 5 years. There was no difference in glycaemic status between those with or without DR at 5 years highlighting the relevance of early history of glycaemic exposure in our subjects to future incidence of DR.

Finally, in 45 T2DM subjects with DR at diagnosis, fasting, postprandial glucose and HbA_{1c} along with fasting, postprandial β-cell responsiveness at diagnosis were all independently associated with DR progression.

Thus, this study has indicated that hyperglycaemia resulting from pancreatic β-cell deficiency contributes to the risk of development and progression of DR. The data emphasises the need for earlier diagnosis of T2DM and cautious normalization of glycaemia to eliminate glucotoxicity on the already impaired β-cell function. The evidence indicates the potential value of supporting β-cell function aiming to achieve near-normal glycaemia and thus preventing the onset and progression of DR in subjects with T2DM.

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Abbreviations

ACCORD	Action to Control Cardiovascular Risk in Diabetes
ACE	Angiotensin-Converting Enzyme Inhibitor
ACR	Albumin: Creatinine Ratio
ADED	Advance Diabetic Eye Disease
ADVANCE	Action in Diabetes and Vascular Disease: Preterax and Diamicron Modified Release Controlled Evaluation
AGE	Advanced Glycation End-products
AIR_G	Acute Insulin response to Glucose
ANOVA	Analysis of Variance
AUC	Areas under the Curve
BDR	Background Diabetic Retinopathy
BMI	Body Mass Index
BP	Blood Pressure
CAD	Coronary Artery Disease
CI	Confidence Interval
CIR	Corrected Insulin Response
CPR	Calculating Pancreatic Response
CSMO	Clinically Significant Macular Oedema
CWS	Cotton Wool Spots

DCCT	Diabetes Control and Complications Trial
DD	Disc Diameter
DI	Disposition Index
DM	Diabetes Mellitus
DIRECT	Diabetic Retinopathy Candesartan Trials
DME	Diabetic Macular Oedema
DPP	Diabetes Prevention Program
DR	Diabetic Retinopathy
DRS	Diabetic Retinopathy Study
DRSSW	Diabetic Retinopathy Screening Service for Wales
ETDRS	Early Treatment Diabetic Retinopathy Study
EURODIAB	Epidemiology and Prevention of Diabetes
EX	Exudate
FFA	Free fatty acids
FIELD	Fenofibrate Intervention and Event Lowering in Diabetes
FPG	Fasting Plasma Glucose
FPI	Fasting Plasma Insulin
FSIVGTT	Frequently Sampled Intravenous Glucose Tolerance Test

GDM	Gestational Diabetes Mellitus
GIR	Glucose Infusion Rate
GP	General Practitioner
HDL	High-Density Lipoprotein
HM	Haemorrhage
HOMA	Homeostasis Model Assessment
IDF	International Diabetes Federation
IGT	Impaired Glucose Tolerance
IGF-1	Insulin like Growth Factor
IQR	Interquartile range
IR	Insulin Resistance
IRI	Immunoreactive Insulin
IRMA	Intra Retinal Microvascular Abnormality
IVGTT	Intravenous Glucose Tolerance Test
LADA	Latent Autoimmune Diabetes of Adulthood
LDL	Low-Density Lipoprotein
M_0	Fasting β-cell responsiveness
M_1	Postprandial β-cell responsiveness
Ma	Microaneurysm
MTT	Meal Tolerance Test
MODY	Maturity-onset DM of the young

NEFA	Non-esterified fatty acids
NHS	National Health Service
NICE	National Institute for health and Care Excellence
NM	Non-Mydriatic
No DR	No Diabetic Retinopathy
NGT	Normal Glucose Tolerance
NPDR	Non-Proliferative Diabetic Retinopathy
NVD	New Vessels on the optic Disc
NVE	New Vessels Elsewhere
OHA	Oral Hyperglycaemic Agents
OR	Odds Ratio
PDX-1	Pancreas Duodenum Homeobox-1
PDR	Proliferative Diabetic Retinopathy
PKC	Protein Kinase C
PPDR	Pre-Proliferative Diabetic Retinopathy
PPG	Postprandial Plasma Glucose
PPI	Postprandial Plasma Insulin
PRP	Pan Retinal Photocoagulation
QA	Quality Assurance
RAS	Renin-Angiotensin System

ROS	Reactive Oxygen Species
SBP	Systolic Blood Pressure
S_G	Glucose Effectiveness
S_I	Insulin Sensitivity
SD	Standard Deviation
T1DM	Type 1 Diabetes Mellitus
T2DM	Type 2 Diabetes Mellitus
TCh	Total Cholesterol
UCP-2	Uncoupling Protein 2
UG	Ungradeable
UK	United Kingdom
UKPDS	United Kingdom Prospective Diabetes Study
UMA	Urinary Microalbumin
VA	Visual Acuity
VB	Venous Beading
VDAT	Veterans Affairs Diabetes Trial
VEGF	Vascular Endothelial Growth Factor
WEQAS	Welsh External Quality Assessment Scheme
WESDR	Wisconsin Epidemiologic Study of Diabetic Retinopathy
WHO	World Health Organisation

Chapter 1

Introduction

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1.4.1 Summary

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1.1 General Introduction

There is a worldwide epidemic of Diabetes Mellitus (DM) (IDF Diabetes Atlas (7th edition) (IDF 2015), with an estimated prevalence worldwide in the adult population of around 415 million in 2015 and this is predicted to increase to 642 million by 2040. In high-income countries approximately 90% of the adult population have Type 2 DM (T2DM). Worldwide healthcare costs are rising with 12% of the global health expenditure related to DM and its complications, which accounts for most (approximately 80%) of the total expenditure. Future increases in global health expenditure will be driven by population growth expected in low and middle income countries, where 75% of diabetic subjects live, accentuated by increasing urbanisation and adverse lifestyle changes. DM is currently the fifth most common reason for death in the world - around 1 in 8 people between 20 and 79 years old have their death attributed to DM and this figure is expected to rise. The life expectancy on average is reduced by (Prince CB 2002) more than 20 years for people with Type 1 DM (T1DM) and up to 10 years for people with T2DM. Further the Framingham Heart Study also reports that men and women with DM ≥ 50 years lived on average 7.5 and 8.2 years less than their non diabetic equivalents (Franco et al. 2007).

Since 1996, the number of people with DM in the UK has risen from 1.4 million to 3.5 million , with DM prevalence in the UK estimated to rise to 5 million by 2025 (https://www.diabetes.org.uk/Documents/Position%20statements/DiabetesUK_Facts_Stats_Oct16.pdf). That means 1 in every 17 people having diabetes (includes diagnosed and undiagnosed). It is also predicted that up to 630,000 people in the UK

have DM but not diagnosed. In 2015, the number of people diagnosed with diabetes in the adult population across the UK was: England: 2,913,538, Northern Ireland: 84,836, Scotland: 271,312 and Wales: 183,348. (England-http://www.hscic.gov.uk/catalogue/PUB18887 et al.) The majority of these cases are of T2DM, which has been linked to increasing cases of obesity. 90% of adults with DM have T2DM and 2 % of children with 85% of all subjects with DM having T2DM. The prevalence of T2DM especially has been increasing at a high rate and this is now one of the world's most common long-term health conditions.

Diabetes is currently classified into: Type 1 DM (secondary to β-cell destruction, usually resulting in absolute insulin deficiency), T2DM (secondary to a progressive insulin secretory defect on the backdrop of insulin resistance (IR). Other types include Gestational diabetes mellitus (GDM), which is diagnosed in the 2nd or 3rd trimester of pregnancy and specific types of DM due to other causes, e.g., monogenic diabetes syndromes (such as neonatal DM and maturity-onset DM of the young [MODY]) and diseases of the exocrine pancreas (such as cystic fibrosis). Other agents which can cause or precipitate diabetes include drugs such as glucocorticoids, those used in the treatment of HIV/AIDS or after organ transplantation, or chemical pollutants such as food toxins/preservatives (American Diabetes Association 2015). However in 2015, Leslie et al. have noted that the current classification does not completely capture the different disease forms. (Leslie et al. 2016). They note that both T1DM and T2DM have common features encapsulated by adult-onset autoimmune diabetes and MODY. Thus they have outlined evidence that the use of laboratory testing could improve disease

classification and the efficacy of treatment for major types of DM (Leslie et al. 2016).

The pathogenesis of T2DM is multifactorial (Stumvoll M et al. 2005) and presents at different stages of dysglycaemia reflecting varying degrees of insulin deficiency and insulin resistance. There are different phenotypes of T2DM. These include individuals who have lost weight due to a relative or absolute lack of insulin secretory capacity (insulinopaenia); however, the majority are overweight or obese and they are predominantly insulin resistant (Beck-Nielsen H and Groop LC 1994; Kahn CR 1994; Robertson RP 1995). It is difficult to determine the exact cause in an individual subject without detailed evaluation. Furthermore, the clinical expression of the disease may arise through genetic and/or environmental influences. Hyperglycaemia itself impairs pancreatic β cell function and exacerbates IR, leading to a vicious cycle (Li Y et al. 2004). This is summarised in Figure 1.1

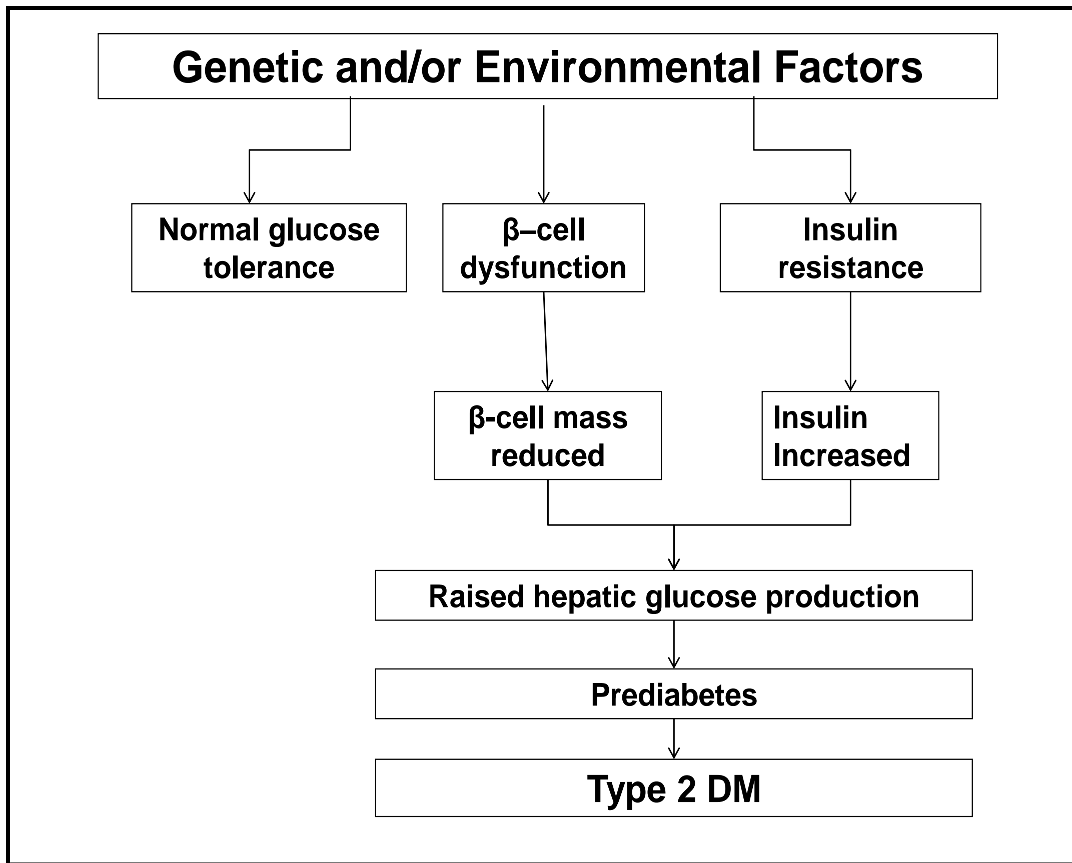

Figure 1.1: Proposed sequence of the key pathological features of T2DM *[Adapted from (Leahy JL 2005)].*

Maintenance of normal glucose homeostasis is precise. In the fasting/basal state 50% of glucose uptake occurs in brain, 25% in splanchnic tissues (liver and gastrointestinal tissues) both of which are insulin-independent mechanism (De Fronzo RA 2004). The final 25% occurs in insulin dependent tissues i.e. muscle and adipose tissue. Post glucose intake, resulting in an increase in plasma glucose concentration, there is stimulation of both insulin release and glucose uptake by splanchnic (liver and gastrointestinal tissues) and peripheral tissues (muscle) whilst suppressing predominantly endogenous hepatic glucose production. The liver has an important role in maintaining euglycaemia via glycogenolysis when plasma glucose is low, supported by the generation of glucose from non-carbohydrate carbon substrates such as pyruvate, lactate, glycerol, glucogenic amino acids, and fatty acids (both even-chain and odd-chain). Plasma glucose levels are controlled by hepatic and renal glucose production with the liver contributing approximately 80% and the kidneys 20% (Stumvoll M et al. 1997; Ekberg K et al. 1999). Insulin inhibits hepatic glucose production by inhibiting glucagon secretion, reducing plasma non-esterified fatty acid levels, reducing the amount of gluconeogenic precursors supplied to the liver, and changing the neural input to the liver (Girard J 2006). Insulin also increases glucose uptake for storage and thereby reduces plasma glucose (Figure 1.2).

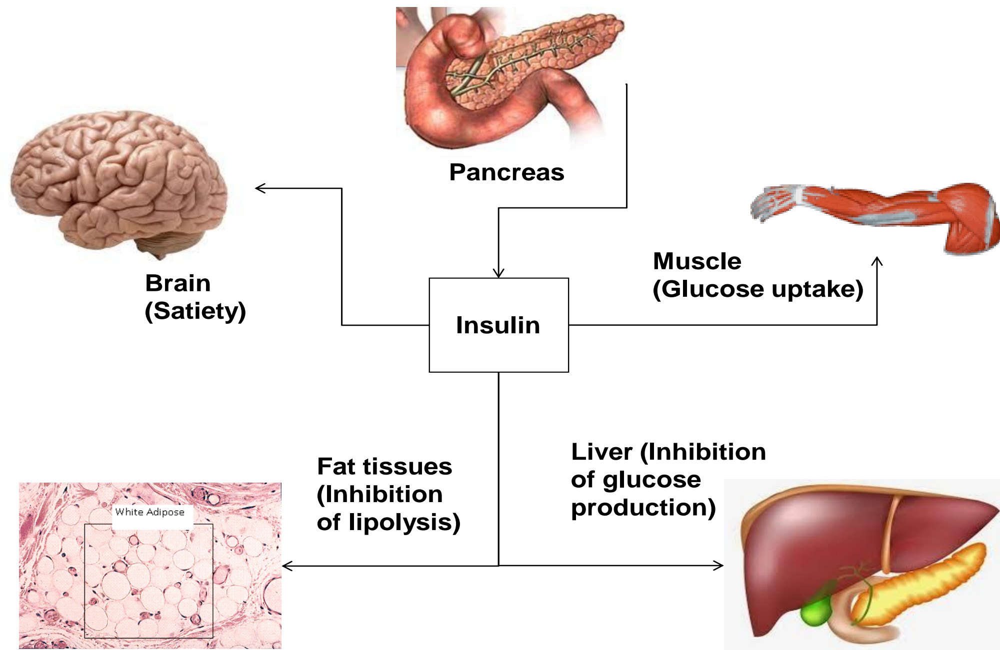

Figure 1.2: Schematic representation of normal effects of insulin. *Insulin secretion from the pancreas normally reduces glucose output by the liver, enhances glucose uptake by skeletal muscle, and suppresses fatty acid release from fat tissue [Adapted from (Stumvoll M et al. 2008)].*

Association of T2DM with central abdominal obesity is well recognised. Obesity leads to IR with increased levels of non-esterified fatty acids (NEFA) which are transported to the liver, where they reduce glucose utilisation, and stimulate hepatic gluconeogenesis resulting in increased glucose production (Pickup J and Wiliiams G 1997). Fat cells also play an important role in the pathophysiology of glucose intolerance through multiple pathways. NEFAs are stored as triglycerides in adipocytes and serve as an energy store during fasting and when they become resistant to the anti-lipolytic effects of insulin (Groop LC et al. 1989), this leads to elevation of fasting plasma NEFA (Fraze E et al. 1985), in parallel with increasing hyperglycaemia. Chronic elevation of NEFA, in addition to stimulating hepatic gluconeogenesis (Ferrannini E et al. 1983), induces liver and muscle IR (Roden et al. 1996) and also causes impairment of insulin secretion (Carpentier et al. 2000) referred to as lipotoxicity. Dysfunctional fat cells also secrete inflammatory, atherosclerotic adipocytokines which lead to IR (Bays et al. 2004), instead of secreting insulin-sensitising adipokines (e.g. adiponectin). Furthermore enlarged adipocytes become insulin resistant which decreases their ability to store fat, and this excess lipid finally leads to muscle and liver IR (Bays et al. 2004). Roy Taylor's group stated the twin cycle hypothesis which postulated that a chronic positive caloric balance in the background of existing peripheral IR lead to hyperinsulinaemia and increase accumulation of intrahepatic triglyceride and start a vicious cycle of lipo and glucotoxicity (Taylor 2008) (Figure 1.3). They further state that, T2DM is a potentially reversible condition where sustained weight loss achieved by the 40% who responded to a very low calorie diet by achieving fasting plasma glucose of <7 mmol/L led to remission of DM for at least 6 months in (Steven et al. 2016).

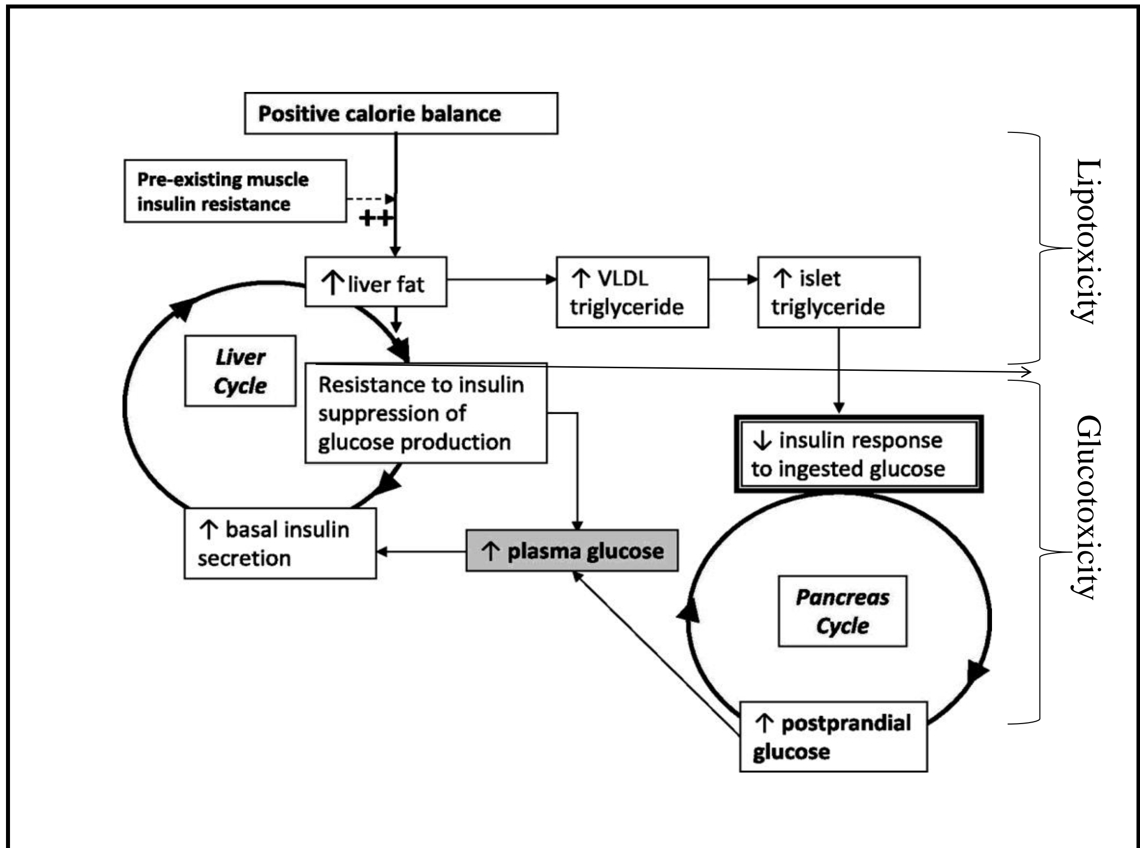

Figure 1.3: Vicious cycle of lipo and glucotoxicity*: During long-term excess calorie intake, especially in the presence of muscle insulin resistance, the raised plasma insulin levels will expedite chronic excess calorie storage from carbohydrate via de novo lipogenesis [Adapted from (White et al. 2016)].*

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Increased glucose production by the liver contributes to fasting and postprandial hyperglycaemia, which are hallmarks of T2DM. An important feature of this pathologic response is impaired insulin action in the liver a result of impaired β-cell function and hepatic IR. Hyperglucagonaemia also plays an important role in dysregulated hepatic glucose production and consequent abnormal glucose homeostasis (Mitrakou A et al. 1992). Plasma glucagon concentrations are inappropriately elevated in diabetic subjects, and suppression of the pancreatic α-cells by hyperglycaemia is blunted (D'Alessio D 2011). This leads to greater hepatic glucose production in the fasting state and attenuated reduction after meals. Studies in animal models show that reduction of glucagon action can mitigate effects of hyperglycaemia, even in the face of severe hypoinsulinaemia. Currently there are no definitive treatments for diabetic subjects yet available that act specifically on the glucagon signalling pathway but relatively newer agents including glucagon-like peptide-1 (GLP-1) receptor agonists inhibit plasma glucagon secretion and this possibly contributes to their action to lower blood glucose.

Of note, insulin has anti-inflammatory and anti-oxidant properties which are intrinsic to the pathophysiology of DM. This has been well demonstrated by (Figure 1.4) Dandona et al. (Dandona P et al. 2007).

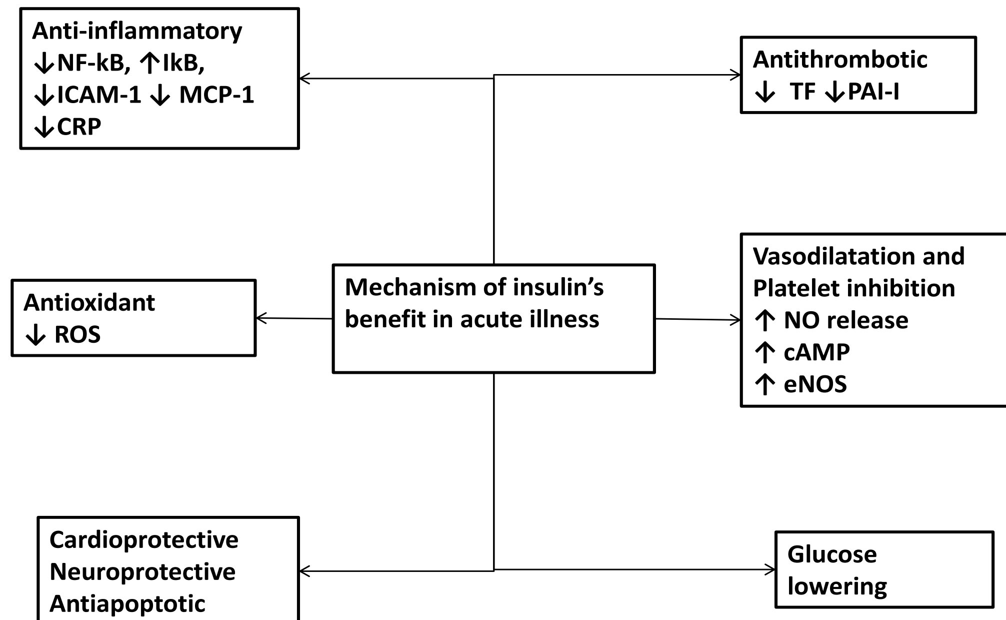

Figure 1.4 Current view of the action of insulin. *The anti-inflammatory, anti-apoptotic, cardio-protective, and neuroprotective effects of insulin have been demonstrated in humans and in animal models. The vasodilatory, reactive oxygen species (ROS)–suppressive, antiplatelet, antithrombotic, and profibrinolytic effects have been demonstrated in humans. cAMP - cyclic adenosine monophosphate; CRP - C-reactive protein; eNOS - endothelial nitric oxide synthase; ICAM - intracellular cell adhesion molecule; I_B _ inhibitor _B; MCP - monocyte chemo-attractant protein; NF-κB - nuclear factor–κB; NO - nitric oxide; PAI - plasminogen activator inhibitor; TF - tissue factor[Adapted from (Dandona P et al. 2007)].*

During normal glucose homeostasis, the pancreatic β-cell adapts to insulin resistance (IR) by enhancing insulin secretion. A curvilinear relationship exists between normal β-cell function and Insulin Sensitivity (IS) (Bergman RN 1989). When the β-cell is unable to adapt sufficiently, Impaired Glucose Tolerance (IGT) or T2DM develops (Figure 1.5). Studies on Pima Indians have reported that β-cell dysfunction is critical in the pathogenesis of T2DM (Weyer C et al. 1999) and β-cell dysfunction leading to abnormality in insulin secretion occurs early in the pathophysiology of DM and can often be demonstrated in 1st degree relatives of subjects with T2DM, who have Normal Glucose Tolerance (NGT).

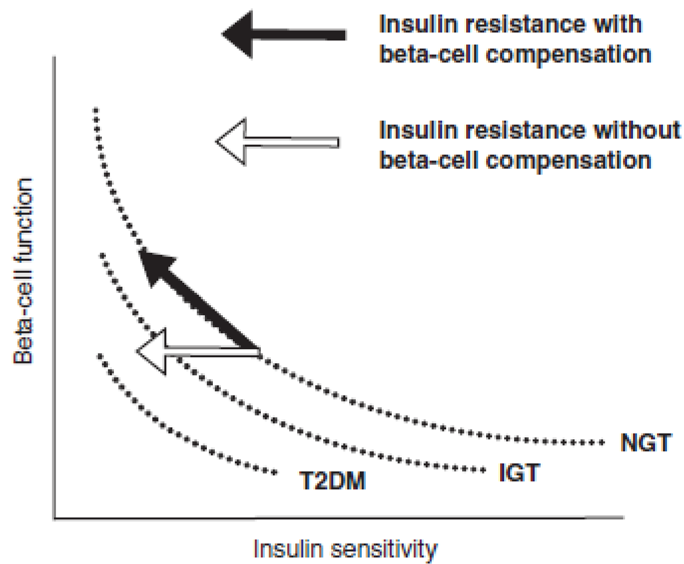

Figure 1.5: Hyperbolic relationship between β cell function and insulin sensitivity. *In subjects with normal glucose tolerance a quasi-hyperbolic relationship exists between β-cell function and insulin sensitivity. With deviation from this hyperbola, deterioration of glucose tolerance occurs. Abbreviations: IGT, impaired glucose tolerance; NGT, normal glucose tolerance; T2DM, type 2 diabetes mellitus (Stumvoll M et al. 2008).*

Reduced β-cell mass either through genetic and/or β-cell cytotoxic factors predispose to glucose intolerance. As the blood glucose level rises above normal, acquired defects in the glucose homeostasis system occur to further impair the β cell's glucose responsiveness to meals. In response to an intravenous bolus of glucose, an impairment of the first phase insulin response is clearly evident in the pre-diabetic state of impaired glucose tolerance. The elevated blood glucose, in conjunction with the excess fatty acids, a typical feature of obesity and IR, causes additional deterioration in β-cell function progressing over time to overt DM (Leahy JL 2005). In the early setting of T2DM, there is an initial enhancement of β-cell function/secretion in response to the increased fasting and postprandial hyperglycaemia (Stumvoll M et al. 2003 Apr).

Hovorka and colleagues examined newly presenting subjects with T2DM and found a close inverse relationship between β-cell responsiveness and FPG (Hovorka R et al. 2001) and showed that pancreatic responsiveness was reduced in the fasting state by approximately 50% and during meal simulation by approximately 80% (Hovorka R et al. 1998). The estimated β-cell function was impaired in subjects with normal body weight and a higher FPG (UK Prospective diabetes study group V 1988). Previously the UKPDS in addition reported a lower Insulin Sensitivity (measured by HOMA methodology) in obese, male, sedentary Caucasian subjects with newly diagnosed T2DM. On a similar note, Owens et al suggested a concentration-dependent inhibitory effect of chronic fasting hyperglycaemia on the ability of the β-cell to further respond to an acute increase in plasma glucose, following an intravenous glucose challenge. Thus these newly diagnosed Caucasian subjects with

T2DM were characterised by dysfunctional β-cell function, resulting in a quantitative and qualitative deficit in insulin secretion, accompanied by a relative hyper-proinsulinaemia (Owens DR et al. 1996) Proinsulin is increased in the fasting and postprandial state in the earlier stages of diagnosis but at the later stages of T2DM this falls.

DeFronzo et al. reported that the upper tertile of individuals with NGT have a 50% decline in β-cell function. However, subjects in the upper tertile of IGT are maximally/near-maximally insulin resistant, have lost 70–80% of their β-cell function (Ferrannini E et al. 2005; Abdul-Ghani MA et al. 2006a; Abdul-Ghani MA et al. 2006b) and have approximately a 10% incidence of DR. Therefore, preservation of the remaining 20–30% of β-cell function is critical to prevent future development of T2DM (DeFronzo RA and Abdul-Ghani MA 2011). Maneschi et al has also shown that in subjects with T2DM, the residual β-cell function is important for the degree of diabetic control but failed to establish a direct relationship between the degree of insulin deficiency and presence of diabetic microangiopathy (Maneschi F et al. 1982).

The current epidemic of obesity leads to a state of IR and causes stress on the β cells to enhance insulin secretion to overcome the state of IR with normal glucose tolerance, maintained as long as β-cell compensation is viable. However, with progression of time, β cells are unable to offset the effects of IR with further enhancement of insulin secretion, and initially postprandial plasma glucose and then

fasting plasma glucose levels increase leading to a diagnosis of T2DM. Therefore, it is progressive β-cell failure that predicts the rate of progression of T2DM. This has been illustrated by the Starling's Curve of the Pancreas (DeFronzo et al. 2013) by DeFronzo et al. The plasma insulin response depicts the classic 'Starling's Curve of the Pancreas' and is illustrative of the natural history of T2DM (Figure 1.6). In summary, increased insulin resistance is important in the early stages of T2DM; however there is relentless decrease in β-cell function across the whole spectrum of T2DM.

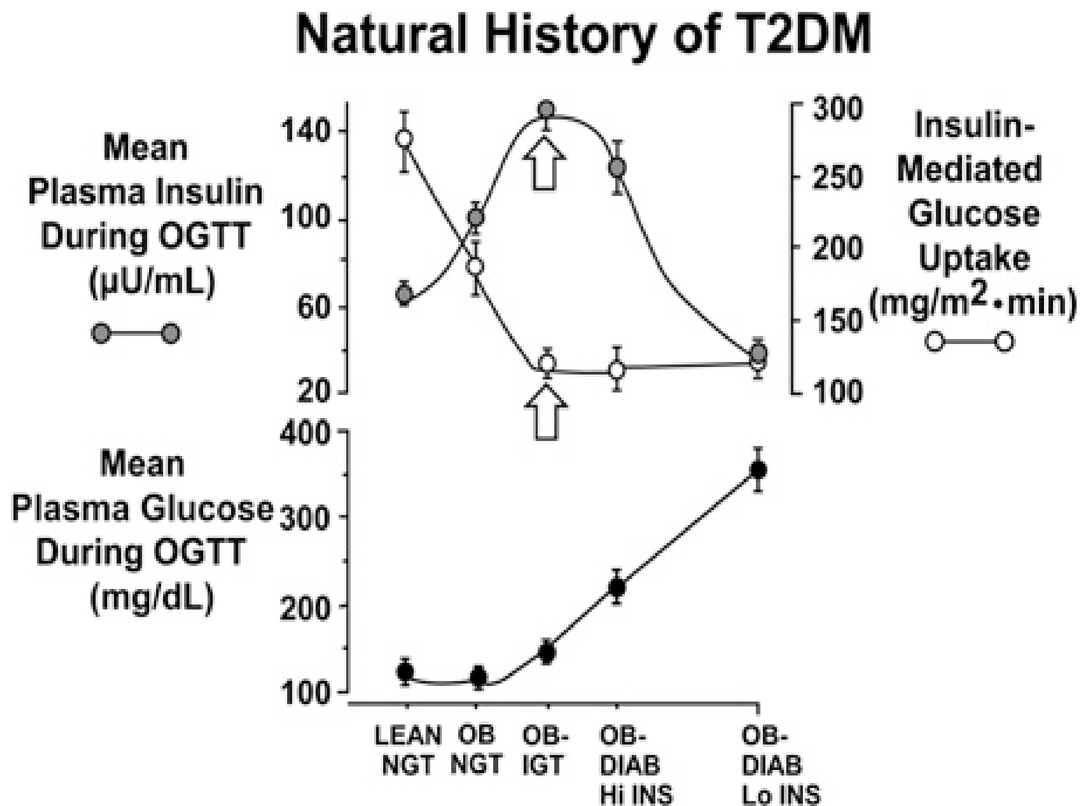

Figure 1.6 Starling's Curve of the Pancreas (DeFronzo et al. 2013) *shows the progressive increase of mean plasma glucose during the OGTT from lean NGT to obese diabetic subjects. The rise and fall in the mean plasma insulin reflects the early β-cell compensation followed by β-cell exhaustion. The insulin-mediated glucose uptake in these subjects shows a steady initial decline and then a significantly reduced basal steady state which is illustrative of the natural history of T2DM. The two white arrows are indicative of the transition point from IGT to T2DM in these parameters. (NGT: Normal Glucose Tolerance, IGT: Impaired Glucose Tolerance, OB: Obese, Diab: Diabetes Ins: Insulin)*

The defects in glucose homeostasis that occur as the diabetes metabolic environment evolves were first identified by studies showing that in intensively-treated subjects with T2DM, when blood glucose values were brought as close to normal as possible, improved β-cell function was noted (Turner RC et al. 1976), with later studies also showing some reversal of IR. This reversal effect is unrelated to the type of treatment—it does not matter how the glucose level is lowered (Kosaka K et al. 1980), and is most effective early in the course of the disease. Several studies have shown substantial recovery of glucose tolerance after short-term insulin infusions or high dose sulfonylurea therapy in newly diagnosed subjects with T2DM (Peters AL and MB. 1996; Li Y et al. 2004). However Weng et al. showed that early intensive insulin therapy in subjects with newly-diagnosed T2DM has better outcomes on the recovery and maintenance of β cell function and sustained glycaemic remission compared with subjects who were treated with Oral Hypoglycaemic Agents (OHA) (Weng J et al. 2008).

Monnier et al. demonstrated that the deterioration of glucose homeostasis in subjects with T2DM progressed from postprandial to fasting hyperglycaemia following a 3 step process. The 1st step related to the three diurnal post-meal periods considered as a whole the 2nd step occurred during the morning period, and the 3rd and final step corresponded to sustained hyperglycaemia over the nocturnal fasting periods. This describes the key stages in the evolution of T2DM (Monnier L et al. 2007).

In T2DM, hyperglycaemia results in enhanced oxidative stress due to the formation of excess reactive oxygen species (ROS) formation leading to β-cell damage i.e. glucose toxicity (Owens DR et al. 1996). Normally β cells have only low amounts of catalase and superoxide dismutase, proteins which metabolize ROS (Robertson RP et al. 2003). ROS activate NF-kB, which is pro-apoptotic. It has also been observed in an animal model of diabetes that pancreas duodenum homeobox-1 (PDX-1), a regulator of insulin gene transcription, is diminished by hyperglycaemia, another possible mechanism of "glucose toxicity." In addition, altering uncoupling protein-2 (UCP-2) by high glucose leads to uncoupling of oxidative glucose metabolism from ATP formation in the mitochondrion resulting in lower ATP (Patane G et al. 2002) and consequently reduced insulin secretion.

Although free fatty acids (FFA), also termed NEFA, can acutely increase insulin secretion, chronic FFA overload diminishes β-cell function. T2DM subjects often have increased FFA due to IR to (adipocyte) lipolysis. This FFA increases linearly with Fasting Plasma Glucose (FPG). It is now clear that high glucose inhibits β-cell fatty acid oxidation, which may lead to accumulation of long-chain coenzyme A (LC-CoA) (Robertson RP et al. 2004). This has been suggested to interfere with normal potassium channel activity, or to lead to activation of UCP-2, which would lead to uncoupling of oxidative glucose metabolism from ATP formation in the mitochondrion leading to lower ATP.

To reiterate one of the most controversial topics within the field of T2DM over many years - is this disease of IR or β cell dysfunction? The confusion for many years was that once T2DM and even IGT had been diagnosed, both defects were invariably present. Attempts to investigate earlier in the course of the disease by studying individuals at high-risk who were still normoglycaemic, high-risk ethnic groups such as Pima Indians, those whose parents both had T2DM, and women who previously had had gestational DM - often reported that IR was present, but not β-cell dysfunction (Martin BC et al. 1992), concluding that IR was the initial (and thus dominant) defect in this disease in these population groups.

However, these early studies based their conclusions regarding unimpaired β-cell function on experimental techniques that were generally misinterpreted. IR was relatively easily measured by several methods - glucose clamping that is labour intensive and usually only done with a limited number of subjects, or several computer models that could be done with large groups. In contrast, the assessment of β-cell function is much more complex. The insulin response to intravenous glucose normally occurs in a biphasic pattern and the amount of insulin released is highly responsive to the prevailing glucose value. As glucose tolerance moves from normal to minimally impaired, the insulin secretion that occurs within the first 30 minute of eating (first phase) becomes markedly attenuated, resulting in an elevated prandial rise in glycaemia, i.e., this is the physiologic definition of impaired glucose tolerance. It is this postprandial hyperglycaemia that causes the insulin secretion after the first 30 minute (second phase) to be higher as a compensatory response during the relatively early stages of T2DM. The early studies typically assessed β-

cell function by measuring the insulin value pre and 2 hours after a meal. Consequently, it was concluded, based on the 2-hour insulin value being higher than normal, that there was no β-cell dysfunction in the early stages of T2DM; thus IR only was assumed to be responsible for the supernormal 2 hour insulin value. This misinterpretation was eventually corrected by studies that also measured 30-minute post-meal insulin values, or acute insulin responses to intravenous glucose, and showed that defective early phase insulin secretion occurred before the onset of T2DM (Perley MJ and DM. 1967; Gerich JE 1998).

More informative were large cross-sectional and natural history studies in terms of the relative importance of β-cell dysfunction versus IR in this disease. They confirmed the findings of the earlier studies that IR occurs early in the disease, typically when glucose values are still within the normal glucose tolerance range. The reason is multifactorial - in some related to a genetic abnormality that affects insulin sensitivity, and others from lifestyle factors such as obesity, lack of exercise, high-fat diets, aging, etc. Thereafter, however, IR does not change much - once present, it remains present. Thus, it is not worsening of IR that causes blood glucose values to go from normal to IGT to T2DM, but worsening of β-cell function. The natural history studies of T2DM invariably reported a biphasic pattern of β-cell function, hyperinsulinaemia early on maintaining blood glucose values normal to mildly impaired, and then a falling insulin level (so-called β-cell failure) resulting in rising glycaemia (Lillioja S et al. 1988; Lillioja S et al. 1993), (Starling Curve of the Pancreas). As glycaemia starts to rise, the acquired β-cell dysfunction occurs (defective first phase insulin secretion), and glycaemia worsens even more, often to

overt T2DM. Thus, whereas IR is an important contributory pathogenic element in T2DM, current dogma is that the β-cell determines the level of glycaemia in individuals who are genetically at risk for T2DM.

It was argued some years ago that IR caused the β-cell failure through exhaustion, i.e., continued stimulation of an otherwise normal β-cell eventually causes it to become permanently dysfunctional. However, that concept does not fit the facts. Many highly insulin-resistant subjects never get T2DM; only about a third of morbidly obese subjects and a third of those with Cushing's disease or acromegaly develop T2DM. Also, puberty, pregnancy, and aging are periods of profound IR — most of us do not get T2DM because our β cells are able to continuously compensate. Thus, it seems that a necessary part of T2DM is a compromised β-cell compensatory ability and many consider the disease to be a failure of β-cell compensation (Cavaghan MK et al. 2000; Ehrmann DA et al. 2004) or an inability to sustain an adequate β-cell response in the face of increasing insulin resistance.

The most recent studies have returned to the question of which comes first, IR or β cell dysfunction, because of development of more precise experimental techniques to assess β-cell function. One of the most utilized is the disposition index, which is based on the principle that β-cell function normally varies dependent on the degree of insulin sensitivity, i.e. the insulin response to a meal or other stimulus in an insulin-sensitive person such as a marathon runner is normally considerably less than for normoglycaemic IR subjects. The curve of this relationship is hyperbolic

(Bergman RN et al. 1981; Kahn SE et al. 1993) and is called the disposition index. It is important to realize everyone goes through times of IR (puberty, pregnancy, aging), but most do not get T2DM because of β-cell compensation.

IR is an early and characteristic feature of the natural history of T2DM in high-risk populations but overt T2DM develops only when the β cells are unable to appropriately augment their insulin secretion to compensate for the defect in insulin action. Furthermore there is an insulinopaenic group of subjects with T2DM (more common in south African populations (Joffe BI et al. 1992) and Swedish middle aged men (Grill V et al. 1999) whose insulin sensitivity is normal at the onset of T2DM, whereas insulin secretion is severely impaired (De Fronzo RA 2004).

Thus in summary, even when blood glucose levels are minimally above normal in the pre-diabetes stage then the glucose responsiveness of the β-cell to a carbohydrate challenge is impaired. As glucose intolerance progresses in the presence of excess fatty acids, a typical feature of obesity and insulin resistance, then β-cell function deteriorates even further to overt DM (Leahy JL 2005).

The chronic state of hyperglycaemia caused by both IR, and relative impairment in insulin secretion leads to long-term complications affecting various organ systems in the body. They can be divided into those affecting larger blood vessels (macrovascular) and smaller blood vessels (microvascular). The former include

Coronary Artery Disease, Cerebrovascular and Peripheral Vascular Disease while the latter involves Retinopathy, Nephropathy and Neuropathy.

Most of the mortality and morbidity of DM arise from these complications and lead to the financial burden on the National Health Service (NHS). 10% of the NHS budget for England and Wales is spent on DM, which is an estimated 14 billion pounds of which 11.7 billion pounds relates to T2DM (Kanavos P et al. 2012). The St. Vincent declaration published in 1989 (Diabetes Care and Research in Europe 1989), by the World Health Organisation (WHO) in collaboration with the European Association for the Study of Diabetes (EASD), set down targets for improvements in diabetes care including reductions in the incidence of complications. The aims of the St. Vincent declaration included a 30% reduction in the incidence of new-onset blindness, a 30% reduction in the incidence of renal failure and a 50% reduction in the number of major amputation (Diabetes Care and Research in Europe 1989).

1.2 Diabetic Retinopathy

Recent analysis suggests that there are approximately 93 million people with Diabetic Retinopathy (DR), 17 million with proliferative DR, 21 million with diabetic macular oedema, and 28 million with vision-threatening DR worldwide (Yau et al. 2012). DR is the most common and feared microvascular complication of DM and was until recently regarded as the most prevalent cause of visual impairment in the working-age (16-64 years) population in developed countries (Heng LZ et al. 2013) (Bunce C and Wormald R 2008). However, a recent report has

indicated that in the United Kingdom (UK), DR has been overtaken by inherited retinal conditions as the leading cause of blindness in the working age group which the authors suggested was possibly a result of DR screening programmes and improved diabetes care (Liew G et al. 2014). Liew et al. further report that there has been a reduction in certified blindness from retinopathy/maculopathy from 17.7 % in 1999-2000 to14.4 % in 2009-2010. Thus the setting up of the screening services has led to a positive outcome though the St. Vincent declaration had not been met.

The early detection and treatment of modifiable risk factors known to influence its onset and progression is imperative. Approximately 17-18% of subjects with T2DM have DR at presentation, 40-60% have some DR after 20 years of known DM duration, with 10% developing sight threatening lesions e.g. proliferative DR and/or exudative maculopathy (Stefansson E et al. 2000). Diabetic maculopathy is the commonest cause of visual loss in individuals with DM due to the much higher proportion of individuals with T2DM.

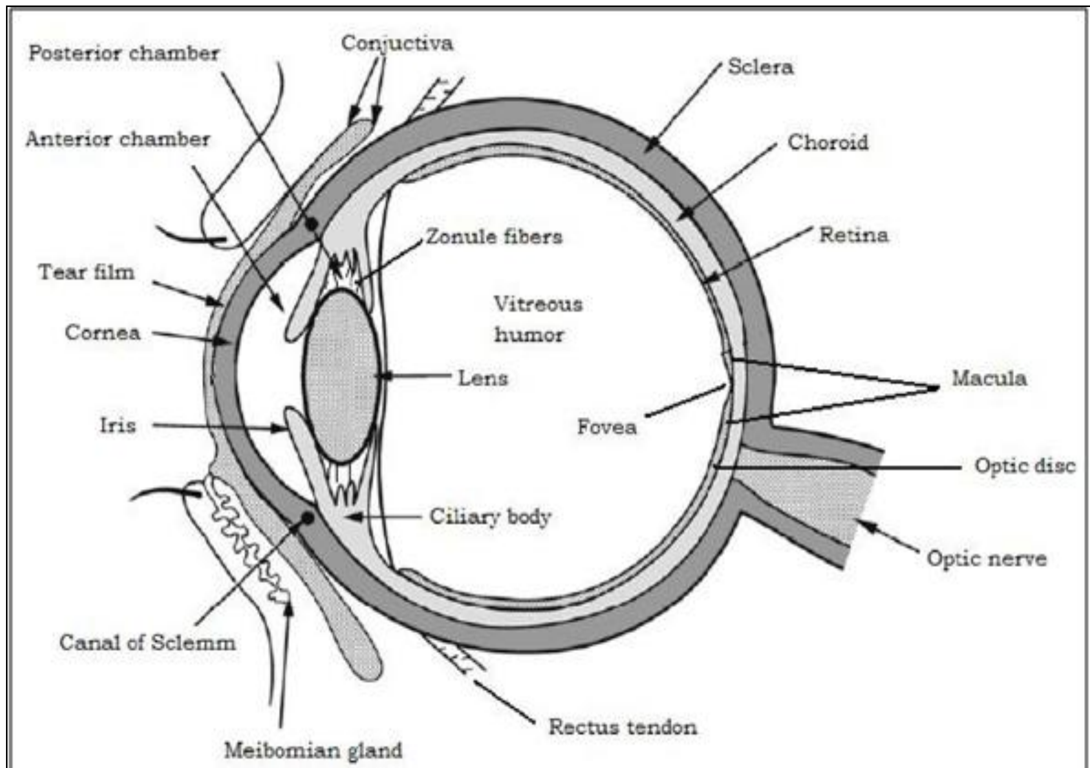

Figure 1.7: Cross sectional diagram representing the internal structure of the eye (Gupta D) *showing the structures in the anterior and posterior chamber of the eye with the fovea and macula located at the posterior pole.*

A cross sectional diagram representing the internal structure of the eye is shown in Figure 1.7. In it we see the retina, which is the inner-most, light sensitive layer of the eyeball at the back of the eye. It gathers light, codes the information as an electrical signal and transmits it via the optic nerve to the processing area of the brain (Denniston AKO and Murray PI 2014). It is supplied by numerous small blood vessels and contains photoreceptors which are light-sensitive cells. The retina consists of 95% of neural tissue and 5% vascular tissue (Figure 1.8) It is essentially a neurovascular unit comprising the layers depicted (Figure 1.8) (Antonetti DA et al. 2012a). This neural retina is a thin (150-400 microns) layer of transparent neural tissue continuous with the non-pigmented layer of the ciliary body anteriorly. The retina comprises photoreceptors (rods, cones), integrators (bipolar, horizontal, amacrine, ganglion cells), an output pathway (nerve fibre layer) and support cells (Muller cells). On histological examination, the retina is typically divided into multiple layers: three layers mainly contain nuclei (outer/inner nuclear layers and ganglion cell layer) and two layers mainly contain synaptic connections (outer and inner plexiform layers) (Denniston AKO and Murray PI 2014).

The macula is an oval-shaped pigmented yellow spot near the center of the retina of the eye. It has a diameter of around 6 mm and near its center is the fovea, which is a small pit that contains the largest concentration of cone cells/photoreceptors in the eye and is responsible for central and high resolution vision (Kanski JJ and Bowling B 2011). A transparent, jelly-like substance that forms the main bulk of the eyeball is the vitreous humour. It maintains the shape of the eye and also refracts light onto the retina.

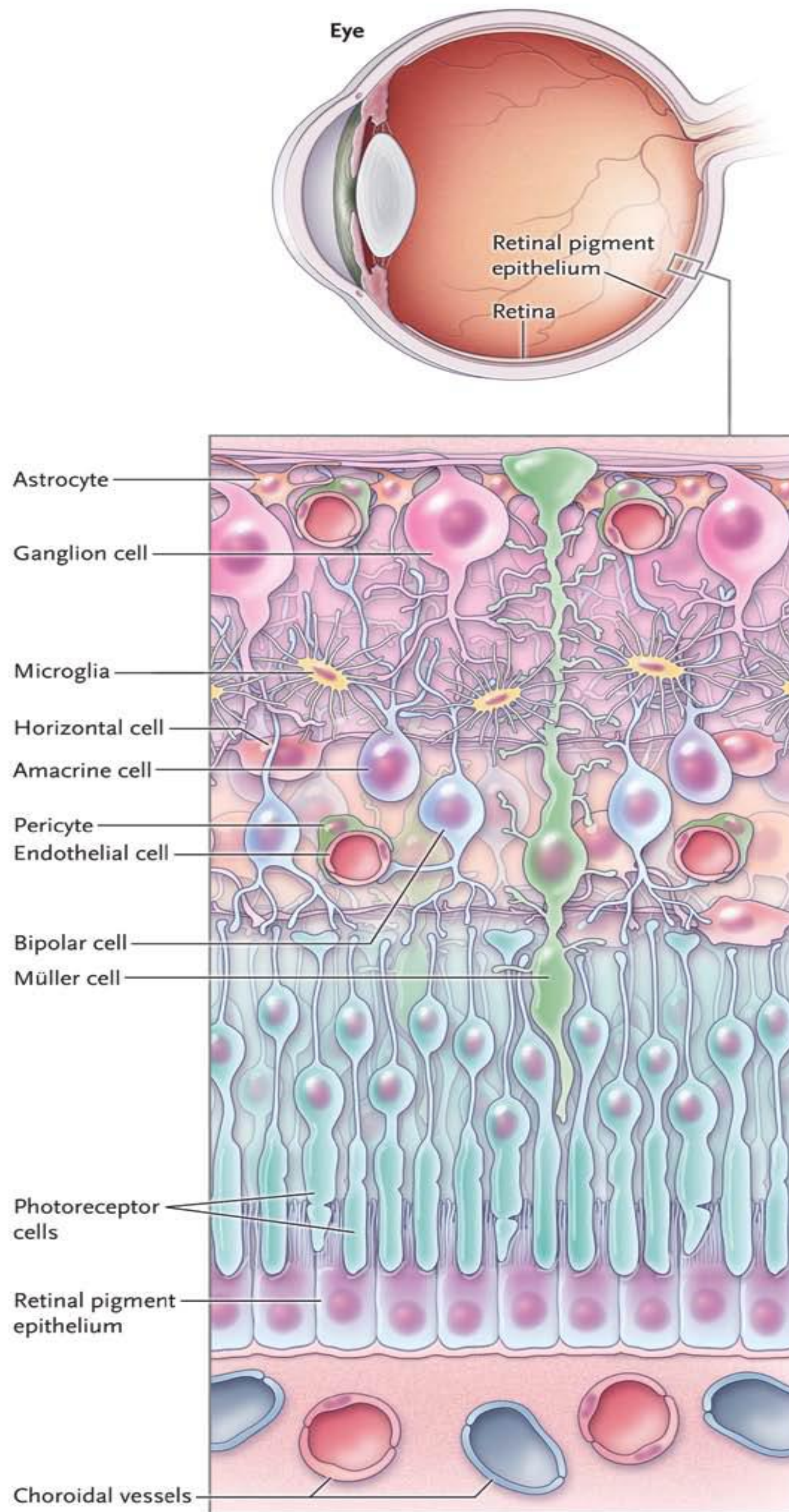

***Figure 1.8 The neurovascular unit of the retina* (Antonetti DA *et al.* 2012a)** *showing the various nerve fibre, vascular and cellular layers.*

Diabetic macular changes in the form of yellowish spots and full or partial thickness extravasations through the retina were observed for the first time by Eduard Jäger (Wolfensberger TJ and Hamilton PA 2001) and published in 1855. Jaeger's findings were not accepted until 1872, when Edward Nettleship published his paper "Oedema or cystic disease of the retina," providing the first histopathological proof of "cystoid degeneration of the macula" in subjects with DM (Nettleship E 1872). Soon afterwards in 1876, Wilhelm Manz described proliferative vascular changes and the importance of tractional retinal detachments and vitreous haemorrhages in individuals with diabetes (Manz W 1876). Arthur James Ballantyne in 1943 demonstrated that DR represents an unique form of vascular disease (Ballantyne AJ and Loewenstein A 1943). In T1DM early changes appear by five years post diagnosis; however, in T2DM, DR was diagnosed in about 19% during the 1st year after DM diagnosis in UK general practices (Kostev K and Rathmann W 2013).

DR is mainly a lesion of the retinal capillaries. Prior to the development of any clinically visible lesions, histological changes appear i.e. basement membrane thickening, death of pericytes which leads to the formation of microaneurysms (Figure 1.9), loss of vascular smooth muscle cells and endothelial cell proliferation. Microaneurysms are the earliest ophthalmoscopically visible lesion in persons with diabetes regarded as pathognomonic of diabetes (Figure 1.9). These earliest lesions are seen only on fluorescein angiograms, which show up as small areas of non-perfusion. Fluorescein angiography demonstrates areas of non-perfusion and the presence of microaneurysms (Figure 1.11). The response to non-perfusion of some capillaries (Figure 1.10) is dilatation of others (Kohner EM 1993). When dilatation is localised, as more commonly seen, a microaneurysm forms, but dilatation can be generalised, and this is more commonly seen at the posterior pole in the macular region.

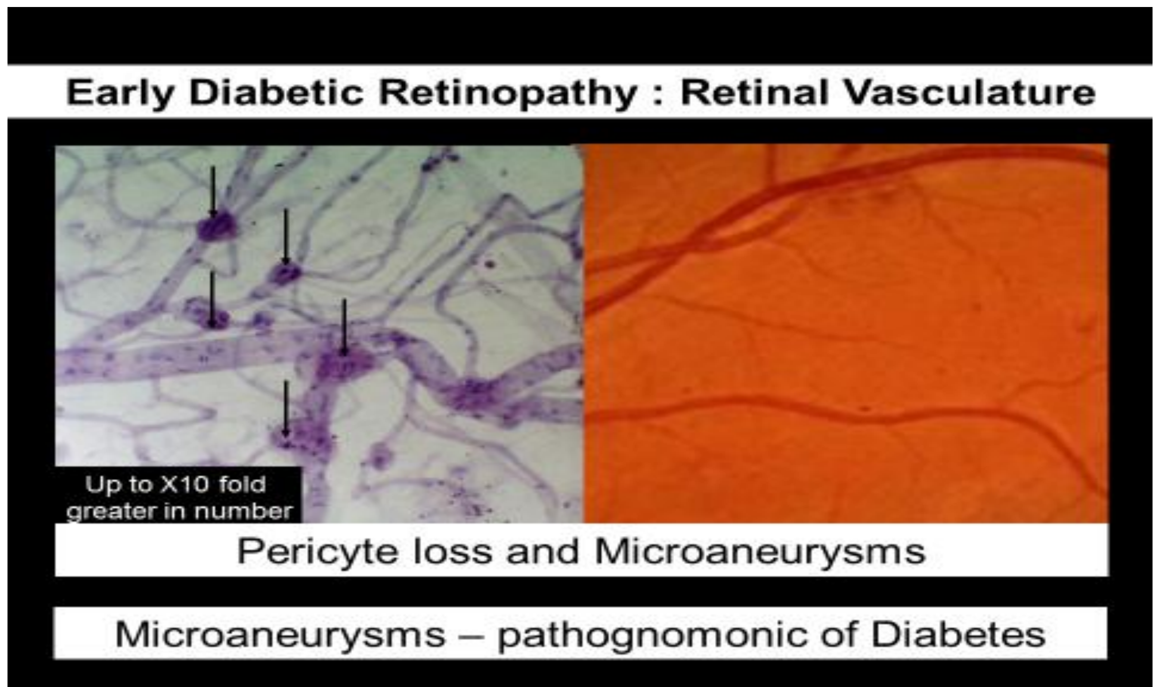

Figure 1.9: Retinal vasculature changes depicting early DR i.e. pericyte loss and microaneurysms. Black arrows: microaneurysms.

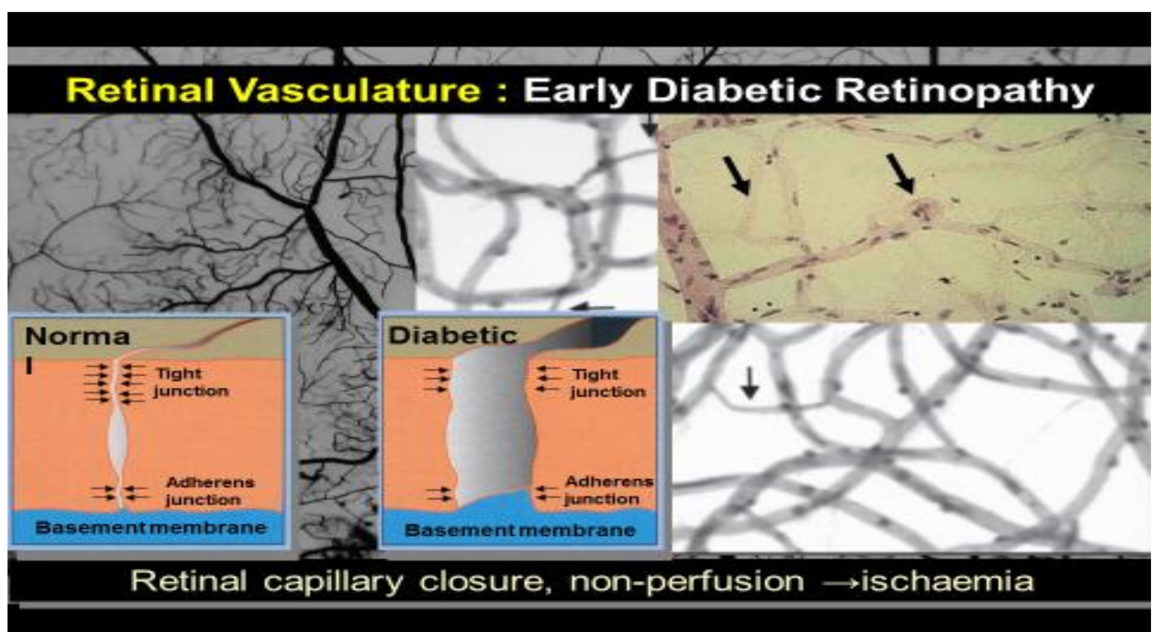

Figure 1.10: Retinal vasculature changes depicting early DR i.e. retinal capillary closure leading to non-perfusion and thus to ischaemia. Black arrows: Upper right panel: microaneurysms, Lower right panel: pericyte loss.

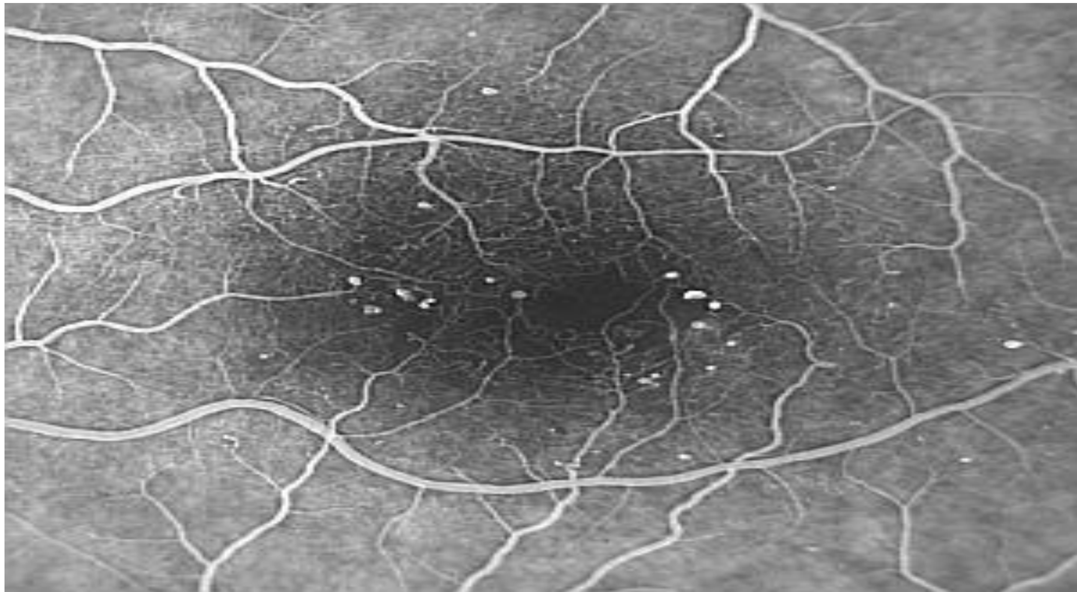

Figure 1.11: Fluorescein angiogram with microaneurysms fluorescing (Goatman K 1997)

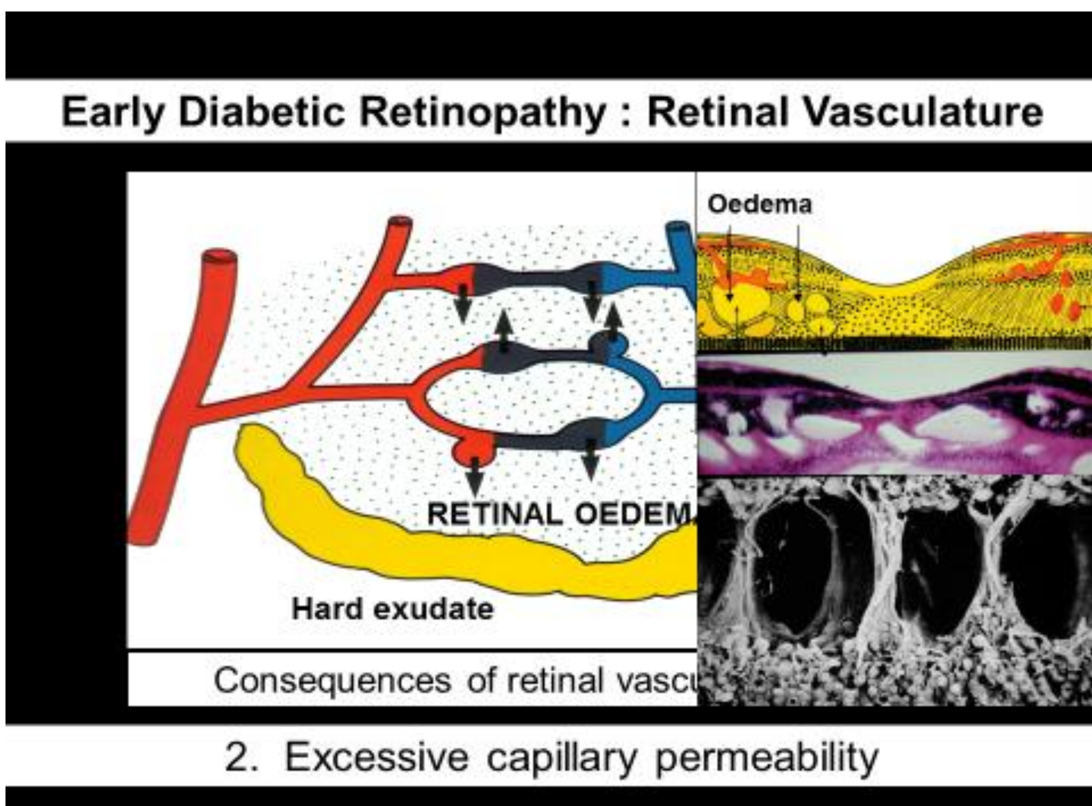

Figure 1.12: Retinal vasculature changes depicting DR i.e. excessive capillary permeability leading to retinal oedema and hard exudate formation. Black arrows left panel: leakage. Black arrows right panel: retinal oedema. Lower right panel: High resolution image of intraretinal cyst formation leading to retinal oedema.

Dilated capillaries are usually incompetent and leaky. This leakage from a reduced number of dilated capillaries leads to exudative and oedematous forms of the sight-threatening diabetic macular oedema (Figure 1.12). With increasing number of capillaries becoming occluded the larger vessels become affected. As arteries are involved, there is always a large area of capillary non-perfusion. When this occurs suddenly, cotton wool spots are formed; when it occurs gradually, only a featureless atrophic retina is visible (Kohner EM 1993). Large blot haemorrhages usually form at the interface of the perfused and ischaemic areas of the retina. Dilated capillaries are seen in this largely avascular area and are known as intra-retinal microvascular abnormalities (IRMAs). These are commonly found near ischaemic cotton wool spots, which remain as dilated capillaries or progress to form new vessels (neovascularisation), especially when present together with other lesions, that include abnormal dilatation and beading of the veins. Whereas venous dilatation occurs relatively early in DR and is non-specific, the formation of venous tortuosity in the form of loop formation, beading, and reduplication indicates severe retinal ischaemia in the surrounding retina which causes dilatation (Kohner EM 1993). This constellation of lesions suggest imminent new vessel formation, which represents the stage of Pre-proliferative Diabetic Retinopathy (PPDR)

The next stage of development is the appearance of new vessels (Proliferative DR) is usually formed from veins initially in the more ischemic retinal periphery or on the optic disc. The new vessels themselves do not cause visual symptoms; it is bleeding from the new vessel that is responsible for visual impairment in most subjects with T1DM. They arise secondary to large areas of ischaemia under the influence of VEGFs. When the new vessels break through the internal limiting membrane they become attached to the posterior surface of the vitreous, which they utilise as a scaffold upon which to further proliferate (Kohner EM 1993). The retracting vitreous then pulls on these newly-formed friable blood vessels causing pre-retinal haemorrhage. In response to the bleeding and the formation of fibrous tissue, retinal traction and detachment may occur. The visual loss related to these is sudden and unexpected. This sudden visual loss is in contrast to the visual loss seen in diabetic macular oedema, where vision fails gradually with fluid accumulating at the fovea. Patients with T1DM may lose vision from macular oedema, but this is occasionally profound or (especially when associated with renal failure) causes blindness (Kohner EM 1993). In older T2DM subjects, macular oedema is associated with ischaemia or extensive formation of hard exudates and is the main cause of visual loss due to DR. Individuals with mild-to-moderate Non-proliferative DR (NPDR) have impaired contrast sensitivity and visual field defects that cause difficulty with driving, reading, and managing diabetes and other activities of daily living. Visual acuity, as determined with the use of Snellen/Log MAR charts, declines when the central macula is affected by oedema, ischemia, epiretinal membranes, or retinal detachment (Antonetti DA et al. 2012a). The various stages of DR are summarised in Fig 1.13 and evolution of Diabetic Retinopathy in Fig 1.14.

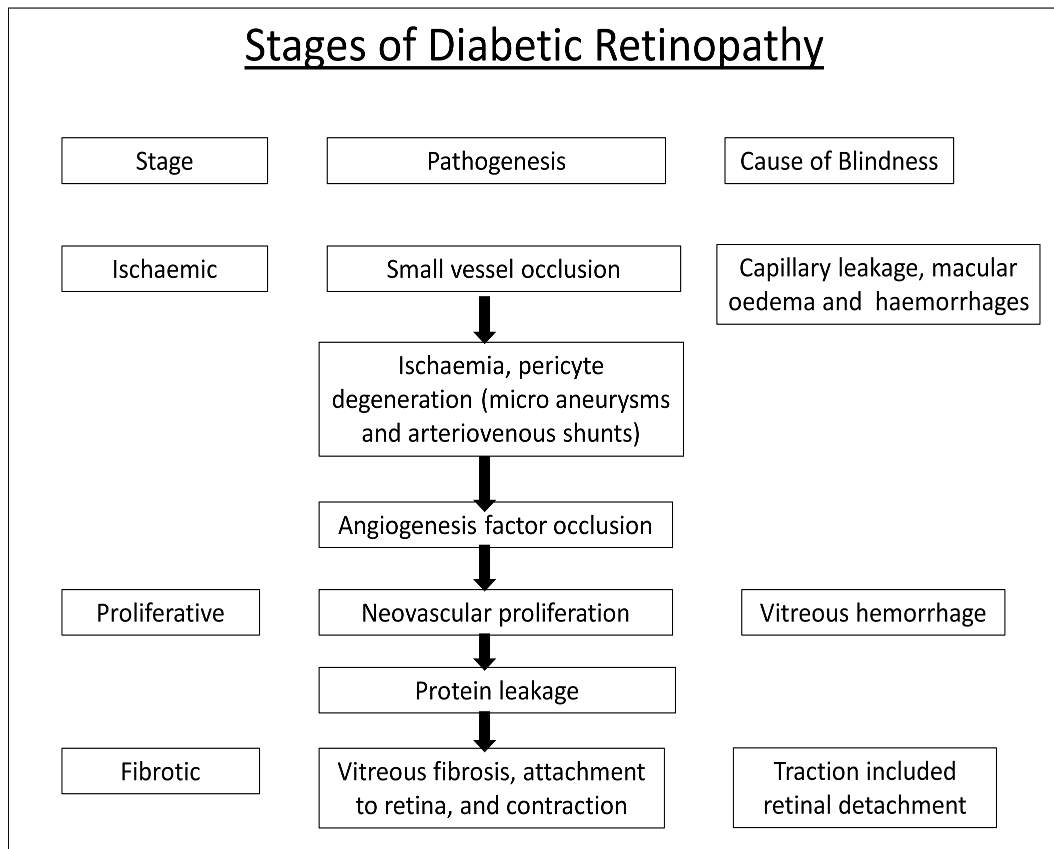

***Figure 1.13: Represents the stages associated with Diabetic Retinopathy** in relation to the underlying pathogenesis and the associated possible causes of blindness. [Adapted from (*https://diabetesalert.wordpress.com/2014/05/25/stages-of-diabetic-retinopathy/)].

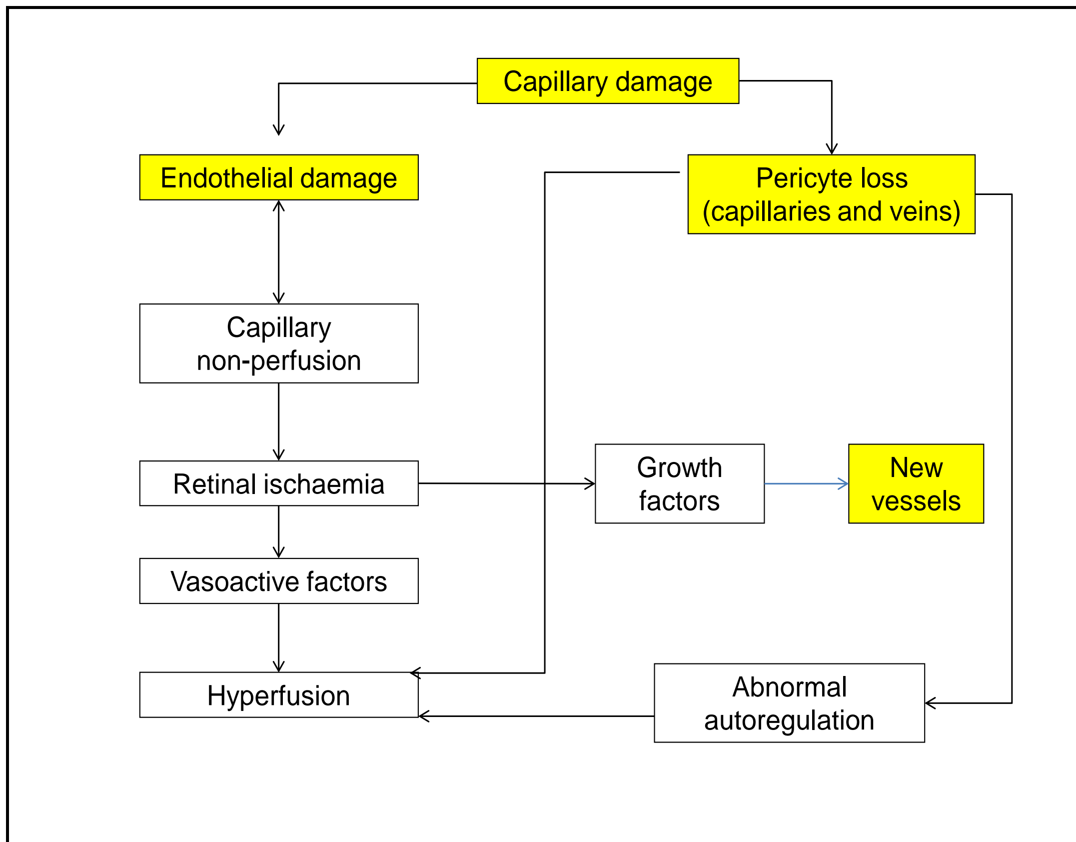

Figure 1.14: Evolution of Diabetic Retinopathy: *Adapted from* (Kohner EM 1993). *The highlighted boxes represent the key stages in the pathogenesis of DR (A detailed schematic representation is shown in Figure 1.25)*

Classification of DR used in this thesis was based on the Diabetic Retinopathy Screening Service for Wales (DRSSW) grading protocol (Table1.1), which is an enriched version of the UK National DR grading protocol (Harding S et al. 2003). Classification was as follows:

8181**Table 1.1: Grading Protocol for the Diabetic Retinopathy Screening Service for Wales**

R0	No Diabetic Retinopathy
R1	Background Diabetic Retinopathy (BDR)
R1.1	Mild BDR • < 5 Mas > 1 DD from fovea • < 4 Hms > 1 DD from fovea • 3 Mas < 1 DD from fovea • ≤ 3 MA < 1 DD from fovea with VA better than 6/12 • exudates > 2 DD from fovea with or without CWS (< 5)
R1.2	Moderate BDR • ≥ 5 MAs > 1 DD from fovea • ≥ 4 < 8 HMs > 1DD from fovea • > 3 MAs < 1 DD from fovea with VA > 6/12 • Circinate or grouped exudates > 2 DD from fovea but within arcades • Questionable IRMA *only in the presence of MA/HM*
R2	Severe BDR (Pre-Proliferative DR, PPDR) • ≥ 8 blot haemorrhages *per eye* (superior and inferior hemi-fields) • Venous irregularities, beading, reduplication, venous loops (but not on their own) • Definite IRMA • With or without CWS (but not CWS on their own)
R3	Proliferative DR (PDR)/Advance Diabetic Eye Disease (ADED) • New vessels on disc (NVD) • New vessels elsewhere (NVE) • Pre-retinal haemorrhage • Vitreous haemorrhage • Pre-retinal fibrosis • Traction retinal detachment
M0	**No** Maculopathy
M1	Possible Maculopathy • Exudates < 2 DD >1DD from fovea • > 3 Mas <1 DD from fovea with VA < 6/12 • Hm < 2DD from fovea
M2	Definite Maculopathy • Exudates < 1 DD from fovea • Retinal thickness changes < 1 DD from

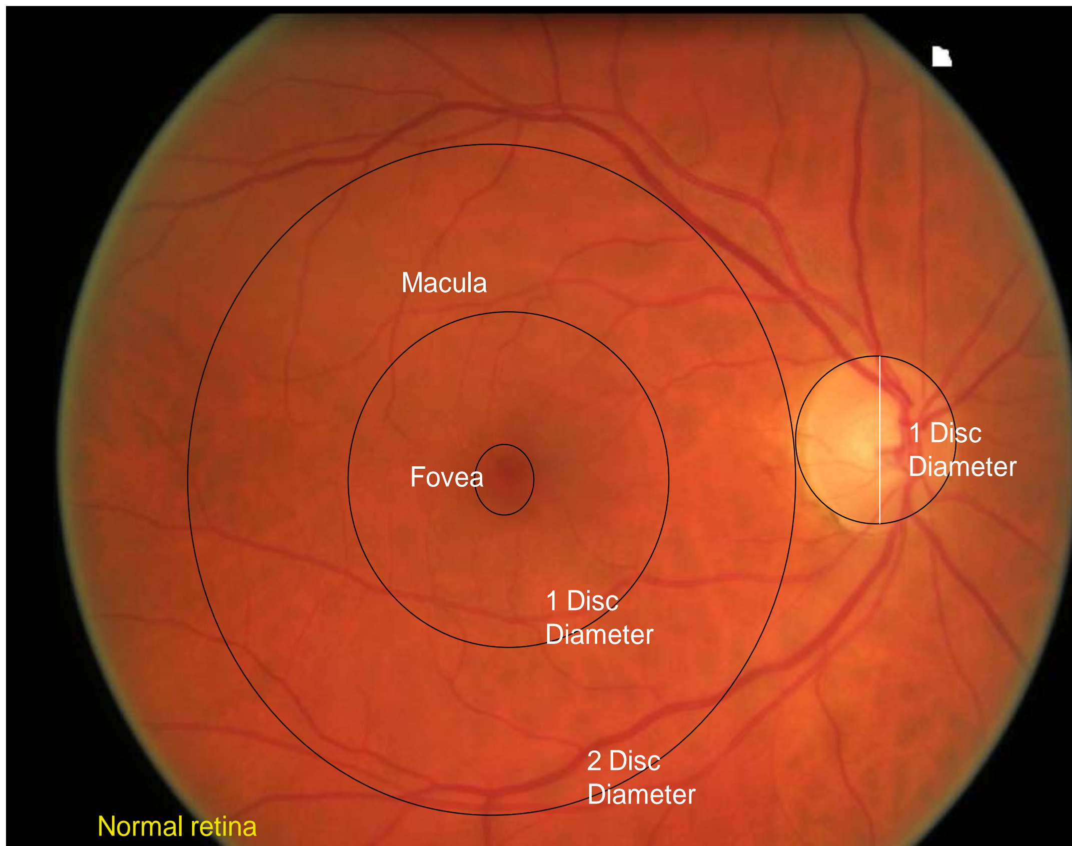

Figure 1.15: Normal Retina (Right Eye) 45 degree field with the fovea as a depression in the retinal surface at the centre of the macula.

Figures 1.15 to 1.19 illustrate the normal retina and the different grades of severity of DR.

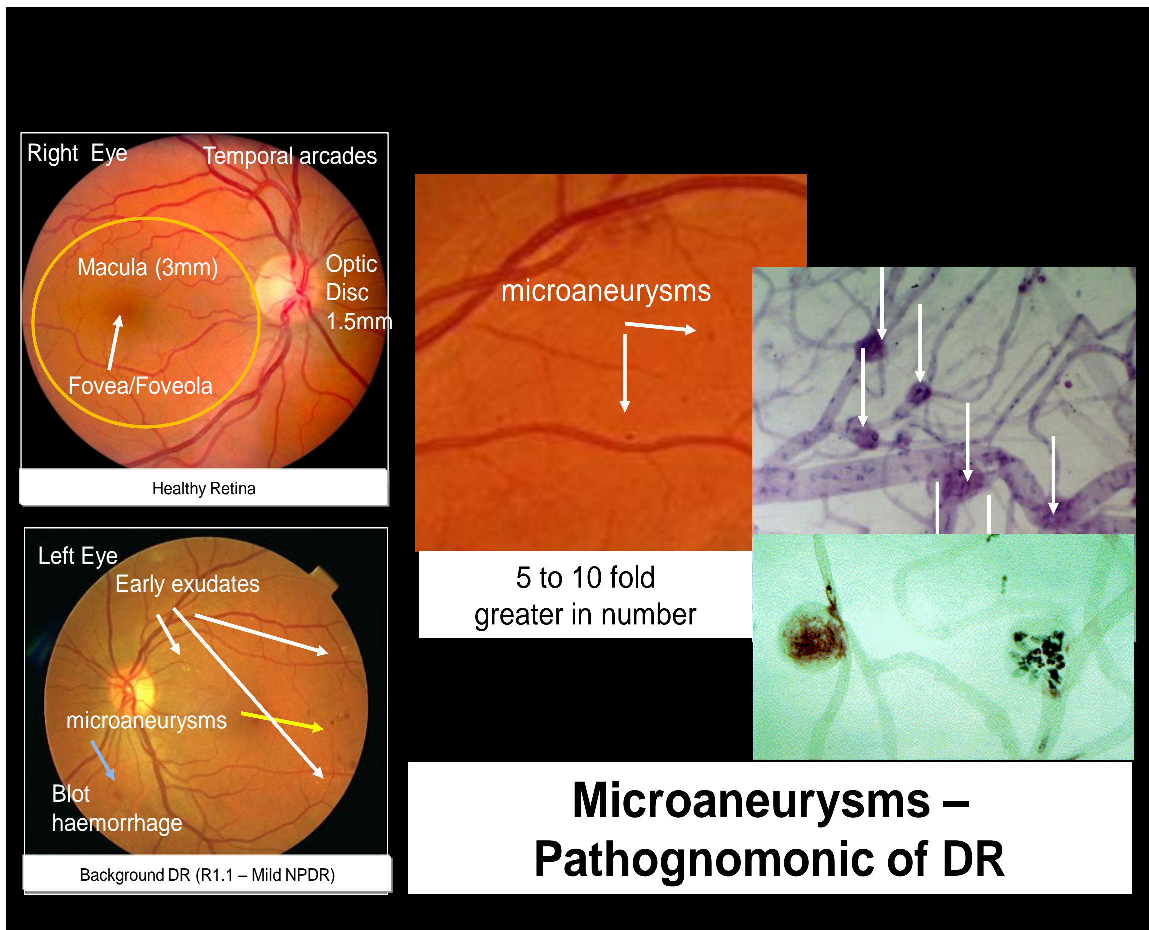

Figure 1.16: Background DR *Pathologic processes: multiple microaneurysms, early exudates and blot haemorrhage*

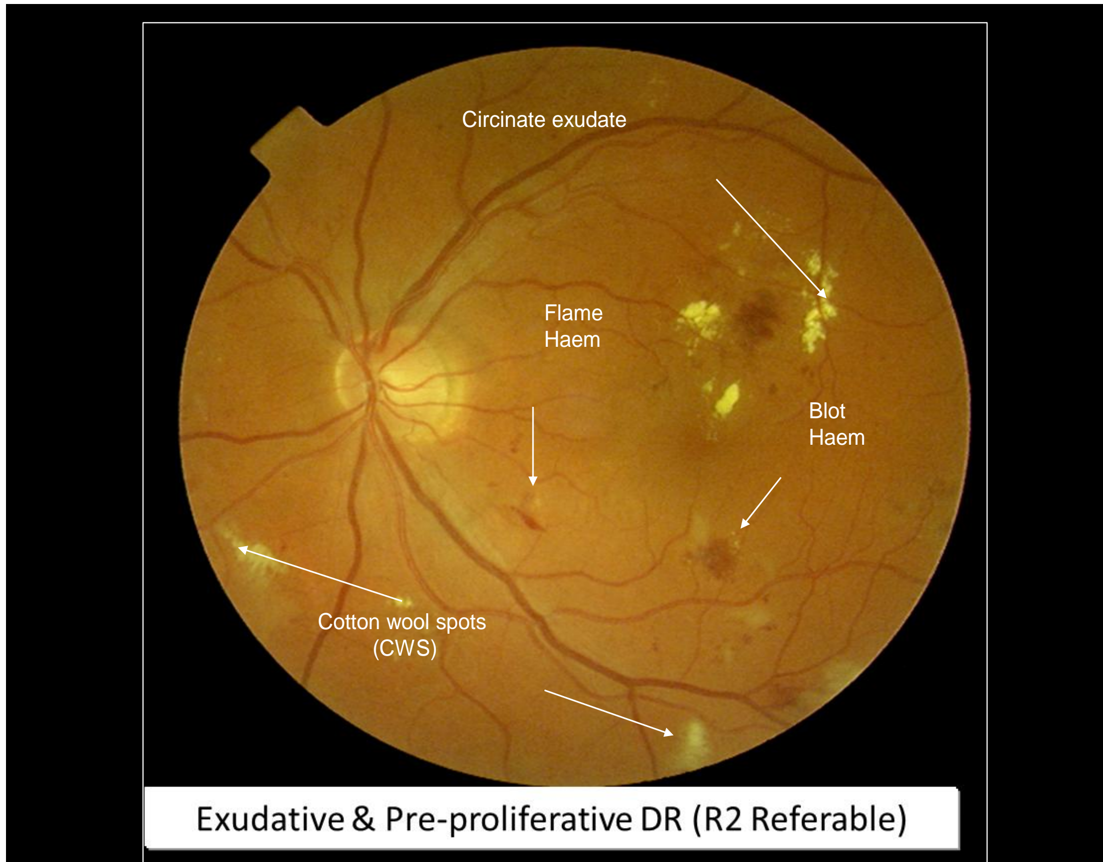

Figure 1.17: Pre-proliferative DR *Pathologic processes: multiple blot and flame haemorrhages, cotton wool spots and hard exudates.*

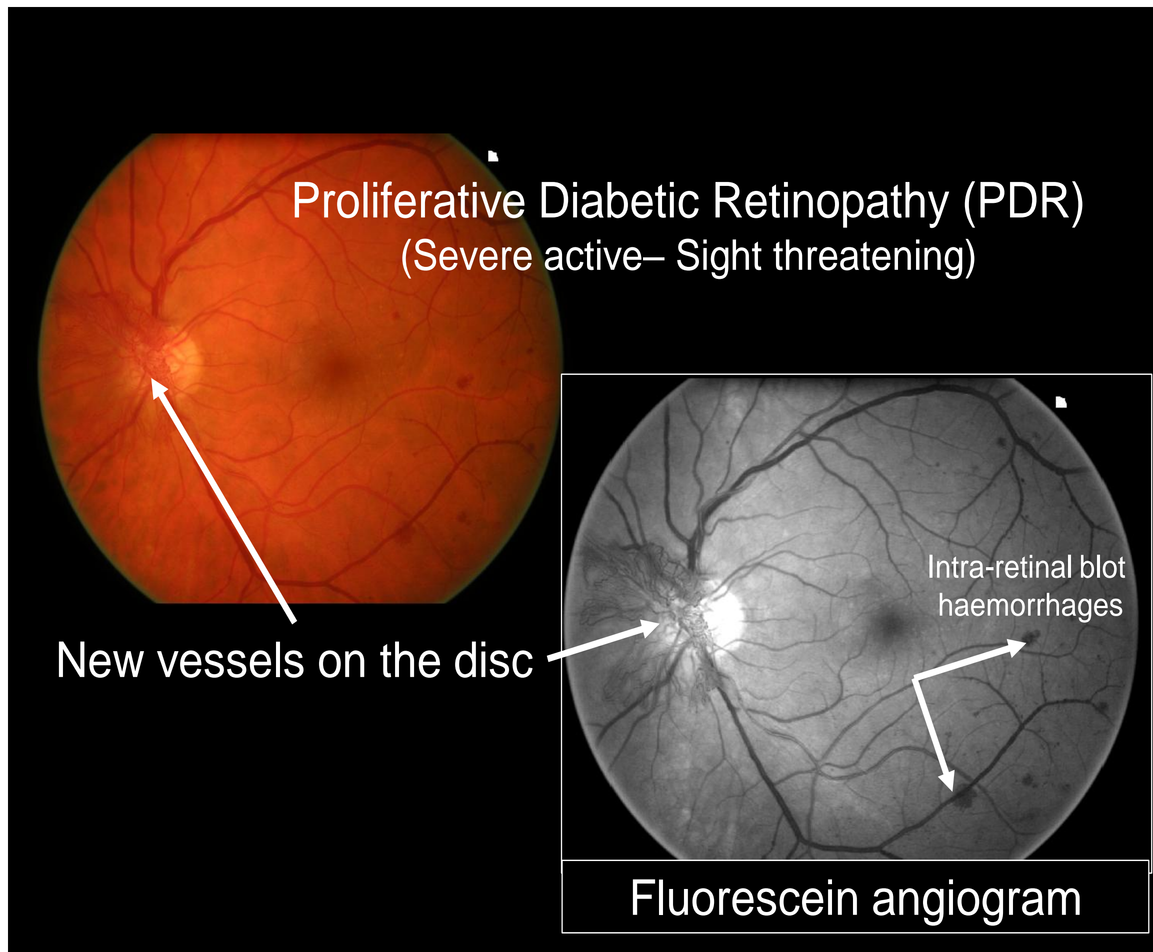

Figure 1.18: Proliferative DR *Pathologic processes: Proliferation of new blood vessels and intra-retinal blot hemorrhages*

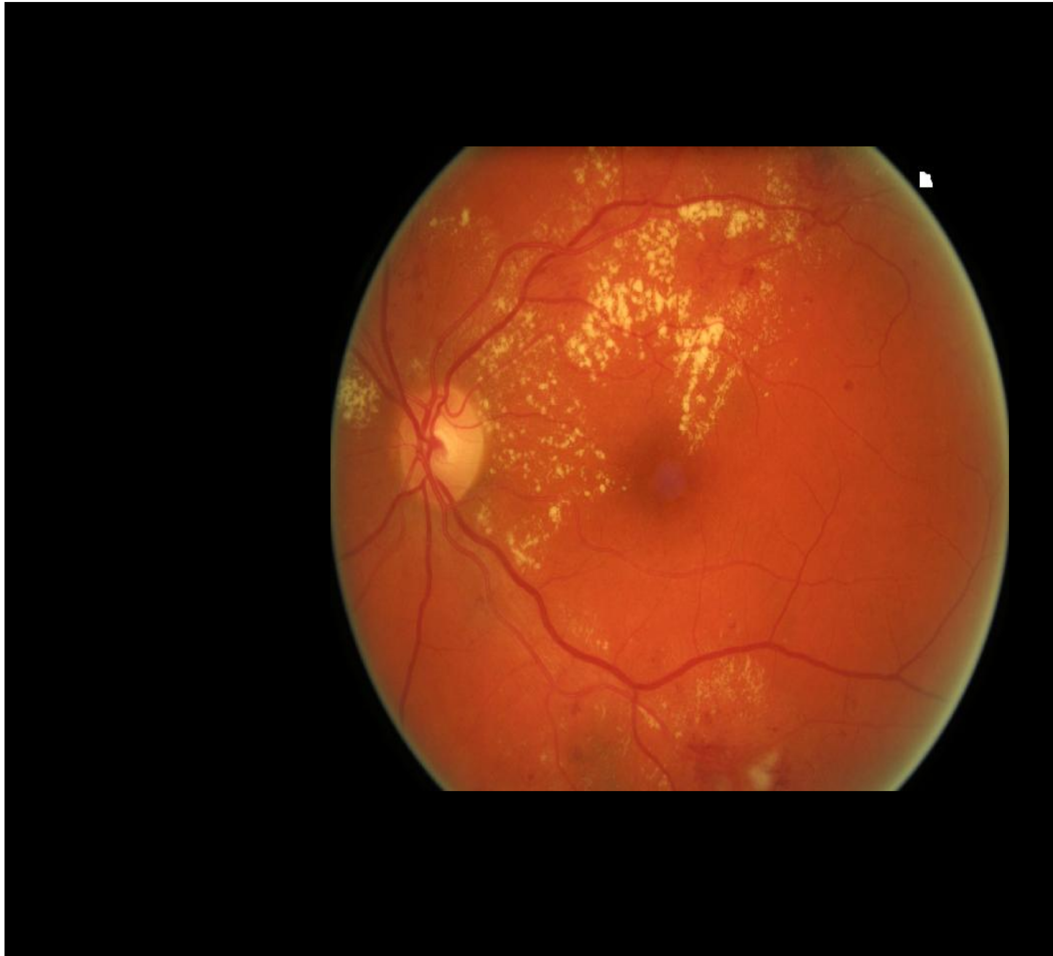

Figure 1.19: Extensive Maculopathy including exudative changes *Pathologic processes: Excessive vascular permeability*

The WHO's first Global Report on diabetic retinopathy has reported that about 8.5% of adults globally have DM (World Health Organization 2016). Thus, diabetic macular ooedema (DME) and PDR have increased and cause Visual Acuity (VA) loss, leading to approximately 2.6% of cases of global blindness in 2010 (Bourne RR et al. 2013; World Health Organization 2016). However the leading cause worldwide in 2010 for blindness remained cataract (33%) with uncorrected refractive error (21%), and macular degeneration (7%) following suit and for moderate and severe vision impairment were uncorrected refractive error (53%), cataract (18%), and macular degeneration (3%) (Bourne RR et al. 2013).

Over the last few decades, development of clinically significant DME or PDR represented a threshold for administering ocular-specific treatment, with laser therapy being the main treatment for both. Laser treatment for DME minimises the risk for progressive visual loss by about 50% (Early Treatment Diabetic Retinopathy Study Research Group 1991a), and application of appropriate Pan Retinal Photocoagulation (PRP) for PDR (Figure 1.19 b) minimises the risk for severe vision loss (The Diabetic Retinopathy Study Research Group 1981). Diabetic Retinopathy Study (DRS) (Diabetic Retinopathy Study Group 1978) and the Early Treatment Diabetic Retinopathy Study (ETDRS) (Early Treatment Diabetic Retinopathy Study Research Group 1985) showed the beneficial effects of retinal photocoagulation, which significantly reduced the severe visual loss due to PDR and DME (Figure 1.20 a, b) and these studies led to guidelines (The Royal College of Ophthalmologists 2012) and screening programs for the timely detection and treatment of DR.

Since the initial studies it has been shown that when DR approaches PDR, PRP should proactively be considered to prevent progression to high risk PDR. In ETDRS very severe NPDR (ETDRS 53E) had a 48.5% risk of progressing to high risk PDR within 1 year and recommendations stated that even where follow-up was possible PRP treatment should be considered in these eyes because they showed increased risk of severe visual loss and need for vitrectomy (Davis MD et al. 1998b). Earlier laser has been recognised to prevent progression to high risk DR, and that PDR has higher risk of blindness was reported in both DRS and ETDRS. However the balance of risks with laser modalities available at that time meant that laser intervention was recommended only when DR approached high risk PDR. With modern laser techniques, PRP is often done before the development of PDR (Lövestam-Adrian M et al. 2003).

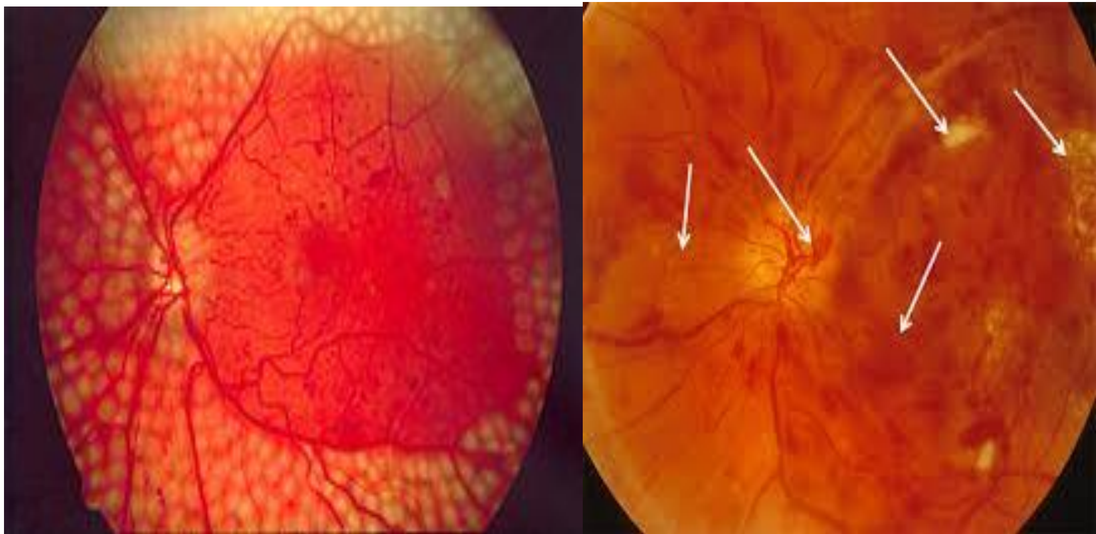

Figure 1.20: Appearance of retina after photocoagulation treatment *a) Scatter Photocoagulation b) Pan-retinal Photocoagulation with new vessel formation* (white arrows) on the disc (NVD) and elsewhere (NVE) (https://upload.wikimedia.org/wikipedia/commons/3/3f/Fundus_photo_showing_scatter_laser_surgery_for_diabetic_retinopathy_EDA09.JPG)

a) **b)** **c)**

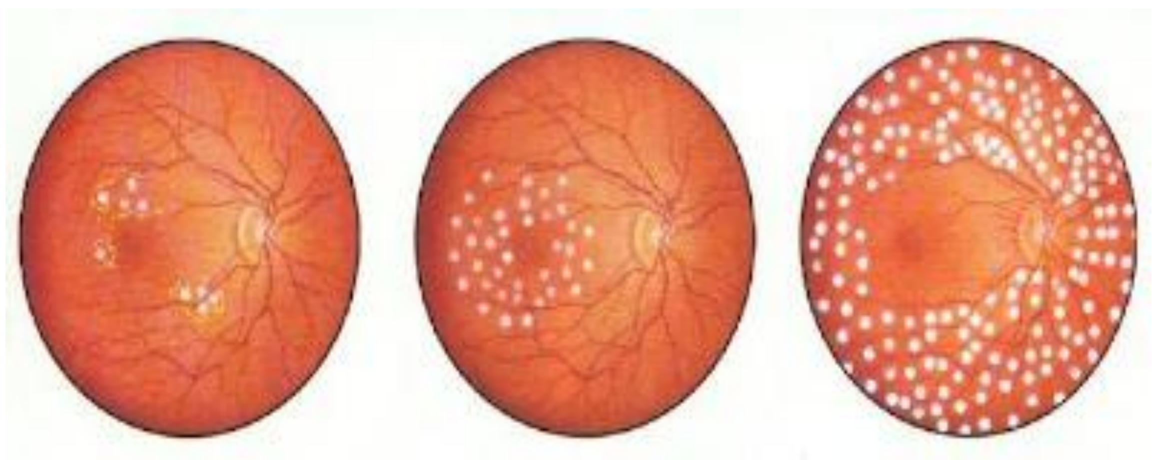

Fig 1.21: Types of Retinal Photocoagulation *(a) Focal treatment is used to treat macular oedema due to focal leakage b) Grid treatment is used to treat macular oedema due to diffuse leakage c) Pan-retinal treatment may be used to treat PPDR and PDR* (http://www.theeyepractice.com.au/optometrist-sydney/eyes_diabetes_diabetic_retinopathy_and_possible_treatments)

Although laser treatment is a recognised therapy for DME, its efficacy may be limited and it may have untoward effects. Macular laser treatment for DME may not improve VA (Elman MJ et al. 2010; Nguyen QD et al. 2012; Korobelnik J.F et al. 2014) and Pan Retinal Photocoagulation can also lead to peripheral visual field defects, night-visual impairment, and reduction of contrast sensitivity. Often additional PRP is needed in 45% of patients with DME and vitrectomy in about 5% of cases (Ferris F 1996; Gross JG et al. 2015).

Inhibitors of vascular endothelial growth factors (VEGF) (Ranibizumab, Bevacizumag, Aflibercept) administered directly into the eye via intravitreal injection block the activity of vascular endothelial growth factors. These agents have revolutionized the treatment of DME and PDR over the past 10 years and in many subjects treatment has changed dramatically from laser to medical options. Randomized controlled trials have reported that anti-VEGF agents are effective for treating both centre-involved DME leading to VA loss (Nguyen QD et al. 2012) (Korobelnik J.F et al. 2014; Wells JA et al. 2015) and less severe stages of PDR (Gross JG et al. 2015) used in combination with laser treatment.

Starting ocular-specific treatments at earlier stages of DR is now advocated as it is clinically relevant to treat in earlier stages of DR from patients' perspective. DR severity graded by the Diabetic Retinopathy Severity Scale (DRSS) (Early Treatment Diabetic Retinopathy Study Research Group 1991b) indicate that worsening of two or more steps on the DRSS leads to an increased risk for subsequent vision loss

(Klein R et al. 2001). Thus for diabetic subjects when an eye has developed DME or PDR, the patient has possibly already had a significant decrease in his/her visual function.

Anti-VEGF pharmacologic agents currently used to treat DME (Diabetic Retinopathy Clinical Research Network et al. 2015) and milder forms of PDR (Writing Committee for the Diabetic Retinopathy Clinical Research Network et al. 2015) show additional benefits in substantially altering the natural history of progressive DR which worsening over time. The treatment slows progression to more advanced DR stages, improving DR status in many eyes and slowing the underlying disease process central to DR itself, which is progressive retinal non-perfusion. Population-based analyses and clinical trial data show that the threshold to initiate ocular-specific anti-VEGF treatments for DR is being lowered to earlier stages of DR such as severe BDR/ PPDR .

Subjects with recently diagnosed T2DM have a much lower life time risk of PDR, macular oedema, and visual impairment as compared with patients from earlier periods (Kempen JH et al. 2004 Apr; Sloan FA et al. 2008 Nov; Klein R and BE. 2010). This reduction may reflect improved management of glycaemia, blood pressure, and lipid levels (Klein R and BE. 2010). These improvements have resulted from the introduction of new devices for self-monitoring of blood-glucose levels and the administration of insulin, new medications (e.g., statins and hypoglycaemic agents), surgical interventions (including vitrectomy), an increased awareness of the

need for intensive control of glycaemia and blood pressure, and the implementation of educational and screening programs.

1.3 Risk factors affecting diabetic retinopathy

Various risk factors associated with the development and progression of DR include non-modifiable factors like duration of DM (Klein R et al. 1984; Zhang X et al. 2010), and modifiable risks like hyperglycaemia (Klein R et al. 1984; Kohner EM et al. 1998; UK Prospective diabetes study group (UKPDS) 1998; Zhang X et al. 2010), hypertension (Klein R et al. 1984; Klein R et al. 1989b; Kohner EM et al. 1998; UK Prospective Diabetes Study Group 2004), dyslipidaemia (Chew EY et al. 1996; Cusick M et al. 2003) and BMI (Klein R et al. 1984)

1.3.1 Hyperglycaemia

Pirart conducted a large prospective survey on 4440 patients between 1947 and 1973, including 2795 patients from diagnosis of DM with metabolic control being monitored by a urine glucose score. It provided strong early evidence that poor glycaemic control was associated with an increased risk of DR (Pirart J 1977 Dec,). The association between chronic hyperglycaemia and retinopathy has been studied in detail with a review by Colwell in the 1960's commenting on the association between poor glycaemic control and retinopathy (Colwell JA 1966 Jul). Similarly, another review by Knowles (Knowles HCJr 1964) also commented on the relationship between dysglycaemia and microvascular disease.

Introduction of home blood glucose monitoring and assessment of glycaemic control, led to keynote prospective studies to explore the relationship between glucose control and diabetes complications. The Diabetes Control and Complications Trial (DCCT) in Type 1 DM (The Diabetes Control and Complications Trial Research Group 1993 Sept 30) and the UK Prospective Diabetes (UKPDS) in T2DM (UK Prospective diabetes study group (UKPDS) 1998) have both provided definitive evidence that microvascular complications of DM are reduced by improved glycaemic control. The UKPDS and DCCT, along with their 10-year follow-up, have further demonstrated the benefits of early and sustained improvement in glycaemic control with respect to DR (The Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications Research Group 2000 Feb; Holman RR et al. 2008) i.e. the legacy effect/metabolic memory.

One of the major effects of chronic hyperglycaemia of DM is enhanced glycosylation of proteins. Glucose binds (irreversibly) to tissue proteins leading to structural and chemical changes in their properties leading to the formation of advanced glycosylation end products (AGE) (Brownlee M et al. 1988 May 19). HbA_{1c} (Haemoglobin A_{1c}) being one such glycosylated protein is used to monitor chronic blood glucose control in DM. Red blood cells (RBC) contain haemoglobin which, in the presence of glucose in the plasma, is constantly glycosylated to $HbA_{1c.}$ The lifespan of RBC is approximately 120 days, and the rate of glycosylation depends on the blood glucose concentration over this time period. Thus the level of HbA_{1c} indicates the average glycaemia over the previous 3 months (average age of RBC) (Krall LP and RS. 1989). However, to be more specific, about 50% of the

HbA_{1c} represents the glycaemic exposure over the preceding month with 25% in the 2nd month prior to the HbA_{1c} measurement and the final 25% over the 3rd month prior to the HbA_{1c} monitoring (Tahara Y and Shima K 1993). Other conditions related to haemoglobinopathy and haemolysis is also known to affect the integrity of the HbA_{1c} measurement, as the red cell turnover increases and thus the amount of glycosylated haemoglobin is decreased.

It is believed that tissue damage from glycosylation leads to both microvascular and macrovascular complications of DM (Andreani D et al. 1991; Brownlee M 1992 Dec,). AGE accumulation in proteins renders significant structural and functional changes to the vascular system. Collagen also manifests these morphological changes as glycosylated collagen. The accumulation of AGE leads to generation of reactive oxygen species, which trap soluble proteins such as albumin and immunoglobulin G (Figure 1.22). When these proteins are trapped by glycated collagen, they retain their ability to form immune complexes in situ which may explain the characteristic diabetic tissue changes i.e. thickening of the capillary basement membrane in the retina and kidney (Creutzfeldt W and Lefebvre P 1988). Capillary basement membrane thickening, endothelial cell proliferation and capillary closure are some initial changes of DR (Ashton N 1974; Kohner EM 1993). The binding of AGE to endothelial cells increases permeability (Esposito C et al. 1989) and leads to capillary leakage in the retina (Antonetti DA et al. 2012b). Fluorescein angiography was the first technique to document the abnormal leakage of fluorescein through the retinal vessels (Cunha-Vaz JG and Maurice DM 1967). The technique is still used in the diagnosis and aids in the management of DR.

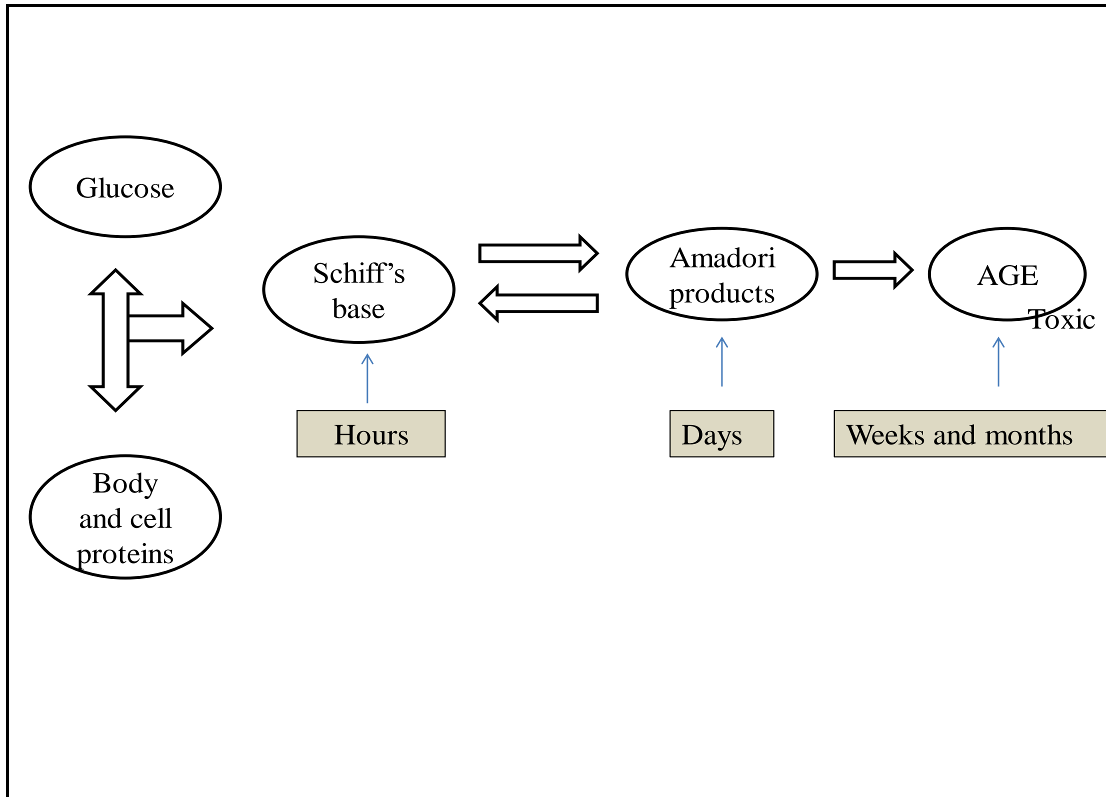

Figure 1.22: Formation of advanced glycation end products (AGEs) *[Adapted from (Tarr JM et al. 2013)] Glucose irreversibly binds to tissue proteins leading to structural and chemical changes in their properties. This leads to the characteristic diabetic tissue changes i.e. thickening of the capillary basement membrane in the retina.*

Hyperglycaemia also leads to the activation of the polyol pathway of glucose metabolism (Andreani D et al. 1991). Glucose is enzymatically reduced to sorbitol by aldose reductase intracellularly. Sorbitol is then oxidised to fructose (Figure 1.23). Sorbitol penetrates cell membranes slowly and the increased flux of glucose via the polyol pathway results in abnormally raised sorbitol concentrations. This increases the osmotic potential of cells and induces oedema or swelling. The early stages of DR show damage and degeneration of the pericyte cells, which support the walls of the retinal capillaries. Hyperglycaemia activates the polyol pathway in the mural pericyte cells, which contain the enzyme aldose reductase, leading to accumulation of sorbitol to damaging concentrations and pericyte death (Kador PF et al. 1990). Unfortunately, agents which inhibit aldose reductase (e.g. Alrestatin, Epalrestat) have not altered the development of retinopathy. Epalrestat is the only commercially available inhibitor till date. In addition, some other ARIs such as Sorbinil and Ranirestat had been advanced into late stage of clinical trials and found to be safe for human use. However clinical trials of ARIs had little therapeutic success in DR (Grewal et al. 2016).

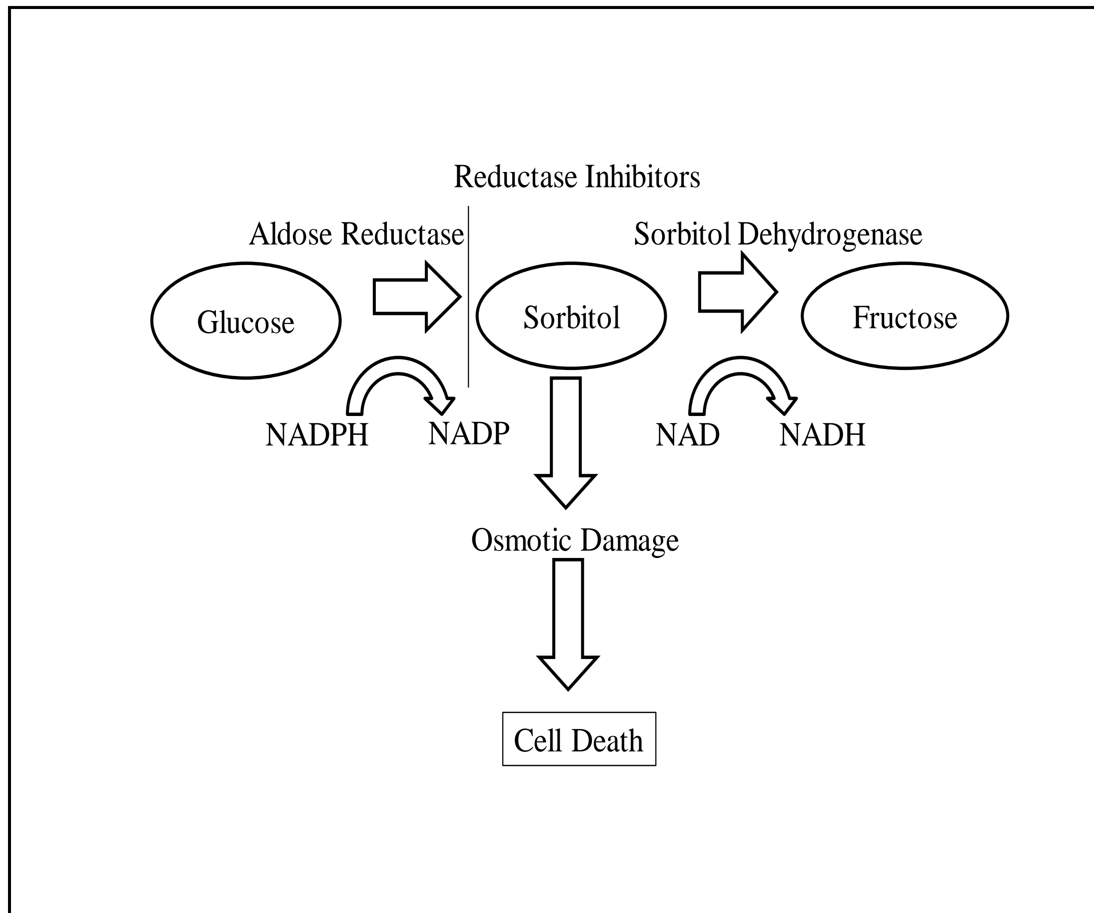

Figure 1.23: Polyol pathway*[Adapted from (Tarr JM et al. 2013)] Glucose is enzymatically reduced to sorbitol by aldose reductase intracellularly which is then oxidised to fructose. Sorbitol penetrates cell membranes slowly. This increase in the osmotic potential of cells induces oedema and cell death.*

Retinal capillary pericytes have a contractile role (Kelley C et al. 1987) and therefore their loss contributes to increased sheer forces due to the lack of deformability i.e. more stiff and less pulsatile (Davis MD 1992). Blood flow in the normal retina is maintained by autoregulation. The retinal blood vessels constrict or dilate in response to changes in local perfusion pressure and metabolic requirements (Alm et al. 1987). In diabetic subjects alterations in blood flow (hyper-perfusion due to hyperglycaemia) in the retina have been measured (Fallon TJ et al. 1987; Grunwald JE et al. 1990). In addition there in increased viscosity of blood in diabetic subjects and capillary closure contribute to these alterations (McMillan DE 1983). These alterations also lead to increased shear stress within the retinal vessels and consequent damage to the endothelium. Basement membrane thickening increases the rigidity of the retinal blood vessels and may contribute to the loss of autoregulation (Tooke JE 1989; Andreani D et al. 1991).

Hyperglycaemia also causes retinal hyper perfusion (Grunwald JE et al. 1990) and rapid normalisation of high blood glucose levels may lead to worsening of DR in those with previous poor glycaemic control with evidence of diabetic retinopathy (Henricsson M et al. 1997). The sudden changes in retinal blood flow damage the abnormal retinal capillaries and thus it is advisable to gradually reduce glucose levels in poorly controlled diabetic subjects especially where there is evidence of DR present.

Thus to summarise chronic hyperglycaemia is a major initiator of diabetic vascular complications. Elevated glucose, via various mechanisms such as increased production of advanced glycation end products, activation of protein kinase C (Figure 1.24) stimulation of the polyol pathway and enhanced ROS generation, regulates vascular inflammation, altered gene expression of growth factors and cytokines, and platelet and macrophage activation, thus playing a central role in the development and progression of diabetic vascular complications (Yamagishi S and Imaizumi T 2005). The evidence shows that inhibition of different PKC isozymes is not sufficient to normalise vascular endothelial growth factor (VEGF)-induced barrier damage of retinal endothelial cells. However PKC-β inhibition prevents hyperglycemia-induced VEGF expression in retinal pericytes, suggesting that PKC inhibitors should be administered before increased VEGF expression is established in the diabetic retina. Though initial studies have indicated that treatment of diabetic patients with ruboxistaurin, a specific inhibitor of PKC-β, may reduce visual loss in patients with diabetic retinopathy, the overall benefit seems to be small (Deissler and Lang 2016).

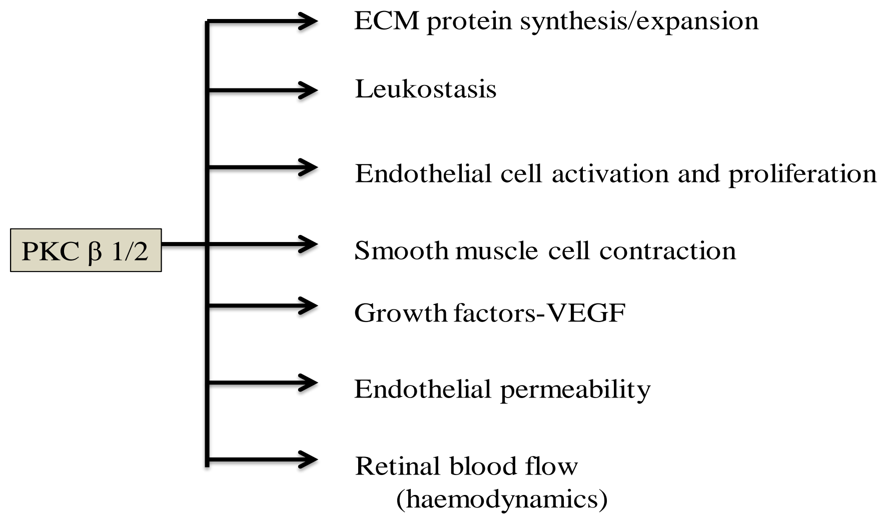

Figure 1.24: Regulation of pathophysiological processes in Diabetic Retinopathy by protein kinase C (PKC) *[Adapted from (Tarr JM et al. 2013)].*

DR is a multifactorial disease involving several pathological mechanisms, including increased oxidative stress, inflammation, the polyol pathway leading to sorbitol accumulation, production of advanced glycation end products (AGEs) and activation of the protein kinase C (PKC) pathway (Figure 1.25).

These pathways can in turn activate the production of cytokines and many vasoactive factors, such as vascular endothelial growth factor (VEGF) and pigment epithelium-derived factor (PEDF), which are vital in mediating the structural and functional changes of DR. Clinically significant DME can occur in the late stages of DR with NPDR or PPDR and is the most common cause of vision loss (Robinson R et al. July 2012).

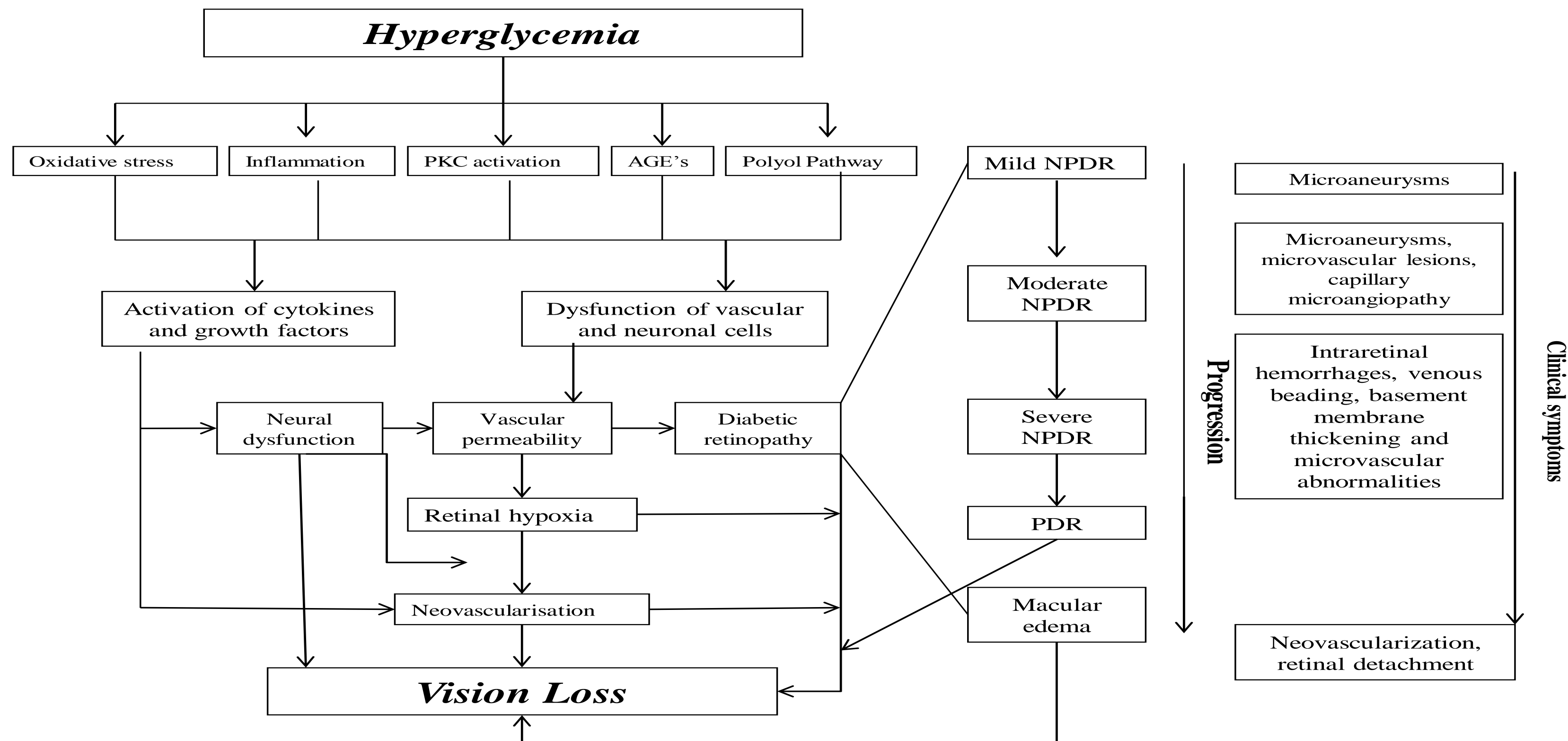

Figure 1.25: Key factors involved in the pathogenesis of DR and the clinical symptoms evident at different stages of DR *(Robinson R et al. 2012)*.

1.3.1.1 Clinical evidence of Hyperglycaemia as a risk factor for diabetic retinopathy

Overall hyperglycaemia as represented by HbA_{1c} contributes to both initiating and promoting all these mechanisms and if hyperglycaemia is targeted and normalised then the cycle of damage can be prevented and risk of incidence and progression of DR will be significantly reduced. Both DCCT and UKPDS have stated that DR was not completely prevented, even in patients with near normal glycaemia, although no one achieved absolutely normal glucose profiles. The UKPDS demonstrated a 25% reduction in the rate of development of microvascular complications in the intensively-controlled group. It also showed that incidence [relative risk in the middle $1/3^{rd}$ (HbA_{1c} 6.2 %-7.4%) was 1.4 and 2.5 in the top 1/3rd] and progression [relative risk in the middle $1/3^{rd}$ was 4.1 and 8.1 in the top $1/3^{rd}$ ($HbA_{1c} \geq 7.5\%$)] of DR was strongly associated with HbA_{1c} (Stratton IM et al. 2001).

Investigating the possible association between hyperglycaemia and the presence of DR has, over the years, involved measurement of various metabolic indices, predominantly HbA_{1C} (Stratton IM et al. 2006) and/or fasting plasma glucose (Miki E and Kikuchi M 1994). In the Diabetes Prevention Program (DPP), subjects with impaired glucose tolerance and recent onset T2DM were studied, and the investigators found a higher baseline HbA_{1c} and systolic blood pressure (SBP) amongst those with DR, but no difference in insulin secretion (estimated by the Corrected Insulin Response, CIR methodology) (Diabetes Prevention Program Research Group 2007). Also the Kumamoto study showed that intensive glycaemic control utilising multiple daily insulin injection therapy in individuals with T2DM

delays the onset and the progression of DR. (Ohkubo Y et al. 1995). In 2005 Shiraiwa et al. showed that postprandial hyperglycaemia (and postprandial hypo-insulinaemia) were possible predictors for incident DR in Japanese T2DM subjects who were not on insulin treatment (Shiraiwa T et al. 2005a).

Multiple factors are clearly involved. The Action to Control Cardiovascular Risk Factors in Diabetes (ACCORD) trial assessed the effects of intensive glycaemic, lipid and hypertensive therapy on cardiovascular events and the progression of DR (ACCORD study group and Accord Eye Study Group 2010). Amongst these, intensive glycaemic control was noted to significantly reduce the risk of progression of DR after 4 years. In the Accord follow-on study, analysing T2DM subjects with established cardiovascular disease and duration of DM >10 years, the investigators showed that early intensive glycaemic control continued to reduce DR progression (ACCORD Study Group 2016).

To try to establish the effect of the modification of these various parameters, the Steno-2 trial an intensified, multifactorial intervention of modifiable risk factors compared intensive to conventional treatments in persons with T2DM and microalbuminuria (Gaede P et al. 2003) and showed a 57% risk reduction of DR for individuals in the intensive treatment group over an 8-year period. The Steno-2 follow up study went on to demonstrate a median of 7.9 years of gain of life at 21.2 years of follow-up after 7.8 years of intensified, multifactorial, target-driven treatment of T2DM with microalbuminuria. This increase in lifespan was matched

by time free from incident cardiovascular disease (Gaede P et al. 2016). The Veterans Affairs Diabetes Trial (VADT) (Azad et al. 2014) similarly tested the hypothesis that intensive glycaemic control would be associated with better eye outcomes in subjects with poorly controlled DM and higher plasma C-peptide levels. The fasting C-peptide measured here has been used as a measure of pancreatic reserve and reflecting on to the glycaemic control. The incidence and progression of DR was assessed by grading seven-field stereoscopic fundus photographs at baseline and 5 years later in 858 of 1,791 participants of the study. Post adjustment for all covariates, risk of progression (but not incidence) of DR increased by 30% for each 1% increase in baseline $HbA_{1c.}$ The incidence of DR was reduced by 67.2% with each 1 pmol/ml increment in baseline C-peptide. Baseline C-peptide was also an independent inverse risk factor for the progression of DR, with a reduction of 47% with each 1 pmol/ml increase in C-peptide. Thus the study demonstrated that poor glucose control at baseline was associated with an increased risk of progression of DR over 5 years. Importantly for the first time a higher C-peptide at baseline was associated with reduced incidence and progression of DR.

Focusing more on blood glucose control, the ADVANCE trial (Zhu CH et al. 2013) evaluated the effects of intensive blood glucose control on microvascular complications in patients with T2DM by comparing the therapeutic effects of intensive and standard treatment in patients with T2DM. Direct ophthalmoscopy and seven-field stereoscopic retinal photography were used to examine the fundi at baseline, and repeated after 5 years of treatment. The severity of DR did not progress in subjects in the intensive group, but worsened in the standard group (P= 0.0006).

Thus intensive therapy proved to be able to maintain stable vision and proved that intensive control of blood glucose can diminish the incidence and/or slow the progression of DR in patients with T2DM.

The association of hyperglycaemia and DR in established type 2 Diabetes Mellitus (T2DM) subjects is well accepted. However, the independent association between β-cell status, insulin sensitivity and DR in newly diagnosed treatment naïve T2DM subjects remained previously unreported.

1.3.2 β-cell function

In the UKPDS, β-cell function was estimated based on the fasting glucose and insulin levels by the homeostasis model assessment HOMA-B (Matthews DR et al. 1985) and demonstrated a strong relationship between DR and impaired β-cell function (Kohner EM et al. 1998). Since the increasing hyperglycaemia of T2DM is associated with progressive deterioration of β-cell function, (U.K. Prospective Diabetes Study Group 1995) it is possible that the assessment of β-cell function provides a better guide to the severity of DM than the measurement of FPG and glycosylated haemoglobin levels at a single clinic visit. [The lesser association of DR with these glycaemia indices might in part be because some subjects had already restricted their diet between the time when the diagnosis was suspected by their primary care physician and their first clinic visit]. The UKPDS also reported that the severity of DR at diagnosis of T2DM was related in both sexes to higher FPG, lower fasting serum insulin levels, and reduced β-cell function (HOMA-B). Increased

alcohol consumption was related to increased severity of DR in men and leaner women had more severe eye lesions (Kohner EM et al. 1998). A community based study in Taiwan has also demonstrated that both β cell dysfunction and IR (HOMA methodology) were associated with DR in established T2DM patients (Tung TH et al. 2007).

1.3.3 Insulin Resistance

Rather than simply glucose level itself, Maneschi et al suggested in 1983 that increase in IR may contribute to the pathogenesis of DR (Maneschi F et al. 1983). Similarly, over the last decade, there have been several other reports associating IR with DR (Tung TH et al. 2007) (Katsumori K et al. 1995; Suzuki M et al. 2000; Nakano S et al. 2003).

Suzuki et al. measuring insulin sensitivity by euglycaemic glucose clamp method found that the frequency of advanced DR (PPDR and PDR) was more frequent in the IR group than in the insulin-sensitive group. Insulin sensitivity expressed as glucose utilization (glucose clearance) was also found to be significantly lower in T2DM subjects with DR compared to subjects without DR after adjustment for age, BMI, FPG, and known duration of diabetes. Furthermore, IR was more severe in those subjects with both DR and nephropathy; thus they concluded that IR associated with T2DM is closely associated with the progression of microangiopathies (Suzuki M et al. 2000).

Further on the consideration of insulin resistance, Katsumori et al. undertook a study to determine whether patients with IR syndrome (IRS - insulin resistance, hyperinsulinaemia, hyperglycaemia, obesity, dyslipidaemia, hypertension), were a high-risk population for macro- and microvascular diseases in Japanese T2DM and in subjects with borderline glucose-intolerance. The prevalence of late-stage DR (PDR/maculopathy) in the IRS group was significantly higher than that in the other group (12.3% vs. 2.4%, respectively, $P<0.005$). Macroalbuminuria, but not microalbuminuria, was also significantly higher in the IRS group (12.3% vs. 3.6%, $P < 0.02$). Thus they concluded that IRS preferentially increased the development of CAD, and was also involved in the progression of microvascular diseases including diabetic retinopathy (Katsumori et al. 1995).

Not only is IR important for risk of development of retinopathy, Nakano et al measuring IR from the Glucose Infusion Rate (GIR) during an euglycaemic hyperinsulinaemic clamp study, demonstrated that a decrease in the GIR was associated with increasing severity of DR, neuropathy and nephropathy (Nakano S et al. 2003). They also demonstrated that IR is inversely correlated to urinary C-peptide secretion or pancreatic β-cell function thus suggesting that pancreatic β-cell function was unable to compensate for increased IR to maintain euglycaemia in T2DM subjects (Nakano S et al. 2003) as previously reported (Kahn SE et al. 1993). Genetic factors have also been shown to be involved in Insulin secretion and IR in T2DM (Ferrannini E 1998; Gerich JE 1998).

However, there is not complete agreement as to the role of IR as, in contrast to all the above studies, Stolk et al. when determining IR using the insulin to glucose ratio 2 hours after an oral glucose load showed no difference in subjects with or without DR (Stolk RP et al. 1995).

1.3.4 Hypertension

Elevated BP enhances blood flow and leads to retinal capillary endothelial cell damage in eyes of people with DM (Kohner EM 1989). This has been further observed in clinical studies thus showing an association between hypertension and the presence and severity of DR in people with DM (Fujisawa T et al. 1999; Gillow JT et al. 1999).

Hypertension has been associated with both the development and progression of DR (Klein R et al. 1984; Klein R et al. 1989b; Kohner EM et al. 1998; UK Prospective Diabetes Study Group 2004) within the UKPDS showing that the severity of DR at diagnosis of T2DM to be related to a higher systolic and diastolic BP (Kohner EM et al. 1998). The UKPDS, in a subset of hypertensive patients, showed that stringent BP control reduced the risk of developing macrovascular and microvascular complications of DM (Turner R et al. 1996) and that a 10 mm Hg decrease in updated mean SBP was associated with a 13% risk reductions of microvascular complications ($P < 0.0001$). Thus in patients with T2DM, the risk of diabetic complications has been strongly associated with raised BP and any reduction in BP is likely to reduce the risk of complications, with the lowest risk being in those with

SBP of <120 mmHg (Adler AI et al. 2000). The UKPDS has also reported that the 6-year incidence and progression of retinopathy were both significantly related to BP (Kohner EM et al. 1996).

In studying how hypertension may be a risk factor for diabetic complications, Rassam et al. demonstrated impairment in retinal vascular autoregulation in response to raised systemic BP in diabetic subjects, more so at an elevated blood glucose level, thus providing an additive mechanism for the detrimental effect of hypertension on DR (Rassam SM et al. 1995). The DPP, where subjects with IGT and recent onset T2DM were studied, also found a higher baseline SBP amongst those with DR (Diabetes Prevention Program Research Group 2007). This had built on data from the Santa Barbara County Diabetic Retinopathy Screening Feasibility Study where the mean SBP was found to be higher in patients with all types of DR with the relationship remaining significant when smokers and non smokers were separated although there was no significant difference noted in mean SBP between patients with severe DR (PPDR and PDR) and those with BDR (Lewis JM et al. 1994).

In contrast however, recently the Accord Eye Study Group has shown that intensive BP control does not reduce the rate of progression of DR (ACCORD study group and Accord Eye Study Group 2010). Of note in the Accord Eye Study Group, the target SBP was maintained at <140 mm Hg for the standard group and <120 mm Hg for the intensive group, however for the UKPDS the targets were <180 mm Hg and

<150 mm Hg respectively. This might be a possible explanation of the difference in outcomes. Thus the association of hypertension is reasonably well-established with DR though not all studies have found it to have the same importance as a risk factor.

1.3.5 Dyslipidaemia and its treatment

The contribution of dyslipidaemia (Chew EY et al. 1996; Cusick M et al. 2003) to the development and progression of DR is documented in the EURODIAB study where the serum total triglyceride concentration was significantly higher in those with moderate and severe non-NPDR and PDR in T1DM subjects than in those without DR (Sjolie AK et al. 1997). Similarly in the ETDRS elevated serum triglyceride was independently associated with the development of high risk PDR in both T1DM and T2DM subjects (Davis MD et al. 1998a).

Cholesterol levels have also been implicated - in the WESDR study in subjects using insulin (irrespective of age at onset) a higher total serum cholesterol was associated with increased odds of having retinal hard exudates (Klein BE et al. 1999). Also the ETDRS estimated that there was twice the risk of having hard exudates at entry into the study and 1.5 times the risk of developing hard exudates during the 4 year study period if there was increased serum total cholesterol and low density lipoprotein cholesterol (LDL-cholesterol) at baseline/point of entry to the study, but not with other lipoprotein fractions and the total triglyceride levels (Chew EY et al. 1996). In contrast however, there was no relationship between total cholesterol and high density lipoprotein cholesterol (HDL-cholesterol) and the presence of DR (hard

exudates) in the older onset age group not requiring insulin therapy (Klein BE et al. 1991). Cholesterol as also been studied as one of multiple risk factors and he STENO-2 study found a 67% significant reduction in DR in the intensive intervention group involving control of multiple risk factors, including glycaemic control, BP, cholesterol and microalbuminuria over 4 years, which was sustained at 8 years (Gaede P et al. 1999; Gaede P et al. 2003). However these findings have not been universally consistent (Duncan LJ et al. 1968), Though this study with clofibrate showed a highly significant decrease in hard waxy exudates it did not cause appreciable improvement in visual acuity/improvement in vascular retinal lesions. The initial severity of the exudative lesions was also not related to the fasting serum cholesterol or triglyceride levels and there was further no correlation between the effect of clofibrate on exudates and serum lipids. The study however had modest numbers with twenty-three patients and twenty-five controls which might be a contributing factor to the outcome.

Interestingly the use of fibrates has been shown in some studies to have a beneficial effect on retinal exudates (Harrold BP et al. 1969; Dorne PA 1977; Freyberger H et al. 1994; Keech AC et al. 2007; ACCORD study group and Accord Eye Study Group 2010). Treatment with fibrates in people with T2DM was independently associated with a reduced rate of progression to a first diagnosis of DR (Morgan CL et al. 2013) and macular oedema. Duncan et al. (Duncan LJ et al. 1968) also noted a highly significant decrease in hard waxy exudates ($p \leq 0.0001$) in subjects treated with fibrates. The Fenofibrate Intervention and Event Lowering in Diabetes study (FIELD) reported that individuals with T2DM treated with fenofibrate, in addition to

therapies for hyperglycaemia and other risk factors for DR were less likely to need laser therapy than controls (Keech A et al. 2005). There was also less progression of pre-existing DR with fenofibrate. However, in individuals without DR at baseline there was no significant reduction in the development of DR (Keech AC et al. 2007).

A retrospective matched cohort study found that treatment with fibrates was associated with a 20% reduction in the rate of first onset of DR (Morgan CL et al. 2013), but this reduction in the onset of DR did not appear to be attributable to the lipid lowering effects of fibrates. Other nonlipid related mechanisms that may explain the effect of fibrates on DR include the anti-inflammatory, antioxidant and anti-VEGF properties of fibrates (Poynter ME and Daynes RA 1998; Delerive P et al. 1999). Fenofibric acid has also been reported to prevent the disruption of the retinal pigment epithelium cells, prevention of breakdown of the blood brain capillary barrier and down-regulation of vascular endothelial growth factor (VEGF) (Meissner M et al. 2004; Trudeau K et al. 2011; Villarroel M et al. 2011). In addition fibrates also possess neuroprotective properties (Bordet R et al. 2006). However, no data is available to indicate which features of DR progressed or whether any regression was seen. In interpreting these studies with their clinical implications (Wright and Dodson 2011), it must be noted that DR was not the primary endpoint by design, a tertiary endpoint in FIELD, and DR endpoints recorded in a sub study cohort of the ACCORD study population. Hence, the exact beneficial action of fenofibrate on DR remains to be elucidated.

1.3.6 Other risk factors

1.3.6.1 Genetics

Genetic and environmental factors both appear to affect the development of DR. Evidence indicating significant correlations between the severity of DR in family members has been reported in the DCCT study (The Diabetes Control and Complications Trial Research Group 1997) and by others (Alcolado 1998). An increased risk is seen in identical twins of affected probands (Leslie RD and Pyke DA 1982). Heritability has been estimated to be as high as 27% for any DR and 52% for PDR (Cho H and Sobrin L 2014) demonstrated by Looker et al. (Looker HC et al. 2007) and Hietala et al. (Hietala K et al. 2008). There are current studies assessing genome-wide associations offering a better understanding to the genetic architecture of DR susceptibility (Liew et al. 2009; Cho H and Sobrin L 2014). DR is a however a polygenic disorder and linkage analyses, candidate gene association studies and genome-wide association studies (GWAS) performed till date hasn't identified any widely reproducible risk loci for DR (Cho H and Sobrin L 2014). Combined analysis of data from multiple GWAS is emerging as an important next step to explain the unaccounted heritability. Important factors to future discovery of genetic underpinnings of DR are precise DR ascertainment, focus on more heritable disease forms (PDR), stringent selection of control participants with relation to diabetes duration, and methods that allow combining of existing datasets from various ethnicities to achieve sufficient sample sizes to detect variants with modest effect sizes.

1.3.6.2 Ethnicity

Ethnic origin differences in the prevalence of DR have been a focal point of interest in recent research (Cheung et al. 2010). An association between ethnicity with the prevalence of DR, presence of any DR and also severe/referable stages of DR have previously been reported to be higher in non-Caucasian persons when compared with Caucasians by Ross et al. (Ross SA et al. 2007), Stolk et al. (Stolk RP et al. 2008) and Raymond et al. (Raymond NT et al. 2009). The studies did adjust for confounding variables. Though this variation in frequency may reflect true differences in prevalence, lack of uniformity in study designs, protocols for examination and documentation of DR may explain some of the reported differences. There also remains a possibility that differences in environmental and genetic risk factors as well as other covariates could have a marked impact on frequency.

1.3.6.3 Smoking

The reporting of the effect of smoking on the incidence of DR is varied. Walker at al. in a cross-sectional study of 193 adult patients demonstrated that smoking was related to DR in men but not in women, although in the latter group the prevalence of smoking was low (Walker JM et al. 1985). In a sample of 181 diabetics, a statistical association of smoking was also established with PDR (Paetkau ME et al. 1977). Interestingly however the UKPDS reported in newly-diagnosed T2DM subjects that development of DR was strongly associated with not smoking. In those who already had DR, progression was also associated with not smoking (Stratton IM

et al. 2001). Thus smoking status was inversely related to the incidence and progression of DR.

This was in variation to other published findings (Madsbad S et al. 1980; Owens D R et al. 1988; Lewis JM et al. 1994; Guillausseau PJ et al. 1998) which showed no association of DR with smoking. Some of these other studies were from a smaller population group (129 T2DM subjects) (Owens D R et al. 1988) and 163 subjects (Madsbad S et al. 1980) noting that no difference might not be truly reflective of a bigger population cohort as in the UKPDS trial. However Moss et al studied a larger cohort of diabetic subjects in USA and in these individuals baseline smoking history was categorized by status and pack-years smoked while diabetic. After controlling for known risk factors for the incidence and progression of DR, pack-years smoked was of borderline significance (P= 0.052) in predicting incidence of DR in younger-onset subjects. Smoking was not associated with incidence in older-onset subjects or with progression or progression to PDR in any of the groups. (Moss SE et al. 1991). Moss et al further reported the association between cigarette smoking and the incidence and progression of DR by studying a large population-based cohort study who participated in baseline, 4-year, and 10-year examinations. Neither smoking status nor pack-years smoked showed significant associations with increased risk of DR. Thus cigarette smoking was not noted to be a risk factor for the long-term incidence of DR in these two studies of larger population cohort. (Moss SE et al. 1996). These studies supported the earlier work of Klein et al. in 1983 that showed that there was no association between smoking history and DR. There was also no established relationship between the risks or severity of DR and number of cigarettes

smoked daily, or the number of pack-years smoked while diabetic. Overall, these data suggest that there is no excess risk of DR in smokers or ex-smokers when contrasted with those who never smoked (Klein R et al. 1983). This does suggest that meta-analyses might be needed to clarify the exact relationship of DR with smoking status.

In summary, although a majority of studies have not established a definitive relationship between smoking and incidence and progression of DR, it does not imply that persons with DM who smoke should not stop as cigarette smoking is a risk factor for other complications and associated conditions of DM, particularly cardiovascular and respiratory disease.

1.4 Summary Hypothesis and Aims

1.4.1 Summary

To summarise, the mechanisms for the development of DR are multifactorial, inter-related and complex. Genetic and environmental risk factors interact early on in the disease process to lead to the initial background changes. The effect of the various risk factors on the pathogenesis of DR evolves with the progression of the disease process. A complete and thorough understanding of the various risk factors contributing to the prevalence and incidence of DR will enable early intervention and thereby prevent the complication of DR, which can lead to visual impairment and

have an effect on day-to-day lifestyle. Timely and early interventions are imperative, in order to slow down or impede the development of sight threatening DR.

1.4.2 Hypothesis

The first research hypothesis was that along with the overall glycaemic exposure (routinely expressed by HbA_{1c}), both the fasting and postprandial components of glycaemic exposure act in concordance and independently to affect the prevalence, incidence and progression of diabetic retinopathy. The second hypothesis was that β-cell dysfunction (fasting and postprandial) is the basis of this overall dysglycaemia leading to diabetic retinopathy.

1.4.3 Aims of the Thesis

The aim of this thesis has been to assess the cross sectional and longitudinal relationships between the metabolic and clinical risk factors and DR in a group of newly diagnosed T2DM subjects. Data from standard meal tolerance tests and intravenous glucose tolerance tests in this group provides a unique insight into the dynamic secretory responses to meal and IV glucose stimuli. Subjects were studied at diagnosis of T2DM and at years 1, 2 and 5 years post diagnosis.

The following were analysed:

i) Prevalence of DR in newly diagnosed T2DM subjects (Chapter 3)

ii) Incidence of DR in the subjects with no DR at diagnosis followed up over a 5 year period (Chapter 4)

iii) Progression of DR in the subjects with DR at diagnosis followed up over the 5 year period (Chapter 5)

This thesis examined the impact of the putative risk factors: $HbA_{1c,}$ fasting and postprandial glucose and insulin measured during the MTT and FSIVGTT on each of the above mentioned outcomes. In addition the relationship of fasting and postprandial β-cell responsiveness, Acute Insulin Response to IV glucose (AIR_G), Insulin Sensitivity (S_I), and Glucose Effectiveness (S_G) was also estimated and compared against these outcomes.

Chapter 2

METHODS

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2.1 Study design

2.1.1 Study subjects

661 newly diagnosed treatment naïve subjects, with T2DM (GAD antibody negative) were entered into the study. The subjects were originally referred by primary care physicians on clinical presentation and underwent Oral Glucose Tolerance Test (OGTT) or Fasting Glucose at the Biochemistry department, Llandough Hospital to confirm diagnosis as per WHO criteria (World Health Organisation 1985) (Table 2.1). All study participants were investigated within 1-2 weeks following diagnosis of T2DM and prior to any intervention with either lifestyle advice and/or hypoglycaemic medications. The study was conducted between 1981 and 2007. Following informed consent, subjects were assessed on one or two consecutive days at a metabolic unit during which they all had a general medical examination and underwent a standardised Meal Tolerance Test (MTT) on the 1st day with a smaller group having in addition an Intravenous Glucose Tolerance Test (IVGTT) on the 2nd day. They all also had retinal photographs taken at this time. Ethical approval for the study was obtained from South Glamorgan/Bro Taf Local Research Ethics Committee.

Table 2.1: WHO criteria employed for diagnosis of Type 2 Diabetes Mellitus (World Health Organisation 1985)

Type 2 Diabetes Mellitus	Venous Plasma Glucose (mmol/L)
Fasting and/or	≥ 7.8
2 hours after 75gm oral glucose load	≥ 11.1
Impaired Glucose Tolerance	
Fasting and/or	< 7.8
2 hours after 75gm oral glucose load	≥ 7.8 and ≤ 11.1
Normal Glucose Tolerance	
Fasting and/or	< 7.8
2 hours after 75gm oral glucose load	< 7.8

Table 2.2: Inclusion and exclusion criteria for the study subjects

Inclusion criteria

The patient:

	1	is aged	(30 – 70 years)
either	2	has a fasting blood glucose of	(>7 mmol/L)
or	3	has a random blood glucose of	(>11mmol/L)
	4	is newly diagnosed	
	5	is Caucasian	

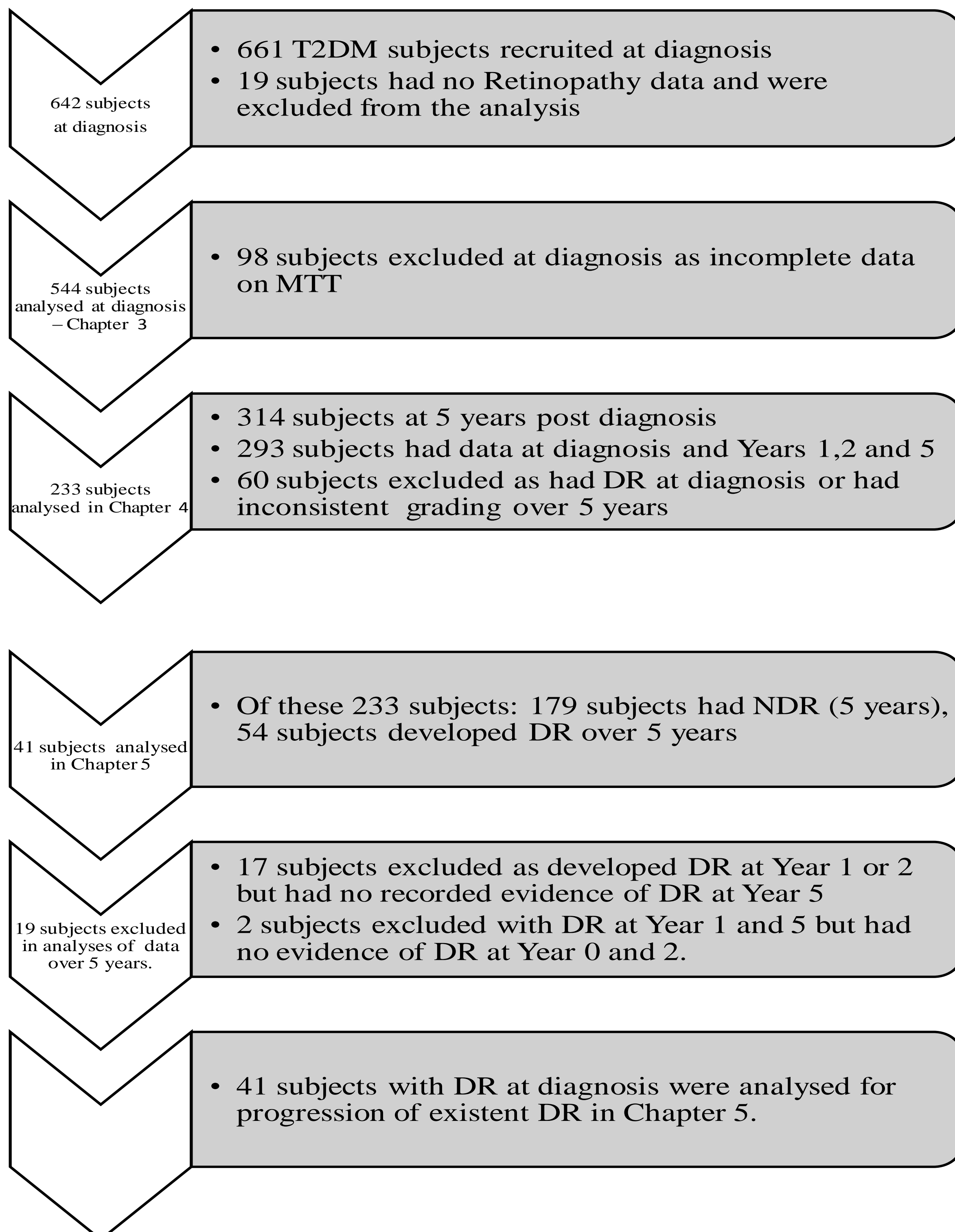
Exclusion criteria

The patient

6	has not commenced treatment for diabetes
7	is not receiving treatment for hypertension
8	is not suffering from a severe concomitant disease
9	is not taking the following concomitant medication: thiazide diuretics any corticosteroids hormone replacement therapy anti-anginal drugs (e.g. beta blockers, calcium antagonists)

These subjects were followed up for a period of 5 years during which time they had further general medical reviews (clinical examination, biochemical and haematological screening) and metabolic and retinopathy assessment at 1, 2 and 5 years. Detailed numbers of subjects studied over the study period is summarized in (Table 2.2). Some subjects were lost to follow up due to non-attendance, illness, relocation, transfer of care to GPs, and death. In addition subjects underwent routine annual reviews during the study period. Over the study period treatment consisted of diet, oral hypoglycaemic agents, insulin therapy or combination therapies as deemed necessary by the responsible health care professional. Regular contact was maintained with subjects and an appointment reminder system helped to minimise study dropouts. I was involved in the follow up studies of the subjects between 2008 and 2010.

Table 2.3: Subjects studied over the study period



2.2 Clinical Methods

2.2.1 Experimental protocol of metabolic challenge tests

The T2DM subjects were admitted to the metabolic unit at the University Hospital of Llandough following a 12-hour overnight fast and remained on bed rest throughout the morning of each of the study days. The subjects were advised to maintain normal diet and be rested for 3 days prior to the tests.

Each subject's height and weight were measured using a standard balance machine. Body Mass Index (BMI) was calculated (weight in kg/height in m^2). Blood pressure was measured after 10 minutes rest in the supine position using a sphygmomanometer. A 17 gauge luer-lock venflon (Ohmeda AB, Helsingborg Sweden) was inserted into an anticubital fossa vein and fasting samples were taken for plasma glucose, HbA_1 (1981-1995), HbA_{1c} (1995 onwards), total serum cholesterol (TC), HDL-C and LDL-C cholesterol, total serum triglycerides (Tg), urea and creatinine concentrations In addition serum immuno-reactive insulin (IRI) (1981-1994), serum specific insulin (ELISA 1990 onwards), C-peptide were measured to assess β-cell function and Insulin Sensitivity (IS).

The study extended over a long period of time during which certain assay changes occurred i.e. glycosylated haemoglobin and insulin assays. HbA_1 measured in the early part of the study was converted to HbA_{1c} utilising the formula (HbA_{1c} =

$0.83HbA_1$ - 0.54) (Cull CA et al. 1997). The insulin assay is described in section 2.2.5.

2.2.2 The Meal Tolerance Test (MTT)

All subjects underwent a standardized Meal Tolerance Test (MTT). This involved consuming a 500-kcal meal (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) (Owens DR et al. 1996). Subjects were required to consume the whole meal within 10 min (0 to 10 minutes). All subjects remained resting in a supine position throughout the test period with no smoking allowed. Blood samples were taken at -30 and 0 (fasting) minutes and thereafter at frequent intervals up to 240 minutes, to estimate plasma glucose, insulin, and C-peptide concentrations. The MTT, considered more physiological than an oral glucose tolerance test (OGTT), was a standardised mixed meal nutrient challenge, to determine the degree of glucose intolerance and pancreatic β-cell response. Approximately 30 seconds before a sample was due, the saline was stopped and approximately 2 mls of blood was withdrawn and subsequently discarded to remove all of the saline and diluted blood from the catheter. Following this a further 5 to 10 mls of blood was drawn for the sample to be assayed. This procedure has been previously validated.

2.2.3 The Insulin Modified Frequently Sampled Intravenous Glucose Tolerance Test (FSIVGTT)

A smaller subgroup of subjects also underwent a ‘Frequently Sampled Intravenous Glucose Tolerance Test’ (FSIVGTT), (Bergman RN et al. 1981) to determine the state of insulin and glucose sensitivity, in addition to the β-cell response. This was carried out on the second day, again after an overnight fast, involving the intravenous administration of 0.3 gm of glucose per kg body weight given at 0 minutes and infused over a 2-minute period. Thereafter, blood samples were taken at minute intervals up to 10 minutes. An intravenous bolus of insulin (Actrapid; Novo Nordisk, Bagsvaerd, Denmark) was then injected at 20 minutes at a dose of 0.05 U/kg insulin and further blood samples were collected at 22, 23, 24, 25, 27, 30, 40, 50, 60, 70, 80, 90, 100, 120 and 180 minutes (Owens DR et al. 1996). At each time point, measurements of plasma glucose, c-peptide and insulin were made. Assays used are described in Section 2.2.5

As for the MTT, the FSIVGTT involved the insertion of an indwelling intravenous cannula into an antecubital fossa vein in the forearm of the subject and connected via a three-way tap to a slow-running saline infusion, to maintain the patency of the cannula and allowing for repeated blood sampling with least inconvenience to the patient.

2.2.4 Assessment of retinopathy

Both eyes were dilated using Tropicamide (1%) and retinal images were obtained through dilated pupils. Two 45° images were taken, one centred on the macula and the second a posterior pole image with the optic disc position one disc diameter from the edge of the image (Figure 2.1). For subjects presenting from 1981 to 1986, ophthalmoscopy and Polaroid images (Canon CR3-45NM) were employed. From 1986 onwards, ophthalmoscopy and 35 mm colour transparencies (CR4-45NM retinal camera, Kodak Ektachrome EPR 64 film) were used. In 1994, the film was changed to Kodak Ektachrome EB 100. Use of digital images was started around 2000 with some overlap and was routinely used from 2002 onwards.

A) B)

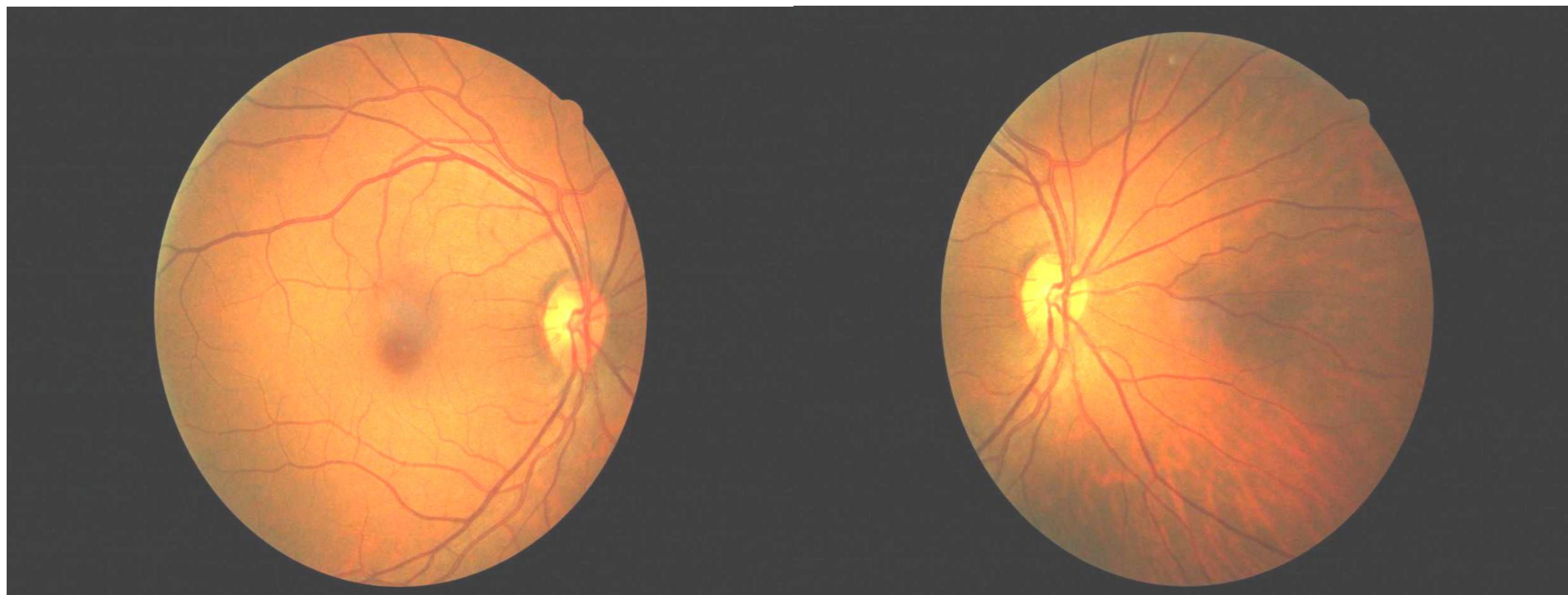


Figure 2.1: Two standard retinal fields A) macular centred and B) nasal (Images from DRSSW)

The retinal images were graded by an experienced diabetologist with the highest grade for both eyes used for classification. All images were then regraded by a senior retinal grader against the newer DRSSW grading protocol and any differences were reconciled by reference to a second diabetologist and ophthalmologist to arrive at the final grading.

Classification of DR was based on the Diabetic Retinopathy Screening Service for Wales (DRSSW) grading protocol (Table 2.3), which is an enriched version of the UK National DR grading protocol (Harding S et al. 2003).

The retinal images were graded as showing: No DR (no diabetic retinopathy changes seen), Background DR (BDR): Mild, moderate and severe BDR including those with possible maculopathy (M1), Pre-Proliferative (PPDR), Proliferative DR (PDR) and Exudative Maculopathy (M2).

Table 2.4: Grading Protocol of Diabetic Retinopathy Screening Service for Wales (DRSSW)

R0	No DR
R1	Background DR (BDR)
R1.1	Mild BDR • < 5 Mas > 1 DD from fovea • < 4 Hms > 1 DD from fovea • 3 Mas < 1 DD from fovea • ≤ 3 MA < 1 DD from fovea with VA better than 6/12 • exudates > 2 DD from fovea with or without CWS (< 5)
R1.2	Moderate BDR • ≥ 5 MAs > 1 DD from fovea • ≥ 4 < 8 HMs > 1DD from fovea • > 3 MAs < 1 DD from fovea with VA > 6/12 • Circinate or grouped exudates > 2 DD from fovea but within arcades • Questionable IRMA *only in the presence of MA/HM*
R2	Severe BDR (PPDR) • ≥ 8 blot haemorrhages *per eye* (superior and inferior hemi-fields) • Venous irregularities, beading, reduplication, venous loops (but not on their own) • Definite IRMA With or without CWS (but not CWS on their own)
R3	PDR/ADED • New vessels on disc (NVD) • New vessels elsewhere (NVE) • Pre-retinal haemorrhage • Vitreous haemorrhage • Pre-retinal fibrosis • Traction retinal detachment
M0	No Maculopathy
M1	Possible Maculopathy • Exudates < 2 DD >1DD from fovea • > 3 Mas <1 DD from fovea with VA < 6/12 • Hm < 2DD from fovea
M2	Definite Maculopathy • Exudates < 1 DD from fovea • Retinal thickness changes < 1 DD from

No DR - no diabetic retinopathy; BDR - background diabetic retinopathy, mild, moderate and severe (PPDR - pre-proliferative diabetic retinopathy); PDR - proliferative diabetic retinopathy; ADED - advanced diabetic eye disease;

MA - microaneurysm; Hm - haemorrhage; Ex - exudate; IRMA - intra-retinal microvascular abnormalities; CWS - cotton wool spots; DD – disc diameter;

The various grades of DR have been illustrated in Chapter 1: Figures 1.14-1.18

2.2.5 Assay methods

Blood was taken into fluoride oxalate for measurement of glucose (YSI 2300, YSI, Hants, UK) and into lithium-heparin for measurement of C-peptide and specific insulin (Andersen L et al. 1993).

Glucose was assayed using 1) the glucose oxidase method (model 2300 Yellow Springs Analyzer, YSI, Inc., Yellow Springs, OH) with the intra-assay coefficient of variation <4%; 2) The Instrumentation Laboratories glucose oxidase assay (ILab 300 plus clinical chemistry analyser) with the intra-assay coefficient of variation ≤ 2.5%.

Specific insulin was measured using a 2-site immunochemiluminometric assay (ICMA); Invitron Ltd, Monmouth UK) comprising monoclonal capture and labelled antibodies. The intra-assay coefficient of variation was < 7.5%.

Although the majority of insulin samples were assayed using the specific insulin assay, some of the earliest collected samples were assayed using a less specific immunoreactive insulin (IRI) radioimmunoassay (Heding, 1972). Cross reactivity with intact proinsulin was approximately 60%, which resulted in a higher measured IRI concentration than for the measured 'specific' insulin.

In order to enable the samples that were assayed using IRI only to be included in the final insulin analysis, a conversion factor was generated. 2290 samples were assayed using both the less specific IRI and the specific insulin assay. The resulting data were plotted and after removal of outliers, a regression equation was generated:

Y = 1.1755X + 58.264, where Y = IRI and X = specific insulin, with R^2 of 0.7472 was rearranged to generate:

Specific Insulin = (IRI – 58.264)/1.1755.

As this equation had the potential to generate negative specific insulin values, after discussion with a statistician the regression line was altered to have an intercept of zero. The subsequent equation Y = 1.3819X with R^2 of 0.7038 was rearranged to give:

Specific insulin = IRI/1.3819.

C-peptide was measured using a 2 site assay (ICMA; Invitron Ltd, Monmouth UK) comprising polyclonal capture antibody and monoclonal labelled antibodies. The intra-assay coefficient of variation was < 8%.

2.3 Metabolic data modelling

2.3.1 Baseline parameters

Glucose, insulin and C-peptide levels: Fasting plasma glucose (FPG), plasma insulin (FPI) and plasma C-peptide levels were measured. The postprandial plasma glucose (PPG), plasma insulin (PPI) and postprandial plasma C-peptide were represented by the 120 minute values and the areas under the curve ($AUC_{0\text{-}240mins}$) estimated up to 4 hours during the MTT.

2.3.2 Homeostasis model assessment (HOMA)

The Homeostasis Model Assessment (HOMA) estimates steady-state β-cell function (% B) and insulin sensitivity (% S), as percentages of a normal reference population. HOMA-B, HOMA-S and HOMA-IR (Insulin Resistance) were calculated employing only the fasting plasma glucose and specific insulin levels obtained at time 0 minutes during the MTT using the Homeostasis Model Assessment (HOMA; version 2.2.2) (Levy JC et al. 1998) Figure 2.2.

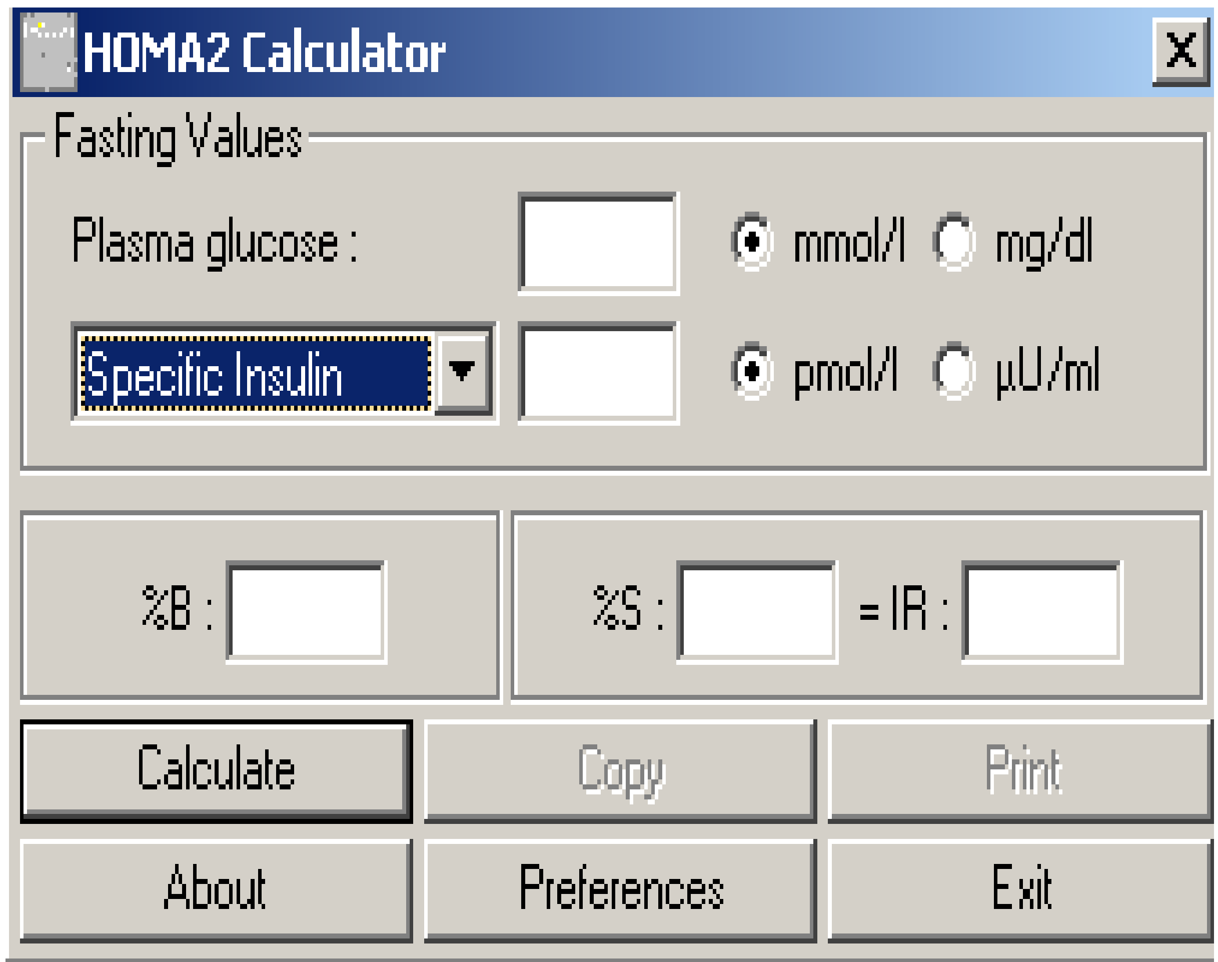


Figure 2.2 shows the HOMA model *utilising the fasting plasma glucose and specific insulin levels obtained at time 0 minutes during the MTT to calculate steady state β-cell function (% B) and insulin sensitivity (% S) and insulin resistance (IR).*

2.3.3 CPR program

The CPR (Calculating Pancreatic Response) program (Hovorka R et al. 1998)-(Figure 2.3) was used to quantify pancreatic ß-cell responsiveness during the MTT. Fasting β-cell responsiveness (M_0) is the ability of fasting glucose to stimulate insulin secretion and postprandial β-cell responsiveness (M_1) is the ability of the postprandial glucose to increase insulin secretion in response to a meal. The M_0 is the C-peptide response to fasting glucose representing fasting prehepatic insulin secretion and the M_1 is the C-peptide response to postprandial glucose representing the increase in prehepatic insulin secretion in response to an increment in postprandial glucose (Hovorka R et al. 1998).

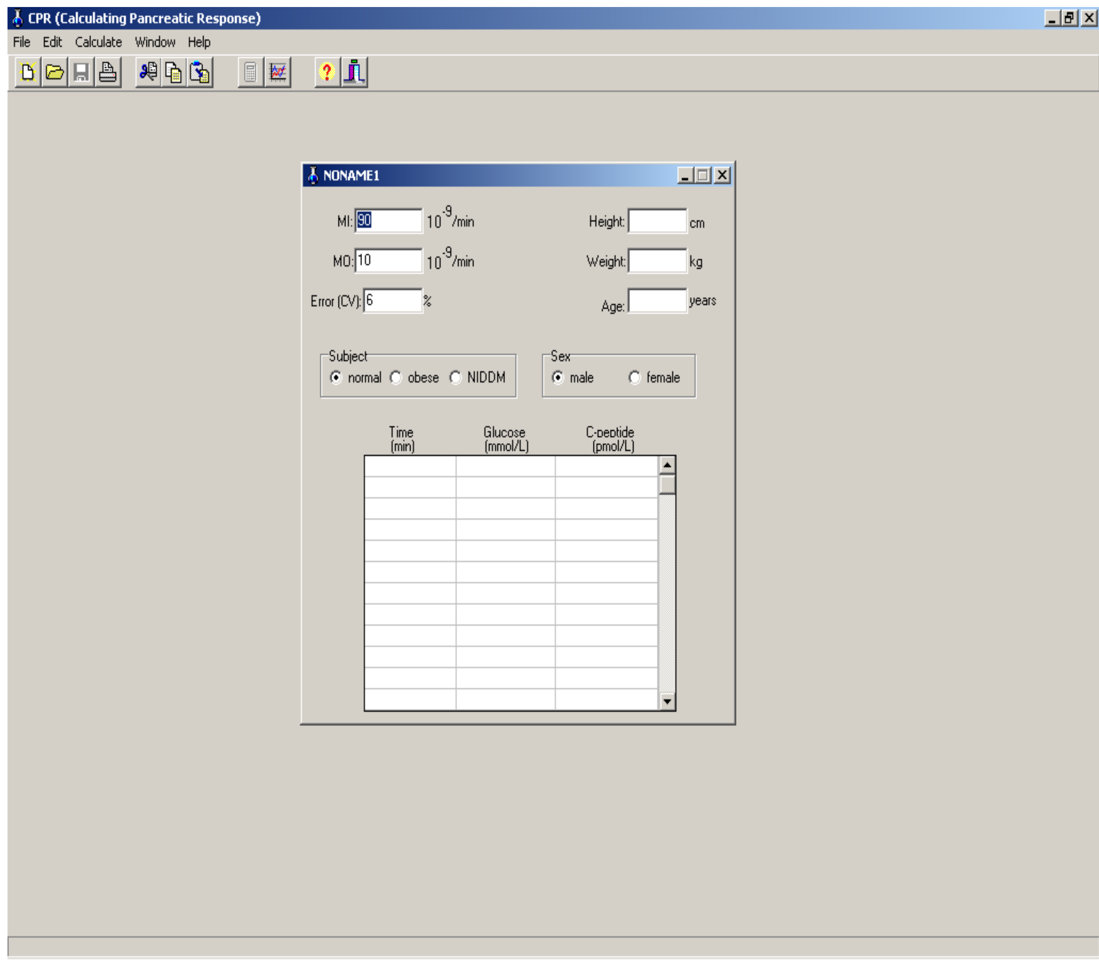


Figure 2.3 shows how the CPR program *quantifies pancreatic ß-cell responsiveness during the MTT. It takes into account the height, weight, age, sex, type of subject (normal/obese/T2DM) along with the glucose (mmol/L) and C-peptide (pmol/L) measurements at 30-minute intervals over a 4-hour period.*

2.3.4 The minimal model (MINMOD)

The acute 'first phase' insulin response to glucose (AIR_G) was calculated as the incremental area under the insulin curve from 0-10 minutes during the FSIVGTT (Kahn SE et al. 1993).

The Minmod program (developed by Bergman and Pacini) using the software IS_CIBA (G Mehring, Novartis) was used (Figure 2.4). 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) (Bergman RN et al. 1985 ; Bergman RN et al. 1992).

Both S_I and S_G are measures of insulin sensitivity; the former measures insulin sensitivity at an incremental insulin concentration, the latter at the basal insulin concentration. Thus S_G reflects how much of the glucose pool is cleared per minute at basal insulin and S_I demonstrates, how much 1 unit of insulin changes the glucose disposal (min^{-1} per mU/L).

The Disposition Index (DI), a measure of the overall ability of the islet cells to secrete insulin normalised to the degree of insulin resistance, was calculated as DI = S_I x AIR_G (Kahn SE et al. 1993).

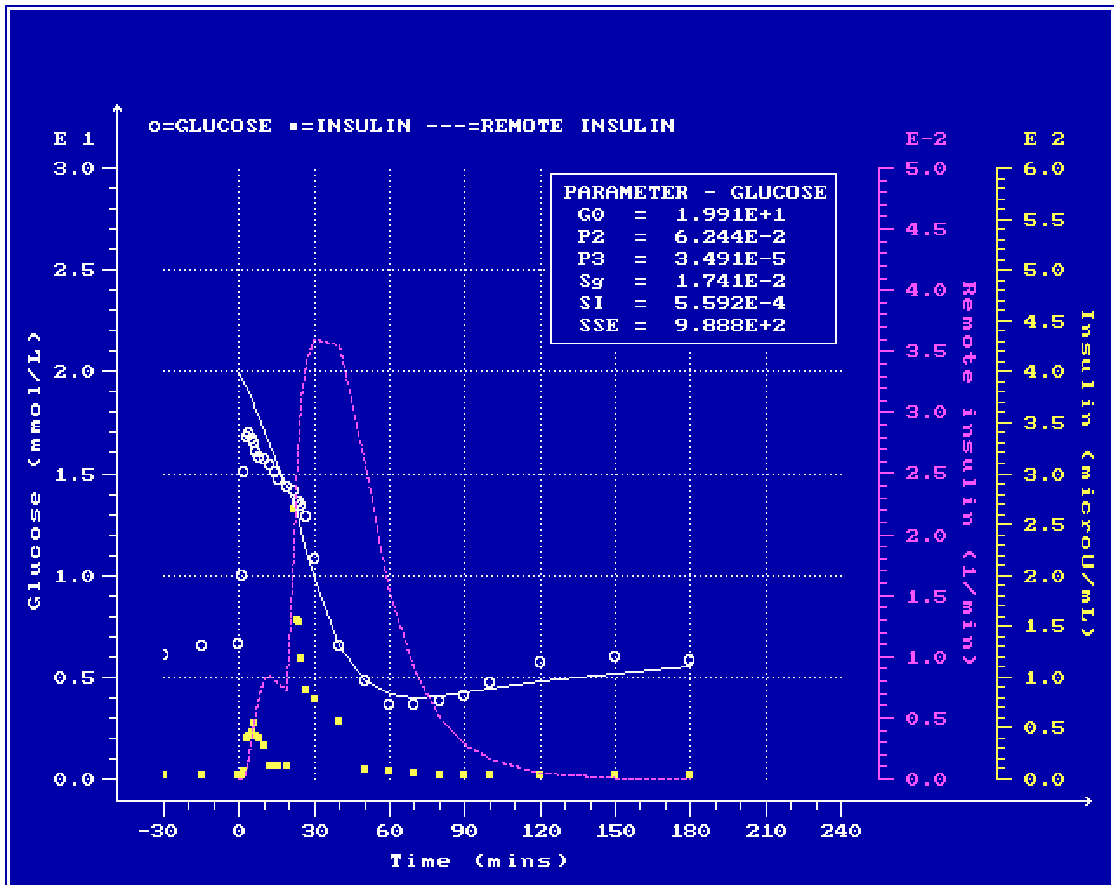


Figure 2.4: IS_CIBA sample analysis *shows how plasma glucose and insulin levels measured at frequent intervals over 180 minutes is utilised by the program to calculate* S_I *and* S_G.

2.4 Statistical Methods

All analyses were carried out using SPSS 20 statistical computer software. $P < 0.05$ has been taken to denote statistical significance throughout this thesis unless otherwise stated.

The AUC was calculated using the "Trapezoidal rule" i.e. [(concentration at $time_1$ x 0.5) + concentration t_2 + concentration t_3 + concentration t_4 ……+ (concentration t_{last} x 0.5)] x sample time interval. Its measured unit is concentration unit x time unit e.g. pmol/l x h or pmol.h/l.

2.4.1 Data structure

Data was collected on all the participant newly diagnosed Type 2 diabetic subjects. Demographic variables and blood chemistry variables were recorded. Other variables i.e. BMI (described in chapter 2.2.1) were calculated from these measures.

Table 2.5: Clinical variables recorded during the study

Recorded Variables	**Units**
Age	years
Weight	kg
Height	m
Blood Pressure	
Systolic blood pressure	mmHg
Diastolic blood pressure	mmHg
Plasma Lipids	
Total Cholesterol (TC)	mmol/L
High Density Lipoprotein Cholesterol (HDL-C)	mmol/L
Total Triglycerides (TG)	mmol/L
Renal function	
Creatinine	mmol/L
Urea	mmol/L
Plasma Glucose	
Fasting Glucose (FPG)	mmol/L
Postprandial Glucose (PPG) (120 mins)	mmol/L
Glycosylated Haemoglobin (HbA_1)	% (1981-1994)
Glycosylated Haemoglobin (HbA_{1c})	% (1995 onwards)
Plasma C-peptide	
Fasting C-peptide (FCP)	nmol/L
Postprandial C-peptide (PCP)	nmol/L
Plasma Insulin	
Fasting Immuno-reactive Insulin (IRI, 0 mins)	pmol/L (1981-1994)
Postprandial Immuno-reactive Insulin (IRI, 120 mins)	pmol/L (1981-1994)
Fasting Specific Insulin (0 mins)	pmol/L (1991 onwards)
Postprandial Specific Insulin (120 mins)	pmol/L (1991 onwards)

Table 2.6: Categorical variables recorded during the study

Categorical Variables	Coding
Sex	0 Female
	1 Male
Smoking Status (SMOK)	0 non-smoker
	1 ex-smoker
	2 current smoker
Family History (FH)	0 no family history
	1 first degree relative with Type 2 Diabetes
Treatment (at 1,2 and 5 years)	0 Diet
	1 Oral Hypoglycaemics
	2 Insulin or Combination therapy

Table 2.7: Derived variables calculated for study subjects

Derived Variables	**Units**
BMI	kg.m^2
MTT derived	
AUC $_{Glucose\ (0-240min)}$	mmol/L
AUC $_{C-peptide\ (0-240min)}$	nmol/L
AUC $_{Insulin\ (0-240min)}$	pmol/L
M_0	*10^{-9} pmol/kg/min
M_1	*10^{-9} pmol/kg/min
HOMA-B	%
HOMA-S	%
HOMA-IR	
FSIVGTT derived	
S_I x 10^{-4}	(microU/ml)$^{-1}$.min^{-1}
S_G x 10^{-2}	min^{-1}
$AIR_{G\ (0-10min)}$	microU/ml. min
DI x 10^{-2}	

2.4.2 General statistical tests

Descriptive analysis of the data with continuous and normally distributed data was recorded as mean ± standard deviation, (SD) and, when not normally distributed, represented by median and interquartile range (IQR). Normality was checked using qq plots. Categorical data has been presented as total numbers (n) and percentages (%).

The Student's t-test has been used to compare the means of two groups and analysis of variance (ANOVA) employed to compare the means of more than two groups for normally distributed continuous data. Mann-Whitney U and Kruskal Wallis tests were used for non-normally distributed continuous variables and Pearson chi-squared test was used for categorical data. P values of <0.05 were taken as statistically significant.

We also calculated the average/mean of all the measured metabolic variables from diagnosis including variables at Years 1, 2 and 5 years post diagnosis (Chapter 4 and 5). These mean averaged metabolic variables of T2DM subjects have been defined as the follow-up indicator of diabetic control over 5 years.

2.4.3 Univariate Logistic Regression Analyses

A substantial numbers of tests were performed, to indicate differences between sub-groups for the identification of possible important explanatory variables. This in

addition to univariate regression methods gave a smaller subset of variables for multivariable analysis. These designated putative risk factors were assessed using logistic regression methods for the presence or absence of DR (Binary outcome). The non-normally distributed variables were log transformed.

Odds ratios with 95% CI have been used to denote the likelihood of an event occurring. An odds ratio equal to one occurs when the odds are the same in two groups and represents no association between the exposure and the disease (Kirkwood BR and Sterne JAC 2003). Odds ratios (OR) less than one are interpreted as the event being less likely to occur for an increase in the predictor variable, whereas odds ratios larger than one are interpreted as the event being more likely to occur.

2.4.4 Multivariate Logistic Regression Analyses

Multiple regression is a technique which analyses how dependant variables are influenced by predictor variables. Thus it is utilised to establish a prediction equation for the dependant variable from a number of predictor variables. All multivariate analyses were adjusted for age, gender, BMI and risk factors like SBP and TC which have previously been reported to have an association (Klein R et al. 1989b; Chew EY et al. 1996; UK Prospective Diabetes Study Group 2004) with DR. In our study a non-correlated subset of clinical and metabolic variables was determined based on statistical and clinical relevance

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Chapter 3
Retinopathy at diagnosis in subjects with Type 2 diabetes mellitus

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3.1 Introduction

A relatively recent analysis based on the 2010 global estimate of persons with DM at 285 million reported that approximately 93 million people had evidence of DR, 17 million with proliferative DR, 21 million with diabetic macular oedema, and 28 million with vision-threatening DR worldwide (Yau et al. 2012). In subjects with T2DM the prevalence estimates for DR ranged from 7 to 55.0% in the USA, 21.0 to 52.0% in the UK and 18.8 to 65.9% in Scandinavia (Williams R et al. 2004).Thus screening, early detection of DR and treatment of known and modifiable risk factors is imperative. Approximately 17-18 % of subjects with T2DM have some lesions of DR at diagnosis, 40 to 60% after 20 years and approximately 10% developing sight-threatening lesions related to either exudative maculopathy and/or proliferative DR after 20 years of known DM duration (Stefansson E et al. 2000).

Various risk factors are associated with the development and progression of DR including hyperglycaemia (Zhang X et al. 2010), duration of DM (Zhang X et al. 2010), hypertension and dyslipidaemia (Klein R et al. 1989b; Chew EY et al. 1996; UK Prospective Diabetes Study Group 2004). 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 (The Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications Research Group 2000 Feb; Holman RR et al. 2008). Furthermore the UKPDS had shown that for every 1% decrease in HbA_{1c}, there was a 37% risk reduction in microvascular complications in T2DM, predominantly DR and 14% reduction in all-cause mortality (Stratton IM et al. 2006) . The ACCORD Eye Study Group has

shown that intensive glucose and lipid lowering, but not intensive blood-pressure control, reduces the rate of progression of DR (ACCORD study group and Accord Eye Study Group 2010). In the ACCORD follow-on study (ACCORDION) in T2DM subjects with established cardiovascular disease and duration of DM>10 years re-affirmed that early intensive glycaemic control continued to reduce the risk of DR progression (ACCORD Study Group 2016).

In individuals with T1DM, the DCCT showed that the total glycaemic exposure (HbA_{1C} and duration of diabetes) explained 11% of the variation in retinopathy risk in the complete cohort (The Diabetes Control and Complications Trial Research Group (DCCT) 1995) so that other factors may potentially explain the other 89% of the variation in risk among subjects independent of HbA_{1C}. These will include environmental, genetic factors as well as glycaemic variation and other measures of glycaemia, on their own or through an inter-correlation 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 (Miki E and Kikuchi M 1994; Stratton IM et al. 2006) with few examining dysglycaemia in more detail and very little reference to the role of β-cell function and insulin resistance. However, in 2005, Shiraiwa et al. suggested that postprandial hyperglycaemia and postprandial hypoinsulinaemia were predictors for incident DR in Japanese T2DM subjects who

were not on insulin treatment (Shiraiwa T et al. 2005a). The Diabetes Prevention Program (DPP) studied subjects with impaired glucose tolerance and recent onset T2DM, and found a higher baseline systolic blood pressure (SBP) and HbA_{1c} amongst those with DR, but found no difference in insulin secretion (measured 30 minutes post glucose challenge) as estimated by the Corrected Insulin Response (CIR) (Diabetes Prevention Program Research Group 2007) calculation. Their prevalence of DR was 8% in the subjects noted to be in the 'pre-diabetic' state and was 12% in the T2DM subjects within 3 years of diagnosis. In contrast a community-based study in Taiwan demonstrated that both β-cell dysfunction and increased insulin resistance (IR) (both measured by the HOMA methodology) were associated with the presence of DR in established T2DM patients (Tung TH et al. 2007). Similarly, over the last decade, there have been other reports associating IR with DR (Katsumori K et al. 1995; Suzuki M et al. 2000; Nakano S et al. 2003).

3.2 Hypothesis and Aim of this analysis:

The first research hypothesis was that along with the overall glycaemic exposure (routinely expressed by HbA_{1c}), both the fasting and postprandial components of glycaemic exposure act in concordance and independently to affect the prevalence, of DR in newly diagnosed type 2 diabetic subjects. The second hypothesis was that β-cell dysfunction (fasting and postprandial) is the basis of this overall dysglycaemia leading to DR in these subjects.

The inter relationship between β-cell function, glucose effectiveness (S_G), insulin sensitivity (S_I), fasting and postprandial dysglycaemia, with the presence of DR in newly-diagnosed subjects with T2DM has not previously been reported. The aims of this study were to investigate the relationship between these and the presence of DR in newly-diagnosed previously-untreated T2DM.

3.3 Methods

In an attempt to minimise the number of confounding factors, such as duration of known T2DM and the use of different treatment modalities, we recruited only newly diagnosed and treatment- naïve T2DM subjects. 661 subjects were recruited at diagnosis. 19 subjects were excluded as they had no retinopathy data. A further 98 subjects were excluded from the analysis as they had incomplete MTT data. Thus 544 newly diagnosed treatment naïve subjects, with T2DM (Group A) were recruited into the study 1-2 weeks after diagnosis of DM prior to any intervention with either lifestyle advice and/or hypoglycaemic medications between 1981 and 2007.

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 modified IVGTT was not used until part way through the study accounting for the smaller sample size. Once the modified IVGTT was used, most subjects (apart from those where the intravenous cannula failed etc.) underwent both tests.

The methods chapter (Chapter 2) states in detail the subject recruitment, experimental protocol, data analysis and basic statistical analysis.

3.3.1 Statistical analysis

Following the initial descriptive analysis and comparison of the means of the two groups, the designated putative risk factors were assessed using logistic regression methods with non-normally distributed variables [(FPG, FPI, PPG, PPI, $AUC_{Glucose\ (0\text{-}240min)}$, $AUC_{Insulin\ (0\text{-}240min)}$, HOMA B, M_0, M_1 and S_g)] log transformed. A non-correlated subset of clinical and metabolic variables was determined based on statistical and clinical relevance. All multivariate analyses were adjusted for age, gender, BMI and putative risk factors systolic blood pressure and total cholesterol which have previously been reported to have an association (Klein R et al. 1989b; Chew EY et al. 1996; UK Prospective Diabetes Study Group 2004) with DR. The p value was calculated using the likelihood ratio test. All analysis were conducted using SPSS 20 with $p < 0.05$ taken as significant (two-tailed). Multivariate analysis was also conducted finally with the models including a parameter of β-cell responsiveness/ β-cell function and including a parameter of glycaemia, adjusting for the above mentioned variables.

3.4 Results

3.4.1 Baseline characteristics of subjects with type 2 diabetes at diagnosis (Table 3.1)

Of the 544 subjects (Group A), (393 male and 151 female, 2.6:1) with a mean age of 54 (SD±10) years, 16.5% (90) had evidence of DR whereas the remaining 83.5% (454) had no DR at the time of presentation. Of those with DR, the majority 84.4% (76) had lesions of BDR (R1.1, R1.2 with or without M1), 15.6% (14) had PPDR (R2) and none displayed exudative maculopathy (M2) or PDR (R3). In the subgroup of patients (Group B, n= 201) who underwent FSIVGTT in addition to MTT, 15% (30) had DR comprising 12.5% (25) with BDR and 2.5% (5) PPDR with none having exudative maculopathy or PDR. The remaining 85% (171) of subjects had no evidence of DR.

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 were significantly leaner i.e. had a lower body weight (p= 0.02) compared to those without DR, although the difference in BMI did not reach significance (p= 0.06). Subjects with DR had a non-significantly higher $HbA_{1c,}$ (p= 0.06). Apart from weight, other baseline characteristics were not significantly different between those with or without DR (Table 3.1).

Table 3.1: Baseline characteristics for subjects with No Diabetic Retinopathy compared to those with Diabetic Retinopathy at time of diagnosis of T2DM. Group A: 544 subjects who underwent MTT, Group B: 201 subjects who underwent MTT and FSIVGTT

Group A	**All subjects**	**No Diabetic Retinopathy**	**Diabetic Retinopathy**	**p value (between NDR and DR)**
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} (%)	7.7 (2.0)	7.7 (2.0)	8.0 (1.8)	0.06
Group B	**All subjects**	**No Diabetic Retinopathy**	**Diabetic Retinopathy**	**p value (between NDR and DR)**
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} (%)	7.6 (1.9)	7.6 (2.0)	7.7 (1.5)	0.80

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

3.4.2 Comparative analysis of metabolic and hormonal data in subjects with type 2 diabetes with and without retinopathy at diagnosis (Tables 3.2 and 3.3)

The metabolic variables during the MTT for group A subjects with or without DR are detailed in Table 3.2. Those subjects with DR in the fasting state had lower plasma insulin concentrations (p= 0.036), estimated β-cell responsiveness i.e. M_0 (p= 0.014) and β-cell function i.e. 'HOMA-B' (p= 0.044), associated with higher fasting glucose levels (p= 0.021). In the postprandial state, individuals presenting with DR had significantly higher postprandial (2 hour) glucose (p= 0.023) with lower postprandial insulin levels (p= 0.001). T2DM subjects with DR had numerically lower but non-significant (p= 0.065) postprandial β-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 $AUC_{Glucose\ (0\text{-}240min)}$ (p= 0.023) and lower $AUC_{Insulin\ (0\text{-}240\ min)}$ (p= 0.001) in comparison to those without DR (Table 2a). The glucose and insulin profiles and indices of β-cell responsiveness (M_0 and M_1) during the MTT in subjects with DR and NDR are illustrated in Figures 1a and b.

The baseline characteristics and the metabolic responses in group B subjects with DR and NDR who underwent FSIVGTT are detailed in Table 3.3. Whereas insulin sensitivity (S_I) was not significantly different between the two groups, 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 disposition index (DI) between those with or without DR.

Table 3.2: Comparison of the metabolic variables during the Meal Tolerance Test in subjects with No Diabetic Retinopathy and those with Diabetic Retinopathy at diagnosis of T2DM

Group A	**No Diabetic Retinopathy (n=454)**	**Diabetic Retinopathy (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**
$AUC_{Glucose\ (0\text{-}240min)}$ (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**
$AUC_{Insulin\ (0\text{-}240min)}$ (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

Table 3.3: Comparison of metabolic variables following Frequently Sampled Intravenous Glucose Tolerance Test in subjects with No Diabetic Retinopathy and those with Diabetic Retinopathy at diagnosis

Group B	**No Diabetic Retinopathy (n=171)**	**Diabetic Retinopathy (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 (1st – 3rd Inter Quartile Range)

FPG = Fasting Plasma Glucose; PPG = Post Prandial Glucose; AUC = Area Under the Curve
S_I = Insulin Sensitivity; S_G = Glucose effectiveness; $AIR_{G\ (0\text{-}10min)}$ = Acute Insulin Response to glucose; DI = Disposition Index

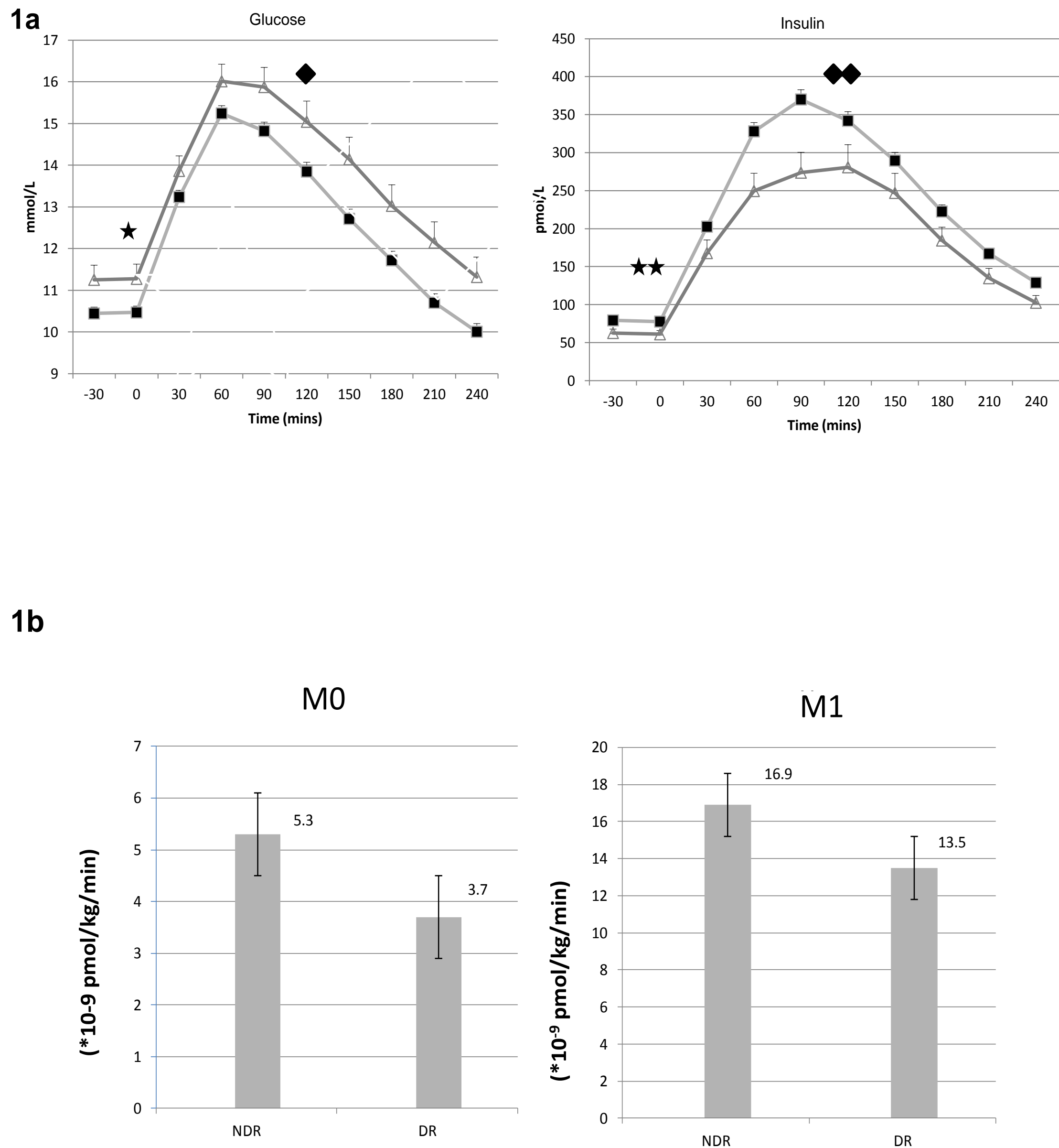

Figure 3.1: Glucose and Insulin Profiles with β cell responsiveness in patients with and without diabetic retinopathy.

1a) *Plasma glucose and insulin profile (mean ± SEM) during MTT in subjects with NDR (Filled squares) (n = 454) and those with DR (Open triangles) (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) (p = 0.014) and Post-prandial (M_1) (p = 0.065) β cell responsiveness (mean ± SEM) during MTT in subjects with NDR and those with DR at diagnosis of T2DM.*

3.4.3 Univariate and multivariate regression analysis (Tables 3.4 and 3.5)

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) show the contribution of fasting, postprandial and overall hyperglycaemic/insulinopaenic exposure in subjects leading to DR at diagnosis. However, in this group of subjects no significant relationship was observed for HbA_{1c} (OR 2.3 [95% CI 0.900, 5.859] p = 0.082) with DR, when adjusted for the mentioned variables although 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. In addition each 1 pmol/L decrease in fasting and postprandial insulin was associated with increased risk of DR by 24% and 34% respectively.

Based on the inter-group differences (Tables 2 and 3), univariate logistic regression was conducted which demonstrated that postprandial glucose, $AUC_{Glucose\ (0\text{-}240min)}$, postprandial insulin, $AUC_{Insulin\ (0\text{-}240min)}$, M_0, HOMA-B and S_G were significantly related to the presence of DR (Table 3.4).

Factors independently associated with DR in multivariate logistic regression analyses when adjusted for age, sex, BMI, total cholesterol and systolic blood pressure are detailed in (Table 3.5). Measures of β-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 independently associated with DR along with S_G (OR 0.20 [95% CI 0.066, 0.602] p= 0.004), reflecting contribution of an "insulin-independent component of glucose tolerance".

When a parameter of β-cell responsiveness/β-cell function and a parameter of glycaemia (HbA_{1c}/FPG/PPG) was included in multivariate logistic regression analyses adjusting for the above mentioned variables the parameters of β-cell responsiveness/β-cell function were significantly associated with the presence of DR, thus possibly reflecting that in this group of patients, failing β-cell function is the driving force behind the total glycaemic exposure whether it be from FPG or PPG or HbA_{1c} (Table 3.5).

Table 3.4: Univariate and multivariate logistic regression depicting variables independently associated with the presence of DR

	Number	Crude OR (95% CI)	p	Adjusted OR (95% CI)	p	OR (95% CI)	p
				(for age and sex)		(fully adjusted **)	
HbA1c (%)	506	2.329 (0.931, 5.823)	0.071	2.515 (0.997, 6.346)	0.051	2.296 (0.900, 5.859)	0.082
Fasting Glucose (mmol/L)	544	2.078 (0.982, 4.400)	0.056	2.238 (1.051, 4.765)	**0.037**	2.23 (1.038, 4.791)	**0.040**
Postprandial Glucose (mmol/L) (120 mins)	543	1.944 (1.004, 3.763)	**0.049**	2.054 (1.058, 3.987)	**0.033**	2.093 (1.063, 4.123)	**0.033**
$AUC_{Glucose\ (0\text{-}240min)}$ (mmol/L)	544	2.081 (1.021, 4.242)	**0.044**	2.196 (1.075, 4.487)	**0.031**	2.252 (1.087, 4.664)	**0.029**
Fasting Insulin (pmol/L)	494	0.782 (0.607, 1.007)	0.057	0.784 (0.607, 1.012)	0.061	0.759 (0.585, 0.986)	**0.039**
Postprandial Insulin (pmol/L) (120 mins)	534	0.681 (0.526, 0.882)	**0.004**	0.686 (0.529, 0.890)	**0.005**	0.664 (0.511, 0.863)	**0.002**
$AUC_{Insulin\ (0\text{-}240min)}$ (pmol/L)	534	0.625 (0.465, 0.840)	**0.002**	0.631 (0.468, 0.850)	**0.002**	0.612 (0.453, 0.828)	**0.001**
M_0 (*10^{-9} pmol/kg/min)	540	0.693 (0.514, 0.934)	**0.016**	0.697 (0.517, 0.940)	**0.018**	0.658 (0.484, 0.894)	**0.007**
HOMA-B (%)	494	0.750 (0.582, 0.968)	**0.027**	0.745 (0.577, 0.963)	**0.025**	0.739 (0.570, 0.958)	**0.022**
S_G x 10^{-2} (min^{-1})	201	0.206 (0.069, 0.618)	**0.005**	0.211 (0.070, 0.642)	**0.006**	0.200 (0.066, 0.602)	**0.004**

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

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

FPG = Fasting Plasma Glucose; PPG = Post Prandial Glucose; AUC = Area Under the Curve, S_G = Glucose effectiveness

Table 3.5: Univariate and Multivariate logistic regression depicting variables independently associated with the presence of DR

	Number	Crude OR (95% CI)		Adjusted OR (95% CI)		Adjusted OR (95% CI)	
a)				(fully adjusted *)		(adjusted for * and HbA1c ϴ)	
M_0 ($*10^{-9}$ pmol/kg/min)	540	0.693 (0.514, 0.934)	**0.016**	0.658 (0.484, 0.894)	**0.007**	0.647 (0.470 – 0.891)	**0.008**
HOMA-B (%)	494	0.750 (0.582, 0.968)	**0.027**	0.739 (0.570, 0.958)	**0.022**	0.719 (0.550 – 0.940)	**0.016**
b)				(fully adjusted *)		(adjusted for * and FPG ©)	
M_0 ($*10^{-9}$ pmol/kg/min)	540	0.693 (0.514, 0.934)	**0.016**	0.658 (0.484, 0.894)	**0.007**	0.655 (0.482 – 0.891)	**0.007**
HOMA-B (%)	494	0.750 (0.582, 0.968)	**0.027**	0.739 (0.570, 0.958)	**0.022**	0.739 (0.570 – 0.958)	**0.022**
c)				(fully adjusted *)		(adjusted for * and PPG ®)	
M_0 ($*10^{-9}$ pmol/kg/min)	540	0.693 (0.514, 0.934)	**0.016**	0.658 (0.484, 0.894)	**0.007**	0.655 (0.482 – 0.891)	**0.007**
HOMA-B (%)	494	0.750 (0.582, 0.968)	**0.027**	0.739 (0.570, 0.958)	**0.022**	0.739 (0.570 – 0.958)	**0.022**

* 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; PPG = Post Prandial Glucose

3.5 Discussion

Our analysis shows 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. Although HbA_{1c} was higher in the subjects with DR, it fails to reach significance ($p= 0.06$). Shiraiwa et al. studied Japanese T2DM subjects known to have DM but not on insulin treatment (Shiraiwa T et al. 2005a) and established postprandial hyperglycaemia but not HbA_{1c} to be independently correlated with the presence of DR (Shiraiwa T et al. 2005a) and stated postprandial hyperglycaemia as a possible predictor for incident DR in their subjects. Contrary to our findings, two recent studies from the 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 (Kostev K and Rathmann W 2012; Looker HC et al. 2012). The DPP study involving newly diagnosed T2DM subjects has also reported a higher HbA_{1c} amongst those with DR (Diabetes Prevention Program Research Group 2007).

Our findings have also demonstrated that the presence of DR is independently associated with reduced fasting and postprandial β-cell responsiveness, resulting in both fasting and postprandial hyperglycaemia. In this analysis there is an independent association of M_0 and HOMA-B with presence of DR by measuring β cell functio(Hovorka R et al. 1998)n in response to a standardised meal challenge, employing both the CPR program (Hovorka R et al. 1998) and the HOMA methodologies. Similarly the relationship between DR with β cell function (HOMA-

B) has been seen in a community-based study in Taiwan by Tung et al. (Tung TH et al. 2007) which involved 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 association between fasting β-cell dysfunction and DR in established T2DM patients as reported by Tung et al (Tung TH et al. 2007) is in agreement with our findings in our newly-diagnosed, treatment naïve, T2DM subjects. The UKPDS has also reported that the severity of retinopathy at diagnosis of T2DM was related in both sexes to reduced β-cell function with lower serum insulin levels, higher fasting plasma glucose, systolic and diastolic blood pressure (Kohner et al. 1998). These findings contrasts with the DPP study involving newly diagnosed T2DM subjects, where no difference in insulin secretion estimated by the CPR was found (Diabetes Prevention Program Research Group 2007). This is possibly because the loss of the first phase insulin response was not captured in DPP as it was the 30 minute insulin that was measured.

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 (Best JD et al. 1996). 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 (Ebeling P et al. 1988; Henriksen JE et al. 1994). Thus, in the presence of significant β-cell dysfunction and resultant insulinopaenia, a relatively poor S_G will further worsen glycaemia. In addition, the insulin-

independent component of glucose tolerance was reduced and independently associated with the presence of DR at diagnosis. This might explain our findings, where the newly diagnosed T2DM subjects with worse S_G are more likely to present with DR.

This analysis indicates significant contributions of β-cell dysfunction, fasting and postprandial hyperglycaemia/insulinopaenia and reduced glucose effectiveness to the presence of DR. Thus it adds to the evidence base of contributory factors towards development of diabetic retinopathy. Several epidemiological studies have confirmed the association between hyperglycaemia and the development of late diabetic complications (The Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complication (DCCT/EDIC) Study Research Group 2005; Holman RR et al. 2008). 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 (The Diabetes Control and Complications Trial Research Group (DCCT) 1995). Recent research has also suggested that postprandial glucose levels and glucose variability, may confer additional risks for the development of micro- and macro vascular diabetic complications (Brownlee M and Hirsch IB 2006; Hanefeld M and Temelkova-Kurktschiev T 2002).

Mechanistically, this may be explained by reports that hyperglycaemia contributes towards DR by increasing the expression of several growth factors (e.g. platelet derived growth factor and vascular endothelial growth factors) by inducing apoptosis of retinal cells (Mizutani M et al. 1996; Barber AJ et al. 1998). Similarly physiological concentrations of insulin have been shown to rescue cultured optic nerve cells from apoptosis and to be necessary for survival of retinal ganglion cells in culture medium (Barber AJ et al. 2001).

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 differed from other reports because it comprised both newly diagnosed, treatment naïve participants with T2DM, and thus lacked confounding effects of therapeutic interventions. By contrast, other cross-sectional studies have associated insulin sensitivity (assessed by euglycaemic clamp) with the presence or severity of DR (Katsumori K et al. 1995; Suzuki M et al. 2000; Nakano S et al. 2003). The numbers of subjects and controls in those studies were modest and the subjects recruited had established T2DM that was being treated with a variety of hypoglycaemic agents, both oral agents and insulin preparations. It is therefore unclear whether the association that they found was entirely independent of the underlying confounders such as duration and treatment modalities.

Thus, in our cohort of newly diagnosed T2DM subjects, a leaner group of subjects, (which could be a manifestation of the relative insulinopaenia of subjects presenting with DR) with a reduced β cell secretory/responsiveness and hyperglycaemia, presented at diagnosis with DR. These individuals did not have a significant contribution from diminished insulin sensitivity at the point of diagnosis.

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 diagnosis. Thus, we were able to rule out confounding factors like duration of DM and treatment modalities. 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 insensitivity. An independent association for the presence of DR with HbA_{1c} in subjects with newly diagnosed T2DM within the first year of their diagnosis (Diabetes Prevention Program Research Group 2007) (Kostev K and Rathmann W 2012; Looker HC et al. 2012) is well- recognized. However, our study fails to show this association with chronic glycaemia as represented by HbA_{1c}. A limitation of our study was that over its duration, because of developments in the measurement of HbA_{1c}, we employed two different assays, which may account for this difference. However, our study provides a detailed analysis of the metabolic and hormonal responses to different carbohydrate challenges in our population of T2DM subjects. The DPP reported more than 12% of subjects with T2DM had DR within approximately 3 years of diagnosis (Diabetes Prevention Program Research Group

2007). 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. In our study, diagnosis of DM was not through a standardized or uniform screening program; therefore, duration of DM prior to the study is unknown and may have been substantial especially during the early part of this study.

3.6 Summary

In our population of newly diagnosed treatment-naïve T2DM subjects:

- The prevalence of DR was noted to be associated with relatively worse fasting β-cell responsiveness and function with resultant relative insulinopaenia.
- The contribution of the insulin independent (as manifested by reduced S_G) component of glucose tolerance to the prevalence of DR was further noted.
- Thus as a consequence of the above mentioned we have exhibited a contribution of both fasting and postprandial hyperglycaemia and hypoinsulinaemia to the prevalence of DR.

Therefore, assessment of β-cell function may help to identify those subjects with T2DM at risk of developing DR who may benefit from early intensive treatment with current or newer therapies which help preserve β-cell function in order to achieve and maintain good diabetes control and limit progression to complications of DM.

Chapter 4

Diabetic Retinopathy status in subjects with newly diagnosed type 2 diabetes with no diabetic retinopathy at diagnosis 5 years post diagnosis

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4.6 Summary

4.1 Introduction

In 2004 Williams and colleagues published a systematic review on the incidence of DR, PDR and maculopathy in T1DM, T2DM or mixed cohorts based on 153 articles (Williams R et al. 2004). The incidence of DR in individuals with T2DM was 66.9% over 10 years in the USA and 22.0% over 6 years in the UK. Identifying the risk factors involved in the development and progression of DR is important in order to be able to define strategies in an attempt to prevent visual impairment and blindness caused by this complication. Despite differences in methodologies and populations studied, a number of studies have demonstrated that the prevalence and incidence of DR are influenced predominantly by the duration and type of DM, glycaemic and blood pressure control, dyslipidaemia and the use of insulin (Appendix Table 1). Other less consistent risk factors include ethnicity, age of onset of diabetes, gender, pregnancy and genetic makeup (Stewart LL et al. 1993). In the 1970's Kajinuma and colleagues also suggested that a past history of obesity during the preceding five year follow-up period increased the occurrence of DR (Kajinuma et al. 1983).

From the perspective of risk factors the overall glycaemic exposure in DM seems to be the cornerstone for the development and progression of DR, compounded by the duration of DM and hyperglycaemia (Keen H et al. 2001; Yoshida Y et al. 2001; Tapp RJ et al. 2006; Perol J et al. 2012). Most studies have assessed glycaemic control using measures of glycosylated haemoglobin in the form of HbA1c (Klein R et al. 1995; Stratton IM et al. 2001; Voutilainen-Kaunisto RM et al. 2001; Yoshida Y et al. 2001) and FPG (Voutilainen-Kaunisto RM et al. 2001; Janghorbani M et al.

2003; Tapp RJ et al. 2006; Tapp RJ et al. 2008) with a lesser number including the 2 hour PPG (Voutilainen-Kaunisto RM et al. 2001; Shiraiwa T et al. 2005b). In a group of Japanese subjects with T2DM, Takao et al. (Takao T et al. 2011) demonstrated that fasting plasma glucose variability when estimated (using standard deviation) over a prolonged period of time (27-44 years, mean 33 years) is an independent risk factor for PDR. Chen and colleagues further showed that T2DM subjects who had poor glycaemic control at or near the time of diagnosis and who remained poorly controlled over the subsequent four year follow-up period had a three times higher rate of development of diabetic retinopathy (31.0%) than those with better glycaemic control throughout the study (5.5%) (Chen MS et al. 1995).

However, the relationship between indices of β-cell function, glucose effectiveness (S_G) and insulin sensitivity (S_I), with the progression to DR in newly diagnosed subjects with T2DM, reviewed over a prolonged period of time (up to 5years) remains inadequately addressed.

4.2 Aim of analysis

The aim of this analysis was to examine the association between β-cell function, glucose effectiveness (S_G) and insulin sensitivity (S_I) with the progression to DR in newly diagnosed and treatment naïve T2DM who had no evidence of DR at the time of diagnosis and who were observed for up to 5 years.

4.3 Methods

Data was available on a total of 314 subjects at Year 5, with 293 having data at both diagnosis and Year 5. 179 subjects had no evidence of DR at baseline and throughout the study period. Of the remaining 114, a total of 60 subjects were excluded from analysis i.e. 41 subjects who had DR at Year 0, 17 subjects who developed DR at Year 1 or 2 but had no recorded evidence of DR at Year 5 and 2 subjects with DR at Year 1 and 5 but with no evidence of DR at Year 0 and 2.

Therefore, the number of subjects with NDR at diagnosis, who progressed to DR over the 5 year period, was 54 of whom 12 developed DR at Year 1, 15 developed DR at Year 2 and the remaining 27 first developed DR at Year 5. Data involving 233 subjects have been included in the analyses involving 179 subjects who remained without DR and 54 who developed DR during course of the 5-year observation period. Thus, to monitor incidence, we compared those with NDR at baseline and throughout the 5 years (n= 179) with those subjects who developed DR during the 5-year follow-up (n= 54). Subjects were examined at diagnosis, Year 1, Year 2 and Year 5 from diagnosis. The subject recruitment, experimental protocol, data analysis and basic statistical analysis are described in detail in Chapter 2.

Appendix Table 2 shows the comparison of baseline characteristics of subjects who were not followed up over 5 years compared to those who were followed up over 5 years and shows that there was no significant difference in them.

4.3.1 Statistical analysis

Following the initial descriptive analysis and comparison of the means of the two groups, a non-correlated subset of clinical and metabolic variables were determined, based on statistical and clinical relevance. The designated putative risk factors were assessed using logistic regression methods with non-normally distributed variables [(FPG, FPI, PPG, PPI, $AUC_{Glucose\ (0\text{-}240min)}$, $AUC_{Insulin\ (0\text{-}240min)}$, HOMA B, M_0, M_1 and S_g)] log transformed. All multivariate analyses were adjusted for age, gender, BMI and risk factors that included systolic blood pressure and total cholesterol, which have previously been reported to have an association with DR. All analysis were conducted using SPSS 20 with $p<0.05$ taken as statistically significant (two-tailed).

We calculated the average/mean of all the measured metabolic variables from diagnosis, including variables at Years 1, 2 and 5 years post diagnosis and have defined them as the follow-up indicator of diabetic control over 5 years.

4.4 Results

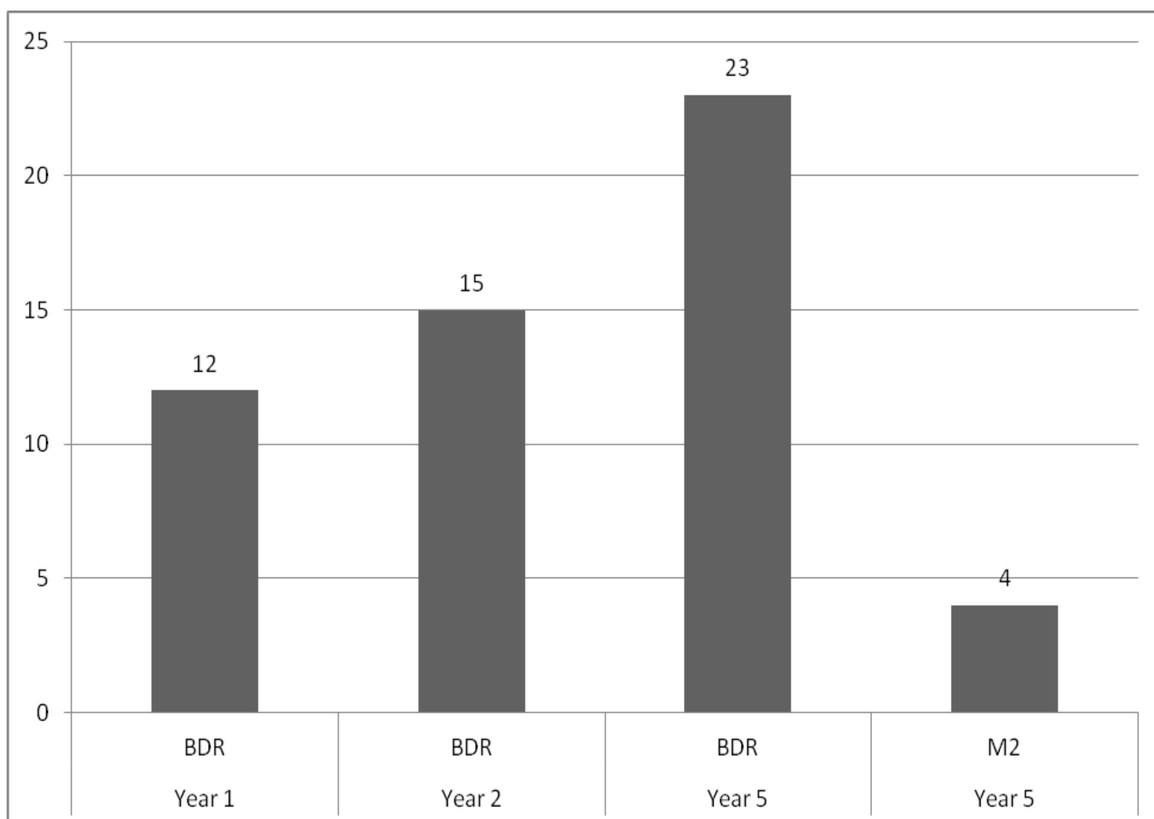
4.4.1 Baseline characteristics of subjects with T2DM at 5 years post diagnosis:

Data involving 233 subjects have been included in the analyses involving 179 subjects who remained without DR and 54 who developed DR during the course of the 5-year observation periods. Of the 233 subjects (Group A) (145 male and 88 female, 1.65:1) with a mean age of 54 (SD±9) years, 76.8% (179) never developed

DR with 23.2% (54) developing DR by 5 years after diagnosis. Of the ones who developed DR, BDR was seen in 12 (22%) after 1 year, 15 (28%) after 2 years and in 27 (50%) after 5 years. Of those with DR at 5 years, the majority of 93% (50) had lesions of BDR including M1 with BDR. Only 1.7% (4) had exudative maculopathy and none progressed to PPDR or PDR (Figure 4.1).

In the subgroup of 76 subjects (Group B) who underwent FSIVGTT at diagnosis in addition to a MTT, 76.3% (58) subjects had no evidence of DR at Year 5. In contrast, 23.7% (18) developed DR comprising predominantly of BDR 88.9% (16) with 11.1% (2) having exudative maculopathy. There were 6 subjects with BDR at Year 1 and 13 in Year 2.

Figure 4.1: Subjects with NDR at diagnosis who developed each level of DR at years 1, 2 and 5.



BDR: Background Diabetic Retinopathy

M2: Definite Maculopathy

4.4.2 Comparison of baseline data for subjects with type 2 diabetes who did or did not develop DR at 5 years.

Baseline characteristics including age, weight, BMI, systolic and diastolic blood pressure, total cholesterol and HbA_{1c} of the patients with DR and NDR in Year 5 in groups A and B are detailed in Table 4.1. Group A subjects with DR at Year 5 presented with a significantly higher $HbA_{1c,}$ ($p = 0.017$) at baseline. There was a trend to a greater percentage of males in the NDR group, but this was not statistically significant. Other baseline characteristics were not significantly different between those with or without DR at Year 5. In Group B, HbA_{1c} was higher in those with DR at Year 5 but the difference did not reach statistical significance. None of the other baseline characteristics were significantly different between those with or without DR at 5 years from diagnosis

There was no significant difference in the glycaemic parameters or β-cell function at diagnosis in the smaller subset of subjects who develop DR at Year 1, 2 and 5. However the subjects who develop DR earlier have lower measured fasting insulin levels at diagnosis. (Appendix Table 3)

Table 4.1: Baseline characteristics in subjects with No Diabetic Retinopathy (NDR) throughout 5 years since diagnosis compared to those who develop Diabetic Retinopathy (DR) by 5 years from diagnosis of T2DM. Group A: 233 subjects who underwent MTT, Group B: 76 subjects who underwent FSIVGTT

Group A	**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 (%)	8.2 (2.1)	8.1 (2.1)	8.8 (1.8)	**0.017**
Group B	**All subjects**	**No Diabetic Retinopathy**	**Diabetic Retinopathy**	**Comparison of NDR and DR (p value)**
Number	**76**	**58**	**18**	**-**
Age at presentation (years)	54 (9)	53 (9)	56 (9)	0.27
Male Sex	76	79	67	0.27
Weight (kg)	91(17)	91(17)	91(19)	0.97
BMI ($kg.m^2$)	31 (6)	31 (5)	32 (7)	0.51
Systolic blood pressure (mmHg)	133 (17)	133 (17)	133 (17)	0.95
Diastolic blood pressure (mmHg)	81 (10)	81 (10)	80 (10)	0.56
Total Cholesterol (mmol/L)	5.6 (1.2)	5.5 (1.2)	5.8 (1.1)	0.37
HbA1c (%)	7.8 (2.1)	7.6 (2.2)	8.3 (1.9)	0.15

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

The metabolic variables observed during the MTT for Group A subjects with or without DR during the 5 year period are detailed in Table 4.2a

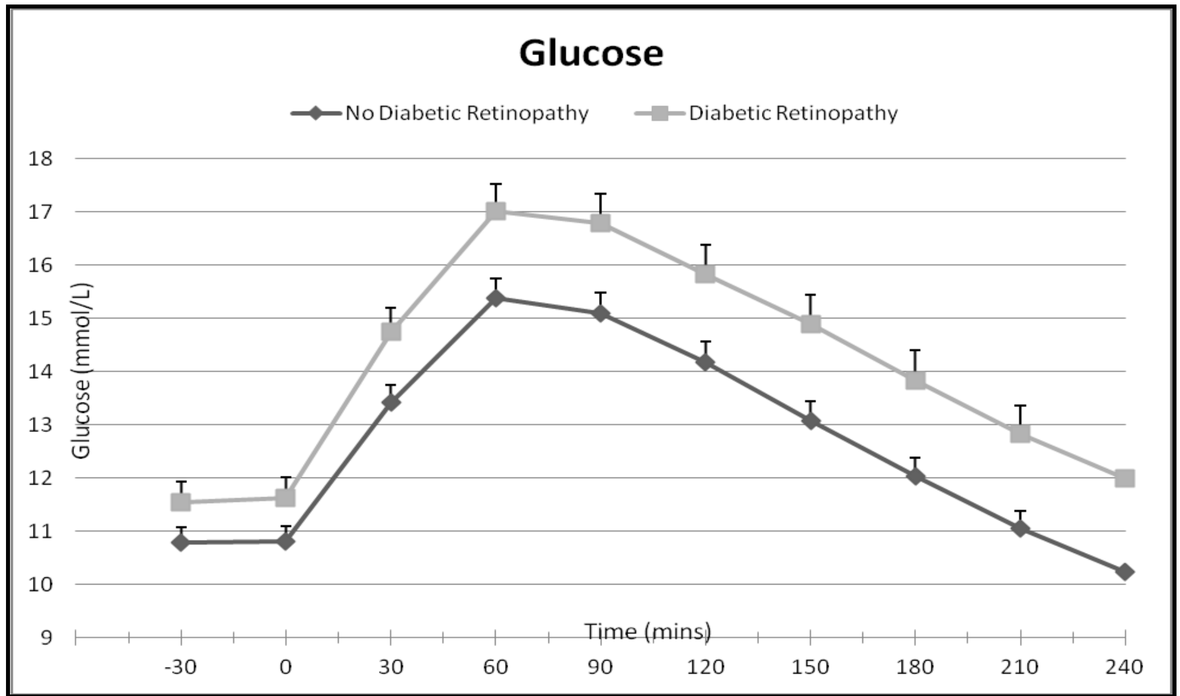
Table 4.2a: Comparison in subjects who underwent a Meal Tolerance Test with No Diabetic Retinopathy throughout 5 years since diagnosis compared to those who develop Diabetic Retinopathy by 5 years from diagnosis of T2DM by their **parameters at diagnosis**.

Group A	**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 $_{Glucose\ (0-240min)}$ (mmol/L)	11.6 (8.6-14.6)	13.7 (11.2-15.6)	**0.007**
HbA1c (%)	7.4 (6.4-9.6)	8.6 (7.8-10.0)	**0.017**
Fasting Insulin (pmol/L)	61.2 (40.0-97.0)	56 (28-92)	0.177
Postprandial Insulin (pmol/L) (120 mins)	270 (145-428)	185 (94-391)	**0.044**
AUC $_{Insulin\ (0-240min)}$ (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)	**<0.0001**
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

During the MTT, those with DR at Year 5, compared to those without DR, had at baseline a higher HbA_{1c} (p= 0.017) and fasting glucose levels (p= 0.031) with a reduced (p= 0.025) basal ß-cell secretory function (M_0) and non-significantly lower fasting insulin concentrations (p= 0.177), although the estimation of overall β-cell function (HOMA-B) was significantly reduced (p= 0.044) at diagnosis. In the postprandial state, individuals with DR at Year 5 had a highly significantly poorer estimated postprandial β-cell responsiveness to the test meal i.e. M_1 (p= 0.000) and lower postprandial insulin levels (p= 0.044) associated with a higher postprandial (2 hour) glucose level (p= 0.009).

Over the 4-hour MTT study period, subjects with DR had significantly higher $AUC_{Glucose\ (0\text{-}240min)}$ (p= 0.007) and lower $AUC_{Insulin\ (0\text{-}240\ min)}$ (p= 0.042) in comparison to those without DR at Year 5 (Table 4.2a).

The glucose and insulin profiles and indices of ß-cell responsiveness (M_0 and M_1) during the MTT in subjects with DR and NDR are illustrated in Figures 4.2, 4.3 a and b.



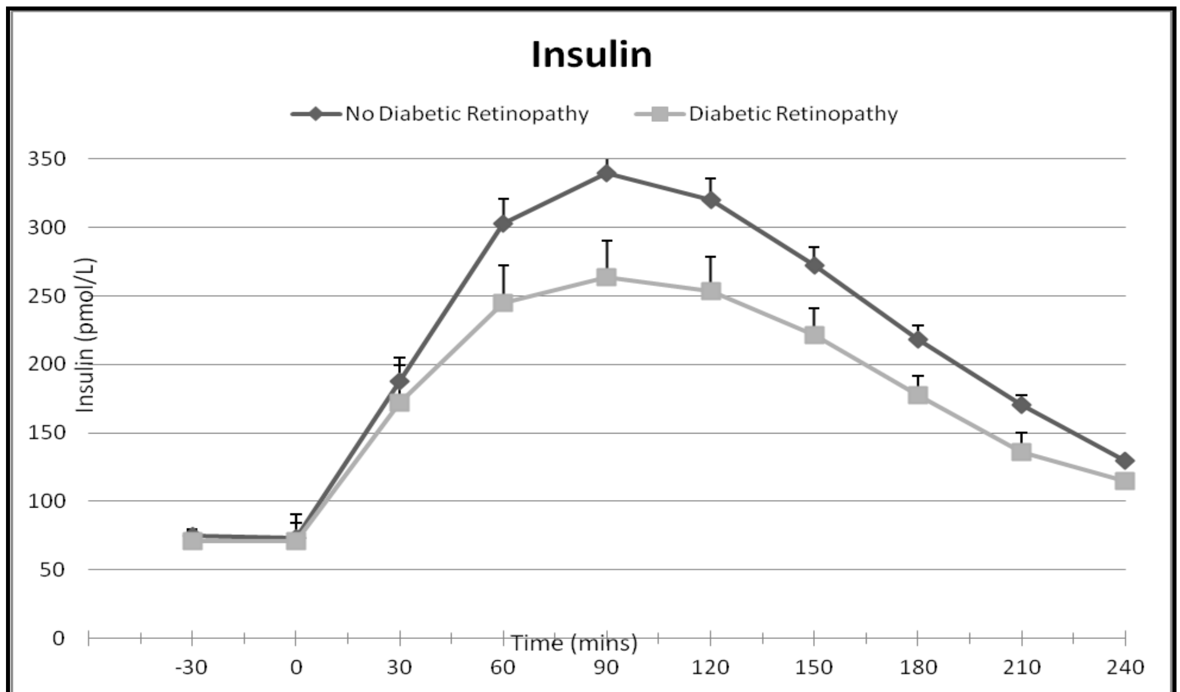
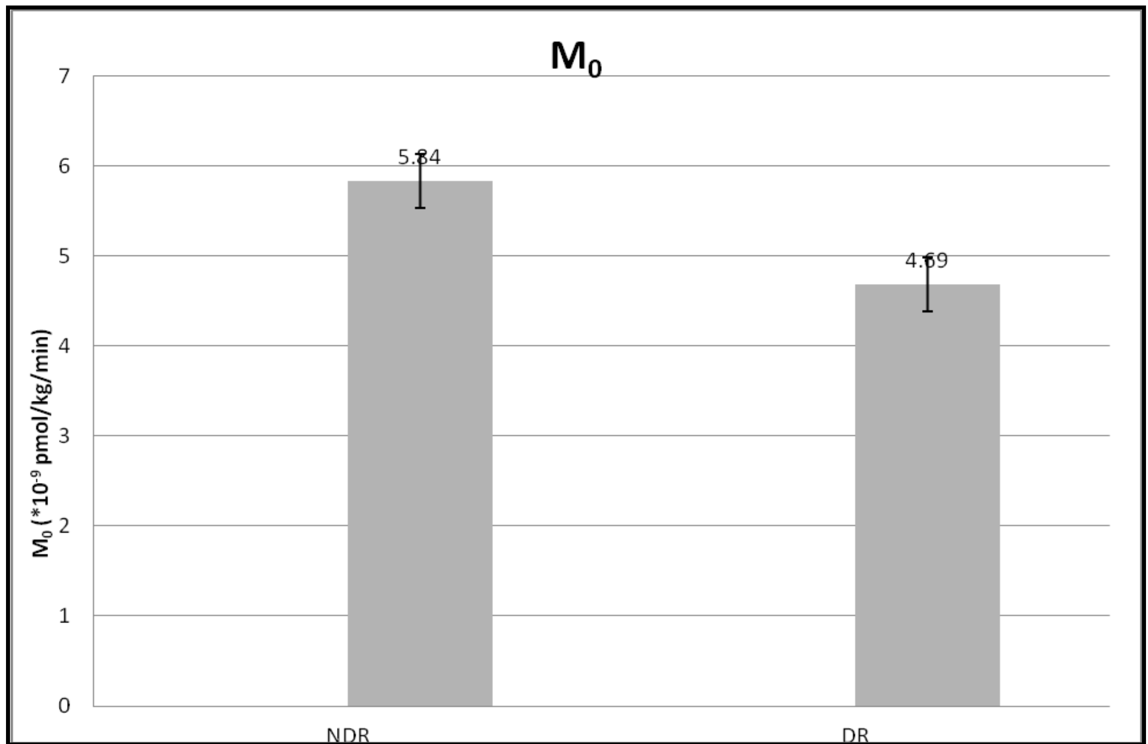


Figure 4.2 a and b: Baseline (at diagnosis) Glucose and Insulin Profiles (mean ± SEM) in subjects with and without diabetic retinopathy at 5 years post diagnosis. Differences between NDR (n=179) and DR (n=54): Fasting Glucose (p= 0.031), 2hr Postprandial Glucose (p= 0.009), Fasting Insulin (p= 0.177) and 2hr Postprandial Insulin (p= 0.044).



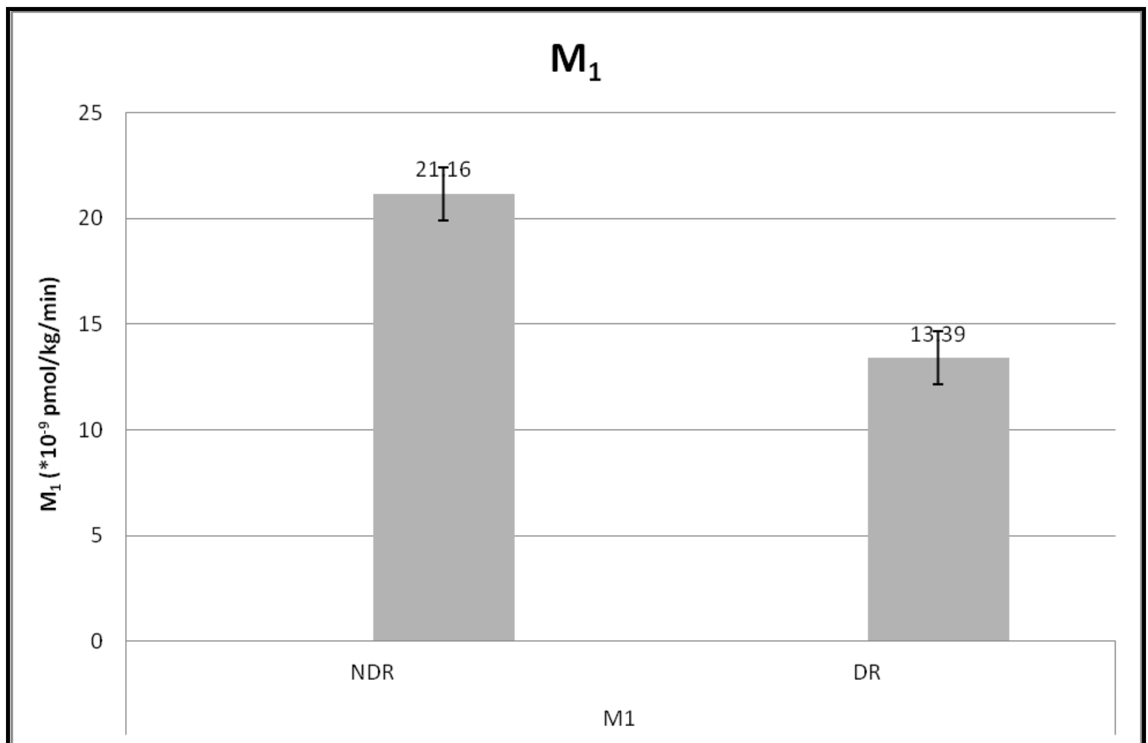


Figure 4.3 a and b: Baseline (at diagnosis) Fasting (M_0) and postprandial(M_1) β-cell responsiveness (mean±SEM) in subjects with and without diabetic retinopathy by 5 years post diagnosis. Significant differences between NDR and DR: Fasting ($p= 0.025$) and postprandial ($p= 0.000$) β-cell responsiveness

The baseline (at diagnosis) β-cell responsiveness, insulin sensitivity in those subjects (group B) who underwent FSIVGTT and developed DR or remained NDR at Year 5 are detailed in Table 4.2b. Whereas insulin sensitivity (S_I) was not significantly different between the two groups, the AIR_G ($p = 0.024$) and S_G ($p = 0.036$) were significantly reduced in those with DR compared to those without DR at Year 5. There was no difference in the insulin sensitivity or DI between those with or without DR.

Table 4.2b: Comparison of metabolic variables following Frequently Sampled Intravenous Glucose Tolerance Test in subjects with No Diabetic Retinopathy throughout 5 years since diagnosis compared to those who develop Diabetic Retinopathy by 5 years from diagnosis of T2DM by their **parameters at diagnosis.**

Group B	**No Diabetic Retinopathy (n=58)**	**Diabetic Retinopathy (n=18)**	**p value**
S_I x 10^{-4} [(microU/ml)$^{-1}$.min^{-1}]	0.7 (0.4-1.5)	1.1 (0.6-1.8)	0.108
S_G x 10^{-2} (min^{-1})	1.6 (1.3-1.9)	1.3 (0.9-1.6)	**0.036**
$AIR_{G\ (0-10min)}$ (microU/ml. min)	121.6 (64.6-192.9)	68.3 (43.7-113.6)	**0.024**
DI x 10^{-2}	64.6 (32.7-153.3)	71.6 (42.8-102.2)	0.735

Data expressed as median (1st – 3rd IQR)

FPG = Fasting Plasma Glucose; PPG = Post Prandial Glucose; AUC = Area Under the Curve

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

4.4.3 Univariate regression analysis

Based on the inter-group differences (Table 2a and 2b), univariate logistic regression analysis was conducted, which demonstrated that HbA_{1c}, fasting and postprandial glucose, $AUC_{Glucose\ (0\text{-}240min)}$, and S_G were positively related to the development of DR by Year 5 whereas β-cell function/secretory capacity represented by M_0, M_1, HOMA-B, postprandial insulin, $AUC_{Insulin\ (0\text{-}240min)}$, were negatively related to the appearance of DR within the 5-year observation period (Table 4.3).

Table 4.3: Univariate and Multivariate logistic regression depicting variables independently associated with development of Diabetic Retinopathy by 5 years from diagnosis of T2DM.

		Univariate		Multivariate	
	Number	Crude OR (95% CI)	p	OR (95% CI)	p
				(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 $_{Glucose\ (0\text{-}240min)}$ (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 $_{Insulin\ (0\text{-}240min)}$ (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**
M_1 (*10^{-9} pmol/kg/min)	224	0.48 (0.33, 0.70)	**0.000**	0.46 (0.32, 0.68)	**0.000**
HOMA-B (%)	224	0.70 (0.50, 0.98)	**0.040**	0.60 (0.41, 0.87)	**0.007**
S_G x 10^{-2} (min^{-1})	76	0.15 (0.27, 0.85)	**0.032**	0.15 (0.27, 0.85)	**0.032**

** 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, S_G = Glucose effectiveness

4.4.4 Multivariate regression analysis

Factors associated with DR in univariate logistic regression analyses were adjusted for age and sex, BMI, total cholesterol and systolic blood pressure and are detailed in (Table 4.3). The p value was calculated using the likelihood ratio test.

HbA_{1c} (OR 4.48 [95% CI 1.26, 15.96] p = 0.021), fasting glucose (OR 2.78 [95% CI 1.02, 7.64] p= 0.045), postprandial glucose (OR 3.44 [95% CI 1.34, 8.86] p= 0.011), $AUC_{Glucose\ (0\text{-}240min)}$ (OR 3.62 [95% CI 1.34, 9.76] p= 0.011), fasting insulin (OR 0.59 [95% CI 0.36, 0.94] p= 0.027), postprandial insulin (OR 0.66 [95% CI 0.46, 0.96] p= 0.031) and $AUC_{Insulin\ (0\text{-}240min)}$ (OR 0.53 [95% CI 0.34, 0.83] p= 0.006) highlights the contribution of fasting, postprandial and overall hyperglycaemic and insulinopaenic status of subjects, when assessed at diagnosis, who subsequently at 5 years had developed DR.

Measures of β-cell secretory function i.e. M_0 (OR 0.59 [95% CI 0.39, 0.91] p= 0.015), HOMA-B (OR 0.60 [95% CI 0.41, 0.87] p= 0.007) and M_1 (OR 0.46 [95% CI 0.32, 0.68] p= 0.000) are independently associated with the development of DR along with S_G (OR 0.15 [95% CI 0.27, 0.85] p= 0.032), which reflects an insulin-independent component of glucose tolerance.

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. Also each 1 pmol/L decrease in fasting and postprandial insulin was associated with increased risk of DR by 41% and 34% respectively.

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

The characteristics of patients at Years 1, 2 and 5 including age, weight, BMI, systolic and diastolic blood pressure, total cholesterol and HbA_{1c} of the patients with DR and NDR in Year 5 in groups A are detailed in tables 4.4, 4.5 and 4.6.

Table 4.4: Year 1 characteristics of subjects with No Diabetic Retinopathy (NDR) throughout 5 years compared to those with Diabetic Retinopathy (DR) by 5 years of diagnosis of T2DM. Group A: Subjects who underwent MTT.

	All subjects	**NDR**	**DR**	**p value**
Number	**233**	**179**	**54**	
Age at presentation (years)	55.0 (9.4)	55.0 (9.8)	56.0 (8.2)	0.661
Male Sex (%)	175 (75)	139 (77.7)	36 (66.7)	0.075
Weight (kg)	85.9 (16.2)	85.4 (16.4)	87.4 (15.3)	0.343
BMI ($kg.m^2$)	28.8 (6.5)	28.7 (6.0)	29.3 (8.1)	0.231
Systolic blood pressure (mmHg)	131 (16)	130 (16)	133 (14)	0.238
Diastolic blood pressure (mmHg)	80 (10)	79 (10)	81 (11)	0.224
Total Cholesterol (mmol/L)	5.1 (1.1)	5.1 (1.1)	5.2 (1.0)	0.476
HbA_{1c} (%)	6.6 (1.2)	6.5 (1.1)	6.9 (1.4)	**0.011**

Data expressed as Mean (± SD); Sex: Number (%); BMI = Body Mass Index (kg/m2)

Table 4.5: Year 2 characteristics of subjects with No Diabetic Retinopathy (NDR) throughout 5 years compared to those with Diabetic Retinopathy (DR) by 5 years of diagnosis of T2DM. Group A: Subjects who underwent MTT.

	All subjects	**NDR**	**DR**	**p value**
Number	**233**	**179**	**54**	
Age at presentation (years)	56.0 (9.4)	56.0 (9.8)	57.0 (8.2)	0.661
Male Sex (%)	175 (75)	139 (77.7)	36 (66.7)	0.075
Weight (kg)	87.3 (16.6)	87.0 (16.6)	88.4 (16.6)	0.528
BMI ($kg.m^2$)	29.1 (6.9)	28.8 (6.8)	30.3 (7.3)	0.119
Systolic blood pressure (mmHg)	129.3 (15.7)	128.6 (14.5)	131.7 (19.0)	0.461
Diastolic blood pressure (mmHg)	78.3 (9.3)	78.0 (9.0)	79.3 (10.1)	0.386
Total Cholesterol (mmol/L)	5.0 (1.1)	5.0 (1.1)	5.1 (1.0)	0.660
HbA_{1c} (%)	6.9 (1.5)	6.7 (1.4)	7.3 (1.5)	**0.016**

Data expressed as Mean (± SD); Sex: Number (%); BMI = Body Mass Index (kg/m2)

Table 4.6: Year 5 characteristics in subjects with No Diabetic Retinopathy (NDR) throughout 5 years compared to those with Diabetic Retinopathy (DR) by 5 years of diagnosis of T2DM. Group A: Subjects who underwent MTT.

	All subjects	**NDR**	**DR**	**p value**
Number	**233**	**179**	**54**	
Age at presentation (years)	59.0 (9.4)	59.0 (9.8)	60.0 (8.2)	0.661
Male Sex (%)	175 (75)	139 (77.7)	36 (66.7)	0.075
Weight (kg)	85.1 (23.0)	85.0 (22.7)	85.4 (24.2)	0.528
BMI ($kg.m^2$)	29.1 (7.2)	29.0 (7.1)	30.0 (7.8)	0.163
Systolic blood pressure (mmHg)	135.6 (17.3)	135.2 (16.8)	136.9 (18.8)	0.675
Diastolic blood pressure (mmHg)	79.1 (10.1)	78.7 (10.5)	80.4 (8.8)	0.527
Total Cholesterol (mmol/L)	5.2 (1.2)	5.2 (1.2)	5.1 (1.1)	0.604
HbA_{1c} (%)	7.5 (1.6)	7.4 (1.6)	8.0 (1.6)	**0.009**

Data expressed as Mean (± SD); Sex: Number (%); BMI = Body Mass Index (kg/m2)

At Years 1, 2 and 5, group A subjects who developed DR at Year 5 presented with a significantly higher HbA_{1c}, ($p= 0.011$, $p= 0.016$ and $p= 0.009$) (Tables 4.4, 4.5 and 4.6). Other baseline characteristics were not significantly different between those with or without DR at Year 5.

The metabolic variables in Year 1, 2 and 5 during the MTT for group A subjects with DR or no DR are detailed in Tables 4.7, 4.8 and 4.9.

Table 4.7: Comparison of the metabolic variables at **1 year** 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**	**-**
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**
$AUC_{Glucose\ (0\text{-}240min)}$ (mmol/L)	8.3 (7.2 – 10.2)	9.8 (8.1 – 11.1)	**0.006**
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
$AUC_{Insulin\ (0\text{-}240min)}$ (pmol/L)	214.3 (134.8 – 313.8)	183.4 (113.0 – 289.5)	0.158
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
HOMA-IR	1.7 (1.0 – 2.4)	1.6 (1.1 – 2.3)	0.870

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

AUC = Area Under the Curve

Those with DR at Year 5, compared to those without, had a higher HbA_{1c} (p= 0.001), a higher fasting glucose (p= 0.003) along with higher postprandial (2 hour) glucose (p= 0.005) in Year 1 (Table 4.7). Over the 4-hour MTT study period, subjects with DR had significantly higher $AUC_{Glucose\ (0\text{-}240min)}$ (p= 0.006) in comparison to those without DR at Year 5 (Table 4.7), 1 year post diagnosis.

Table 4.8: Comparison of the metabolic variables at **2 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**	**-**
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**
AUC $_{\text{Glucose (0-240min)}}$ (mmol/L)	8.6 (7.3 – 10.7)	9.9 (8.0 – 12.4)	**0.017**
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
AUC $_{\text{Insulin (0-240min)}}$ (pmol/L)	208.0 (131.0 – 333.0)	186.0 (101.1 – 334.1)	0.312
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
HOMA-IR	1.7 (1.1 – 2.5)	1.5 (1.0 – 2.7)	0.617

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

AUC = Area Under the Curve

Those with DR at Year 5, compared to those without, had a higher HbA_{1c} (p=0.0016) in Year 2 (Table 4.8), with a lower estimated postprandial β-cell responsiveness i.e. M_1 (p= 0.009) and a higher postprandial (2 hour) glucose (p= 0.005) (Table 4.8).

Over the 4-hour MTT study period, subjects with DR had significantly higher $AUC_{Glucose\ (0\text{-}240min)}$ (p= 0.017), in comparison to those without DR at Year 5 (Table 4.8), 2 years post- diagnosis. A higher HbA_{1c} (p= 0.009) at year 5 was the only remaining significant association with DR, 5 years post-diagnosis (Table 4.9)

Table 4.9: Comparison of the metabolic variables at **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**	**-**
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
AUC $_{Glucose\ (0-240min)}$ (mmol/L)	9.5 (7.4 – 12.7)	10.3 (7.9 – 13.7)	0.222
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
AUC $_{Insulin\ (0-240min)}$ (pmol/L)	199.5 (118.1 – 312.7)	161.4 (84.6 – 304.0)	0.240
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
HOMA-IR	1.6 (1.0 – 2.9)	1.7 (1.1 – 2.6)	0.991

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

AUC = Area Under the Curve

4.4.6 Univariate regression analysis of Year 1, 2 and 5 Parameters

Based on the inter-group differences (Tables 4.4 – 4.9) univariate logistic regression was conducted which demonstrated that after one year post-diagnosis of T2DM the HbA_{1c} (p= 0.028), fasting glucose (p= 0.006), postprandial glucose (p= 0.006) and $AUC_{Glucose\ (0\text{-}240min)}$ (p= 0.011), were all significantly related to the development of DR by Year 5 (Table 4.10). Similarly, after 2 years post-diagnosis HbA_{1c} (p= 0.011), postprandial glucose (p= 0.007), estimated postprandial β-cell responsiveness i.e. M_1 (p= 0.011) and $AUC_{Glucose\ (0\text{-}240min)}$ (p= 0.011) lead to development of DR with no contribution from any of the measured parameters 5 years post-diagnosis (Table 4.10),

Table 4.10: Univariate and Multivariate logistic regression depicting variables (Year 1 to 5) independently associated with development of Diabetic Retinopathy by 5 years from 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 $_{Glucose\ (0\text{-}240min)}$ (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 (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 $_{Glucose\ (0\text{-}240min)}$ (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

4.4.7 Multivariate regression analysis of Year 1, 2 and 5 Parameters

Independently-associated risk factors for the development of DR in univariate logistic regression analyses were adjusted for age and sex, BMI, total cholesterol and systolic blood pressure (Table 4.10). The p value was calculated using the likelihood ratio test.

At the end of the first year (Year 1), HbA_{1c} (OR 6.20 [95% CI 1.04, 36.82] p= 0.045), fasting glucose (OR 7.71 [95% CI 1.78, 33.29] p= 0.006), postprandial glucose (OR 4.57 [95% CI 1.52, 13.69] p= 0.007), $AUC_{Glucose\ (0\text{-}240min)}$ (OR 5.00 [95% CI 1.43, 17.50] p= 0.012) demonstrate the contribution of continuing fasting, postprandial and overall hyperglycaemic exposure at Year 1 leading to DR by 5 years.

Similarly at the end of the second year (Year 2) HbA_{1c} (OR 5.69 [95% CI 1.16, 27.94] p= 0.032), postprandial glucose (OR 4.83 [95% CI1.60, 14.64] p= 0.005), $AUC_{Glucose\ (0\text{-}240min)}$ (OR 4.15 [95% CI 1.25, 13.76] p= 0.020), further confirmed the contribution of continuing fasting, postprandial and overall hyperglycaemic exposure at Year 2 leading to DR by 5 years.

Independent association with DR for measures of β-cell secretory capacity were seen at Year 1 i.e. HOMA-B (OR 0.49 [95% CI 0.26, 0.87] p= 0.016) and at Year 2 M_1 (OR 0.59 [95% CI 0.41, 0.86] p= 0.006).

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

These mean values of T2DM subjects with DR and NDR by Year 5 are detailed in Table 4.11.

Table 4.11: 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 $_{Glucose\ (0\text{-}240min)}$ (mmol/L)	9.6 (8.1 – 11.4)	10.5 (9.5 – 11.7)	**0.015**
HbA_{1c} (https://www.diabetes.org.uk/Documents/Position%20statements/DiabetesUK_Facts_Stats_Oct16.pdf)	7.0 (6.3 – 8.0)	7.6 (6.9 – 8.2)	**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 $_{Insulin\ (0\text{-}240min)}$ (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

Those with DR at Year 5, compared to those without, had a significantly higher mean averaged HbA_{1c} (p= 0.003) over 5 years since diagnosis, and a higher mean averaged postprandial (2 hour) glucose (p= 0.007) over 5 years. Over the 4-hour MTT study period, subjects with DR had significantly higher mean averaged $AUC_{Glucose\ (0\text{-}240min)}$ (p= 0.0015) in comparison to those without DR at Year 5 (Table 4.11) over 5 years post diagnosis. There was no significant difference in the mean averaged Insulin levels between the two groups. Those with DR by Year 5, compared to those without were also associated with a lower mean averaged estimated postprandial β-cell responsiveness i.e. M_1 (p= 0.001).

4.4.9 Univariate regression analysis of Mean averaged metabolic parameters over 5 years

Based on the inter-group differences (Table 4.11), univariate logistic regression was conducted, which demonstrated that the cumulative HbA_{1c} (p= 0.004), postprandial glucose (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 4.12). Estimated postprandial β-cell responsiveness i.e. M_1 (p= 0.001) over the 5 years was also highly significantly related to the development of DR.

Table 4.12: 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.

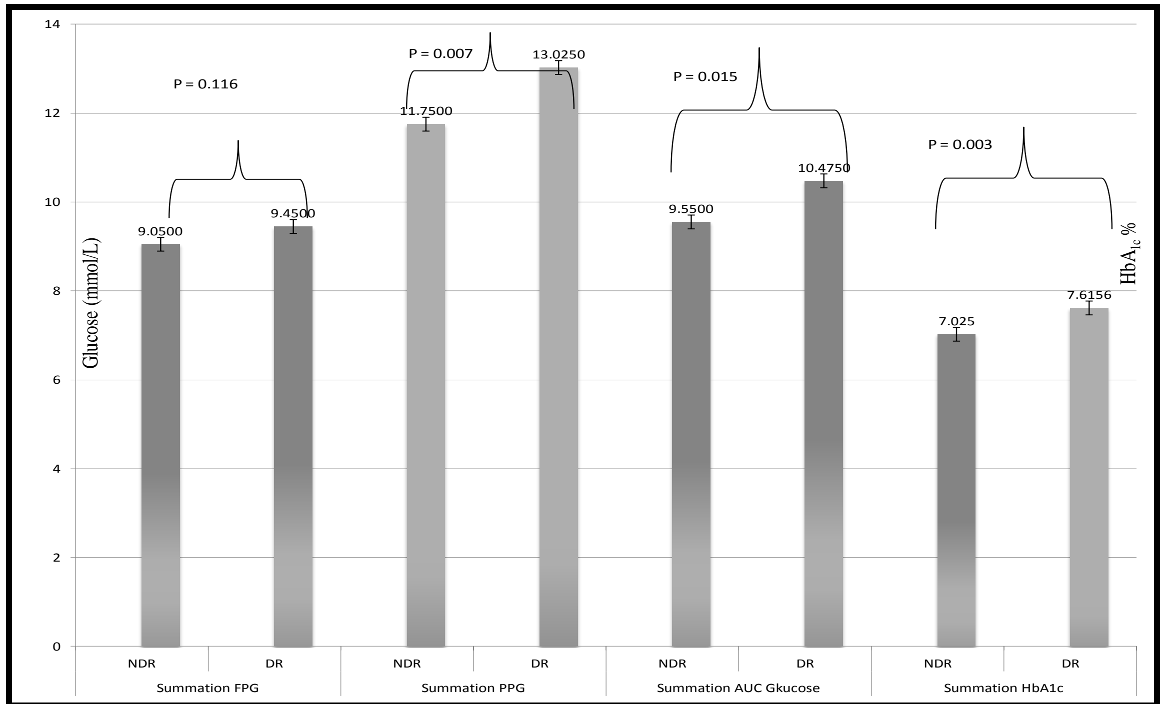
		Univariate		Multivariate	
	Number	Crude OR (95% CI)	p	OR (95% CI)	p
				(fully adjusted **)	
Mean HbA1c (%)	233	1.51 (1.14 – 2.01)	**0.004**	1.48 (1.12 – 1.97)	**0.008**
Mean Fasting Glucose (mmol/L)	230	1.15 (0.96 – 1.38)	0.143	1.13 (0.93 – 1.36)	0.227
Mean Postprandial Glucose (mmol/L) (120 mins)	230	1.15 (1.02 – 1.30)	**0.022**	1.15 (1.02 – 1.30)	**0.023**
Mean $AUC_{Glucose\ (0\text{-}240min)}$ (mmol/L)	230	1.17 (1.02 – 1.35)	**0.023**	1.18 (1.02 – 1.36)	**0.027**
Mean M_0 ($*10^{-9}$ pmol/kg/min)	227	0.89 (0.78 – 1.02)	0.100	0.81 (0.68 – 0.96)	**0.014**
Mean M_1 ($*10^{-9}$ pmol/kg/min)	224	0.93 (0.89 – 0.97)	**0.001**	0.93 (0.86 – 0.97)	**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

4.4.10 Multivariate regression analysis of Mean averaged metabolic parameters over 5 years

Factors 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 4.12). Measures of β-cell function – M_0 (OR 0.81 [95% CI 0.68, 0.96] p= 0.014) and M_1 (OR 0.93 [95% CI 0.86, 0.97] p= 0.001) show independent association with DR. HbA_{1c} (OR 1.48 [95% CI 1.12, 1.97] p= 0.008), Postprandial glucose (OR 1.15 [95% CI 1.02, 1.30] p= 0.023), $AUC_{Glucose\ (0-240min)}$ (OR 1.18 [95% CI 1.02, 1.36] 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 mentioned variables (Table 4.12). Figures 4.4a and 4.4b illustrate the mean Fasting, Postprandial and AUC Glucose and Insulin Profiles and mean HbA_{1c} over the 5 year study period (Years 0, 1, 2, and 5) in patients with and without diabetic retinopathy by 5 years post-diagnosis of T2DM.



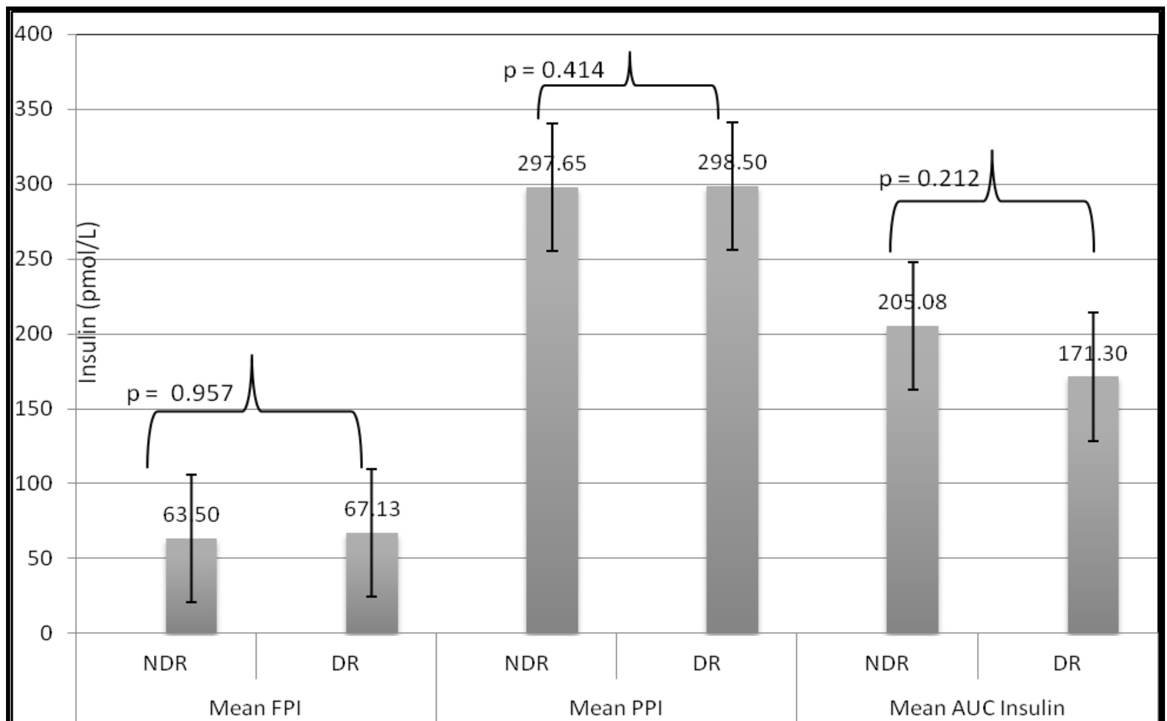
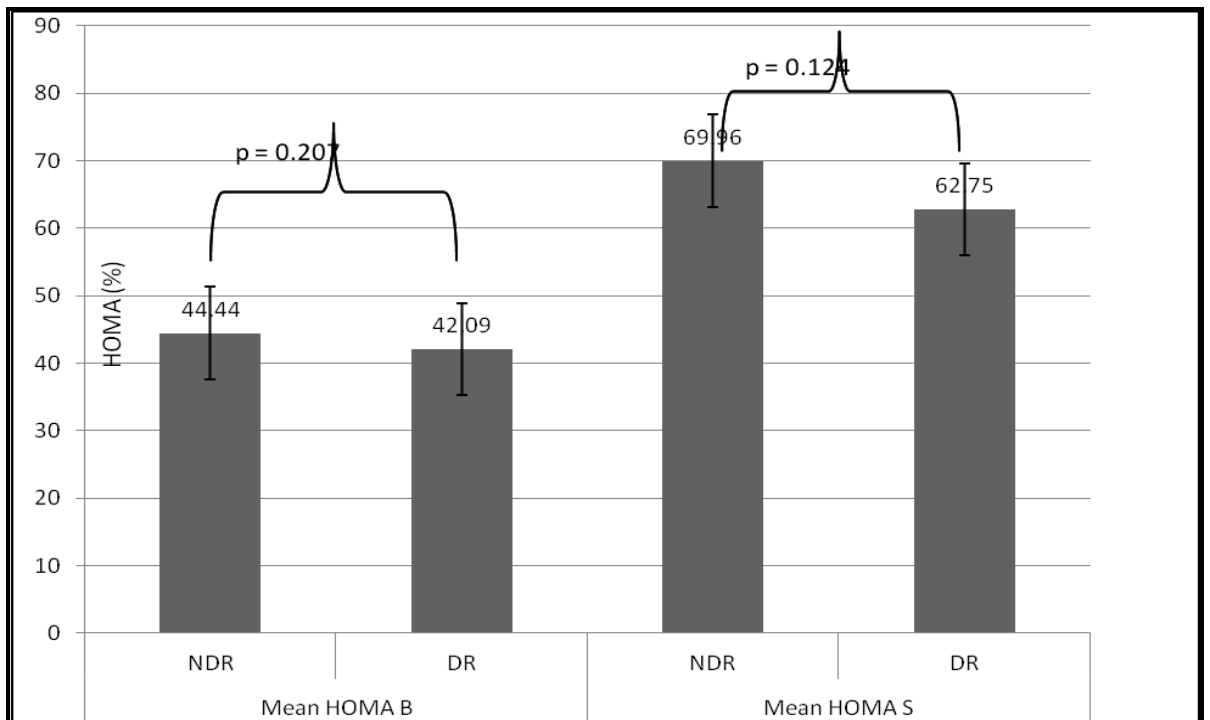


Figure 4.4 a and 4.4b: Mean Fasting (FPG), Postprandial (PPG), AUC Glucose $_{(0\text{-}240\ min)}$, Mean HbA1c and Mean Fasting Insulin (FPI), Postprandial (PPI) and AUC Insulin $_{(0\text{-}240\ min)}$ Profiles (mean ± SEM) combined over the 5 year study period in subjects with and without diabetic retinopathy by 5 years post-diagnosis of T2DM.

Figure 4.5 a and 4.5 b illustrate the mean HOMA-B and S and mean fasting and postprandial β-cell responsiveness over 5 years with in patients with and without diabetic retinopathy by 5 years post-diagnosis of T2DM.



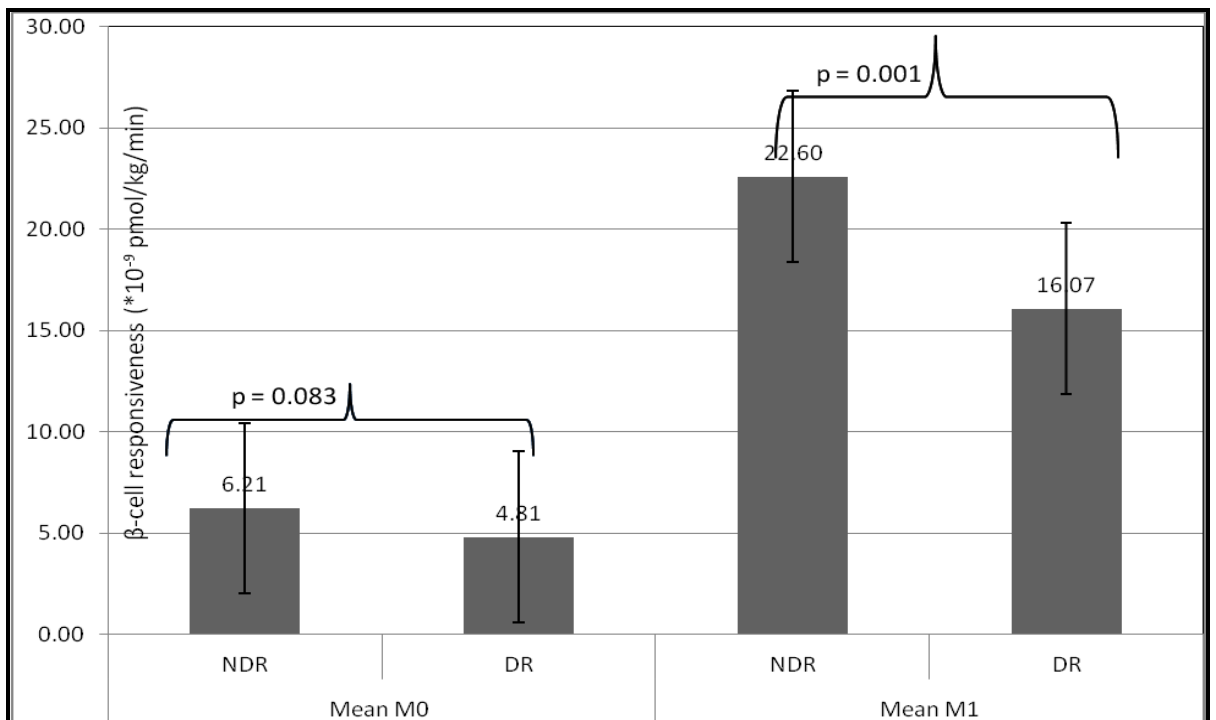


Figure 4.5 a and 4.5 b: Mean HOMA-B and S and Mean fasting (M_0) and postprandial (M_1) β-cell responsiveness (mean±SEM) over 5 years in subjects with and without diabetic retinopathy by 5 years post diagnosis of T2DM.

4.5 Discussion

Our analysis indicates a strong and independent association between incident DR and glycaemic control at the time of diagnosis represented by HbA_{1c}, fasting and the 2-hour postprandial hyperglycaemia, as well as the overall 4-hour glucose ($AUC_{(0-240min)}$) response to the standardised test meal. We have further shown that calculated measures of β-cell function during fasting (M_0) and HOMA-B and in response to the test meal (M_1), conducted at the time of diagnosis, were independently associated with DR along with S_G, which reflects the contribution of an “insulin-independent component of glucose tolerance”. These findings suggest that by the time of diagnosis of T2DM, subjects have lost a substantial part of their β-cell function, and are at a greater risk of developing DR in a further 5 years time. In addition, the data emphasise the contribution of both postprandial and overall glycaemic exposure over the 5-year follow up period to the development of DR i.e. the cumulative effect of poor glycaemic control and β-cell dysfunction. The degree of dysglycaemia is related to a lower fasting and postprandial β-cell responsiveness at diagnosis leading to the postprandial and overall glycaemic exposure over the 5-year study period.

In our study, involving patients who were newly-diagnosed with T2DM, those who went on to develop DR by 5 years had a significantly higher baseline HbA_{1c} (8.6%) compared to those who did not (7.4%). This association remained significant on multivariate logistic regression after adjusting for age, sex, BMI, SBP and total cholesterol. Thus for every 1% rise in HbA_{1c} at diagnosis, there was a four times greater likelihood of developing DR in the 5 years follow-up. There was also a

significant and independent association between the HbA_{1c} level at Years 1 and 2 and the 5-year mean HbA_{1c} with the development of DR over the 5-year observation period. However, although the HbA_{1c} at Year 5 showed a significant univariate association, once adjusted for the above-mentioned variables, it did not retain its significance.

Similar to our study, in which 23.2% of the subjects developed DR over 5 years, the UKPDS who also recruited newly diagnosed subjects with T2DM showed that their study participants had a DR incidence of 22% over 5 years (Stratton IM et al. 2001). They had also demonstrated that glycaemic control at diagnosis and overall glycaemic exposure were significant contributory factors to the incidence of DR. The Wisconsin Epidemiologic Study of Diabetic Retinopathy (WESDR) also reported that in individuals with both younger and older onset DM over 6 years, glycaemic control, as measured by glycosylated haemoglobin, was associated with an increased risk of development of DR (Klein R et al. 1995). However in contrast to our findings, they did not find a relationship between DR incidence and β-cell function, as defined by plasma C-peptide measurement. Our study is also consonant with that of Yoshida et al. studying T2DM subjects with no DR at the initial visit where they also found a significant contribution of baseline overall glycaemic exposure (HbA_{1c}) and duration of DM (Yoshida Y et al. 2001) to the development of DR. This is a robust finding as Looker et al. (Looker HC et al. 2003) studying the Pima Indians found a slightly lower cumulative incidence than ourselves at 16.8% but overall glycaemia was again found to have a significant contribution. In addition, more recent studies also have confirmed the role of HbA_{1c} in the incidence of DR

(Semeraro F et al. 2011) (Rudnisky CJ et al. 2012). In 130 Korean patients with T2DM who had NDR at baseline, age, known duration of DM, mean FPG, and HbA_1 levels during the follow-up period of 5.3 years were higher in the patients who developed DR. Cox proportional hazards analysis established mean HbA_1 as the only independent risk factor for the development of DR (Kim HK et al. 1998).

In the Verona Diabetes Study, by the time of the second eye examination (4-5 years follow up) 124 patients had either developed new DR (79 patients) or progressed to a more severe degree of DR (45 patients). By multivariable logistic regression analysis, the development of DR was independently predicted by the average glycaemia over time, expressed as glycated haemoglobin or mean FPG (Zoppini G et al. 2009). These results suggest that in elderly T2DM subjects, the magnitude of hyperglycaemia independently predicts the development of DR. A Taiwanese study also showed that glycaemic control as measured by mean FPG and mean HbA_{1c} was significantly related with the incidence of DR over a 4-year study period (Chen MS et al. 1995). Therefore the analysis of our study supports the association of both baseline and overall glycaemic exposure and the development of DR.

Both the fasting and postprandial hyperglycaemic and insulinopaenic responses during a standardised MTT, as well as the overall 4-hour ($AUC_{(0\text{-}240min)}$) response to the meal at the time of diagnosis, are strongly related to the development of DR by 5 years. In our study, the FPG at diagnosis, and at one year post-diagnosis, had an independent association with the development of DR by 5 years in our T2DM

subjects. FPG at Year 2 and 5 and Mean FPG although higher in subjects with DR compared to those without, did not reach statistical significance. FPI at diagnosis was higher in subjects without DR and after adjusting for the above mentioned variables showed a significant contribution to the development of DR. FPI at year 1, 2, 5 and the mean FPI for the 5 years showed no association with the development of DR. The contribution of fasting insulinopaenia at diagnosis contributing independently to incident DR has not been documented before. Thus this is reflective of a failing fasting β-cell function leading to an insulinopaenic milieu and leading to a higher fasting hyperglycaemic exposure causing development of DR.

In the same vein as our study, an epidemiological study from Mauritius showed baseline FPG to contribute independently to a 6 year incidence of DR (Tapp RJ et al. 2006). In their newly-diagnosed DM subjects at baseline, the incidence of NPDR was 19.1% with no incident cases of PDR. Their 6-year incidence of DR of 23.8% (sight-threatening in 0.4%) was very much in line with our 5-year incidence of 23.2%. The Atherosclerosis Risk in Communities (ARIC) study (studying DM subjects aged 45-64 years from USA with a 3 year DR incidence of 10.1%) (Wong TY et al. 2007) and the Blue Mountains Eye Study (studying an older Australian population-based cohort over 5 year) (Cikamatana et al. 2007) both similarly showed the association of baseline FPG with incident DR. Baseline FPG similarly showed significant contribution to incident DR in other international studies including a 4 year study in Iran (Manaviat MR et al. 2008) and Japan (Araki et al. 1993). A slightly longer study involving Oklahoma Indians with a mean follow-up time of 12.7 years reported a fasting plasma glucose level >11.1 mmol/L (200 mg/dl)

increased the risk of DR 3.6 times that for a level <7.8 mmol/L (140 mg/dl) (Lee ET et al. 1992).

A data-pooling analysis of nine studies from five countries with 44,623 participants aged 20-79 years with gradable retinal photographs was published by Colagiuri. They demonstrated glycaemic thresholds for DR from receiver-operating characteristic curve analyses to be 6.6 mmol/l for FPG, 13.0 mmol/l for 2-hour PPG, and 6.4% for HbA_{1c} (Colagiuri et al. 2011). Massin et al. also reported the positive predictive values for DR, increasing sharply from 108 mg/dl (6.0 mmol/L) for FPG and from 6.0% for HbA1c levels and propose these thresholds to identify those at risk of DR 10 years later (Massin P et al. 2011). These two studies further consolidate the importance of achieving a FPG around 6.0-6.6 mmol/L to minimise the risk of DR.

On a slightly different note, a study in T2DM Japanese subjects showed that long-term fasting plasma glucose variability (CV-FPG) was a risk factor for PDR independent of the mean FPG or HbA_{1c} in people with T2DM. PDR development was also significantly associated with HbA_{1c} more than 5 years earlier and with the mean FPG more than 10 years earlier (Takao T et al. 2011). This study has indicated that FPG variability is an additional risk factor independent of the mean FPG or HbA_{1c} for the development of PDR in people with T2DM. This is consistent with their previous report that FPG variability is a risk factor independent of the mean FPG or HbA1c for the development of mild moderate and severe NPDR in these

same subjects (Takao T et al. 2010). These observations support a legacy effect and are consistent with the results of the DCCT/EDIC (White NH et al. 2008) and UKPDS 80 (Holman RR et al. 2008). On a similar note, although our study does not establish an association of mean FPG over a period of 5 years with DR, the independent association of FPG at diagnosis and Year 1 with development of DR by 5 years post diagnosis is reflective of a delayed effect of good early glycaemic control.

In our study, we found that the 2 hour PPG following a standardised meal at the time of diagnosis, 1 and 2 years post diagnosis and the mean PPG over 5 years all have an independent association with the development of DR by 5 years. The difference in the 5-year PPG however, although noted to be higher in subjects with DR, compared to those without DR, fails to reach statistical significance. PPI at baseline indicated a significant contribution to the development of DR by 5 years. However, the PPI at years 1, 2, 5 and the mean FPI over the 5 years observation period did not shown any association with the development of DR in our population of T2DM subjects. This post-prandial insulinopaenic and glycaemic effect is important as the relevance of the postprandial glycaemic exposure has not been extensively documented before. However, Shiraiwa et al. in the past decade has shown an independent correlation of postprandial plasma glucose 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 (Shiraiwa T et al. 2005b). Ohkubo et al. has also proposed that achieving a glycaemic threshold of $HbA_{1c} < 6.5\%$, FPG< 110

mg/dl and 2 hour PPG< 180 mg/dl would prevent the onset and progression of diabetic microangiopathy (Ohkubo Y et al. 1995).

As in our study Voutilainen-Kaunisto et al. examined newly-diagnosed T2DM subjects over 10 years in a prospective study in Finland, grading DR using 45° fundal photographs at diagnosis baseline and after 5 and 10 years. Their frequency of DR increased sharply after 5 years with 55% of subjects showing evidence of DR at 10 years. In these subjects, (Voutilainen-Kaunisto RM et al. 2001) FPG, 2-hour PPG and HbA_{1c} at 5 years but not baseline, predicted development of DR at the 10-year follow-up. In contrast to our study, their fasting insulin and fasting C-peptide levels failed to show an association with DR development (Voutilainen-Kaunisto RM et al. 2001). 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 contribution of metabolic memory. They further demonstrated that their risk for developing DR was 7.7 times greater with elevated PPG levels compared with 4.2 times for elevated FPG (4th quartile values compared to 1st quartile glucose values). Our study establishes a greater contribution of postprandial and overall glycaemic exposure to the development of DR than FPG, which is concordant with the Finnish study. A similar 10-year study in Pima Indian adults also showed development of DR to be directly related to higher FPG and 2-hour PPG (Gabir MM et al. 2000).

The analysis of our study further reports that the $AUC_{Glucose\ (0\text{-}240min)}$ at the time of diagnosis and 1 and 2 years post-diagnosis and Mean $AUC_{Glucose\ (0\text{-}240min)}$ over a 5-

year follow-up period have an independent association with the development of DR over this period of time. AUC $_{Glucose\ (0\text{-}240min)}$ at Year 5, although higher in subjects with DR, fails to reach statistical significance. AUC $_{Insulin\ (0\text{-}240min)}$ at diagnosis also showed a significant contribution to the development of DR by 5 years, but AUC $_{Insulin\ (0\text{-}240min)}$ at years 1, 2, 5 and Mean AUC $_{Insulin\ (0\text{-}240min}$) showed no association with the development of DR. This shows that the glycaemic exposure over 4 hours, measured during the MTT, has a significant contribution to the development of DR. This glycaemic exposure shows a contribution from both the fasting and postprandial glycaemic insult on the microvasculature.

In our study, newly-diagnosed T2DM subjects who went on to develop DR by 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 this 5-year time period. A significant contribution was also noted from a lower fasting β-cell function at Year 1 and lower postprandial β-cell responsiveness at Year 2 to the development of DR. There was no noted association with any parameters of insulin resistance/sensitivity.

Whereas we have studied β-cell function prospectively and early in the course of known disease, Suzuki et al retrospectively studied 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 >10 years. 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 low pancreatic β-cell insulin secretory capacity may be a risk factor for the development of PDR (Suzuki K et al. 1989).

In our study the AIR_G and S_G were significantly reduced in those with DR compared to those without DR at Year 5 but there was no difference in the insulin sensitivity or DI between those with or without DR. The independent association of the insulin-independent (as manifested by reduced S_G) component of glucose tolerance to the incidence of DR has not been noted before.

Our analysis also 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 (Hovorka R et al. 1998) and the HOMA methodologies. This analysis of newly-diagnosed T2DM subjects indicates that a 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 and insulinopaenic 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, do have an effect on the incidence of DR. In our data there is no noted effect of antihypertensive or insulin use over the 5 years on development of DR by Year 5. However, our subjects on oral hypoglycaemic medications at Year 1, 2 and 5 have a

significantly greater chance of developing DR by Year 5. A similar significant association was noted with uses of lipid-lowering medication at Year 5 in our subjects. There was however no difference in the outcome on the basis of use of Metformin or Sulphonylurea in our subjects.

The long-term contribution of the postprandial component in our study (mean PPG) is stronger than the mean FPG on the development of DR. Of note is that most parameters of glycaemic control and β-cell secretory status at diagnosis, and soon after, appear to be the main contributors to the development of DR, with no significant contribution from the HbA_{1c} and other glycaemic parameters at year 5. This is compatible with the early history of glycaemic exposure leading to a delayed effect 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.

As mentioned previously, in our study the pre-diagnostic duration of DM might be slightly longer than other studies, which is possibly reflected in the slightly greater percentage of subjects presenting with DR. Following treatment introduction, the percentage of subjects on OHG agents developing DR is significantly higher, thus reflecting possibly a more advanced/severe stage of the disease process. The numbers of subjects on insulin were limited and therefore it is not possible for this to have a meaningful association with the outcome. Therefore, the need to diagnose T2DM as early as possible in order to intervene and lower the glycaemic exposure is

paramount, as Colagiuri has demonstrated glycaemic thresholds for diabetes-specific retinopathy to be about 6.4%.

4.6 Summary

In this chapter, our study of newly diagnosed T2DM subjects indicates that most parameters of glycaemic control and β-cell secretory status at diagnosis and over the 5-year period of observation appear to be the main contributors to the development of DR. We have shown that:

- The factors that contribute significantly to the development of DR include a lower fasting and postprandial β-cell responsiveness at diagnosis and over the 5-year study period.
- The significant contribution of the increased postprandial and overall glycaemic exposure over the first 5 years from diagnosis.
- 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.
- The early history of a lower glycaemic exposure not leading to DR development by 5 years in our study is indicative of better early glycaemic milieu at diagnosis leading to a long-term risk reduction for DR.

Chapter 5
Follow-up at 5 years of subjects with type 2 diabetes with retinopathy at diagnosis

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5.1 Introduction

5.2 Aim of the study

5.3 Methods

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5.6 Summary

5.1 Introduction

In the previous chapters (Chapters 3 and 4), the relevance of β-cell dysfunction, fasting, postprandial and overall glycaemia has been demonstrated on the prevalence and incidence of DR in newly diagnosed subjects with T2DM without DR at diagnosis over the 5-year follow-up study. In this chapter the influence of similar β-cell and metabolic indices on the future course of existing DR in newly-diagnosed subjects with T2DM over the same follow-up time period of 5 years is examined. The relationship between HBA_{1c} (Klein R et al. 1995; Kim HK et al. 1998; Looker HC et al. 2003) and FPG (Lee ET et al. 1992; Chen MS et al. 1995; Cikamatana et al. 2007; Zoppini G et al. 2009) on DR progression is well documented with the contribution of PPG more recently recognised (Voutilainen-Kaunisto RM et al. 2001; Shiraiwa T et al. 2005b) although not widely reported. However, the contribution of fasting and postprandial β-cell function is rarely reported.

5.2 Aim of the study

The aim of this chapter is to analyse the association between (a) β-cell function, (b) insulin sensitivity, (c) glycaemic status ($HbA_{1c,}$ fasting and postprandial glucose and insulin) and the future of DR over a 5-year period in newly-diagnosed and treatment-naïve type 2 diabetic subjects with DR at diagnosis

5.3.1 Subjects and Methods

Of the 293 subjects who had data over the 5 years from diagnosis i.e. at baseline, Year 1, Year 2 and Year 5, 41 subjects had evidence of DR at diagnosis and will be the subject of the analysis presented in this chapter. The data on 233 subjects with no DR at diagnosis has been detailed in the preceding chapter (Chapter 4).

The subject recruitment, experimental protocol, data analysis and basic statistical analysis are described in detail in chapter 2.

5.4 Results

5.4.1: Grades of DR over the 5-year study period

The grades of DR for the 41 subjects over the 5-year study period are detailed in table 5.1.

Table 5.1: Grades of DR over the 5 years

	Diagnosis	**Year 1**	**Year 2**	**Year 5**
NDR		10	10	7
BDR	31	17	19	19
Maculopathy	7	4	6	9
PPDR	3	4	3	3
PDR	-	1	2	3
Missing data	-	5	1	-
Total	41	36 (5)	40 (1)	41

NDR: No DR, BDR: Background DR, PPDR: Preproliferative DR, PDR: Proliferative DR.

As the number of subjects within each grade was small, they were classified into two severity categories of DR (Table 5.2) as per the European Field Guide (Kohner EM and Porta M 1990).

i) Non-Sight Threatening Diabetic Retinopathy (NSTDR), which included Background Diabetic Retinopathy (BDR) and Pre-proliferative Diabetic Retinopathy (PPDR),

ii) Sight Threatening Diabetic Retinopathy (STDR), which included Proliferative Diabetic Retinopathy (PDR) and Definite Maculopathy (M2).

Table 5.2: Categories of DR over the 5 years

	Diagnosis	Year 1	Year 2	Year 5
NDR		10	10	7
NSTDR	34	21	22	22
STDR	7	5	8	12
Missing data	-	5	1	-
Total	41	36 (5)	40 (1)	41

NDR: No DR, NSTDR: Non-Sight Threatening Diabetic Retinopathy, STDR: Sight Threatening Diabetic Retinopathy

They were also further sub-classified according to whether the categories of:

i) DR remained same over 5 years – “Same” (n = 25)

ii) DR progressed same over 5 years – “Progressors” (n = 7) and

iii) DR regressed over 5 years – “Regressors” (n = 9)

Of the 25 subjects who maintained the same status in relationship to NSTDR/STDR: 18 subjects had BDR, 2 had PPDR and 5 had maculopathy. [Of the18 subjects who had BDR 17 remained unchanged with 1 developing PPDR by Year 5; 2 with PPDR– 1 remained PPDR and 1 regressed to BDR by Year 5; 5 with Maculopathy– 3 remained unchanged and 2 developed PDR by Year 5]

<u>Of the 9 “Regressors”:</u> 7 subjects with BDR at diagnosis had NDR at Year 5 and 2 subjects with Maculopathy at diagnosis reverted to BDR and PPDR at year 5.

<u>Of the 7 “Progressors”:</u> there were 6 subjects with BDR and 1 with PPDR and they progressed to develop Maculopathy (6) and PDR (1) over the 5-year study period.

The 41 subjects were studied at diagnosis, Year 1, 2 and 5 and for the total exposure over the 5-year follow-up period for the purpose of comparing these 3 groups of subjects.

5.4.2 Baseline characteristics of subjects with T2DM and DR at the time of diagnosis followed up over a 5-year period

The baseline characteristics of subjects with T2DM and DR at the time of diagnosis who were followed up over a 5-year period according to the evolution of their DR are represented in Table 5.3.

Of the 41 subjects (34 male and 7 female) with a mean age of 54 (SD ± 8) years, 25 (61%) remained at the same stage of DR over the 5 years, 9 (22 %) regressed, and 7 (17 %) progressed from their baseline level of DR.

At baseline, subjects who progressed by Year 5 presented with a significantly higher $HbA_{1c,}$ (p= 0.00) (Table 5.3). Other baseline characteristics were not significantly different between the 3 groups. However there was a distinct trend that the progressors were leaner with a lower weight and BMI and also had a higher diastolic BP, but these parameters did not reach statistical significance.

Table 5.3: Baseline characteristics of subjects with Diabetic Retinopathy whose grade of DR regressed over the 5 year time period since diagnosis compared to those whose grades of DR remained same or progressed.

	All subjects	**Regressors**	**Same**	**Progressors**	**p value (between groups)**
Number (%)	**41 (100)**	**9 (22)**	**25 (66)**	**7 (17)**	**-**
Age at presentation (years)	54 (8)	57 (10)	53 (8)	55 (8)	0.489
Male Sex (%)	34 (83)	8 (89)	20 (80)	6 (86)	0.812
Weight (kg)	82 (17)	90 (23)	81 (14)	73 (18)	0.290
BMI ($kg.m^2$)	29 (6)	31 (7)	29 (5)	26 (5)	0.207
Systolic blood pressure (mmHg)	137 (17)	143 (16)	133 (15)	143 (23)	0.400
Diastolic blood pressure (mmHg)	85 (12)	86 (12)	82 (10)	93 (16)	0.226
Total Cholesterol (mmol/L)	5.4 (1.4)	6.1 (2.0)	5.2 (1.2)	5.3 (1.1)	0.629
HbA1c (%)	8.5 (1.7)	6.7 (1.5)	8.6 (1.5)	10.1 (0.8)	**0.000**

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

5.4.3 Comparative analysis of the metabolic parameters at diagnosis for the three groups of subjects with type 2 diabetes mellitus and diabetic retinopathy.

The metabolic variables during the MTT at baseline, for the subjects who remained at the same stage of DR, progressed or regressed by 5 years after diagnosis are detailed in Table 5.4. Those who progressed by Year 5, compared to those who did not, had a higher HbA_{1c} (p= 0.000), a lower estimated fasting β-cell responsiveness i.e. M_0 (p= 0.003) and β-cell function i.e. 'HOMA-B' (p= 0.001). The progressors had the lowest β-cell responsiveness and function followed by the subjects who remained at the same category, with the regressors having the highest β-cell responsiveness and function at diagnosis. The lower fasting insulin concentrations (p= 0.036) resulted in a higher fasting glucose (p= 0.003) at diagnosis in the progressors compared to the other categories.

In the postprandial state, those individuals whose DR progressed by Year 5, compared to those who did not progress had a lower estimated postprandial β-cell responsiveness i.e. M_1 (p= 0.012) associated with a higher postprandial (2 hour) glucose (p= 0.036) and lower postprandial insulin levels (p= 0.006). The progressors again had the lowest estimated postprandial β-cell responsiveness followed by the subjects who remained at the same category, with the regressors having the highest postprandial β-cell responsiveness at diagnosis. This is appropriately reflected in the trends exhibited by the postprandial glucose and insulin.

Over the 4-hour MTT study period, subjects who progressed had a significantly higher $AUC_{Glucose\ (0\text{-}240min)}$ ($p= 0.001$) and lower $AUC_{Insulin\ (0\text{-}240\ min)}$ ($p= 0.018$) in comparison to those who remained at the same level of DR or whose DR regressed over the 5-year period (Table 5.4).

The plasma glucose, HbA_{1c}, insulin profiles during the MTT and indices of β-cell responsiveness (M_0 and M_1) and HOMA B and S in subjects with existent DR at diagnosis and according to progression or not are illustrated in Figures 5.1 a, b, 5.2 and 5.3.

Table 5.4: Comparison in subjects with Diabetic Retinopathy whose grade of DR remains same throughout 5 years since diagnosis compared to those whose grades of DR Regressed or Progressed by 5 years from diagnosis of T2DM by their **parameters at diagnosis**

	Regressors	**Same**	**Progressors**	**p value**
Number	**9**	**25**	**7**	
Year 0 Parameters				
Fasting Glucose (mmol/L)	9.2 (7.5 - 12.3)	12.5 (10.1 - 14.7)	16.0 (14.3 - 17.5)	**0.003**
Postprandial Glucose (mmol/L) (120 mins)	12.1 (10.2 - 16.6)	16.2 (13.7 - 17.7)	20.9 (16.9 - 23.2)	**0.015**
$AUC_{Glucose (0\text{-}240min)}$ (mmol/L)	9.3 (8.0 - 12.6)	13.9 (11.3 - 16.2)	19.2 (15.5 - 20.0)	**0.001**
HbA1c (%)	6.5 (6.1 - 7.9)	8.8 (7.1 - 9.8)	10.2 (9.3 - 10.8)	**0.000**
Fasting Insulin (pmol/L)	65.0 (40.3 - 93.8)	44.0 (33.5 - 75.5)	32.0 (21.5 - 43.1)	**0.036**
Postprandial Insulin (pmol/L) (120 mins)	358.0 (146.5 - 744.8)	162.0 (106.8 - 226.0)	103.3 (79.0 - 133.5)	**0.006**
$AUC_{Insulin (0\text{-}240min)}$ (pmol/L)	220.7 (99.0 - 499.7)	121.0 (88.8 - 154.7)	87.8 (59.8 - 102.6)	**0.018**
HOMA-B (%)	43.6 (20.9 - 67.8)	19.6 (13.6 - 28.1)	10.0 (6.7 - 15.5)	**0.001**
HOMA-S (%)	56.5 (42.2 - 92.8)	76.6 (44.5 - 110.2)	102.6 (52.1 - 132.2)	0.244
HOMA-IR	1.8 (1.1 - 2.4)	1.3 (0.9 - 2.3)	1.0 (0.8 - 1.9)	0.244
M_0 ($*10^{-9}$ pmol/kg/min)	6.8 (3.9 - 10.4)	3.4 (2.8 - 4.6)	2.4 (0.6 - 3.2)	**0.003**
M_1 ($*10^{-9}$ pmol/kg/min)	29.5 (14.5 - 41.6)	11.3 (8.9 - 19.0)	7.3 (1.5 - 12.8)	**0.012**

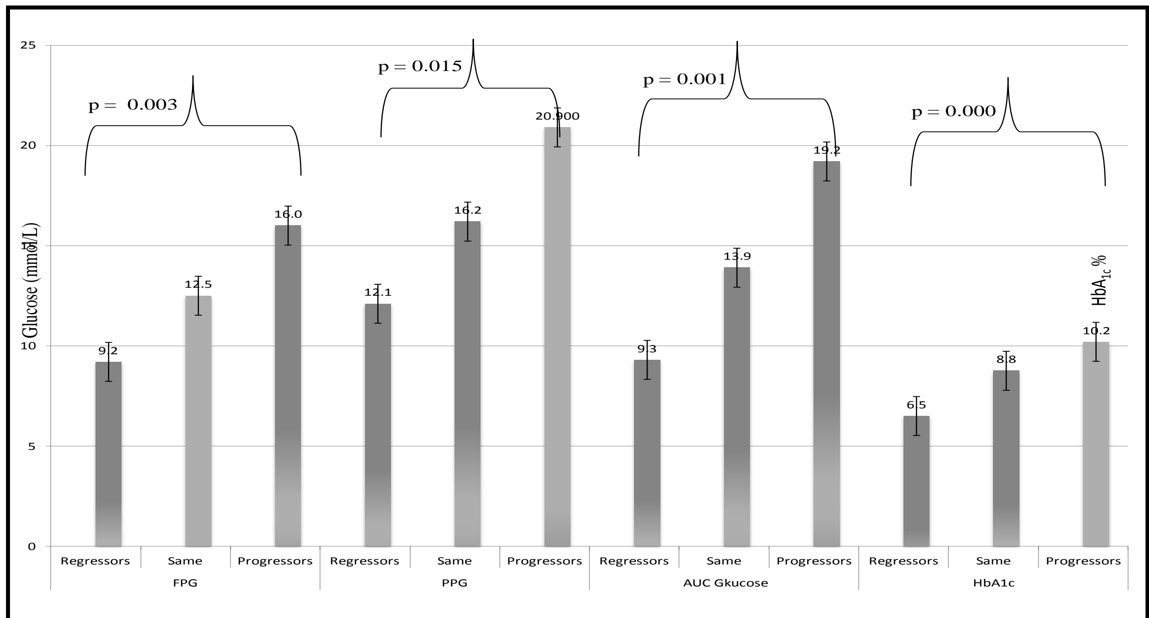

Figure 5.1 a: Plasma Glucose [Fasting (FPG), Postprandial (PPG) and AUC $_{(0-240min)}$ (Area under the Curve) during MTT] and HbA_{1c} at diagnosis (mean±SEM) in subjects with Diabetic Retinopathy at diagnosis who regressed, remained at the same stage or progressed by 5 years post diagnosis.

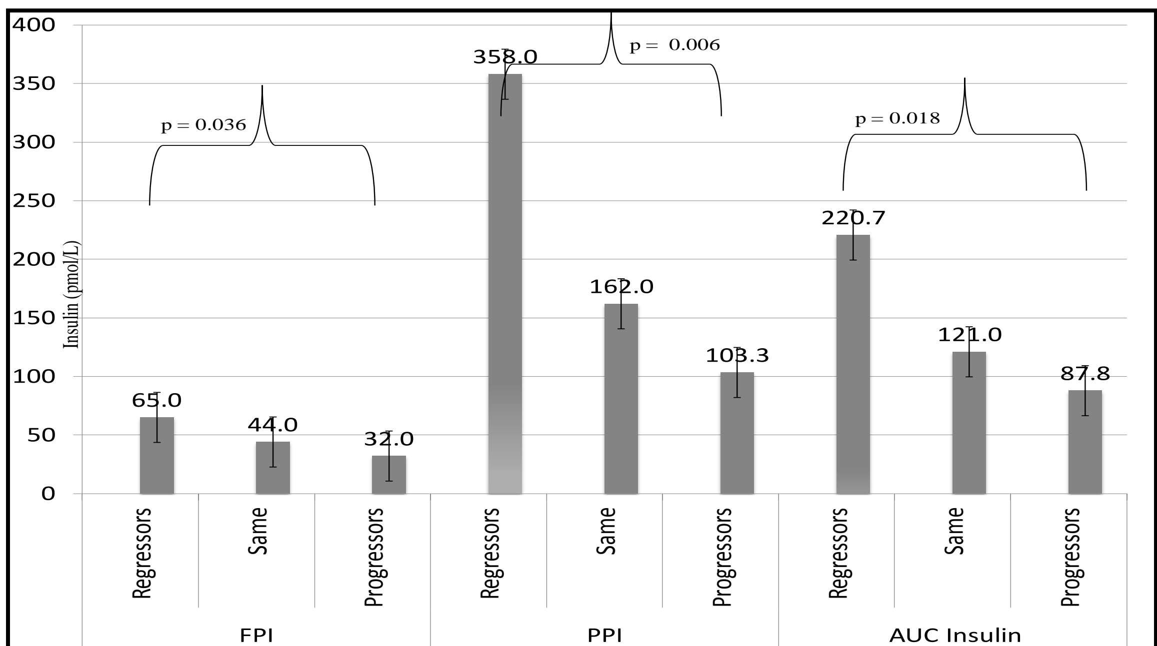

Figure 5.1 b: Plasma Insulin Profiles [Fasting (FPI), Postprandial (PPI) and AUC $_{(0-240min)}$ (Area under the Curve) during MTT] at diagnosis (mean±SEM) in subjects with Diabetic Retinopathy at diagnosis who regressed, remained at the same stage or progressed by 5 years post-diagnosis.

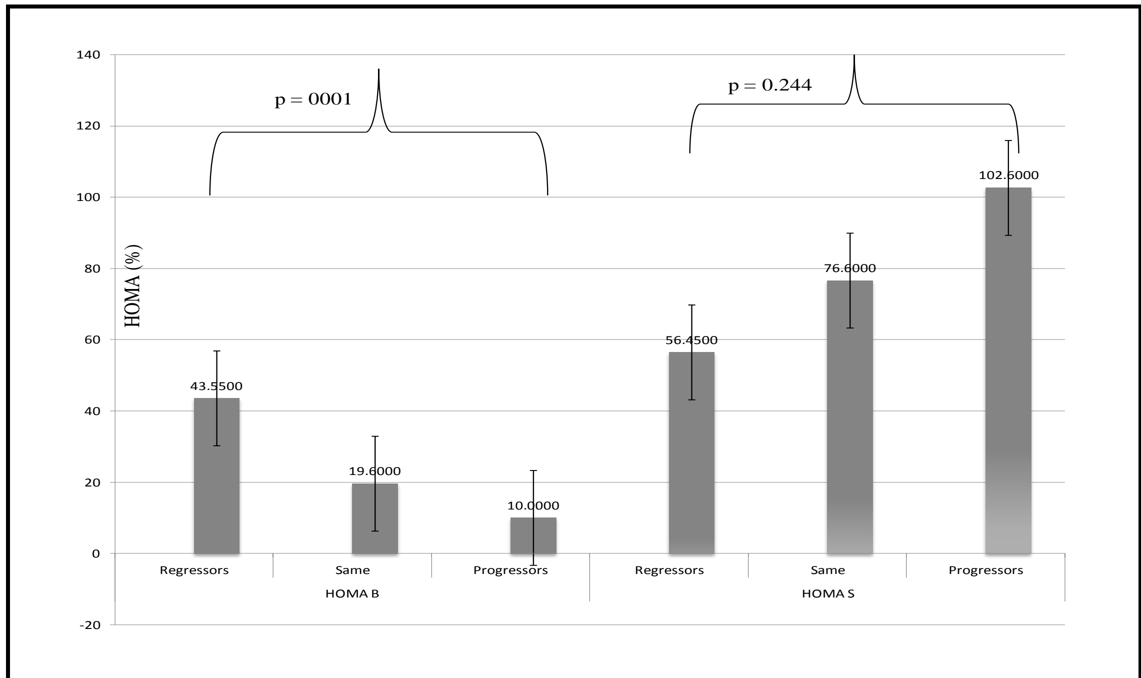

Figure 5.2: β-cell function (HOMA B) and Insulin sensitivity (HOMA S) (mean±SEM) in subjects with Diabetic Retinopathy at diagnosis who regressed, remained at the same stage or progressed by 5 years post diagnosis.

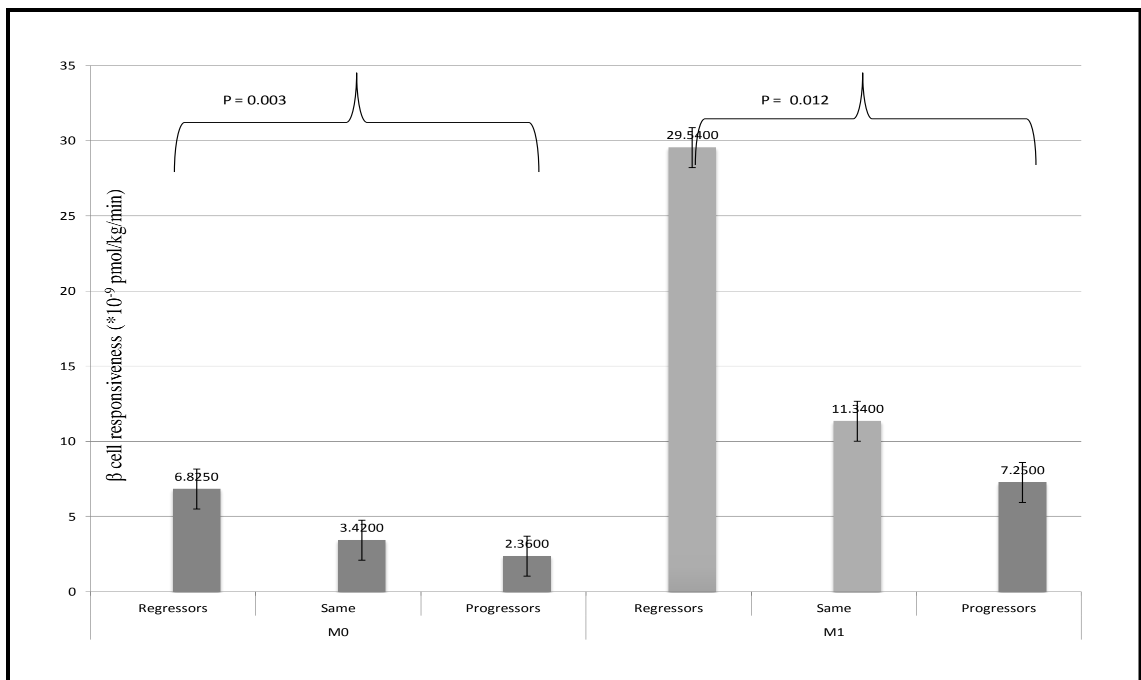

Figure 5.3: Fasting and postprandial β-cell responsiveness (M_0 and M_1) (mean±SEM) in subjects at diagnosis (subjects with diabetic retinopathy at diagnosis) who regressed, remained at the same stage or progressed by 5 years post diagnosis.

5.4.4 Univariate regression analysis

Based on the inter-group differences (Table 5.4) univariate logistic regression was undertaken. It demonstrated that HbA_{1c} and $AUC_{Glucose\ (0\text{-}240min)}$ was significantly higher in the progressors compared to those who remained at the same category and had a positive/direct relationship. Postprandial insulin, $AUC_{Insulin\ (0\text{-}240min)}$, M_0, HOMA-B and M_1 were all significantly lower in the progressors compared to those who remained in the same category and had a negative/inverse relationship.

It also showed that in subjects whose grade of DR progressed, compared to those whose grade regressed, there was a significant positive/direct association with HbA_{1c}, fasting glucose, postprandial glucose and $AUC_{Glucose\ (0\text{-}240min)}$, It also exhibited a significant negative/inverse relationship with the fasting insulin, postprandial insulin, $AUC_{Insulin\ (0\text{-}240min)}$, M_0, HOMA-B and M_1 (Table 5.5).

Table 5.5: Univariate and Multivariate logistic regression depicting variables independently associated with progression or regression of Diabetic Retinopathy by 5 years from diagnosis of T2DM.

	Univariate Analysis		Univariate Analysis		Multivariate Analysis		Multivariate Analysis	
	Crude OR (95% CI)	p	Crude OR (95% CI)	p	OR (95% CI) (Adjusted) **	p	OR (95% CI) (Adjusted) **	p
Year 0 Parameters	Regressors vs. Same		Regressors vs. Progressors		Regressors vs. Same		Regressors vs. Progressors	
HbA1c (%)	2.75 (1.23, 6.14)	**0.014**	8.10 (2.25, 29.17)	**0.001**	-	-	-	-
Fasting Glucose (mmol/L)	1.40 (1.00, 1.87)	0.059	2.12 (1.28, 3.54)	**0.004**	2.41 (1.11, 5.23)	**0.027**	3.74 (1.53, 9.16)	**0.004**
Postprandial Glucose (mmol/L)	1.19 (0.95, 1.48)	0.124	1.58 (1.13, 2.20)	**0.007**	1.66 (1.03, 2.68)	**0.039**	2.23 (1.26, 3.93)	**0.006**
AUC $_{Glucose\ (0-240min)}$ (mmol/L)	1.39 (1.05, 1.85)	**0.023**	2.09 (1.34, 3.25)	**0.001**	2.05 (1.10, 3.81)	**0.023**	3.12 (1.50, 6.51)	**0.002**
Fasting Insulin (pmol/L)	0.99 (0.97, 1.01)	0.190	0.93 (0.88, 1.00)	**0.035**	0.99 (0.96, 1.02)	0.530	0.93 (0.87, 1.00)	0.064
Postprandial Insulin (pmol/L)	0.99 (0.99, 1.00)	**0.033**	0.97 (0.95, 0.99)	**0.025**	0.97 (0.94, 1.01)	0.114	0.95 (0.91, 0.99)	**0.025**
AUC $_{Insulin\ (0-240min)}$ (pmol/L)	0.99 (0.98, 0.99)	**0.035**	0.96 (0.93, 0.99)	**0.022**	0.98 (0.96, 1.01)	0.122	0.94 (0.89, 0.99)	**0.011**
M_0 ($*10^{-9}$ pmol/kg/min)	0.71 (0.52, 0.97)	**0.032**	0.26 (0.10, 0.67)	**0.005**	0.33 (0.11, 1.02)	0.054	0.09 (0.02, 0.48)	**0.005**
M_1 ($*10^{-9}$ pmol/kg/min)	0.92 (0.86, 0.99)	**0.022**	0.82 (0.69, 0.96)	**0.017**	0.90 (0.81, 1.00)	0.055	0.80 (0.66, 0.98)	**0.031**
HOMA-B (%)	0.96 (0.92, 0.99)	**0.046**	0.75 (0.61, 0.93)	**0.008**	0.89 (0.79, 1.00)	0.053	0.66 (0.49, 0.91)	**0.010**

5.4.5 Multivariate regression analysis

Factors 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 5.5). The p value was calculated using the likelihood ratio test.

Fasting glucose: Regressors vs. Same - OR 2.41 [95% CI 1.11, 5.23] p= 0.027), Regressors vs. Progressors - OR 3.74 [95% CI 1.53, 9.16] p= 0.004). Postprandial glucose: Regressors vs. Same - OR 1.66 [95% CI 1.03, 2.68] p= 0.039), Regressors vs. Progressors - OR 2.23 [95% CI 1.26, 3.93] p= 0.006). $AUC_{Glucose\ (0\text{-}240min)}$: Regressors vs. Same - OR 2.05 [95% CI 1.10, 3.81] p= 0.023), Regressors vs. Progressors - OR 3.12 [95% CI 1.50, 6.51] p= 0.002). The data show a significant contribution of dysglycaemia to the evolution of the various stages of DR in type 2 diabetic subjects who present with DR at diagnosis (Table 5.5).

Postprandial insulin (OR 0.95 [95% CI 0.91, 0.99] p= 0.025) and $AUC_{Insulin\ (0\text{-}240min)}$ (OR 0.94 [95% CI 0.89, 0.99] p= 0.011) also show the contribution of a deficient insulin secretory response to a standard meal at the time of diagnosis. This led to fasting and postprandial hyperglycaemia resulting in progression of DR by 5 years, in contrast to those who regressed when adjusted for the mentioned variables (Table 5.5).

Measures of β-cell function M_0 (OR 0.09 [95% CI 0.02, 0.48] p= 0.005), HOMA-B (OR 0.66 [95% CI 0.49, 0.91] p= 0.010) and M_1 (OR 0.80 [95% CI 0.66, 0.98] p= 0.031) show independent association with progression of DR compared to those who regressed. However the difference between regressors and those who remained at the same category was not statistically significant.

5.4.6 Comparative analysis of subjects with type 2 diabetes mellitus (with diabetic retinopathy at diagnosis) at years 1, 2 and 5 from diagnosis

Demographic characteristics at years 1, 2 and 5 including age, weight, BMI, systolic and diastolic blood pressure, total cholesterol and HbA_{1c} of the subjects who remained at the same stage of DR, progressed or regressed by 5 years after diagnosis were not significantly different between the 3 groups. (Appendix Table 2 to 4)

The Years 1 and 2 metabolic variables during the MTT for the subjects who remained at the same stage of DR, progressed or regressed by 5 years after diagnosis are detailed in Table 5.6. Those who progressed by Year 5, compared to those who did not, had a lower estimated fasting β-cell responsiveness at Year 1 M_0 ($p= 0.001$) and Year 2 M_0 ($p= 0.008$) and a lower estimated postprandial β-cell responsiveness at Year 1 M_1 ($p= 0.012$), causing a higher Year 2 fasting glucose ($p= 0.020$) and a higher Year 2 postprandial (2 hour) glucose ($p= 0.049$) (Table 5.6). Year 1, fasting and postprandial glucose show a similar trend but failed to attain statistical significance.

Over the 4-hour MTT study period, subjects who progressed had significantly higher Year 1 $AUC_{Glucose\ (0-240min)}$ ($p= 0.024$) and Year 2 $AUC_{Glucose\ (0-240min)}$ ($p= 0.032$) in comparison to those who remained the same or regressed by Year 5 (Table 5.6).

Table 5.6: Comparisons by metabolic parameters, 1 and 2 years post-diagnosis between subjects with Diabetic Retinopathy whose grade of DR remained the same throughout 5 years from diagnosis compared to those whose DR grades Regressed or Progressed by 5 years post-diagnosis

	Regressors	**Same**	**Progressors**	
Year 1 and 2 Parameters	Median (IQR)	Median (IQR)	Median (IQR)	p value
Number	**9**	**25**	**7**	
Yr 1 Fasting Glucose (mmol/L)	7.1 (6.8 – 7.3)	7.9 (6.9 – 9.0)	9.1 (7.4 – 12.9)	0.079
Yr 1 Postprandial Glucose (mmol/L)	9.7 (7.6 – 11.1)	11.4 (9.4 – 13.2)	12.5 (11.4 – 17.4)	0.056
Yr 1 $AUC_{Glucose\ (0\text{-}240min)}$ (mmol/L)	7.5 (6.9 – 9.5)	9.2 (8.3 – 10.0)	10.8 (10.2 – 15.6)	**0.024**
Yr 1 HbA1c (%)	6.1 (5.2 6.7)	6.6 (5.7 – 7.2)	6.0 (5.3 – 8.3)	0.371
Yr 2 Fasting Glucose (mmol/L)	6.9 (6.1 – 8.4)	8.8 (7.8 – 10.4)	9.6 (9.2 – 12.4)	**0.020**
Yr 2 Postprandial Glucose (mmol/L)	10.0 (9.0 – 12.7)	13.1 (10.0 – 15.3)	14.7 (12.8 – 16.3)	**0.049**
Yr 2 $AUC_{Glucose\ (0\text{-}240min)}$ (mmol/L)	8.3 (6.7 – 10.2)	10.3 (8.3 – 12.8)	12.2 (10.9 – 15.0)	**0.032**
Yr 2 HbA1c (%)	6.3 (5.0 – 6.8)	6.7 (6.2 – 7.6)	5.7 (5.6 – 8.3)	0.226
Yr 1 M_0 ($*10^{-9}$ pmol/kg/min)	10.2 (7.0 – 13.0)	5.9 (4.2 – 8.5)	2.8 (1.5 – 4.1)	**0.001**
Yr 1 M_1 ($*10^{-9}$ pmol/kg/min)	30.1 (20.6 – 53.5)	21.8 (12.3 – 28.1)	8.9 (2.0 – 27.0)	**0.044**
Yr 2 M_0 ($*10^{-9}$ pmol/kg/min)	8.6 (6.0 – 14.0)	4.5 (2.6 – 6.9)	3.0 (1.3 – 4.8)	**0.008**
Yr 2 M_1 ($*10^{-9}$ pmol/kg/min)	32.7 (20.0 – 57.5)	17.3 (10.7 – 30.8)	15.2 (3.0 – 26.4)	0.115

Data expressed as median (1st – 3rd IQR), AUC = Area Under the Curve

5.4.7 Univariate and Multivariate regression analysis of Year 1, 2 and 5 Parameters

Based on the inter-group differences (Table 5.6), univariate logistic regression was conducted. This demonstrated that Year 2 fasting glucose (p= 0.029), Year 1 M_0 (p= 0.016), and Year 2 M_0 (p= 0.039) were significantly related to regression of DR compared to those who remained at the same grade of DR by Year 5 (Table 5.7). It also showed that subjects whose grade of DR progressed, compared to those whose grade regressed, had a significant relationship at Year 1 of $AUC_{Glucose\ (0-240min)}$ (p= 0.028) and M_0 (p= 0.018). Also at Year 2 assessment, fasting glucose (p= 0.018), postprandial glucose (p= 0.042) and $AUC_{Glucose\ (0-240min)}$ (p= 0.022) were significantly related (Table 5.7)

Factors associated with DR in univariate logistic regression analyses when adjusted for age, sex, BMI, total cholesterol and systolic blood pressure showed that none of the metabolic factors had any significant contribution to the afore-mentioned outcome.

Table 5.7: Univariate logistic regression depicting variables independently associated with progression or regression of Diabetic Retinopathy by 5 years from diagnosis of T2DM

	Univariate analysis		Univariate analysis	
	Crude OR (95% CI)	p	Crude OR(95% CI)	p
Year 1 and 2 Parameters	Regressors vs. Same		Regressors vs. Progressors	
Yr 1 AUC $_{Glucose (0-240min)}$ (mmol/L)	1.69 (0.93, 3.09)	0.087	3.87 (1.16, 12.96)	**0.028**
Yr 2 Fasting Glucose (mmol/L)	2.13 (1.08, 4.19)	**0.029**	2.64 (1.19, 5.90)	**0.018**
Yr 2 Postprandial Glucose (mmol/L)	1.30 (0.96, 1.75)	0.086	1.61 (1.02, 2.53)	**0.042**
Yr 2 HbA1c (%)	2.16 (0.99, 4.73)	0.054	1.92 (0.71, 5.22)	0.203
Yr 2 AUC $_{Glucose (0-240min)}$ (mmol/L)	1.55 (0.99, 2.42)	0.056	1.92 (1.10, 3.36)	**0.022**
Yr 1 M_0 ($*10^{-9}$ pmol/kg/min)	0.65 (0.46, 0.92)	**0.016**	0.18 (0.04, 0.74)	**0.018**
Yr 1 M_1 ($*10^{-9}$ pmol/kg/min)	0.95 (0.91, 1.00)	0.065	0.89 (0.78, 1.00)	0.052
Yr 2 M_0 ($*10^{-9}$ pmol/kg/min)	0.76 (0.59, 0.99)	**0.039**	0.55 (0.32, 0.93)	**0.026**
Yr 2 M_1 ($*10^{-9}$ pmol/kg/min)	0.96 (0.92, 1.01)	0.098	0.93 (0.85, 1.02)	0.118

(AUC = Area Under the Curve)

5.4.8 Comparative analysis of subjects with type 2 diabetes mellitus at diagnosis (with diabetic retinopathy) utilising the means of all metabolic parameters over the 5-year study period

We calculated the average/mean of all the measured metabolic variables from diagnosis including variables at Years 1, 2 and 5 years post-diagnosis. These mean averaged metabolic variables of T2DM subjects have been defined as the follow-up indicator of diabetic control over 5 years. These mean metabolic values of T2DM subjects, whose stage of DR remained the same, progressed or regressed by Year 5 are detailed in Table 5.8.

Those who progressed by Year 5, compared to those who did not, had a significantly higher mean averaged fasting plasma glucose (p= 0.010), higher mean averaged postprandial (2 hour) glucose (p= 0.024) and a lower mean averaged estimated fasting i.e. M_0 (p= 0.005) and postprandial β-cell responsiveness i.e. M_1 (p= 0.015) over 5 years post-diagnosis of T2DM.

5.4.9 Univariate and Multivariate regression analysis of Mean averaged metabolic parameters over 5 years

Based on the inter-group differences (Table 5.8) univariate and multivariate logistic regression was conducted on the significant variables but did not show any significant contribution to the measured outcome.

Table 5.8: Comparison of the mean averaged metabolic variables over a 5-year period (Year 0, 1, 2 and 5) during the Meal Tolerance Test (subjects with Diabetic Retinopathy) whose grade of DR remained the same throughout 5 years from the time of diagnosis compared to those whose grades of DR Regressed or Progressed by 5 years from diagnosis of T2DM

	Regressors	**Same**	**Progressors**	
Average values (0-5 years)	Median (IQR)	Median (IQR)	Median (IQR)	p value
Number	**9**	**25**	**7**	
Mean Fasting Glucose (mmol/L)	8.4 (7.4 – 8.7)	9.5 (9.2 – 10.2)	13.2 (11.4 – 15.2)	**0.010**
Mean Postprandial Glucose (mmol/L) (120 mins)	11.3 (10.7 – 12.3)	13.0 (11.3 – 14.2)	17.8 (15.6 -19.1)	**0.024**
Mean M_0 ($*10^{-9}$ pmol/kg/min)	8.7 (5.9 - 10.3)	5.0 (4.1 – 6.7)	2.7 (2.5 – 2.9)	**0.005**
Mean M_1 ($*10^{-9}$ pmol/kg/min)	29.3 (21.8 – 42.2)	19.4 (13.2 - 23.0)	8.0 (6.9 – 9.3)	**0.015**

5.5 Discussion

This analysis shows a significant independent association between the progression of existent DR over a period of 5 years with the degree of hyperglycaemia and insulinopaenia observed in response to a MTT, conducted at the time of diagnosis. The fasting, postprandial and overall (HbA_{1c}) glycaemic exposure at diagnosis all exhibits an independent association with DR progression. Furthermore, measures of fasting β-cell function, M_0 and HOMA-B and postprandial β-cell responsiveness (M_1), estimated at diagnosis, also showed an independent association with DR progression. This reflects that our T2DM subjects with poorer β-cell function and with a greater degree of hyperglycaemia and worsening insulin secretion on presentation were at a greater risk of their DR progressing during 5 years of follow-up since diagnosis.

In this study of newly-diagnosed, previously untreated T2DM subjects, who's existent DR progressed by 5 years, had a higher baseline HbA1c (10.1%) compared to those remaining at the same stage of DR (8.6%) and especially those who regressed (6.7%). This association remained significant on univariate logistic regression. However, the HbA_{1c} level at Years 1, 2, 5 post diagnosis and the mean value over the entire 5-year period did not show a significant association with progression of DR

Baseline glycaemic control represented by the glycosylated haemoglobin level but not C-peptide secretion (random value) was highlighted to be important in the

Wisconsin Epidemiologic Study of Diabetic Retinopathy (WESDR), reported by Klein and colleagues (Klein R et al. 1995). They studied individuals with younger and older onset DM over 6 years. This was further examined by Looker et al. who studied 67 Pima Indian subjects with NPDR at baseline and similarly showed a significant association between baseline hyperglycaemia (HbA_{1c}) and progression of DR (Looker HC et al. 2003). Kim et al. studied T2DM Korean subjects and showed a higher mean HbA_1 during the follow-up period in progressors (to PDR) than the non-progressors (Kim HK et al. 1998). Thus, our finding relating HbA_{1c} with progression of DR is in concordance with these studies.

In addition our study clearly demonstrates that both fasting and postprandial hyperglycaemia coexistent with a deficient insulin response during a MTT conducted at diagnosis is associated with progression of DR by 5 years. In our study population, the FPG at diagnosis and at Year 2 was independently associated with progression of DR by 5 years. Although the FPG at Year 1 and 5 failed to reach significance, the mean FPG over the entire study period was significantly associated with progression of DR. FPI at diagnosis was higher in those subjects with DR progression, in comparison to those who remained at the same stage or regressed, but lost it significance after adjusting for the variables. FPI at year 1, 2, 5 and Mean FPI showed no association with DR progression.

In a different population, the Verona Diabetes Study (Italy) in elderly T2DM subjects showed by the second eye examination after 4 to 5 years follow up, 45

patients had progressed to a more severe degree of DR (Zoppini G et al. 2009). Progression of DR was independently predicted by the average glycaemia (glycated haemoglobin or Mean-FPG) over the study period but not by glycaemic variability in the fasting state (CV-FPG). Similarly, the Blue Mountains Eye Study (Australia) determined the 5-year incidence and progression of DR in their population-based cohort (Mean age 66±8 years) during the period 1992–1994. The baseline risk factors associated with retinopathy progression, after adjusting for age and gender, were an increase in fasting blood glucose, and longer diabetes duration, (Cikamatana et al. 2007).

Numerous other studies have also evaluated different glycaemic parameters that relate to progression of retinopathy. In 1986, Chen et al studied 471 T2DM subjects from primary health-care centres in northern Taiwan and over 4 years, the cumulative incidence of DR progression was 30% with the cumulative incidence of progression to PDR being 5.8%. Glycaemic control, as measured by the mean FPG and $HbA_{1c,}$ was significantly related to the progression of DR (Chen MS et al. 1995). In a more prolonged study involving Oklahoma Indians, after a mean follow-up time of 12.7 years, the overall incidence of PDR (354 participants) was 18.6%; 45% of those with BDR at baseline developed PDR. Significant independent predictors of PDR were FPG, duration of diabetes, plasma cholesterol, SBP and therapeutic regimen (Diet<OHA<Insulin) (Lee ET et al. 1992). FPG>11.1 mM (200 mg/dl) increased the risk of DR to 3.6 times that for a level <7.8 mM (140 mg/dl); 74% of those who had BDR and a baseline FPG>11.1 mM (200 mg/dl) developed PDR. (Lee ET et al. 1992). These four studies above corroborate our findings of an

association of baseline dysglycaemia facilitating the progression of DR. Our mean FPG over the 5 years was higher in progressors than those who remained at same stage or regressed, although it lost its significance after adjusting for the other putative risk factors. This is possible because the subjects were started on treatment post-diagnosis during the 5-year study period.

Our study also reports that PPG at diagnosis and at Year 2 and the Mean PPG over 5 years show an independent positive association with the progression of DR during the overall study period analysis, only baseline PPG shows an independent contribution. PPG at Ye. After regression ar 1 and 5 failed to reach statistical significance. This detailed postprandial glycaemic exposure has not been documented before. PPI at diagnosis showed a significant independent contribution to the progression of DR by 5 years, although the PPI at years 1, 2 and 5 and Mean FPI showed no association with the changes in DR. This can potentially be explained by the lower insulin secretion together with a more advanced stage of the disease process.

In support of our findings, Shiraiwa et al. in the last decade have shown an independent correlation between postprandial plasma glucose and insulin with the progression of DR in T2DM Japanese subjects over a 5-year period. PPG was shown to be a stronger predictor than HBA_{1c} in their cohort of patients with T2DM (Shiraiwa T et al. 2005b). A Finnish study also examined subjects with newly-diagnosed T2DM in a 10-year prospective study (Voutilainen-Kaunisto RM et al.

2001). The frequency of retinopathy was determined by grading of 45° fundus photographs at baseline and after 5 and 10 years. The frequency of retinopathy in T2DM subjects increased sharply after 5 years and by 10 years, 55% of diabetic subjects showed signs of DR. The authors reported that FPG, 2 hour PPG and HbA_{1c} at 5 years, but not at baseline, were predictors of DR at 10-year follow-up. However, fasting insulin and fasting C-peptide failed to show any association (Voutilainen-Kaunisto RM et al. 2001). These findings are similar to ours in reporting an association of DR progression with FPG, 2 hour PPG and HbA_{1c}, 5 years prior to its development. Further, Ohkubo et al. studied T2DM Japanese subjects over a 6-year period and concluded that intensive glycaemic control by multiple insulin injection therapy can delay both the onset and progression of DR. They proposed that a glycaemic threshold of HBA_{1c} <6.5%, FPG <110 mg/dl and 2 hour PPG <180 mg/dl prevented the onset and progression of diabetic microangiopathy (Ohkubo Y et al. 1995).

A further observation in our study was that the overall glucose response to a test meal ($AUC_{Glucose\ (0-240min)}$) at diagnosis and at Year 1 and Year 2 post-diagnosis revealed an independent association in those who progressed, compared to those whose DR regressed over the 5-year study time period. However the $AUC_{Glucose\ (0-240min)}$ at Year 5 and mean $AUC_{Glucose\ (0-240min)}$ over the 5-year observation period failed to reach statistical significance. The corresponding insulin response ($AUC_{Insulin\ (0-240min)}$) at diagnosis showed a significant independent contribution to the progression of DR by 5 years but $AUC_{Insulin\ (0-240min)}$ at year 1, 2, 5 and Mean $AUC_{Insulin\ (0-240min)}$ showed no association with progression of DR possibly as therapies

had been introduced. This reflects the finding of the overall glycaemic exposure to DR progression.

In our analysis, the few newly diagnosed T2DM subjects who exhibited DR at diagnosis, followed by progression over 5 years had significantly lower fasting and postprandial β-cell responsiveness during a MTT at diagnosis and also over the entire 5-year study period. The mean values however, failed to retain their significant association post regression analysis. There was also a significant association between lower fasting and postprandial β-cell function at Year 1 and lower fasting β-cell responsiveness at Year 2, with progression of DR. These parameters however lost their significance during multivariate analysis. There was no noted association with any parameters of insulin resistance/sensitivity.

This is an important parameter to consider here as there is limited mention of the influence of fasting and postprandial β-cell function with DR progression in the literature to date, except for the work of Suzuki and colleagues, who retrospectively studied the role of pancreatic β-cell insulin secretory capacity (24-hour urinary C-peptide) in the development of PDR in diabetic subjects with a duration of DM of greater than 10 years. The incidence of PDR during the 10-year follow-up period was highest in the group with the lowest 24-hour urinary C-peptide. These data are consistent with the view that low pancreatic β-cell insulin secretory capacity may be a risk factor for the development of PDR (Suzuki K et al. 1989). Whilst the WESDR showed that both younger and older-onset individuals with diabetes, treated with

insulin, with undetectable or low plasma c-peptide at baseline, had the highest rates of progression of DR, they found no relationship between DR progression and plasma c-peptide (Klein R et al. 1995).

Our findings, utilising a more detailed assessment of β-cell function in both the fasting and postprandial states, have shown that the progression of existent DR by 5 years in newly-diagnosed T2DM subjects was independently associated with a lower fasting and postprandial β-cell responsiveness at diagnosis. The fasting and postprandial insulinopaenia at diagnosis, in subjects who's DR progressed, is a reflection of failing β-cell function at diagnosis resulting in fasting and postprandial hyperglycaemia. Of note is that the majority of parameters (FPG, PPG, $AUC_{Glucose\ (0-240min)}$, PPI, $AUC_{Insulin\ (0-240min)}$, M_0, M_1 AND HOMA-B) at diagnosis contribute to the progression of DR, with no significant contribution from HbA_{1c} and other glycaemic parameters at year 5. This is compatible with the early history of lesser glycaemic exposure leading to a delayed effect in progression of DR. In our study, we established an independent association of M_0, HOMA-B and M_1 with progression of DR by measuring β-cell function in response to a standardised meal challenge, employing both the CPR program (Hovorka R et al. 1998) and the HOMA methodologies

Our analysis has demonstrated that in our subset of subjects with newly diagnosed T2DM, who present with DR at the time of diagnosis, both the fasting and postprandial glycaemic and β-cell responsiveness over a 5-year follow-up period

contribute significantly to the progression of DR. Fasting and postprandial glucotoxicity has an adverse effect on the retina seen at diagnosis and over the 5-year period which significantly increases the risk of progression of DR. Hence it becomes paramount that we aim to improve overall glycaemic control (HbA_{1c}) at diagnosis but try to minimise both its component parts i.e. fasting and postprandial glycaemia. There are currently various antidiabetic agents which have varying effects on the fasting and postprandial glycaemic control. These should be chosen judiciously according to the existing pathophysiology (phenotype) and optimally used from the early years of a "diabetic subject's journey". This aim should minimise the risk of progression of DR, which could have a profound effect on a person's quality of life due to the loss of vision and blindness.

5.6 Summary

- The factors that contribute significantly to the progression of DR include a lower fasting and postprandial β-cell responsiveness at diagnosis.
- The postprandial and $AUC_{Insulin\ (0\text{-}240min)}$ insulinopaenia at diagnosis in subjects who go on to develop DR is a reflection of a failing β-cell function at diagnosis.
- The early history of a significantly lower glycaemic exposure not leading to DR progression by 5 years in our study is indicative of better early glycaemic milieu at diagnosis leading to a long-term risk reduction for DR progression.

Chapter 6

Discussion

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6.1 Discussion

The aim of this thesis has been to assess the possible relationships between the glycaemic and β-cell secretory status and associated clinical risk factors with the presence and progression of DR over a 5-year period in individuals with T2DM. We recruited newly-diagnosed GAD antibody-negative and previously untreated T2DM subjects. The availability of data derived from a standard meal tolerance test and the insulin modified intravenous glucose tolerance test in a relatively large group of subjects with type 2 diabetes provided an unique insight into the dynamic glucose and insulin secretory responses to a nutrient and intravenous glucose challenge. This detailed information gave the opportunity to examine the real-world evolution of T2DM and its relationship with the presence, development and progression of DR. Type 2 DM subjects were studied near the time of diagnosis prior to any dietary or physical changes were made and after 1, 2 and 5 years of follow up. The analysis of the data has identified certain risk factors both at baseline and during the 5-year study period which contribute in a significant manner to the prevalence, incidence and progression of DR in subjects having presented with either no evidence of DR or with DR at diagnosis.

Shortly after diagnosis in our subjects with type 2 diabetes we examined in detail the association between the state of dysglycaemia i.e. overall glycaemia (HbA_{1c}) plus fasting and postprandial plasma glucose, β-cell function and insulin sensitivity with the presence of DR in our T2DM study population. Serial plasma glucose and insulin levels were measured during the standardized mixed meal challenge from which

indices of β-cell function i.e. HOMA-B, fasting (M_0) and postprandial (M_1) β-cell responsiveness and insulin sensitivity ie HOMA-S were derived. In addition, in a subgroup of subjects we also conducted the 'Frequently Sampled Intravenous Glucose Tolerance test' (FSIVGTT) employed to estimate the Acute Insulin Response to intravenous glucose (AIR_G), Insulin Sensitivity (S_I), and Glucose Effectiveness (S_G).

In the current literature as reviewed in detail earlier indicates that most studies have examined the contribution predominantly of overall glycaemic exposure (HbA_{1c}) and to a lesser extent FPG to i) the prevalence of DR in newly-diagnosed T2DM subjects, ii) the incidence of DR in subjects with no DR at diagnosis and iii) progression of DR in subjects with DR at diagnosis over the 5-year study period (Appendix Table 1). However, reference to the association between other elements of glycaemia such as PPG, as well as β-cell function represented by fasting and postprandial insulin levels and fasting and postprandial β-cell responsiveness with the presence or progression of DR is very limited. Therefore in this thesis we have employed a much more detailed evaluation of glucose tolerance, β-cell function and insulin sensitivity using well accepted methodologies.

In the initial analysis, the findings at diagnosis (Chapter 3) showed that the presence of DR is independently associated with a lower fasting β-cell responsiveness and consequent fasting and postprandial hyperglycaemia. In addition the insulin-independent component of glucose tolerance was reduced and was also

independently associated with the presence of DR at diagnosis. Therefore, at the time of clinical diagnosis of type 2 diabetes we report an association between DR and fasting β-cell responsiveness and glucotoxicity both in fasting and postprandial state.

In the second part of our analysis (Chapter 4) we followed up 233 subjects with no DR at diagnosis over a 5-year follow-up period. Our analysis established a strong and independent association between HbA_{1c} fasting and postprandial hyperglycaemia (2 hour PPG and the overall 4 hour glucose ($AUC_{(0\text{-}240min)}$) response to the test meal) at diagnosis and the incidence of DR. over this 5 year observation period. The derived measures of β-cell function HOMA-B and responsiveness when fasting (M_0 and HOMA-B) and in response to the test meal (M_1) along with S_G (insulin-independent component of glucose tolerance) were all independently associated with the development of DR over the 5-year observation period. This suggests that by the time of diagnosis of T2DM, subjects who have a significantly reduced ß-cell function are at risk of developing DR within a 5-year time period as a consequence of hyperglycaemia both fasting and postprandial (2-hour PPG and $AUC_{Glucose\ (0\text{-}240min)}$) over a prolonged period of excess glycaemic exposure (HbA_{1c}). Furthermore our data further emphasises the contribution of both fasting and postprandial components to the overall glycaemic exposure with all glycaemic parameters significantly and independently related to incident diabetic retinopathy over the 5 year observation period. However the long-term contribution of postprandial glycaemia was seen to be greater than the influence of FPG on the development of DR.

This analysis therefore suggests that impaired β-cell responsiveness, both in the fasting and postprandial state at or near the time of diagnosis resulting in increased postprandial and overall glycaemic exposure over the 5-year study period contribute significantly to the development of DR. The extent of insulinopaenia observed at diagnosis in those subjects who go on to develop DR already reflects a significant defect in β-cell function.

Of note is that most parameters of glycaemic control and β-cell secretory status at diagnosis appear to be the main contributors to the development of DR as there was no difference in HbA_{1c} and other glycaemic parameters at year 5 between those who developed retinopathy and those who did not. The early history of a significantly lower glycaemic exposure not leading to incident DR by 5 years in our study is indicative of better early glycaemic milieu at diagnosis leading to a long-term risk reduction for DR incidence.

In our study, we were also able to established an independent association between various measures of β-cell function in response to a standardised meal challenge, employing both the CPR program M_0, and M_1 (Hovorka R et al. 1998) and the HOMA methodology (HOMA-B) with incidence of DR. The incidence of DR was independently associated with a lower fasting and postprandial β-cell responsiveness at diagnosis. This resulted in hyperglycaemia both in the fasting and postprandial state, concurrent with fasting and postprandial insulinopaenia. In addition the insulin-independent component of glucose tolerance estimated at diagnosis was also

reduced and independently associated with the incidence of DR by 5 years in our newly diagnosed T2DM subjects.

The final analysis (Chapter 5) in a small number of subjects with DR at presentation demonstrates an independent association between the progression of DR from baseline over a period of 5 years with the degree of hyperglycaemia and insulinopaenia revealed during the MTT when performed at or near the time of diagnosis. All measured glycaemic parameters including the fasting, postprandial and overall (HbA_{1c}) glycaemic exposure at or near the time of diagnosis exhibited an independent association with DR progression. Indices of fasting β-cell function i.e. M_0 and HOMA-B and the estimated postprandial β-cell responsiveness (M_1) all showed an independent association with DR progression. This reflects that if on presentation of T2DM the poorer the ß-cell function and greater degree of hyperglycaemia the greater the risk of their DR progressing by 5 years since diagnosis.

We also demonstrated that when the fasting and postprandial glycaemic exposures from serial MTTs were averaged over the 5 year follow up period (Years 0, 1, 2 and 5) these were associated with the progression of DR. The persistent state of hyperglycaemia despite the introduction of a small variety of oral hypoglycaemic agents such as metformin and a sulphonylurea to improve the glycaemic control reflected the continuing deficiency in fasting and postprandial β-cell responsiveness over the 5 years observation period.

The analysis conducted in our cohort of subjects with newly-diagnosed T2DM, who presented with DR, both the fasting and postprandial glycaemic β-cell responsiveness at both diagnosis and averaged over a 5-year follow up period contribute significantly to the progression of DR. Therefore, this provides additional evidence to support the relevance of postprandial hyperglycaemia and insulinopaenia, a consequence of deficient postprandial β-cell responsiveness, to the progression of existent DR. The importance of β-cell function in the fasting and postprandial state to overall dysglycaemia has not previously been well explored or documented.

Hence it becomes paramount that we need to attempt to diagnose type 2 diabetes earlier and to target both fasting and postprandial hyperglycaemia to normalising overall glycaemic control to prevent the occurrence of diabetic retinopathy. This is based on our findings that there is clearly a long-term effect present which extends over a period of 5 years at least in support of early glycaemic control. For those with diabetic retinopathy at the time of diagnosis it is accepted that this may be indicative a prolonged pre-diagnostic period of glucose intolerance for up to 7 years. In addition, our study demonstrates to a greater extent than previous investigations, the already severely compromised state of β-cell function at the time of diagnosis, and its continuation despite the reduction in glycaemia and the introduction of sulphonylureas to facilitate insulin secretion. Normalising glycaemia is paramount and there are currently various antidiabetic agents (GLP 1 agonist or SGLT2 inhibitors) who have varying effects on the fasting, postprandial glucose or both and therefore should be chosen according to the dysglycaemic profile. Appropriate

supplementation with insulin may also need to be considered in support of β-cell function and the opportunity to induce remission provided the risk of hypoglycaemia is minimised. The study also highlights the importance of obtaining relevant information to identify the phenotypic characteristics of the patient in an attempt to define the best treatment modality (Precision Medicine). The study also emphasises the importance of good control in patients with type 2 diabetes with or without evidence of diabetic retinopathy with the proviso that improvement in glycaemic control is introduced slowly in those previously under poor control and pre-existing diabetic retinopathy.

Avoiding diabetic retinopathy and its progression to sight threatening diabetic retinopathy is a key priority of diabetes care today. Improved diabetes care as defined by improvement in glycaemic, BP control and fibrate therapy supported by the introduction of diabetic retinopathy screening services has relinquished diabetic retinopathy from the number one cause of blindness in the working age population. Maintaining eye health in persons with diabetes by preventing preventable causes of blindness such as diabetic retinopathy is currently a global priority in view of its devastating impact on the patient, the family and society in general.

6.2 Limitations of Study

Our study period extended from 1981 and 2007, which can be considered as a limitation as the population demographics may change over 3 decades. In addition, during this period, a greater awareness of T2DM came about and thus, this had the

potential to affect the length of preceding unknown duration of T2DM before diagnosis and therefore the effect on β-cell function. However, the longer duration of the study does reflect a detailed analysis of over almost three decades of prevalence, incidence and progression of existent DR in a Caucasian population with T2DM, resident in South East Wales from diagnosis over a five-year period.

Another limitation of our study was that over its duration, because of serial developments in the measurement of HbA_{1c}, we employed two different assays, although a validated conversion factor was employed.

The subjects lost in follow up in our study would be considered as limitation for the longitudinal analysis. However when baseline characteristics of subjects who were not followed up over 5 years were compared to those who were followed up over 5 years there was no significant difference noted (Appendix Table 3). However a greater number of subjects with long-term follow up data do give more robust data.

The Diabetes Prevention Program reported more than 12% of subjects with T2DM had DR within approximately 3 years of diagnosis (Diabetes Prevention Program Research Group 2007). In comparison, in this study 16.5% of the subjects with newly-diagnosed T2DM, presenting with DR was investigated within 1-2 weeks of diagnosis, possibly indicating a slightly longer pre-clinical period in our cohort. This initial analysis was limited by its cross-sectional design thus making it difficult to

confirm a cause and effect relationship. However, the strength of our study lies in the recruitment of subjects within 1-2 weeks after diagnosis, the detailed assessment of dysglycaemia and β-cell function over an extended period of 5 years utilising the same methodology (a standardised meal tolerance test) complemented by an insulin modified intravenous glucose tolerance test at baseline, supported throughout the observation period by detailed analysis of the presence and severity of diabetic retinopathy.

Early diagnosis and early good glycaemic control are both paramount in preventing or delaying the onset and progression of diabetic retinopathy. Protecting and/or enhancing β-cell function without inducing hypoglycaemia is an aim for the future.

6.3 Future areas of research

The literature to date complimented by the analysis of the data in this thesis demonstrates that the degree of fasting and postprandial hyperglycaemic exposure both contribute to i) the prevalence of DR in newly-diagnosed T2DM subjects, along with the ii) incidence of DR in subjects with no DR at diagnosis and iii) progression of DR in subjects with DR at diagnosis over a period of 5 years follow-up. However, the association of PPG is not extensively documented in the current literature, being limited to only 4 studies in non-Caucasian subjects. Therefore, treatment specifically targeting postprandial hyperglycaemic excursions is justified. As β-cell dysfunction, and insulin resistance are major contributors to dysglycaemia in type 2 diabetes the role of insulin supplementation and/or insulin sensitizers need to be considered and

their potential role elucidated in respect to the prevalence, incidence and progression of DR. This should ensure an appropriate and timely management of T2DM to prevent and limit DR and its effect on our day to day living.

As stated our current study is limited by the numbers of subjects involved, the non-random allocation of therapeutic modalities, the relatively poor glycaemic control achieved and the shortness of the observation period. Future studies should take these into consideration. A more extensive involvement of other putative risk factors such as blood pressure and fibrate therapy will need to be taken into consideration.

In the current times, with a greater awareness of T2DM, it should be possible to investigate a study population at an even earlier stage in their T2DM, even to include persons with pre-diabetes, and follow them up over for a longer study period. Thus if the MTTs and FSIVGTTs were continued for an extended study period, it would demonstrate the prevalence, incidence and progression of DR in relation to the phenotypic characteristics of the patients including β-cell function, insulin sensitivity and other glycaemic indices in a more detailed fashion than at present. This would better delineate the natural history of DR in relation to the various above mentioned metabolic indices. Such future analysis would then have the potential to proceed from the more refined phenotypic characteristics of T2DM subjects to explore the genotypic makeup of these subjects and the risk of diabetic retinopathy.

A detailed analysis of the retinal vascular morphology of T2DM subjects may also reveal subtle changes, which have not been captured under the broad categories of grading of DR used in this study. These suggested future studies could accommodate the limitations of this study.

6.4 Summary of key findings

A cross-sectional analysis performed at the time of diagnosis of type 2 DM indicates that the prevalence of DR is associated with both the fasting and postprandial hyperglycaemia in the presence of impaired fasting β-cell function. The lower fasting and postprandial β-cell responsiveness at diagnosis in those with retinopathy was also evident over the 5 year study period despite the fact that glycaemic exposure at 5 years was similar between the groups with or without diabetic retinopathy. Elevated fasting, postprandial and overall glycaemic exposure at baseline were also independently associated with incident DR in those subjects with no evidence of DR at diagnosis.

Finally, in the subjects with newly diagnosed T2DM, already with evidence of DR at the time of presentation, the fasting and postprandial hyperglycaemia and β-cell responsiveness at diagnosis and throughout the 5 years of follow-up period were significantly associated with the progression of existent DR. This reflects the highly probable aetiology and consequences of glucotoxicity both at diagnosis and over the 5-year observation period.

The importance of the ‘long-term effect’ of dysglycaemia is strongly corroborated in this thesis with parameters at or early on in diagnosis having a significant contribution to the outcome after a period of 5 years.

Appendix Table 1: Summary of prospective studies on risk factors affecting the incidence and progression of diabetic retinopathy

	Population	Numbers	Study Duration (Years)	Cumulative Prevalence	Cumulative Incidence	Retinopathy assessment methods	Risk Factors
WESDR (Klein R et al. 1989a)	Southern Wisconsin Age at DM diagnosis >30 years	987	4 (1980-1984)	-	Insulin Users: NDR to DR - 47% No PDR to PDR – 7% DR progressed – 34% Non - Insulin Users: NDR to DR - 34% No PDR to PDR – 2% DR progressed – 2%	Fundus Photographs	-
Suzuki et al. (Suzuki K et al. 1989)	Japan DM duration >10 years	160	10	-	Incidence of PDR ------------------------ Incidence of BDR	Fundoscopic examination through dilated pupils using a direct ophthalmoscope by ophthalmologists	24 hour urinary C-peptide concentration ------------------------ FPG
Lee et al. (Lee ET et al. 1992)	Oklahoma Indians	927	12.7	-	Incidence of PDR – 18.6 %: (45 % of those with BDR developed PDR)	Fundus photographs through dilated pupils using a nonmydriatic camera (Canon 4 – 45 NM)	Duration of DM FPG Plasma Cholesterol SBP Treatment of DM

	Population	Numbers	Study Duration (Years)	Cumulative Prevalence	Cumulative Incidence	Retinopathy assessment methods	Risk Factors
Araki et al. (Araki et al. 1993)	Japan (> 60 years) NDR at 1st visit	110	5	-	Incidence of DR	Fundoscopic examination of the retina through dilated pupils using direct ophthalmoscope	Duration of DM Baseline FPG Baseline HbA_1 Persistent proteinuria
Chen et al. (Chen MS et al. 1995)	Taiwan T2DM	471	4	-	Incidence of DR: 19.2 % Progression of DR: 30 % Incidence of PDR: 5.8 %	Ocular fundoscopic examination by ophthalmoscope	Mean FPG Mean HbA_{1c}
Henricsson et al. (Henricsson M et al. 1997)	Sweden T2DM ≥ 40 years	1378	3.1	-	-	Fundus Photography	HA_{1c} Change of treatment
Kim et al. (Kim HK et al. 1998)	Korea T2DM	130 NDR at baseline 56 NPDR at baseline	5.3	-	Incidence of DR: 44.4/1,000 person-years Progression of DR to PDR: 37.5/1,000 person-years	Annual fundoscopic examination by ophthalmoscope	Mean HbA_1
Gabir et al. (Gabir MM et al. 2000)	Pima Indian adults	5023	10	1999 WHO Criteria: IGT – 1.6 % DM –19.2 %	Incidence of DR	Direct ophthalmoscope through dilated pupils	FPG 2 hour PPG

	Population	Numbers	Study Duration (Years)	Cumulative Prevalence	Cumulative Incidence	Retinopathy assessment methods	Risk Factors
UKPDS 50 (Stratton IM et al. 2001)	United Kingdom T2DM Newly diagnosed	1919	6	NDR (Year 0) – 63% DR (Year 0) – 37%	NDR to DR - 23.2% DR progressed – 29%	Retinal photographs	Development of DR: HbA_{1c} at diagnosis 6 years glycaemic exposure SBP Not smoking Progression of DR: Age Male Sex HbA_{1c} Not smoking
WHO multinational study of vascular disease in diabetes (Keen H et al. 2001)	Type I and II DM	2,877	8.4	-	Any DR – 47.7% PDR – 9.7%	Direct Ophthalmoscopy	Any DR: Age Duration of DM FPG Serum TC SBP BMI Insulin treatment Proteinuria PDR: Age Duration of DM FPG Serum TC BMI Insulin treatment Proteinuria

	Population	Numbers	Study Duration (Years)	Cumulative Prevalence	Cumulative Incidence	Retinopathy assessment methods	Risk Factors
Voutilainen-Kaunisto et al. (Voutilainen-Kaunisto RM et al. 2001)	Finland Newly diagnosed T2DM	133	10	DR: 8.9% - Diagnosis 17.7% - Year 5 55.7% - Year 10 Severe BDR: 9.9% -Year 5 34.1% -Year 10 PPDR: 4.8% - Diagnosis 2.7% -Year 5 15.3% -Year 10	Frequency: 0% - Diagnosis 0.9% - Year 5 7.3% - Year 10 Incidence: 8.8% - 5 years 50% - 10 years	45° Fundus Photographs	Baseline: None 5 year: FPG 1 hour PPG 2 hour PPG HbA_{1c}
Yoshida et al.(Yoshida Y et al. 2001)	T2DM – No DR at 1st visit	787	6.7	-	16.8%	Direct and Indirect Ophthalmoscopy through dilated pupils	Baseline: HbA_{1c} Duration of DM Treatment of DM BMI 5 year: HbA1c Duration of DM
Janghorbani et al. (Janghorbani M et al. 2003)	Iran Type I and II DM	549	5.4	-	89.4/1000 person years (males) 86.6/1000 person years (females)	Indirect Ophthalmoscopy through dilated pupils and then Fundus Photography	Age at 1st review HbA_{1c} FPG

	Population	Numbers	Study Duration (Years)	Cumulative Prevalence	Cumulative Incidence	Retinopathy assessment methods	Risk Factors
Looker et al. (Looker HC et al. 2003)	Pima Indians – Arizona – USA Type I & II	280	4	-	16.8%	Retinal photographs (two standard fields for each eye)	HbA_1 (Overall glycaemia) OHG
Shiraiwa et al. (Shiraiwa T et al. 2005b)	Japan T2DM	151	5	-	Progression of DR	Fundoscopic examination of the retina through dilated pupils by ophthalmologists	PPG PPI
Tapp et al. (Tapp RJ et al. 2006)	Mauritius Type I and II DM	528	6 (1992-98)	-	All: DR – 23.8% STDR – 0.4% Known DM and NDR at baseline: NPDR – 29.2% PDR – 1% Newly diagnosed DM NPDR – 19.1%	Retinal photographs were taken using a TRC-50VT retinal camera in three fields of the right eye (centred on the optic disc; macula (temporal to the optic disc); and nasal to disc).	Baseline: Duration of DM FPG
ARIC (Wong TY et al. 2007)	USA Type I and II DM (Age: 45 - 64)	981	3 (1993 – 1996)	27.2 %	10.1 %	45° Non-mydriatic retinal photograph of only one eye	Mean arterial BP FPG Serum TC Fibrinogen

	Population	Numbers	Study Duration (Years)	Cumulative Prevalence	Cumulative Incidence	Retinopathy assessment methods	Risk Factors
Cikamatana et al. (Cikamatana et al. 2007)	Australia (Older population)	2334	5 (1992-94)	-	Cumulative Incidence of DR: 22.2 % Progression of DR to PDR: 4.1 %	Retinal photographs (Modified ETDRS scale)	Baseline FPG Duration of DM
Manaviat et al. (Manaviat MR et al. 2008)	Iran T2DM without DR	120	4	-	Cumulative Incidence of DR: 5.8 % - 1st year, 20.3 % in 2nd year 24.4% in 3rd year 7.4 % in 4th year Grades of DR: affected by:	Ophthalmoscopic examination	FPG Duration of DM SBP
Zoppini et al.(Zoppini G et al. 2009)	Italy – Verona Diabetes Study T2DM - Elderly	746	4 -5	-	Incidence of DR Progression of DR	Indirect Ophthalmoscopy after pupil dilation and then two field stereoscopic retinal photographs	Average glycaemia over time – HA_{1c} Mean FPG HDL
Takao et al (Takao T et al. 2010)	Japan T2DM with NDR in 1st visit (1966-79)	170	33 (Mean) [27-40]	-	Incidence of NPDR	Annual fundoscopic examination by ophthalmologists	CV – FPG

	Population	Numbers	Study Duration (Years)	Cumulative Prevalence	Cumulative Incidence	Retinopathy assessment methods	Risk Factors
Takao et al. (Takao T et al. 2011)	Japan T2DM with NDR in 1st visit (1966-79)	170	33 (Mean) [27-40]	-	Incidence of PDR	Annual fundoscopic examination by ophthalmologists	CV – FPG FPG (5 years prior to event) HbA_{1c} (10 years prior to event)
Jones et al. (Jones CD et al. 2012)	Central Norfolk – UK Type I and II DM	20,686	1990 - 2006	NDR – 79% NPDR – 18% PPDR – 2.9%	5 Years: NDR to PPDR – 4% NDR to STMO – 0.59% NDR to PDR – 0.68% 10 Years: NDR to PPDR – 16.4% NDR to STMO – 1.2% NDR to PDR– 1.5% 5 Years: BDR to PPD – 23% BDR to STMO – 5.2% BDR to PDR – 6.1% 10 Years: NDR to PPDR – 53% NDR to STMO - 9.6% NDR to PDR – 11%	Two photographs of each eye were taken, one centered on the optic nerve and the other on the fovea, using Canon 45NM or 46NM fundus cameras (Canon UK, Reigate, U.K.) with 45° fields and Orion Eyecap and DRSS digital imaging software.	Age Duration of DM Treatment of DM Hypertension

	Population	Numbers	Study Duration (Years)	Cumulative Prevalence	Cumulative Incidence	Retinopathy assessment methods	Risk Factors
Perol et al. (Perol J et al. 2012)	French population Type I and II DM	254	3	-	14%	OPHIDAT telemedical network	Duration of DM Microalbuminuria Macroalbuminuria
Rudnisky et al. (Rudnisky CJ et al. 2012)	Canada	980 All 777 NDR	7.6	-	NDR to DR - 16.6%	Stereoscopic mydriatic retinal photography	HA_{1c} SBP

Appendix Table 2

Comparison of **baseline characteristics** of subjects who were **not followed up over 5 years** compared to those who were **followed up over 5 years**

	All subjects	**Not followed up over 5 years**	**Followed up over 5 years**	**p value (between groups)**
Number	**544**	**311**	**233**	
Age at presentation (years)	54 (10)	55 (10)	54 (9)	0.52
Male Sex (%)	393 (72)	238 (71)	155 (67)	0.28
Weight (kg)	88 (17)	88 (17)	87 (16)	0.85
BMI (kg.m^2)	30.2 (5.0)	30.5 (5.5)	30.0 (5.2)	0.24
Systolic blood pressure (mmHg)	137 (19)	139 (21)	134 (17)	**0.02**
Diastolic blood pressure (mmHg)	83 (11)	83 (11)	83 (10)	0.78
Total Cholesterol (mmol/L)	5.4 (1.2)	5.4 (1.1)	5.5 (1.2)	0.71
HbA_{1c} (%)	7.7 (2.0)	7.8 (2.0)	7.6 (2.1)	0.37

Appendix Table 3: Baseline characteristics of newly diagnosed type 2 diabetic subjects who developed DR at Years 1, 2 and 5

	Develop DR at Year 1	**Develop DR at Year 2**	**Develop DR at Year 5**	
Number	**12**	**15**	**27**	p value
Fasting Glucose (mmol/L)	12.3 (9.0 - 14.6)	11.5 (9.6 - 14.1)	11.2 (9.6 – 13.4)	0.918
Postprandial Glucose (mmol/L) (120 mins)	16.4 (13.9 - 19.5)	16.4 (13.0 – 18.1)	15.2 (12.8 - 18.2)	0.882
HbA1c (%)	8.7 (8.0 - 10.1)	9.3 (8.5 – 10.1)	8.1 (7.0 – 9.6)	0.271
$AUC_{Glucose\ (0\text{-}240min)}$ (mmol/L)	13.8 (10.6 - 16.4)	13.9 (11.0 – 15.5)	12.8 (11.3 – 15.8)	0.984
Fasting Insulin (pmol/L)	31.0 (13.5 - 53.0)	64.0 (34.8 - 116.5)	58.0 (28.0 – 101.0)	0.022
Postprandial Insulin (pmol/L) (120 mins)	108.5 (57.5 – 174.5)	240.0 (138.0 - 339.0)	246.0 (108.8 - 442.5)	0.097
$AUC_{Insulin\ (0\text{-}240min)}$ (pmol/L)	74.2 (57.6 – 121.7)	156.2 (112.9 – 259.4)	180.4 (70.6 – 284.9)	0.064
HOMA-B (%)	14.1 (5.9 – 31.6)	29.1 (19.3 – 42.3)	30.6 (12.3 – 61.4)	0.093
HOMA-S (%)	116.1 (76.5 - 232.8)	54.9 (29.7- 86.7)	58.9 (35.5 - 102.9)	0.017
HOMA-IR	0.9 (0.4 – 1.3)	1.9 (1.1 – 3.4)	1.7 (1.0 – 2.8)	0.017
M_0 ($*10^{-9}$ pmol/kg/min)	2.4 (1.7 – 6.8)	4.1 (2.3 – 6.3)	4.3 (1.9 – 7.4)	0.057
M_1 ($*10^{-9}$ pmol/kg/min)	7.7 (6.3 – 16.7)	9.9 (7.3 - 14.5)	11.5 (5.9 – 15.5)	0.086

Data expressed as median (1st – 3rd IQR), AUC = Area Under the Curve

Appendix Table 4

Year 1 characteristics in subjects with No Diabetic Retinopathy (NDR) throughout 5 years compared to those with Diabetic Retinopathy (DR) by 5 years of diagnosis of T2DM. Group A: Subjects who underwent MTT.

	All subjects	**NDR**	**DR**	**p value**
Number	**233**	**179**	**54**	
Age at presentation (years)	55.0 (9.4)	55.0 (9.8)	56.0 (8.2)	0.661
Male Sex (%)	175 (75)	139 (77.7)	36 (66.7)	0.075
Weight (kg)	85.9 (16.2)	85.4 (16.4)	87.4 (15.3)	0.343
BMI (kg.m^2)	28.8 (6.5)	28.7 (6.0)	29.3 (8.1)	0.231
Systolic blood pressure (mmHg)	131 (16)	130 (16)	133 (14)	0.238
Diastolic blood pressure (mmHg)	80 (10)	79 (10)	81 (11)	0.224
Total Cholesterol (mmol/L)	5.1 (1.1)	5.1 (1.1)	5.2 (1.0)	0.476
HbA_{1c} (%)	6.6 (1.2)	6.5 (1.1)	6.9 (1.4)	**0.011**

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

Appendix Table 5

Year 2 characteristics in subjects with No Diabetic Retinopathy (NDR) throughout 5 years compared to those with Diabetic Retinopathy (DR) by 5 years of diagnosis of T2DM. Group A: Subjects who underwent MTT.

	All subjects	**NDR**	**DR**	**p value**
Number	**233**	**179**	**54**	
Age at presentation (years)	56.0 (9.4)	56.0 (9.8)	57.0 (8.2)	0.661
Male Sex (%)	175 (75)	139 (77.7)	36 (66.7)	0.075
Weight (kg)	87.3 (16.6)	87.0 (16.6)	88.4 (16.6)	0.528
BMI ($kg.m^2$)	29.1 (6.9)	28.8 (6.8)	30.3 (7.3)	0.119
Systolic blood pressure (mmHg)	129.3 (15.7)	128.6 (14.5)	131.7 (19.0)	0.461
Diastolic blood pressure (mmHg)	78.3 (9.3)	78.0 (9.0)	79.3 (10.1)	0.386
Total Cholesterol (mmol/L)	5.0 (1.1)	5.0 (1.1)	5.1 (1.0)	0.660
HbA_{1c} (%)	6.9 (1.5)	6.7 (1.4)	7.3 (1.5)	**0.016**

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

Appendix Table 6

Year 5 characteristics in subjects with No Diabetic Retinopathy (NDR) throughout the 5 years compared to those with Diabetic Retinopathy (DR) by 5 years of diagnosis of T2DM. Group A: Subjects who underwent MTT.

	All subjects	**NDR**	**DR**	**p value**
Number	**233**	**179**	**54**	
Age at presentation (years)	59.0 (9.4)	59.0 (9.8)	60.0 (8.2)	0.661
Male Sex (%)	175 (75)	139 (77.7)	36 (66.7)	0.075
Weight (kg)	85.1 (23.0)	85.0 (22.7)	85.4 (24.2)	0.528
BMI ($kg.m^2$)	29.1 (7.2)	29.0 (7.1)	30.0 (7.8)	0.163
Systolic blood pressure (mmHg)	135.6 (17.3)	135.2 (16.8)	136.9 (18.8)	0.675
Diastolic blood pressure (mmHg)	79.1 (10.1)	78.7 (10.5)	80.4 (8.8)	0.527
Total Cholesterol (mmol/L)	5.2 (1.2)	5.2 (1.2)	5.1 (1.1)	0.604
HbA_{1c} (%)	7.5 (1.6)	7.4 (1.6)	8.0 (1.6)	**0.009**

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

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Peer Reviewed Publication:

- Sharmistha Roy Chowdhury, Rebecca L. Thomas, Gareth J. Dunseath, Rajesh Peter, D. Aled Rees, Rachel V. North, Stephen D. Luzio, and David R. Owens.

  Diabetic Retinopathy in Newly Diagnosed Subjects with Type 2 Diabetes Mellitus: Contribution of β-Cell Function. *J Clin Endocrinol Metab* 2015: 101, pp. 572-580.

PRESENTATIONS (NATIONAL AND INTERNATIONAL CONFERENCES):

ORAL PRESENTATIONS –

- Metabolic factors affecting the prevalence of Diabetic Retinopathy in newly diagnosed treatment naive subjects with Type 2 Diabetes

  Roy Chowdhury S, Thomas RL, Dunseath GJ, Luzio SD, Owens DR

  28th Anglo Danish Dutch Diabetes Group Meeting, Denmark, May 2010

- Metabolic profiles affecting the 5 year incidence of diabetic retinopathy in Type 2 Diabetes

  Roy Chowdhury S, Thomas RL, Dunseath GJ, Luzio SD, Owens DR

  Diabetes UK, Annual Professional Conference, Glasgow, UK, March 2009

ABSTRACTS AND POSTER PRESENTATIONS:

- **Roy Chowdhury S,** Thomas RL, Dunseath GJ, Luzio SD, Owens DR

  Metabolic factors affecting the prevalence of Diabetic Retinopathy in newly diagnosed treatment naive subjects with Type 2 Diabetes

  Abstract book of 69th Scientific Sessions of American Diabetes Association, New Orleans, USA- June 2009, Reference 874P

- Thomas RL, Dunseath GJ, **Roy Chowdhury S,** Peter R, Chudleigh R, North RV, Hale SL, Owens DR

  Association between postprandial glucose and the presence of diabetic retinopathy at presentation in newly diagnosed persons with Type 2 diabetes

  Abstract book of 68th Scientific Sessions of American Diabetes Association, San Francisco, USA- June 2008, Reference 809P

ACHIEVEMENTS

- Metabolic profiles affecting the 5 year incidence of diabetic retinopathy in Type 2 Diabetes

  The Royal Eye Hospital (London) Best Poster Prize

  The Royal College of Ophthalmologists Annual Congress

  Birmingham May 2009

- Determining a safe screening interval for subjects without diabetic retinopathy

  South Western Ophthalmological Society Meeting

  Best Poster Prize

  Newport, South Wales

  November 2010