Characteristics of Prediabetes, predictors of progression and strategies to prevent Type 2 Diabetes Mellitus in a multiethnic population in the United Kingdom.

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by

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List of abbreviations

25 OHD	25 hydroxy Vitamin D
ADDITION	Anglo-Danish-Dutch study of Intensive Treatment In PeOple with screeN detected diabetes in Primary Care
AGE	Advanced glycosylated end products
AHT	Anti hypertensive treatment
ATP	Adenosine tri phosphate
AUC	Area under the curve
BFI	Big five inventory
BMI	Body mass index
BLSA	Baltimore Longitudinal study of Ageing
BP	Blood pressure
CUPS	Chennai Urban Population study
CVD	Cardiovascular disease
DECODA	Diabetes Epidemiology: Collaborative analysis of Diagnostic criteria in Asians
DECODE	Diabetes Epidemiology: Collaborative analysis of Diagnostic criteria in Europe
DIASCAN	Diabetes Screening in Canada Study
DPP	Diabetes prevention programme
DV	Dependant variable
eGFR	Estimated glomerular filtration rate
ELISA	Enzyme linked immuno sorbent assay
EMIS	Egton medical information system
FDPS	Finnish diabetes prevention study
FFA	Free fatty acids
FH	Family history
FHS	Fasting Hyperglycaemia study
FINDRISC	The Finnish diabetes risk score
FPG	Fasting plasma glucose
FPI	Fasting plasma Insulin
FPIS	First phase insulin secretion
GEMCAS	German primary care: data from the German Metabolic and Cardiovascular Risk Project

GLP 1	Glucagon like polypeptide 1
HADP	Human adiponectin
HbA1c	Haemoglobin A1c
HDL	High density lipoprotein
HGO	Hepatic glucose output
HL	Hosmer Lemeshow test
HOMA	Homeostatic model assessment
HOMA IR	Homeostatic model assessment of insulin resistance
HOMA β	Homeostatic model assessment of beta cell function
HR	Hazard ratio
hs CRP	Highly sensitive C reactive protein
HT	Hypertension
i IFG	Isolated impaired fasting glucose
i IGT	Isolated impaired glucose tolerance
IDF	International diabetes federation
IDPP	Indian diabetes prevention programme
IDS	Islington diabetes survey
IFG	Impaired fasting glucose
IGM	Impaired glucose metabolism
IGR	Impaired glucose regulation
IGT	Impaired glucose tolerance
IL6	Interleukin 6
IPAQ	The International physical activity questionnaire
IQR	Inter quartile range
IR	Insulin resistance
IRD	Incidence rate difference
IRS	Insulin receptor substrate
IV	Independent variable
kATP	Potassium Adenosine tri phosphate
LDL	Low density lipoprotein
LR	Logarithmic rate
LRA	Leicester risk assessment
MS	Metabolic syndrome
NGT	Normal glucose tolerance
NHANES	National health and nutritional examination survey

NPV	Negative predictive value
NSC	National Screening Committee
OGTT	Oral glucose tolerance test
PA	Physical activity
PAI 1	Plasminogen activator 1
PDM	Prediabetes
PGLG	Post glucose load plasma glucose
PP	Post prandial
PPV	Positive predictive value
PY	Person years
QUOROM	The quality of reporting of Meta-analyses
RCT	Randomised controlled trial
RR	Relative risk
SA	South Asians
SPSS	Statistical Package for the Social Sciences
T2DM	Type 2 Diabetes Mellitus
TC	Total cholesterol
TLGS	Tehran Lipid glucose study
TNFα	Tumour necrosis factor α
TRFIA	Time resolved Fluorescent immuno assay
U+E	Urea and electrolytes
VD	Vitamin D
WE	White Europeans
WHO	World Health Organisation

1 Executive Summary

Type 2 Diabetes Mellitus (T2DM) is a chronic multi factorial disorder linked to obesity that is associated with increased morbidity and mortality. T2DM poses a major public health problem with the prevalence expecting to reach 4 million in the United Kingdom by the year 2025.

Upto 50% of people may have established complications at the time of diagnosis of T2DM. However, T2DM is preceded by a latent phase of Prediabetes (PDM) which provides a window of opportunity for primary prevention. PDM is often known as impaired glucose metabolism (IGM) or impaired glucose regulation (IGR). PDM is a collective term for impaired fasting glucose (IFG), impaired glucose tolerance (IGT) and those with combined IFG and IGT. The reported prevalence of these conditions is variable throughout the world. This thesis seeks to address key questions on identification of IGR, determine factors predicting progression to T2DM and thus propose prevention strategies in a mixed ethnic population in the UK using data from the ADDITION Leicester and ADDITION PLUS studies.

ADDITION Leicester is a sub study of the multinational multi centre study-ADDITION Europe. ADDITION study is a randomised controlled trial evaluating the benefits of a multi factorial cardiovascular disease risk factor intervention in a cohort of patients with screen detected T2DM.

The prevalence of PDM was 16% in the study population with IFG, IGT and combined IFG and IGT being 2.8%, 11% and 2.2% respectively. People of South Asian (SA) origin have a significantly higher adjusted prevalence of PDM compared to those of White European (WE) origin (OR: 1.57; 95% CI: 1.24 to 1.98). A risk score tailored to the local population (Leicester risk assessment score) was robust in identifying those at risk of developing T2DM and PDM as well those progressing from PDM to T2DM at 12 months. Subjects with PDM have a unique phenotype placing their cardiovascular disease (CVD) risks between T2DM and normal glucose tolerance. Novel markers of CVD such as Interleukin 6, Adiponectin, Leptin and C-reactive protein are also raised in those with PDM compared to normal.

The risk of progression from PDM to T2DM at 12 months is higher for SA compared to WE (OR: 3.09, 95% CI- 1.58 to 6.02). The diabetes progression rate (cases/100 person-years) for IFG, IGT and combined IFG and IGT were 5.51, 3.13 and 14.46

respectively. The risk of progression for SA people occurs at a lower cut off for BMI and waist circumference. A meta analysis of 13,314 patients from 22 studies with PDM revealed a pooled progression rate (cases per 100 person-years) (95% CI) to be 6.29 (4.29- 9.22), 7.48 (5.00-11.18) and 7.86 (5.51- 11.20) for people with IFG, IGT and combined IFG+IGT respectively.

Presence of CVD, central obesity measured both by waist circumference and BMI, triglycerides, fasting plasma glucose (FPG) and HbA1c significantly predict progression to T2DM at 12 months. Presence of metabolic syndrome with more than 2 additional criteria significantly predicts progression to T2DM. In terms of adipocytokines, TNFα is the only marker, after adjusting for confounders that is significantly associated with progression to T2DM. In terms of follow up of this cohort, we propose a two step method using FPG >6 mmol/L as a screening tool to identify people who can subsequently be screened using an OGTT, reducing the number of OGTT needed to 23.5%.

Our findings suggest using a structured screening programme with a risk score used in parallel to the recommended opportunistic screening for T2DM. The need for ethnic specific cut-off for obesity has been established. Factors such as presence of metabolic syndrome, HbA1c >6%, presence of a single diabetes range glucose value and pre-existing CVD may be used in risk stratification of individuals with PDM. These factors may also be used to guide those who may benefit from Metformin in addition to established life style interventions for PDM.

Our findings provide a contemporary and prospective data on the prevalence of PDM in a multi ethnic UK population and factors predicting progression from PDM to T2DM. A robust strategy using a self assessed risk score is proposed to identify those at risk of developing PDM and T2DM. A step wise ethnic specific algorithm using anthropometric measures is also recommended to enable follow up of those with PDM. These findings have important implications for public health in informing strategies to address the emerging pandemic of T2DM.

2 Introduction and literature review

2.1 Introduction

Type 2 Diabetes Mellitus (T2DM) is a chronic multi factorial metabolic disorder that affects both longevity and quality of life. T2DM is multigenic in aetiology and is linked to obesity and insulin resistance. The number of people in the UK with diabetes is increasing rapidly with 1.4 million in 1996 to 2.5 million in 2008. This figure is projected to rise to 4 million in 2025 (1). Globally this figure is expected to reach 380 million (2). This is partly due to lifestyle changes of the general population and partly due to the demographic shift towards an older population. Up to half of this number have potentially undiagnosed diabetes (3;4). T2DM is preceded by a latent and asymptomatic phase called Prediabetes (PDM). This condition includes the dysglycaemic disorders of both fasting and post prandial glucose namely impaired fasting glucose (IFG) and impaired glucose tolerance (IGT) respectively. PDM can be readily identified through screening and is potentially modifiable through simple and cost effective measures that provide us with an effective way to combat the healthcare burden of diabetes (5).

Despite the evidence for the benefits of screening and early detection for T2DM, the National Screening Committee (NSC) advocates only an opportunistic screening and as part of the NHS Health check programme (6-8). The ADDITION Leicester study (Section 4.6) was designed to evaluate the benefits of a structured screening for T2DM and the cost effectiveness of an algorithmic multi factorial intervention in screen detected T2DM patients.

2.2 Aims of this thesis

This thesis will evaluate optimal method of early identification of those with PDM in a UK multi ethnic population and recommend strategies for follow up.

2.2.1 Early identification of PDM

a. What are the characteristics of people with PDM in different ethnic groups?

b. What is the prevalence of micro and macro vascular complications in people with PDM at diagnosis?

c. What are the differences in the biomarkers between various categories of PDM such as IFG and IGT?
d. Do the adipocytokines differ between subjects with PDM from different ethnic groups?

2.2.2 Follow of people with PDM

a. What is the rate of progression of PDM to T2DM in a UK multi ethnic population?
b. What are the factors predicting this?
c. Is the rate of progression different amongst ethnic groups? If so why?
d. Which simple screening tools can be used to effectively follow up people with PDM?
e. Does a simple risk tool devised using the above data be used to predict progression to T2DM?

2.2.3 Summary of aims

This thesis will review the available evidence for screening for PDM and natural history of people with PDM in terms of progression to T2DM and risk of developing cardio vascular disease (CVD). Moreover the biomedical characters and other non traditional risk markers such as biomarkers in people will PDM will also be analysed. The follow up data will look at the progression risk to T2DM and a feasibility of a risk score in this group. This will contribute to the current available data on PDM but in a prospective manner in a UK Multi ethnic population.

2.3 History

The first technical report for diagnostic criteria of Diabetes Mellitus was published by the World Health organisation in 1965 (9). “Borderline diabetes” and “Chemical Diabetes” were used to classify people with glucose levels in the non diabetes range, and had a higher risk of developing diabetes subsequently (10). But the term IGT was first mentioned only in 1979 to include people who have a plasma glucose between the diabetes range and the normal plasma glucose (11). Again IGT is recognised as a condition predisposing to T2DM but no further measures to manage this condition were described. It was not until 1999 that the term IFG was introduced to explain the dysglycaemic fasting plasma glucose (12). IFG and IGT have been interchangeably included under the conditions non diabetic hyperglycaemia (NDH), impaired glucose regulation (IGR) and impaired glucose metabolism (IGM). It is recommended that the

term PDM may be used to address the disorders of IGR when communicating to non healthcare professionals (13). However, PDM does not inevitably lead to T2DM (14). PDM was recognised as a major healthcare problem in the late 90s and the early 2000 following publication of results of landmark prevention studies for T2DM.

2.4 Epidemiology

It is important to recognise that PDM is asymptomatic and hence people are diagnosed through screening or opportunistically when tested for other conditions. The prevalence of PDM has been variably quoted in different studies. The difference is due to the differing background population, ethnic disparities, varying screening tools and methodologies and differing risk strategies adopted for screening. IFG and IGT are metabolically different conditions. This is considered further in section 2.9.4. Hence the terms isolated IGT (i-IGT) and isolated IFG (i-IFG) are sometimes used to describe mutually exclusive categories.

2.4.1 Prevalence of PDM in different populations

The prevalence of PDM varies in different populations. Certain ethnic groups are at a higher risk for the development of T2DM and PDM such as South Asians (SA). There exists not just an inter ethic variation in terms of prevalence of T2DM and PDM, but also an intra ethnic difference depending on geographical location due to the aforementioned environmental factors (15-17). Migrant SA in the UK are highly predisposed to cardiometabolic conditions compared to their White European (WE) counterparts (18-21). However epidemiological data in other ethnic groups such as Nigerians and Ghanaians in T2DM are scarce (22).

The best evidence for epidemiological purposes has been reported in the DECODE and DECODA studies which combined datasets from 13 European and 10 Asian studies respectively (23-25). Unwin *et al* reviewed these data and concluded that IGT is commoner that IFG in most populations. IFG is commoner in men except in people than older than 70 years. The prevalence of IGT rises with age except a few populations where it plateaus in middle age. On the contrary, the prevalence of IFG plateaus in middle age (40–50 years), with the exception of European women where it rises until 70 years (26). Table 2.1 to 2.4 summarise the prevalence of PDM in various populations.

From Table 2.1 it is seen that prevalence of PDM increases with age in the Oriental race and remains static in the SA ethnic group. One possible reason for this difference is a significant shift towards the older population in Japan and China as these countries have one of the highest longevity thus a higher prevalence with advancing age. But equally SA have a younger age of onset of T2DM and PDM as well as cultural differences between the ethnic groups leading to a much earlier gene- environment interactions.

Table 2.1. Age and sex specific prevalence[$] of PDM in Asian population (23)

Ethnic group	Age group	i-IFG	i-IGT	IFG+IGT	PDM
South Asians	30–39	6.85	11.25	3.5	21.65
	40–49	6	11.1	4.15	21.25
	50–59	6.25	9.6	4.55	20.4
	60–69	4.9	11.9	4.3	21.05
	70–79	4.6	12	6.3	22.9
	80–89	5.85	12.95	5.35	24.15
Japanese and Chinese	30–39	2.8	1.25	2.4	6.4
	40–49	3.7	8	1.9	13.6
	50–59	5.3	9.6	2.15	17
	60–69	4.75	13	4.2	22
	70–79	5.25	15	3.45	23.65
	80–89	5.5	13.9	1.3	20.7

$ Prevalences in % are crude and calculated from Qiao 2003.

Table 2.2 Prevalence of PDM amongst different ethnic groups using WHO 1979 and 1985 criteria

Study	Ref	Criteria	Age/ N	Ethnicity	IFG	IGT	IFG±IGT	i-IFG	i-IGT
IDS[3]	(27)	1	>40/ 1,040	Mixed Ethnic	----	4.10%	----	----	----
Nauru	(28)¥	1		Polynesian	40%	----	----	----	----
Sweden ‡	(29)	2	55–57/ 1,843	Caucasian◊	17.30%	27.90%	7.60%	9.70%	20.30%
NHANES III ‡	(30)	2	40-74/ 2,844	Mixed Ethnic	8.30%	14.9	3.90%	4.40%	11%
India	(31)	2	/ 1,082	South Asians	----	IGT: Males:23.4%, Females 28.2%			
Coventry	(32)	2	≥20/ 1,499	Mixed Ethnic	IGT: 5.7% & 9.8% (Men) 6.8% & 11.2% (Women)♣				
Pakistan	(33)	2	≥25/ 967	South Asians	IGT: 8.2% (Men) 14.3% (Women)				
Pakistan	(34)	2	≥25/ 1,035	South Asians	----	9.40%	----	----	----
Pakistan	(35)	2	≥25/ 1,404	South Asians	IGT: 11.9% (Urban) 11.2% (Rural)				
South Africa	(36)	2	>15/ 866	South Asians	----	5.80%	----	----	----
Hawaii	(37)	2	≥30/ 574	Melanesian	----	15.10%	----	----	----
Saudi Arabia	(38)	2	≥15/ 3,252	Arab	IGT: Rural: 8% & 8% Urban: 10% and 11% •				
FHS[4]	(39)	2	30-65/ 1,580	Mixed Ethnic	----	37%	----	----	----

1. WHO 1979 2. WHO 1985 3. Islington Diabetes Survey 4. Fasting Hyperglycaemia study
◊ Women ♣ Caucasian and South Asian respectively ●Men and women respectively
‡ Adapted from Unwin *et al*. The rates have been calculated from the original article cited (26) ¥ Original article not obtained either due non availability of the journal or the article being in e publication

Table 2.3 Prevalence of PDM by WHO 1999 criteria amongst different ethnic groups

Study	Ref	Age/ N	Ethnicity	IFG	IGT	IFG±IGT	i-IFG	i-IGT
Mauritius ‡	(40)	25–74/ 3713	Mixed Ethnic	7.50%	17.20%	3.30%	4.20%	13.90%
Pima ‡	(41)	≥15/ 5,023	Pima Indian	4.40%	13.20%	2.50%	1.90%	10.70%
Australia ‡	(42)	≥25/ 11,247	Caucasian▲	8.30%	10.60%	2.60%	5.70%	8%
DECODA	(23)	See Table 2.1. IDPP[1] and CUPS[2] included in DECODA						
South Africa	(43)	>15/ 1,025	African Black	1.50%	4.80%	----	----	----
Uzbekistan	(44)	>35/ 1,144	Uzbek	IGT: Semirural: 6% & 9% Urban: 9% and 8% *●				
Greenland	(45)	>30/ 1108	Inuit	----	12.20%	----	----	----
Palestine	(46)	30- 65/ 500	Arab	----	8.60%	----	----	----
GEMCAS[3]	(47)	/ 35,869	Caucasian	2%	----	----	----	----
Denmark	(48)	30-69/ 6,758	Caucasian	4.60%	7.50%	----	4.40%	3.10%

●Men and women respectively ▲ Predominantly * Crude Prevalence ‡ Adapted from Unwin *et al*. The rates have been calculated from the original article cited (26).

1. Indian Diabetes Prevention programme 2. Chennai Urban Population study 3. German primary care: data from the German Metabolic and Cardiovascular Risk Project

Table 2.4 Prevalence of PDM using ADA criteria reported in different ethnic groups

Study	Ref	Criteria	Age/ N	Ethnicity	IFG	IGT	IFG±IGT	i-IFG	i-IGT
Hong Kong ‡	(49)	1	18-66/ 1,486	Chinese	2%	7.20%	1.10%	0.90%	6.10%
DECODE ‡	(50)	1	≥30/ 25,364	Caucasians	10%	11.90%	3.10%	6.90%	8.80%
Canada	(51)	1	≥10	Eeyou	4.70%	---	----	---	---
Nepal	(52)	1	>20/ 1,841	South Asians	IFG: 9.1% (Urban) 1.3% (Rural)				
Kuwait	(53)	1	20-50/ 2,260	Arab	3.40%	1.90%	----	----	----
DIASCAN[3]	(54)	1	≥40/ 9,042	Mixed Ethnic	2.50%	0.60%	----	----	----
United States	(55)	2	12-19/ 1496	Mixed Ethnic	11%	----	----	----	----
MexDiab	(56)	2	30- 65	Hispanic	24.60%	8.30%	10.30%	----	----
TLGS[4]	(57)¥	2	≥40/ 4,018	Persian	----	----	----	----	27.30%

‡ Adapted from Unwin *et al*. The rates have been calculated from the original article cited (26).
† Capillary blood glucose
¥ Original article not obtained either due non availability of the journal or the article being in e publication
1. ADA 1997 criteria
2. ADA 2003 criteria
3. Diabetes Screening in Canada Study
4. Tehran Lipid glucose study

2.4.2 Presence of complications in people with PDM

2.4.2.1 Micro vascular complications

Studies have also shown that vascular complications coexist even in the PDM. It is generally accepted that development of vascular complications and deterioration in glycaemia occur side by side as people progress from PDM to T2DM.

Studies show that a significant proportion of people with chronic idiopathic axonal polyneuropathy (CIAP) have impaired glucose status (26;28;48). In a single centre cross sectional study, two fold higher prevalence of IGT was noted in patients with CIAP compared to age matched controls (58). Similarly a reduction in both motor and sensory conduction velocities have been demonstrated in people with IGT compared to those with NGT. In this study no differences in these neurological parameters were seen between people with IGT and T2DM, perhaps suggesting the need to screen for micro vascular complications even in the IGT stage (59). In a recent review the majority of the studies have shown an association between chronic glucotoxicity and neuropathy but epidemiological data to suggest that this occurs independent of conversion to T2DM is not available (60).

A Finnish study showed that the prevalence of microalbuminuria is 1.3 fold higher in people with IGT compared to normal age matched controls. Further, microalbuminuria precedes the development of T2DM (61). In a study involving 48 Pima Indians with IGT, the hallmark of diabetic nephropathy- hyper filtration as evidenced by a significantly raised glomerular filtration rate (GFR) was associated with progression to T2DM. Hyper filtration was also demonstrated in people with IGT compared to those with NGT (62).In one study involving 1154 people with IGT at least 21.7% were reported to have at least one micro vascular complication (63).

In the Diabetes prevention program, 7.9% of individuals with IFG and/or IGT were reported to have features of diabetic retinopathy (64). In the AusDiab study, 6.7% of people with IFG and/or IGT were reported to have retinopathy amongst 1,027 individuals (65).

The risk of CVD from IFG and IGT and the relationship between glucose and incident CVD events are described in section 2.7 on page 17.

2.5 Diagnostic Criteria

2.5.1 WHO Criteria

The first ever diagnostic criterion for IGT was proposed by the World Health Organisation in 1979 (11). The diagnostic criteria for T2DM was based on the plasma glucose performed 2 hours post 75g load glucose load. The cut-off values were based on data from epidemiological studies. The absolute value is based on the observation that subjects below the cut off rarely developed complications from T2DM and many reverted to normal glucose tolerance (NGT) over a period of time.

The 1985 criteria recommend further research into IGT and its impact on CVD and possibly primary prevention of T2DM.

The various diagnostic criteria for the diagnosis of IFG and IGT are tabulated in Table 2.5.

Fasting plasma glucose (FPG) forms the basis of diagnosis diabetes in the 1999 criteria which is based on the increased prevalence of retinopathy with increasing deciles of FPG, intersection of the bimodal curves of distribution of FPG in the population and the value that corresponds to the previously defined 120 minute post glucose load level (PGLG) (12;66). One problem in defining the normal range of plasma glucose in the traditional way of using the mean and two standard deviations is that the high prevalence of diabetes which is more than 2.5% in the population. In the previous report it has been cited that the PGLG value is based on the fact that people below the cut off rarely develop vascular complications and only a small proportion develop metabolic deterioration. Having defined the normal and the diabetes range plasma glucose, the intermediate range levels were known as IFG and IGT.

In the following sections the principles on which the cut off values of IGT and IFG are based are described. This is discussed under three areas- physiological basis, risk of progression to T2DM and the risk of incident CVD.

Table 2.5. Diagnostic criteria for IGR proposed by various organisations

Criteria	IFG	IGT
WHO 1965 (9;66)†	------	FPG not specified, PGLG 6.1-7.1
WHO 1979 (11)	------	FPG <8.0 and PGLG ≥8.0 and <11.0
WHO 1985 (67)	------	FPG <7.8 and PGLG ≥7.8 and <11.1
WHO 1999 (12)	FPG >6.0 and <7.0 and PGLG <7.8	FPG ≤6.0 and PGLG ≥7.8 and <11.1
ADA 2006 (68)	FPG ≥5.6 and <7.0 and PGLG <7.8	FPG <5.6 and PGLG ≥7.8 and <11.1

† Referred as an intermediate state

2.5.1.1 Validity of 2 hour post glucose load threshold

The scientific basis defining the cut off for IFG and IGT varies. There is no physiological basis of the 7.8 mmol/L cut off for IGT. The cut off predominantly is based on the higher transition rate to T2DM in people with IGT. However various studies have quoted differing rates. One reason for this is the different study population, ethnic disparities, differing rate of follow up and different criteria (WHO 1979 or 1985 or 1999) used to define IGT (11;12;67). However it has been well demonstrated that the risk of developing T2DM in people with IGT is considerably higher compared with people with NGT.

The review by Santaguida *et al* quoted an annualised risk of progression (per 100 person-years) to T2DM from IGT to be 1.83 to 34.12 for people who had IGT alone compared to 4% in people with NGT. This was much higher if IFG co existed with IGT (69). A meta-analysis of six studies reported progression rates (per 100 person-years) to be 5.72 ranging from 3. 58 to 8.73 (70). Elevated FPG and obesity further increase this risk. The 5 year incidence of T2DM was 24% compared to 4% in those with NGT, as reported from a Pima Indian study (71).

The risk of CVD increases with increasing 120 minute post load glucose, though this relationship is not always linear as discussed in the following sections.

In summary the cut off of PGLG for IGT is based on the risk of progression to T2DM compared to NGT.

2.5.1.2 Validity of fasting glucose threshold for IFG

In contrast to IGT, though FPG cut off value for IFG has some physiological basis with respect to insulin response; however, similar to IGT, the epidemiological data supporting progression to T2DM and CVD risk appears to be sparse. The 2006 WHO document on diabetes and intermediate disorders of hyperglycaemia quoted two sets of data which reported beta cell dysfunction with increasing FPG (66). Godsland *et al*, demonstrated a decline in the first phase insulin response at a FPG between 4.97 to 5.14 mmol/L as and the late phase insulin response decline at a FPG of 6.0 mmol/L (72). Piche *et al* demonstrated that insulin sensitivity and markers of beta cell function declined progressively even with the range of normal FPG (73). A similar response has been subsequently demonstrated in the IFG range of FPG independent of the glucose tolerance status (74). The meta-analysis of 18 studies demonstrated the increased CVD risks for elevated FPG in the non diabetes range clearly beyond doubt. The pooled relative risks were 1.27 (95% CI, 1.13-1.43) for the upper most category of FPG vs. lower category, all below the diabetes range. On a dose response curve using twelve studies, there was a possible threshold for increased CVD at 5.6 mmol/L (75). Subsequently the Baltimore Longitudinal study of Ageing demonstrated that the FPG of 6.1 mmol/L may be threshold beyond which all cause mortality rises (76). The linear increase in risk of T2DM for FPG has been demonstrated for a value even as low as 4.8 mmol/L in some studies (77). Due to this linear response, the risk of T2DM varies based on the cut off chosen and the lower the cut off the higher the prevalence of IFG (78-80).

2.5.2 Current ADA and WHO criteria

The European Diabetes Epidemiology group concluded that lowering the cut off of IFG to 5.6 mmol/L has far reaching public health consequences, for example potentially significantly increasing the prevalence of IFG and hence recommended that the cut off be maintained at 6.1 mmol/L as recommended in 1999 by WHO (81). WHO endorsed this in its 2006 intermediate report (66). Subsequently Forouhi demonstrated that the risk of incident T2DM is more strongly related to the original definition of IFG (6.1 - 6.9 mmol/L) as opposed to the ADA classification (5.6 - 6.9 mmol/L) with the 10 year cumulative risk (95% CI) (per 1000 person years - PY) being 6.2 (4.0 to 9.8) and 17.5 (12.5 to 24.5) respectively compared to 2.4 (1.2 to 4.8) for NGT (82).

2.5.3 HbA1c as an additional criteria for the diagnosis of T2DM

The WHO in January 2011 have recommended that a HbA1c≥ 6.5% may be used for the diagnosis of T2DM in addition to the OGTT and plasma glucose values recommended in 1999 (83). The wider impact of this diagnostic criteria on prevalence of T2DM have been discussed in detail from our research group previously (84). However utility of HbA1c in the diagnosis of PDM remains controversial and WHO does not recommend this at present. Detailed discussion of this is beyond the remit of this thesis.

2.6 Prediabetes and progression to T2DM

The majority of studies have suggested a continuous relationship between glucose and risk of developing T2DM, mortality and CVD (14;26;70;75;85;86). Hence IFG and IGT are likely to represent an arbitrary cut off to identify group of people at higher risk of developing T2DM and CVD (See 2.5.1.1and 2.5.1.2). In spite of the large number of subjects used in these combined data sets, there are differences as to methods of glucose measurement (capillary, plasma or whole blood), assay method, risk strategy used to screen people and ethnic disparities. The studies looking at progression from IFG or IGT to T2DM also differed in the criteria used (WHO 1985, WHO 1999 or ADA). These may contribute to some amount of imprecision and variation in the analyses. Different measures used to report the progression rates such as relative risk (RR), hazard ratio (HR), progression rates in cases/person years (PY) makes it difficult to compare or combine the data.

Broadly speaking, data looking at the natural progression from PDM to T2DM comes from two different sources- epidemiological prospective studies and control arm of intervention studies in PDM, the latter involving both pharmacological and lifestyle interventions. The former source is probably the better indicator of the natural history of PDM in the background population as intervention studies often introduce a volunteer bias as detailed in Chapter 3.

A literature search identified 23 manuscripts reporting progression from PDM and 3 meta-analyses. The meta-analysis by Santaguida *et al* is shown in Table 2.6. There were 2,389 individuals with IGT with a follow up period of 16,775 person years with the range of follow up period between 1 and 11 years. The overall progression rate was 5.72/100 PY with a wide range between individual studies between 3.58 to 8.73/ 100 PY (69). The analysis looked at unadjusted annualised RR that included both

epidemiological and randomised controlled trials. The results are tabulated in Table 2.6.

Subsequent to this meta-analysis, the ADDITION study from Denmark reported progression rates for IFG and IGT to be 17.6 and 18.8 cases per 100PY respectively in a follow up of 308 individuals with PDM after 1 year. Subjects with PDM were identified in a stepwise high risk screening strategy (48).

In spite of the methodological differences in the studies, a few general conclusions may be reached. The incidence of T2DM is comparatively higher in individuals who have both IFG and IGT. It tends to be similar in those with i-IFG and i-IGT, although there are ethnic differences. Pima Indians with i-IFG appeared to have a significantly higher risk of progression compared to those with i-IGT (41). Moreover people with i-IFG appear to have a higher risk of progressing to diabetes compared to i-IGT in the meta analysis by Santaguida *et al* (69).

Table 2.6. Progression rates to T2DM for PDM categories by Santaguida *et al*.

Glycaemia	Studies (N)	Participants (N)	Follow up‡	RR†	P value
IGT	17	3948	7.79 (1.60- 8.0)	6.02 (4.66 to 7.38)	<0.0001
i-IGT	3	735	5.47 (5.0- 6.4)	5.55 (3.15 to 7.95)	0.002
IFG	5	1204	5.08 (1.1- 9.0)	4.70 (2.71 to 6.70)	0.0003
i-IFG	3	241	5.47 (5.0- 6.4)	7.24 (5.30 to 9.17)	0.0001
IFG+IGT	3	206	5.47 (5.0- 6.4)	12.21 (4.32 to 20.10)	0.0054

‡ Mean years (Range) † Relative to Normal glucose tolerance/ normal fasting plasma glucose

Table 2.7. Characteristics of studies included in the Edelstein *et al* Meta analysis (70)

Study	N	Mean age (range)	Ethnicity	Follow up duration†	Progression rate (100 PY)‡
Baltimore longitudinal study of ageing (87)	675	59.4 (23.2- 92.5)	Caucasian¥	3.8 (1.2- 9.4)	3.58 (0.26)
Rancho Bernardo study (88)	186	68.0 (52.0- 82.0)	Caucasian	8.2 (7.0-9.0)	4.0 (0.57)
San Antonio Heart study (89)	353	48.3 (25.0- 65.0)	Mixed ethnic	8.2 (8.0 - 9.0)	4.34 (0.42)
Nauru study (90)	305	37.3 (12.0- 75.0)	Micronesian	6.2 (5.0 - 12.0	6.28 (0.53)
San Luis Valley Diabetes study (91)	177	59.7 (31.2- 75.0)	Mixed ethnic	1.9 (1.0- 3.2)	7.29 (0.114)
Pima Indian study (92)	693	43.2 (20.0- 89.3)	Pima Indians	3.9 (1.8- 11.4)	8.73 (0.42)

¥ 95% Caucasian

† Mean years (5th to 95th Percentile)

‡ Mean (SE)

2.7 Prediabetes, CVD and mortality risk

2.7.1 Population data

Both IFG and IGT are associated with other risk factors for CVD such as central obesity, hypertension and hyperlipidaemia. However, there are still unanswered questions as to whether this is an association or causation. The common soil theory proposed that T2DM (and there by hyperglycaemia) and CVD have their origins in a common antecedent condition (93). However the evidence for PDM increasing the risk of CVD is limited; moreover there are very few prospective population based studies. However recent meta analyses have suggested the atherogenic nature of plasma glucose in the non diabetic range to be a risk factor for CVD and mortality. However this relationship varies between fasting and post prandial glucose.

A meta analysis involving over 95,000 individuals using 6 studies for fasting glucose and 7 studies for 2 hour post prandial glucose suggested that compared with a glucose level of 4.2 mmol/l, a fasting and 2-h glucose level of 6.1 mmol/dl and 7.8 mmol/l was associated with a relative cardiovascular event risk of 1.33 (95% CI 1.06-1.67) and 1.58 (95% CI 1.19-2.10) respectively, these represent the current WHO cut off for IFG and IGT respectively (85). A subsequent study quantified the relative risk (RR) of CVD events of 1.26 [95% CI, 1.11-1.43] in subjects with a higher (mid point range: 8.3 to 10.8 mmol/L) compared to those with a lower level (mid point range: 3.8 to 5.9 mmol/L) of post challenge plasma glucose (75). The studies included in this analyses all had varying levels of glucose challenge (50-100g) and may contribute to bias in the combined analysis.

Santaguida *et al* reported an annualised risk estimate (per 100 persons in the exposed group) for any nonfatal CVD event of 11.58 to12.39 and 0.63 to 9.68 for IGT and IFG groups respectively in a systematic review involving 6 studies with follow up duration between 6 to 9 years (69). For all cause mortality these figures were 4.35 to 6.35 and 6.07 to 9.15 respectively. Subjects with both IFG and IGT had a significantly higher CVD event rate compared to those with either i-IFG or i-IGT [unadjusted annualised RR range 5.50 (2.86 to 8.90) to 20.69 (12.51 to 34.22) for all cause mortality].

The DECODE reported HR for CVD to be 1.21 (1.05-1.41) and 1.08 (0.70-1.66) for men and women with IFG respectively,compared to those with NGT. The corresponding values were 1.51 (1.32-1.72) and 1.60 (1.22-2.10) for IGT (7.8-11.1

mmol/L) (50). A multivariate model adjusted for age, center, total cholesterol, body mass index, systolic blood pressure, smoking and sex reported an adjusted RR for CVD and all cause mortality of 1.09 (0.90-1.30) and 1.11 (1.00-1.23) for subjects with IFG range compared to normoglycaemia respectively (94). The corresponding risk was 1.34 (1.14-1.57) and 1.40 (1.27-1.54) respectively for subjects with IGT range 2 hour post challenge glucose. When the model included both fasting and post challenge glucoses, CVD and all cause mortality were attenuated at 1.01 (0.84-1.22) and 1.03 (0.93-1.14) respectively for subjects with IFG. However this had little impact for 2 hour post challenge glucose with RR being 1.32 (1.12-1.56) and 1.37 (1.25-1.51) respectively for CVD and all cause mortality.

The AUSDIAB study after a follow up duration of 6.2 years reported results similar to the DECODE study but RR were reported separately for FPG <5.1 and FPG ≥5.1 mmol/l as the relationship between FPG and events were noted to be J shaped. The adjusted hazard ratios (HR) (95% CI) for all-cause mortality were 1.2 (1.1-1.3), 2.0 (1.3-3.0) and 1.1 (1.0-1.2) for 2hPG, FPG <5.1 mmol/l and FPG ≥ 5.1 mmol/l respectively. Corresponding HRs for CVD mortality were 1.2 (1.0-1.4), 4.0 (2.1-7.6) and 1.3 (1.1-1.4) respectively (95). In a model with both FPG and 2hPG, HR for all cause mortality were 1.2 (1.1–1.3), 2.0 (1.4–3.1) and 1.0 (0.9–1.1) for 2hPG, FPG <5.1 mmol/l and FPG ≥ 5.1 mmol/l respectively. The corresponding figures for CVD mortality were 1.1 (0.9–1.4), (2.2–7.8) and 1.2 (1.0–1.4) respectively.

In a retrospective analyses from a Japanese study the Cox's proportional HR (age adjusted for cardiovascular mortality was found to be 2.219 (95% CI 1.076-4.577) for IGT and 1.136 (0.345-3.734) for IFG compared to subjects with normal glucose after 7 years (96).
In a Taiwanese population based study involving over 23,000 men, subjects with IFG were reported to have a RR of 1.30 (P<0.05) compared to normal fasting glucose for CVD mortality (97).
In summary, the CVD risk is increased in those with both higher fasting and 120 PGLG, however this relationship has been consistently demonstrated for the latter in studies.

2.7.2 Data using surrogate markers

Endothelial dysfunction precedes overt CVD. There are various approaches of measuring endothelial dysfunction of which carotid intimal medial thickness (CIMT) is

one (98). A few longitudinal studies have shown an increased CVD risk if the CIMT is over 1 mm. In the Atherosclerosis Risk in Communities study, involving over 12,000 individuals and 5.2 years of mean follow up, the HR (95% CI) was 2.62 (1.55-4.46) for women and 1.20 (0.81-1.77) for men, comparing CIMT ≥ 1 mm vs. <1 mm (99). The Rotterdam study has shown similar results for men but not for women after 2.7 years of follow up (100). The Prevention Conference V has recommended the use of CIMT as an added risk assessment tool for CVD (101). In cross sectional studies CIMT has been shown to correlate well with presence of myocardial infarction and stroke (102;103).

However the diagnostic role of CIMT in subjects with PDM remains controversial. Studies have shown variable results and large population data remains unavailable (104-108). Present evidence suggests that 2hPG is more significantly associated with CIMT than FPG.

2.8 PDM and associated risk factors for CVD

It is well established that both IFG and IGT are associated with the other traditional risk factors of CVD such as hypertension, dyslipidaemia and metabolic syndrome. Both CVD and T2DM develop on a background of genetic predisposition with unfavourable environmental factors. Thus it is often suggested that the two conditions develop in parallel due to the sharing of common risk factors- the common soil hypothesis (93). Central obesity and insulin resistance play a central role in this development.

However data available to associate the common risk factors with either IFG or IGT are limited, not population based and data for IFG is variable. In a study involving 91 individuals with either IFG and /or IGT, blood pressure, BMI and triglyceride levels were significantly higher in people with IFG and/or IGT compared to normal controls (109). The Taiwan study reported a significantly higher BMI, blood pressure and serum cholesterol in subjects with IFG compared to those with normal FPG (97). However data from the RIAD study showed a similar pattern only in subjects with both IFG and IGT and not in those with i-IFG (108). Two Danish studies showed no particular differences between subjects with IFG and IGT in terms of known CVD risk factors (29;110). In a study from Singapore involving 300 people with IGT who were of similar age compared to those with normal glucose tolerance, people with IGT had an adverse CVD risk profile in terms of blood pressure, triglycerides and HDL cholesterol (111). A simpler way of explaining this clustering of CVD risk factors may be is by metabolic syndrome that is more prevalent in subjects with PDM (112).

An IDF consensus reported both IFG and IGT to be risk factors for development for both T2DM and CVD (26;113).

2.9 Pathogenesis of IGT and IFG

Based on the metabolic characteristics seen in individuals with IFG and IGT, there are various literature reports of differences between subjects with IFG and IGT. The basic physiology of Insulin is described initially followed by the changes seen in people with IFG and IGT.

2.9.1 Basic insulin physiology

2.9.1.1 Anatomy and Physiology of the Islets

The normal adult pancreas contains over 1 million islets of Langerhans scattered throughout the exocrine pancreatic tissue. These clusters of cells contain four different cell types (hormones secreted) –β (Insulin), α (Glucagon), δ (Somatostatin) and PP or F cells (pancreatic polypeptide). β cells are situated in the core of the islets. The islets receive 20% of the blood supply of the pancreas supplied by the branches of the splenic and pancreaticoduodenal arteries. The islets are innervated by the adrenergic fibres from the coeliac plexus, cholinergic fibres from the vagus nerve and peptidergic neurons (114). The cephalic phase of insulin secretion that is triggered by the sight and smell of food is initiated by the vagus nerve that originates from the hypothalamus.

The cells of the islets communicate with each other by the blood suppy as well as tight gap junctions. The hormones have both an endocrine and a paracrine effect on the other cells. Nutrients such as glucose and amino acids such as arginine, Glucagon, other enteroinsular hormones such as the GLP-1 and the vagus have a stimulatory effect whereas hormones such as somatostatin, neuropeptide Y and adrenergic stimulation have an inhibitory effect on insulin secretion (115).

2.9.1.2 Insulin structure and secretion

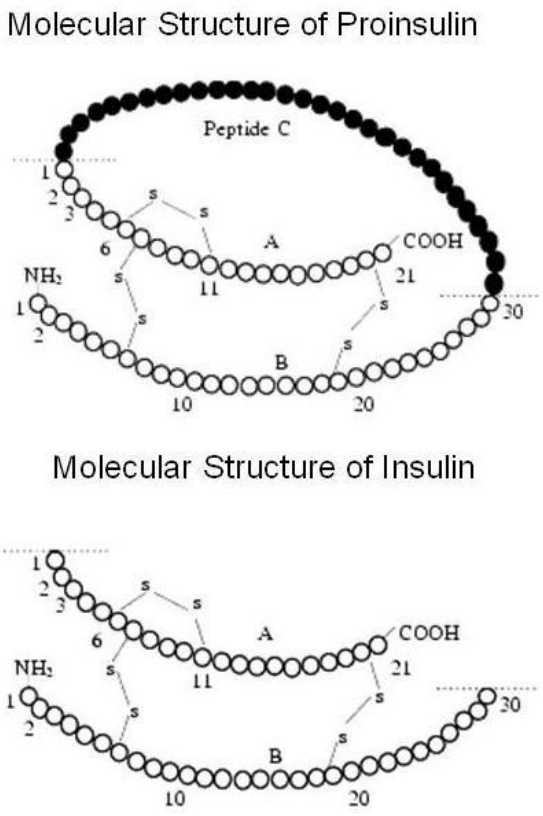
The human insulin molecule consists of two polypeptide chains α and β that contain 21 and 30 amino acid residues respectively. These are interconnected by two disulphide bonds and a similar bond connects the amino acids at positions 6 and 11. Insulin exists as hexamers with zinc ions when stored in the Golgi vesicles in the pancreatic β-cells (Figure 2.1) (116).

Insulin is initially synthesised as Preproinsulin in the rough endoplasmic reticulum that upon translation is rapidly cleaved to form Proinsulin. Proinsulin contains the α and β

chains joined connected by a C-peptide. Proinsulin is transported and stored in the Golgi apparatus and further conversion to Insulin and C-peptide takes place by the carboxy peptidases. Insulin and C-peptide are are then released on appropriate stimulus in equimolar concentration. 95% on the secreted vesicles contain Insulin and the rest contain unprocessed preproinsulin (117).

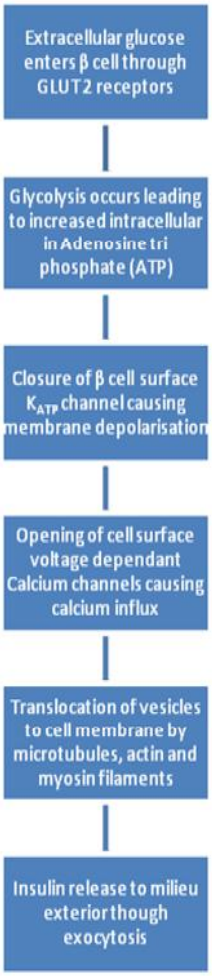
Glucose is the prime secretagogue of insulin secretion. Insulin secretory response curve to glucose is sigmoid shaped with a threshold of 5 mmol/L of extracellular glucose and is maximal around 17 mmol/L. The time course of insulin secretion follows a distinct two phase pattern- the initial acute so called first phase followed by a more sustained and less intense second phase lasting for the duration of the stimulus. The intracellular pathway of insulin secretion is elucidated in Figure 2.2.

Figure 2.1 Molecular structure of Insulin and Proinsulin



Calcium/Calmodulin dependant kinases act as effectors in the downstream pathway of insulin transport and release following a stimulus.

Figure 2.2. Intracellular pathway of Insulin secretion



2.9.1.3 Action of Insulin

The action of insulin is mediated by insulin receptor that belongs to a family of related receptors- Insulin receptor and the Insulin like growth factor-1 receptor. These are all tyrosine kinases and act downstream through the Insulin receptor substrate (IRS) family of downstream proteins. The Insulin receptor is a tetramer composed of two each of the α and β subunits. Binding of the ligand to the receptor causes autophosphorylation of the β subunit that in turn causes phosphorylation of a variety of cytoplasmic proteins to cause the biological actions (118;119).

The biological actions of Insulin are tabulated in Table 2.8.

Table 2.8. Biological actions of Insulin

Adipose tissue
Increased
Glucose entry, fatty acid synthesis and triglyceride deposition
Muscle
Decreased
Protein breakdown and ketogenesis
Increased
Glucose entry and Glycogenesis, protein synthesis and lipogenesis
Liver
Decreased
gluconeogenesis and ketogenesis
Increased
Glycogenesis, protein synthesis and lipogenesis
Vascular (120-124)
Reduction in vascular stiffening
Vasodilatation
Reduced platelet aggregation

2.9.2 Insulin resistance

Insulin resistance (IR) is impairment of physiological actions of insulin, primarily the glucose lowering effect. IR is the key patho physiological abnormality in the development of glucose intolerance and subsequently T2DM. Central obesity appears to be key contributor to IR. Genetic predisposition along with unfavourable environmental factors such as nutritional factors combined with age and reduced physical activity appear to be the cause of obesity in the majority of patients (125-128).

In the initial stages of IR, there is reactive hyperinsulinaemia that compensates for the resistance of the organs that are normally insulin sensitive. This not only compounds IR but also leads to endothelial dysfunction. It is believed that the favourable vascular modifying actions of insulin is blunted in IR states (120;129;130). This whole process

is augmented by the biologically active hormones secreted by the adipose tissue (2.10.2). As patients progress from NGT through IGT to finally T2DM, it has been demonstrated that there is a progressive decline in insulin levels and rise in plasma glucose (131-133). The decline in insulin levels contribute to hyperglycaemia and endothelial dysfunction due to the loss of the favourable vascular actions of Insulin. Rising glucose levels cause reduced β cell function though glucotoxicity (134-138).

2.9.3 Homeostatic model assessment (HOMA) of Insulin Resistance (HOMA-IR) and β cell function (HOMA-β)

IR plays a central role in metabolic syndrome and in PDM. There are several methods of quantitative assessment of IR. The gold standard method is the euglycaemic hyperinsulinaemic clamp method or an intravenous glucose tolerance method both of which are cumbersome.

HOMA was first described by Mathews *et al* in 1985 (134). This is a technique to assess IR with FPG and fasting plasma insulin (FPI) or C-Peptide levels. This is based on steady state levels of FPI and FPG.This model is based on the fact that steady state levels of FPI and FPG are determined by their interactions in a feedback loop between the liver and the beta cells (139). A computer model for various levels of FPI and FPG has been proposed based on Insulin: Glucose interactions based on data from man and animals, for various degrees of Insulin resistance and beta cell deficiency. From the plot of this data, one can estimate the insulin resistance and beta cell function based on FPG and FPI or C-Peptide.

A recent review article summarises the use of HOMA modelling, its physiological basis and its use in clinical research (140).

2.9.4 Pathological changes in IFG and IGT

Broadly, the abnormalities seen in PDM may be that of Insulin secretion (otherwise beta cell function) or that of Insulin action (otherwise IR).Data available in the literature as to the relative contribution of these two entities is variable. Again ethnic disparities have been reported. In general both abnormalities contribute to the pathogenesis of T2DM (141;142).

In the fasting state, basal metabolic requirement is maintained by hepatic glucose output (HGO). Basal insulin secretion is low which determines plasma glucose that is in turn determined by HGO. In the post prandial state, increase in plasma glucose leads to insulin secretion that suppresses HGO, stimulates glycogenesis in the liver

and suppresses lipolysis in the adipose tissue (Table 2.8). This insulin secretion is phasic (2.9.1.2). Many studies have demonstrated inadequate suppression of HGO (143). This in turn is thought to be due to impaired first phase Insulin secretion (FPIS). The importance of this phasic insulin secretion has been extensively reviewed (144). Impaired FPIS is the most consistent finding reported in those with IGT (131;143;145-149). Experimental studies have demonstrated the physiological importance of FPIS, where attenuation of FPIS led to worsened glucose tolerance (150). FPIS is thought to be important to prime the insulin sensitive tissues for subsequent insulin action and for the more consistent second phase insulin secretion (74;151;152). Both subjects with IFG and IGT have a muted FPIS but the latter also have an attenuated late phase insulin secretion. Both groups are insulin resistant, the former due to hepatic resistance with near normal muscle sensitivity, the latter due to muscle insulin resistance (109;153-155).

An Italian study reported that IGT is characterised by increased IR and IFG by impaired insulin secretion in the fasting state (156). Studies have also demonstrated that glycaemic status progression from IFG to IFG+IGT is associated with reduced insulin secretion, in addition to IR (157).

A recent review postulated that IR is initiated by the pathological changes in the adipose tissue and this leads to IR in the muscle and liver. This is followed by reactive hyperinsulinaemia and the changes in beta cells (158). A more recent review demonstrated that subjects with i-IGT and i-IFG have a similar impairment of pancreatic α and β cells function with only the former exhibiting features of IR. This was further explained by differential levels of the incretin hormone effect (159).

2.10 Impaired glucose metabolism- an inflammatory condition?

2.10.1 IGM, atherosclerosis and inflammation

Although individuals with T2DM are undoubtedly more susceptible to all forms of atherosclerotic CVD, there are many unanswered questions surrounding this temporal relationship (160). Established macro-vascular pathology is common at the time of diagnosis of T2DM, suggesting either latency in diagnosis and/or an atherogenic pre diabetes state (161-164). The prevalence of complications may also be related to the duration of T2DM (165). The UKPDS shows that once diabetes is diagnosed, glycaemia is only modestly related to coronary artery disease (166;167). Similarly it has also been shown that the CVD risk in people with IGT is only partly accounted by

glycaemia (168;169). There is some evidence to suggest that this may also be true for micro vascular disease (169). Follow up data from the UKPDS extrapolated that the glucose intolerance may start to progress for up to 12 years sub clinically before the diagnosis of T2DM (170). These data indicate the probability of interplay between glycaemia and other risk factors fairly earlier in the atherogenic process (171).

It is now widely regarded that atherosclerosis is an endothelial inflammatory process initiated by endothelial injury. Endothelial dysfunction and inflammation precede overt cardiovascular events by years. The process involves a complex interaction between the endothelium, inflammatory cells such as the circulating monocytes that convert to active macrophages in intimal layer of the blood vessels (172-174). This process is variably regulated by the chemical mediators secreted not only by these cells but also by the biologically active cells of the adipose tissue- the adipocytes. The risk factors for initiation and progression of this condition are included under the umbrella of metabolic syndrome or syndrome X, of which dysglycaemia is one component (175;176). The emerging evidence is that endothelial dysfunction and inflammation precede overt cardiovascular events (172;174;177-181). Chronic inflammation appears to be the common link between metabolic syndrome and atherogenesis. Abdominal obesity leads to insulin resistance that is accompanied by glucose intolerance and dyslipidaemia that are pro atherogenic (173;182). Endothelial dysfunction also been demonstrated in people with IGM (179-181;183;184).

2.10.2 Role of Adipocytokines

The visceral adipose tissue is no longer merely an organ of energy storage but is the largest endocrine gland secreting numerous biologically active hormones called adipocytokines (185). It is now well established that insulin resistance leads not only to impaired action of insulin on the target tissues such as the muscle, liver and the adipose tissue but also accompanied by dysfunctional adipocytes. This leads to deregulated adipocytokines as well as acute phase proteins that further compound insulin resistance, beta cell dysfunction and endothelial dysfunction which is probably the first step in the initiation on the atherogenic process (173;186-188). In fact a number of such adipocytokines have been implicated in the very early stages of atherogenesis that may serve as a marker for risk stratification in the future (188).

The Inflammatory markers such as Tumour necrosis factor α (TNFα) and highly sensitive C Reactive Protein (hs-CRP) have been shown to be associated with CVD and may serve as markers of underlying atherosclerotic process. It has been shown in

previous studies that CRP rises as patients progress from IGT to T2DM, showing interrelations between glucose intolerance, insulin resistance and the atherosclerotic process (189). Insulin resistance is often manifested by hypertension, elevated insulin, elevated blood glucose, inflammation, dyslipidemia, high triglycerides, low high-density lipoprotein cholesterol (HDL), endothelial dysfunction, and clotting abnormalities. All these physiologic abnormalities predispose to atherosclerosis in IR.

2.10.2.1 Adipocytokines and diabetes mellitus

Adiponectin, Leptin, Resistin, TNFα, Interleukin 6 (IL6) and plasminogen activator inhibitor (PAI-1) are some of adipocytokines that can be measured as markers of endothelial inflammation (173;190;191). Leptin is a peptide hormone that appears to play a critical role in energy balance as a neuroendocrine and an immunomodulatory mediator. Circulatory leptin levels are higher in MS subjects and as a CVD risk marker (192-194). Adiponectin appears to play a protective role in development of T2DM (195-198). Interventions aimed at improving insulin resistance, have shown reduction in the levels of biomarkers that indicate CVD risk reduction (199-201). IL-6 is secreted by the adipose tissue and macrophages, and levels correlate with IR. IL-6 regulates the hepatic acute phase response and hs-CRP is the most widely recognised marker. There is evidence to link hs-CRP to risk of CVD and T2DM (202-204) as well as the direct role of hs-CRP on endothelium (205). PAI-1 has been implicated as a pro atherogenic component of MS (206-209). Recent studies have shown that impaired fibrinolysis is a component of MS increasing incident T2DM risk (210-212).

2.11 Summary

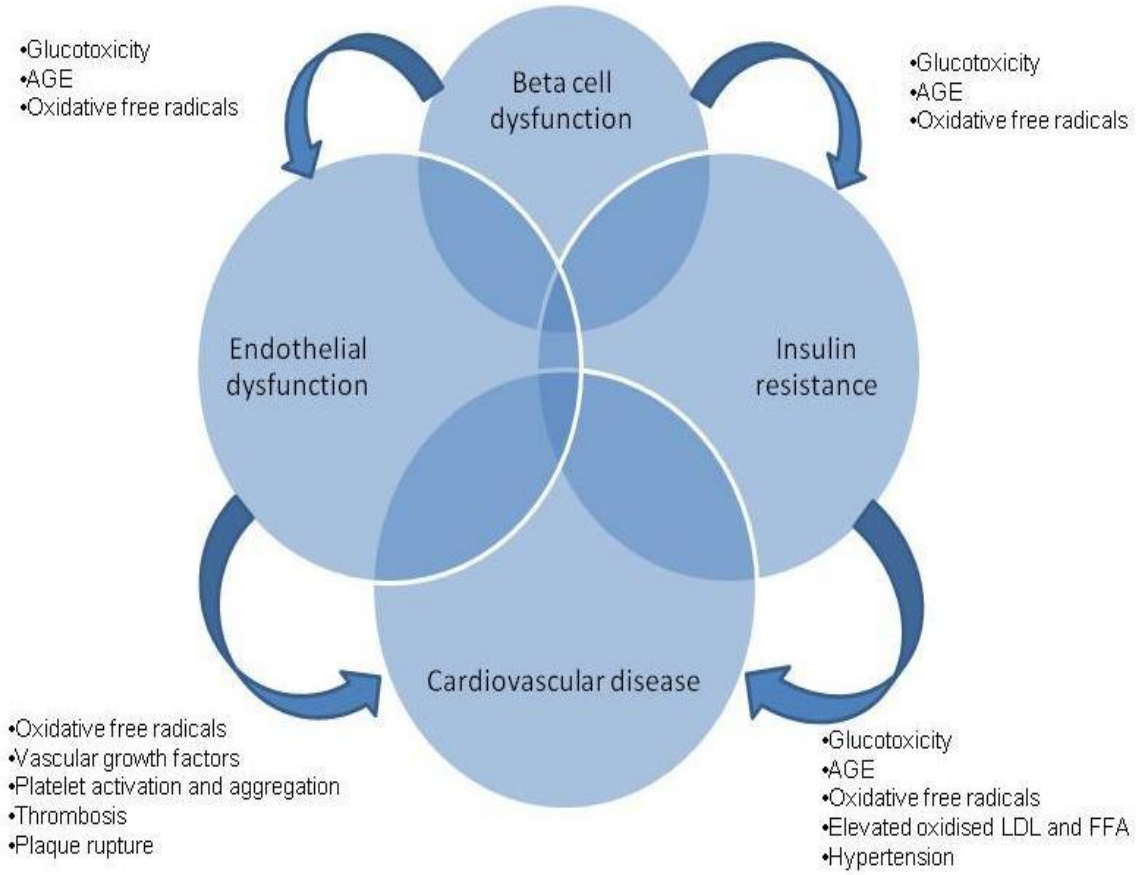
The concept of non diabetic hyperglycaemia was formally recognised only in the late 1970s and since then studies have shown the atherogenic nature of glucose. This causal link appears to be stronger for IGT compared to IFG. IFG and IGT are mutually exclusive conditions of dysglycaemia and presence of both signifies comparatively higher risk of both progression to T2DM and CVD. There are ethnic differences in prevalence of IFG and IGT. IFG and IGT are characterised by both insulin resistance and β cell dysfunction and relative contribution to the physiological state may be determined by the severity and duration of the condition.

The aetiology of T2DM is inter-related with that of atherosclerosis in that both have common risk factors. Chronic sub clinical inflammation appears to be the common link between these conditions. Adipocytokines mediate the development with endothelial and beta cell dysfunction being associated abnormalities in the development of T2DM

and CVD respectively. The inter relationships may be summarised in the model depicted in Figure 2.3.

Various clinical trials have demonstrated the reversible dysglycaemic nature of IFG and IGT with lifestyle interventions being the most effective intervention, though long term sustainability remains to be seen. Various pharmacological interventions using Metformin, Acarbose, Orlistat, Rosiglitazone and Troglitazone have also proven to be helpful. Long term follow up data from the DPP and FDPS shows sustained risk reduction. However this result has not been translated in clinical practice. Trials are ongoing to ascertain the effect of structured education in PDM (213-215). Very few studies have reported long term data on interventions in PDM with respect to the prevention/ reducing risk of CVD (216). Available minimal data suggest no significant beneficial effect; however trials have not been powered for CVD outcome.

Figure 2.3 Interrelationship between glycaemia and CVD risk factors



3 Systematic review and meta analysis

3.1 Background

Systematic reviews and meta analysis are often considered to be the gold standard evidence to assess effectiveness of interventions (217;218). In spite of the challenges and limitations posed by meta analysis of observational studies, they are important for a number of reasons(219). Observational studies provide a tool for studying aetiological association between a risk factor and a disease and can determine the dose response effect. Effectiveness of an intervention in a community practice setting can be studied as opposed to a specialised trial environment. Observational studies can also have heterogeneity in results across different populations and provide data on the association of this variability on the outcomes. Long term adverse effects data such as cancer or CVD can only be determined from case control observational studies as randomised controlled trials (RCT) often do not continue for the duration needed for these incidents to develop or have sufficient sample size. Thus observational studies have their unique role (220). Pooling of all data from observational studies may show an effect that is lost in small individual studies.

Both intensive lifestyle changes and pharmacological interventions have been tested as a therapy for PDM to reduce or delay the onset of T2DM. Almost all of these trials have shown a benefit with the interventions delaying or reducing the risk of progression to T2DM. Some reviews have focussed on the aspect of preventing T2DM (221-224) and some reported factors predicting progression to T2DM (70).

It is well demonstrated that using different criteria for diagnosis of T2DM and PDM identifies people with different phenotypes and CVD risk profiles (24;50;225;226). Hence pooling results from subjects diagnosed using different criteria may introduce selection bias and increase the variation in terms of people at risk of developing T2DM and CVD.

Moreover, the benefits of interventions especially lifestyle changes have often not been translated to routine practice. This is due to four reasons: firstly, people participating in RCTs are often more motivated to change their behaviour and lifestyle which is frequently not the case in clinical practice; secondly utilisation of risk factor based strategies to identify people with PDM in RCTs thus selecting those at highest risk; thirdly availability of dedicated health care professionals for support and advice in an

RCT setting which may not always be feasible in routine practice. Finally trials often exclude people with associated co morbidities such as CVD creating further bias. Following the diagnosis of PDM in the community setting, intervention is usually a leaflet recommending weight loss as a treatment with no follow up and support (13).

This chapter aims to systematically review the literature and pool results on progression rate from PDM to T2DM diagnosed by the present WHO 1999 criteria. Further, we also hypothesize that such incidence rates vary between observational studies and RCT and between different categories of PDM (i.e i-IFG vs. i-IGT vs. IFG+IGT).

3.2 Methodology

3.2.1 Literature search

Medline (1996 to October 2008) and EMBASE (1996 to October 2008) were searched using the search strategy outlined in Appendix 1. The search terms covered T2DM, progression and Prediabetes and its various disorders including the synonyms in various combinations. Expert opinion of a qualified local librarian was sought in designing the search strategy. The references of the articles meeting inclusion criteria were also screened for any potential publication for inclusion.

3.2.2 Study selection

Both epidemiological studies and the control arm of RCT were included to study the natural progression rate from PDM to T2DM. The essential criteria for study selection are outlined in Table 3.1. Two individuals (BS) and (WC) independently assessed the eligibility for inclusion and any discrepancies were resolved by mutual consensus. Abstracts and data presented in conference proceedings were not considered for inclusion.

Table 3.1. Inclusion criteria for studies

1. Diagnosis of both PDM and T2DM be made on WHO 1999/ ADA 1997 criteria
2. T2DM was an outcome measure in RCT
3. Outcome measure be specified after a specified follow up duration
4. Articles published in English

3.2.3 Validity assessment

The author assessed the validity and quality of clinical trials were assessed using the Jadad score (227). STROBE guidelines were used to assess the quality of the observational studies (228;229).

3.2.4 Data extraction

Data extraction was performed with quality assurance of the complete data performed by a second person. Anthropometric and demographic data, duration of follow up and progression rate was collected. As crude data (such as the number of events) were not specified, this was estimated based on the published progression rate in some studies (230).

3.2.5 Data analysis and statistical methods

We calculated the log rate and standard error per 100 PY for each study. To combine these we fitted random effects models to allow for heterogeneity between studies. Sub group analysis was carried out by study type (RCT only, observational studies only) to try and account for possible heterogeneity. The analyses were carried out for IFG, IGT, IFG and IGT, IFG and/or IGT, and any PDM category. The pooled analysis was performed with the help of a qualified departmental statistician.

All analysis was performed on Stata ver 10 (StatCorp, College station, TX, USA) except comparison of incidence rates that was performed using standard Poisson distribution equations (231).

3.2.6 Reporting of results

The reporting of meta analysis was done in accordance with the MOOSE consensus group wherever possible (232).

3.3 Results

3.3.1 Study selection

A total of 2259 and 1198 articles were identified from the Medline and EMBASE literature search respectively. After removing the duplicates a total of 1501 publications were identified for potential inclusion from both databases. Title and abstracts of all the articles were assessed independently and full manuscripts were studied that were potentially relevant to the research question. References and data were reviewed on 96 articles based on the inclusion criteria (Table 3.2, Table 3.3 and Table 3.4). 22 publications were included in the final review (Figure 3.1)

3.3.2 Study characteristics

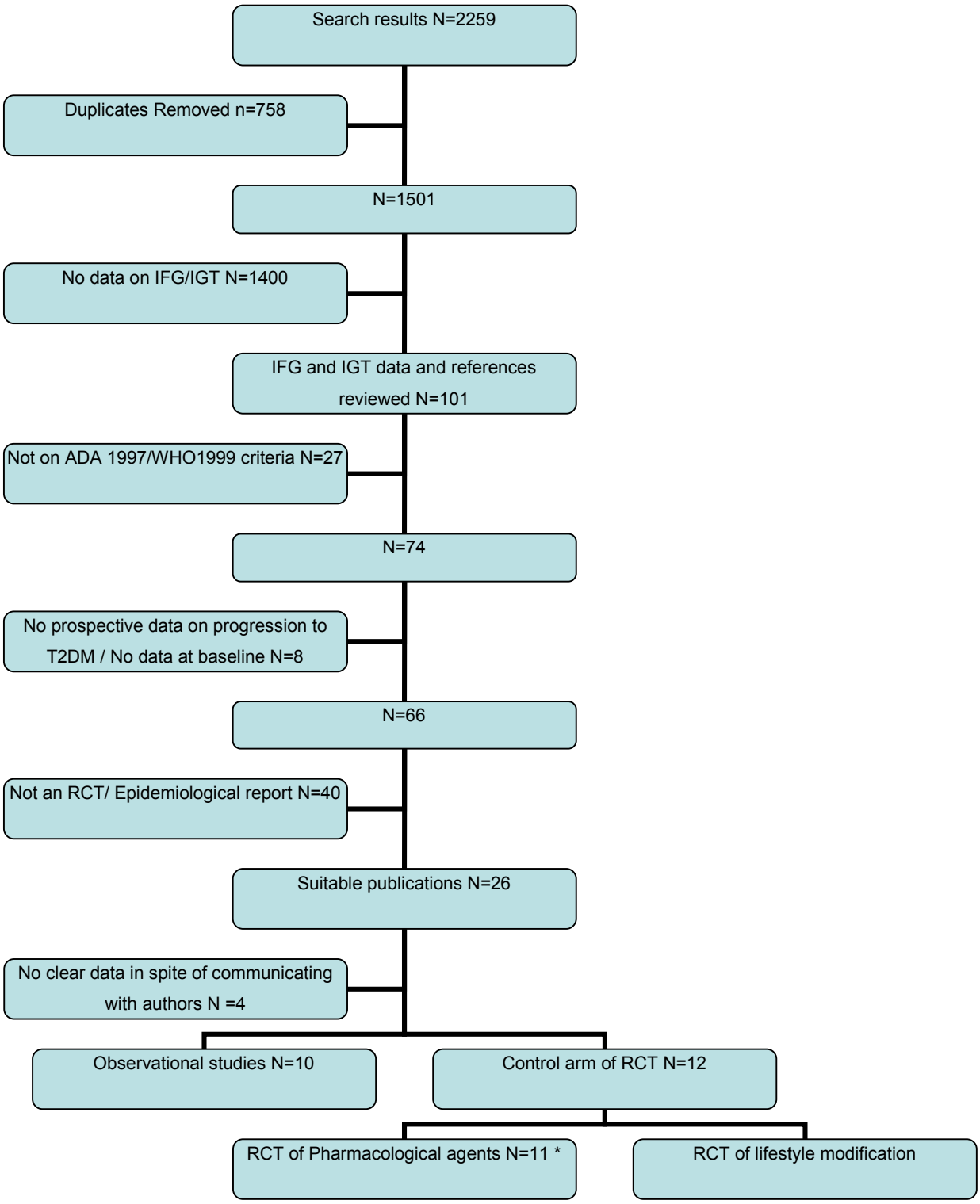
Altogether 22 publications involving 12 RCT (233-245) and 10 cohort studies (40;48;242;246-252) involving a total of 13,314 individuals with PDM were included in the review. We were unable to extract any meaningful data from three studies and unable to obtain any data from the authors (238;253-255).

The studies were widely heterogeneous in terms of ethnic group, follow up duration, baseline BMI and glycaemic status. By definition, the diagnosis of PDM and T2DM is based on ADA 1997 or WHO 1999 criteria in all the studies.

The median age of participants in the studies was 55.0 years (Range: 44.3 to 62.5) reported in 17 papers. The median BMI of the participants was 28.8 (Range: 23.8 to 36.0).

10 studies specified data on people with IFG (8 Cohort and 2 RCT), 14 studies on IGT (6 cohort and 8 RCT) and 3 studies specified with both IFG and IGT (all cohort). In addition, there were 4 studies (2 RCT and 2 cohort) reporting patients with PDM (IFG and/or IGT) in the analysis (i.e not specified if diagnosis IGT or IGT was mutually exclusive). None of the studies specified if the diagnosis of IFG and IGT was an isolated diagnosis or included patients with the other condition.

Figure 3.1. QUOROM flow diagram showing process of selection of studies



* One study had both pharmacological and lifestyle modification arms

3.3.3 Assessment of heterogeneity and publication bias

A funnel plot was constructed to explore the publication bias amongst the studies separately for IFG and IGT studies (Figure 3.2). Funnel plots are an visual aid to detect publication bias systematically. The effect of studies is plotted against the sample size, and a symmetric funnel shaped graph contained within 95% confidence intervals suggest no publication bias (256;257).

The funnel plot shows that there is no obvious publication bias amongst IFG and IGT studies for intra group comparison.

Figure 3.2. Funnel plot showing the plot of logarithm of progression rate and the standard error

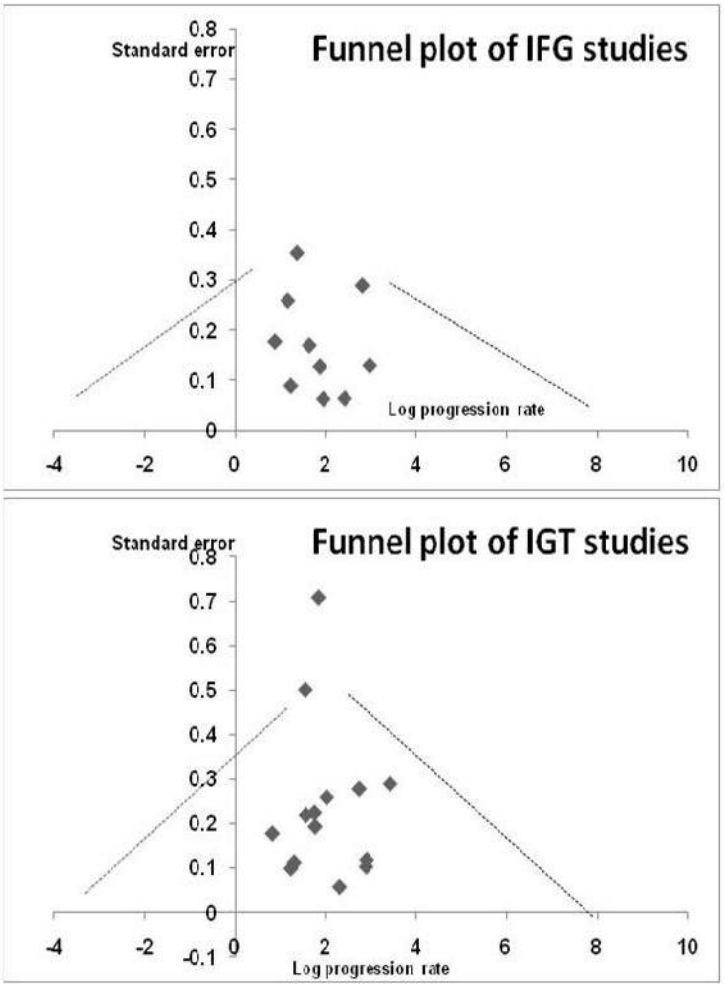


Table 3.2. Characteristics of studies with IFG included in the review

Author	Type	N	Events	Follow up (yrs)	Transition rate	Log transition rate	Standard error (rate)
Chen (246)	2	156	15	3	3.21	1.16	0.26
de Vegt (247)	2	106	35	6.42	5.14	1.64	0.17
Knobler (237)	1	588	256	6.2	7.02	1.95	0.06
Lecomte (249)	2	743	127	5	3.42	1.23	0.09
Meigs (250)	2	20	8	10.2	3.92	1.37	0.35
Nichols (251)	2	926	249	2.39	11.24	2.42	0.06
Rasmussen (48)	2	308	60	1	19.48	2.97	0.13
Rijkelijkhuizen (252)	2	149	62	6.4	6.5	1.87	0.13
Shaw (40)	2	266	32	5	2.41	0.88	0.18
Vermes (245)	1	25	12	2.9	16.55	2.81	0.29

Type of study: 1. RCT 2. Epidemiological cohort study

Table 3.3 Characteristics of studies with IGT included in the review

Author	Type	N	Events	Follow up (yrs)	Transition rate	Log transition rate	Standard error (rate)
de Vegt (247)	2	80	27	5.83	5.79	1.76	0.19
Fang (235)	1	40	15	5	7.5	2.01	0.26
Guerrero-Romero (248)	2	70	20	5	5.71	1.74	0.22
Heymsfield (236)	1	53	4	1.62	4.67	1.54	0.5
Knowler (238)	1	1082	313	2.9	9.98	2.3	0.06
Kosaka (239)	1	356	32	4	2.25	0.81	0.18
Liao (240)	1	32	2	1	6.25	1.83	0.71
Meigs (250)	2	218	81	10.2	3.64	1.29	0.11
Pan (241)	1	127	12	0.31	30.73	3.43	0.29
Ramachandran (242)	1	133	73	3	18.3	2.91	0.12
Rasmussen (48)	2	503	95	1.04	18.13	2.9	0.1
Shaw (40)	2	607	103	5	3.39	1.22	0.1
Tan (243)	2	222	21	2	4.73	1.55	0.22
Tao (244)	1	28	13	3	15.48	2.74	0.28

Type of study: 1. RCT 2. Epidemiological cohort study

Table 3.4 Characteristics of studies with combined IFG and IGT included in the review

Author	Type	Glycaemic status	N	Events	Follow up (yrs)	Transition rate	Log transition rate	Standard error (rate)
de Vegt (247)	2	IFG + IGT	31	20	5.75	11.22	2.42	0.22
Meigs (250)	2	IFG + IGT	27	15	10.2	5.45	1.69	0.26
Shaw (40)	2	IFG + IGT	118	45	5	7.63	2.03	0.15
DREAM Investigators (233)	1	IFG and/or IGT	2646	489	3	6.16	1.82	0.05
DREAM Investigators (234)	1	IFG and/or IGT	2634	658	3	8.33	2.12	0.04
Meigs (250)	2	IFG and/or IGT	265	104	10.2	3.85	1.35	0.1
Shaw (40)	2	IFG and/or IGT	755	180	5	4.77	1.56	0.07

Type of study: 1. RCT 2. Epidemiological cohort study

3.3.4 Study Quality

Amongst the RCTs, five studies were of medium to high quality and seven studies were of low to medium quality. All the trials were randomised and the majority reported on blinding and dropouts. Only two studies reported if allocation of the intervention was concealed and only one study reported the method of generation of randomisation sequence. However it needs to be acknowledged that double blinding and allocation concealment may be difficult in studies that test a lifestyle intervention (n=6) and this scoring system may not be appropriate for these studies. The median Jadad score was 2 (range 1 to 5) out of a maximum possible score of 5. Only one study scored the maximum of 5 (Table 3.5).

Table 3.5. Quality of Studies assessed using JADAD score for RCT

No	Author	Randomisation	Double blinding	Drop outs	Random numbers	Allocation concealment
1	DREAM Investigators	1	1	1	0	0
2	DREAM Investigators (Rosiglitazone)	1	1	1	1	1
3	Heymsfield	1	1	1	0	0
4	Vermes	1	0	0	0	0
5	Knobler	1	1	0	0	0
6	Pan	1	1	0	0	1
7	Ramchandran	1	0	1	0	0
8	Knowler	1	0	1	0	0
9	Fang	2	0	0	0	0
10	Kosaka	1	0	1	1	0
11	Liao	1	0	1	0	0
12	Tao	1	0	1	0	0

The sub headings are scored separately giving a possible total score of 27. The majority of the studies lacked the recommended detail in the title, bias, study size, sensitivity analysis and missing data section (Table 3.6). Few studies did not have the recommended detail on missing data and/or acknowledged the limitations and generalisability of the results to a wider population. Detail on funding sources was not mentioned in two studies. The other sections were present in almost all the studies. For the observational studies, were of moderate to high quality. The median score of the studies was 20 (IQR: 18.25 to 23). None of the studies scored the maximum score.

Table 3.6. Quality of studies assessed using STROBE checklist for observational studies

S No	Author	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
1	Guerrero-Romero	X	√	√	√	√	√	√	X	√	X	X	√	√	X	X	X	√	√	√	√	√	X	√	X	√	X	√
2	Lecomte	X	√	√	√	√	X	√	√	√	X	X	√	√	√	X	X	√	√	√	√	√	X	√	X	√	X	√
3	Meigs	√	√	√	√	√	√	√	√	√	X	X	√	√	√	X	X	√	√	√	X	√	X	√	√	√	√	√
4	Nichols	X	√	√	√	√	√	√	√	√	X	X	√	X	√	√	X	√	√	√	√	X	√	√	√	√	X	√
5	Rasmussen	√	√	√	√	√	√	√	√	√	X	X	√	X	√	√	X	√	√	√	√	X	X	√	X	√	X	√
6	Rijkelijkhuizen	X	√	√	√	√	√	√	√	√	X	X	√	√	√	√	X	√	√	√	X	√	X	√	√	√	X	X
7	Shaw	X	√	√	√	√	√	√	√	√	X	X	√	X	√	X	√	√	X	√	X	√	X	X	X	√	√	√
8	de Vegt	X	√	√	√	√	√	√	√	√	√	X	√	√	√	√	X	√	X	√	√	√	√	√	X	√	√	X
9	Chen	√	√	√	√	√	√	√	√	√	X	X	√	√	√	X	X	√	X	√	√	√	√	√	√	√	X	√
10	Tan	X	√	√	√	√	√	√	√	√	√	X	√	√	√	√	X	√	√	√	√	√	√	√	X	√	√	√

Q1-Title, Q2-Abstract, Q3- Background, Q4-Objectives, Q5-Study design, Q6-Setting, Q7- Participants, Q8- Variables, Q9-Data sources, Q10- Bias, Q11- Study size, Q12- Quantitative variables, Q13- Confounders, Q14- Subgroups, Q15- Missing Data, Q16- Sensitivity, Q17- Participants, Q18- Descriptive data, Q19- Outcome data, Q20- Results, Q21- Results absolute, Q22- Results others, Q23- Key results, Q24- Limitation, Q25- Interpretation, Q26- Generalisability, Q27- Funding.

3.3.5 Progression rate

The rate of transition to T2DM varied from 2.3 to 30.7 cases per 100 PY amongst all the studies. Studies were significantly heterogeneous throughout (p<0.0001). Progression rate varied between 2.4 to 19.5 cases per 100 PY in the IFG group, 2.3 to 30.7 cases per 100 PY in the IGT group and 5.4 to 11.2 cases per 100 PY in those with both IFG and IGT.

Data from the studies was pooled using random effects model to take into account differences between studies. The progression rate for different categories of PDM (i.e. IFG, IGT and Both) are tabulated separately for RCT and cohort studies in Table 3.8.

The progression rate for all the studies (n=25) was 7.67 (6.31 to 9.33) cases per 100 PY, with a significant effect of heterogeneity (I^2 =95.73%, p<0.0001).

The pooled transition rate (95% CI) for people with IFG was calculated to be 6.29 (4.29- 9.22), IGT to be 7.48 (5.00-11.18) and people with both IFG and IGT to be 7.86 (5.51- 11.20) cases per 100 person years (Table 3.7).

Table 3.7. Pooled analysis of all the included studies between different categories of PDM

Population	N (studies)	Cases per 100 person years	95% CI	Heterogeneity P value
IFG	10	6.29	4.29 to 9.22	<0.0001
IGT	14	7.48	5.00 to 11.18	<0.0001
Both	3	7.86	5.51 to 11.20	0.10
And / or	4	5.59	4.13 to 7.56	<0.0001

For all the studies, the incidence rate difference (IRD) (95% CI) between IGT and IFG+IGT was 1.14 cases per 100 PY (0.02- 2.30, $\chi 2$= 1.92, P=0.16) and that between IFG and IFG+IGT was 1.37 cases per 100 PY (0.42- 2.31, $\chi 2$= 2.83, P=0.0925).

Table 3.8. Data pooled using random effects model for different categories of PDM

Population	N (studies)	Cases per 100 person years	95% CI	Heterogeneity P value	Interaction between subgroup p value
			IFG		
RCT only	2	10.29	4.46 to 23.73	0.004	0.26
Epi only	8	5.53	3.30 to 9.26	<0.0001	
			IGT		
RCT only	6	8.42	3.98 to 17.82	<0.0001	0.87
Epi only	7	5.98	3.30 to 10.85	<0.0001	
			Both		
RCT only	0	-	-	-	-
Epi only	3	7.86	5.51 to 11.20	0.10	
			And / or		
RCT only	2	7.17	5.34 to 9.63	<0.0001	0.01
Epi only	2	4.32	3.51 to 5.33	0.08	

.

3.3.5.1 Transition rates between RCT and cohort studies

The progression rate for any category of PDM for epidemiological cohort studies was 6.74 (4.80 to 9.47) and that in RCT studies was 8.25 (6.52 to 10.44) cases per 100 PY. The IRD between the RCT and cohort studies was 4.36 (4.30- 4.42) cases per 100 PY ($\chi 2$= 138.4, p<0.0001) in the IFG group. The IRD in those with IGT was 3.66 (3.55-3.78) cases per 100 PY ($\chi 2$=62.94, p<0.001). Such analysis was not performed on people with both IFG and IGT as no RCT studies were found. When PDM is considered as a whole group, observational studies had a significantly lower

progression rate compared to RCT (IRD= -2.17, 95% CI: 2.13 to 2.22, $\chi 2$= 91.209, p<0.001).

3.4 Discussion

This analysis shows a pooled progression rate from PDM to T2DM of 7.67 cases per 100 person years (6.31 to 9.33) with a significant effect of heterogeneity. The progression rate was significantly higher for those with both IGT and IFG compared to IGT or IFG alone. The progression rate in those in a randomised controlled trial was significantly higher than those in epidemiological studies for both IGT and IFG.

Conducting a meta analysis in the presence of significant heterogeneity is potentially a limitation and even a criticism of the study. However, views amongst the scientific community varies, some advocate pooling of the data in spite of heterogeneity amongst the studies (258) whilst others suggesting a narrative approach to the systematic review (219). To account for heterogeneity, a random effects model method has been used. Particular reasons for heterogeneity may be due to very different population in the studies whose results have been pooled with people of different ethnic origin with varying social backgrounds and different demographic characters. All these are likely to have an impact on the risk of progression to T2DM. Though the diagnostic criteria to diagnose PDM are uniform throughout the studies, the baseline cardiovascular risk factor profile is likely to differ, assay methods are also likely to vary which was not described in 8 studies (37%). The screening strategy used to identify these subjects with PDM is also different, some were recruited from population based studies but most adopted a risk factor based screening programmes. These differences are likely to have led to a selection bias.

This analysis has three unique features. Firstly, it has been demonstrated that progression rates in people under trial conditions and those seen in the community in observational studies are different. People who participate in the RCT perhaps perceive themselves to be at a considerable risk of developing T2DM and are motivated to take part in clinical trials and are amenable to behavioural modification. We have thus shown in a pooled analysis, that the risk of progression to T2DM seen in clinical practice is different to those seen in RCT.

Secondly, it has also been demonstrated for the first time to our knowledge in a systematic review that progression rates are different amongst various categories of

PDM. This is important for policy makers to risk stratify people with PDM to offer intervention to those at higher risk.

Thirdly, to avoid confounders and bias, this meta-analysis is the first of its kind to include studies with uniform diagnostic criteria.

Our pooled analyses results are similar to the ones reported previously. The progression rate reported for people with IGT in a somewhat different set of included studies was 7.07 cases per 100 PY (95% CI: 4.31 to 11.59) (259). Similarly, in a previously published systematic review, the progression rate from IGT to T2DM was 5.72 cases per 100 PY (Range: 3.58 to 8.73), in six prospective studies, with participants ranging from 178 to 693 and a follow up duration between 2 to 27 years (70). There was wide range in the oral glucose load used across the spectrum of studies included in this review. In another meta-analysis, the pooled annualised relative risk per 100 cases (95% CI) were 4.70 (2.71 to 6.70) for IFG group, 6.02 (4.66 to 7.38) for IGT group and 12.21 (4.32 to 20.10) for IFG+IGT group. In this analysis, the number of studies included was 5, 36 and 3 respectively for the groups (69).

It has to be acknowledged that benefits of any intervention are likely to vary outside the trial setting.

Lifestyle interventions are often assumed to be free of adverse events; however adherence to the prescribed program outside the clinical setting still needs evaluating. Cost benefit analysis regarding provision of such a programme, health care professionals needed to deliver, training and quality assurance of such an intervention in a wider community setting needs further research. Structured education facilitating lifestyle changes may play a role in addressing these issues (213-215). Moreover, the psychological morbidity accompanied by a diagnosis of PDM and benefits of an intervention on these health quality outcomes needs to be evaluated in a community setting (13).

Previous systematic reviews have shown that pharmacological and lifestyle interventions such as physical activity and dietary changes reduce the risk of progression from PDM to T2DM (222;260). It is widely accepted that sustained efforts are needed in both dietary changes and improvement in physical activity to achieve this risk reduction. There are some expert consensus recommending the criteria for pharmacological treatment for PDM (261).

3.4.1 Limitations

We acknowledge the wide heterogeneity and publication bias seen in the trials to be a weakness in the review. However as this variability was present in all the studies, comparisons of pooled estimates between groups, confounding of results by heterogeneity can be reasonably assumed to have little impact. The majority of the studies were carried in a Caucasian population. Hence to generalise the results to a wider multi ethnic population is difficult especially the intensity of risk reduction with interventions. A further limitation is that we did not include non English articles due to lack of access to interpretation resources.

3.5 Conclusion

IFG and IGT are two different disorders of glucose metabolisms and these conditions are mutually exclusive. People either have IFG only or IGT only or both. The rates of progression from these conditions to T2DM vary and those with both IGT and IFG have the highest risk. These transition rates vary between those reported from clinical trials and observational studies, the latter perhaps is a representation of population included in the studies. Results from this systematic review provide up to date data on progression rates from PDM to T2DM and also stratifying this risk amongst those with PDM. Such a strategy is vital for planning health care policy to target public health measures to those at high risk. The interventions for PDM not only must delay the progression to T2DM but provide beneficial effects on the ultimate end point- both micro and macro vascular complications. Long term data are needed to assess this effect.

Contributors: BS (B Thiagarajan Srinivasan) is the primary author for this chapter; WC (Dr. Winston Crasto) is a qualified physician who is a Clinical Research fellow, Department of Cardiovascular sciences, University of Leicester who contributed to selection of studies, quality assessment and quality assurance of the data. Dr Laura Gray is a qualified statistician, Department of Health sciences, University of Leicester who performed the pooled data analysis. Mrs Mary Edmunds-Otter and Mrs Sarah Sutton are librarians who helped with designing of the search strategy and performing the literature search.

4 Screening and Epidemiology of Prediabetes

4.1 Introduction to screening

Screening is a public health measure that involves identifying people with a particular health condition without any symptoms. Screening can be targeted primarily towards people at high risk (targeted or high risk screening) or the whole population may be offered screening (variably termed as population-based screening or mass screening or universal screening). Screening for a health condition may be performed when people come into contact with the health care professional for an unrelated problem (opportunistic screening). High risk screening may be performed in a step wise fashion to minimise exposure to the complex tests and may utilise risk scores in this regard.

4.2 Screening for Type 2 Diabetes Mellitus and Prediabetes

Expert consensus for screening for a health condition are, the disease must be common in the population, easy to identify through simple tests and there must be effective intervention for the condition (3;262;263). T2DM conforms to all these suitability criteria (8). It has been recognised that the symptoms of early diabetes are non-specific and difficult to identify and may be dismissed by the patients. Over half of the population who satisfy the criteria for T2DM are unaware of the condition (3;263). The UKPDS and other studies, have demonstrated that over 50% have some evidence of tissue damage related to T2DM even at the time of diagnosis, suggesting a prolonged latent phase of this condition and/or the presence of an atherogenic PDM phase (161-164;264). It is widely believed that the diagnosis of T2DM is preceded by its onset by around 10 years (265;266). Some evidence points to the fact that earlier detection of T2DM may improve the long term outcomes (267).But routine screening for T2DM is still not advocated by the NSC except in the context of screening for vascular disease in the recent NHS vascular check programme (6).

4.3 Present evidence for routine screening

In spite of the latency of T2DM diagnosis, the present evidence for routine screening for T2DM is still considered to be controversial. The NSC advocates opportunistic screening targeted at a population at risk of Diabetes (7). Such a strategy is currently being rolled out throughout the UK as part of the NHS health Checks Programme aimed at identifying people with vascular disease as part of which diabetes screening will be performed (6).

Increasing prevalence of T2DM, availability of effective treatment modalities, availability of simple tests to identify people at risk and presence of a potentially modifiable pre atherogenic PDM phase makes it an interesting proposition to adopt routine screening for T2DM. However, evidence for clinical and cost effectiveness for this strategy is lacking.

The ADDITION study is a structured universal screening programme for T2DM that will report on the feasibility of universal screening of the adult population and benefits of intervention in this screen detected cohort. This study differs from previous intervention studies in T2DM in that the subjects have a unique phenotype as they have screen detected T2DM compared to conventional newly diagnosed T2DM patients (268;269). The study will also use the OGTT that is considered to be the gold standard test for the diagnosis of T2DM (Section 4.6 below).

4.4 Screening tests for T2DM

In spite of the evidence for the potential benefit of identifying PDM in the population and the availability of interventions such as intensive lifestyle changes, there is no uniformly agreed strategy on the tests that can be used to identify this cohort effectively. At present, OGTT is considered to be the gold standard test to diagnose all glycaemic disorders (i.e. T2DM, IFG and IGT). The OGTT is laborious, expensive, time consuming and has a low rate of up take to be used as a screening tool. Moreover, OGTT have a high inter and intra observer variability between 50% and 75% (270;271). Due to these reasons, other modalities of screening have been devised such as screening using capillary blood glucose (either random or fasting), risk scores to identify people at high risk and use of HbA1c or a combination of these tests.

In a study involving 154 subjects with IGT with a median follow up of 5.8 years, even people who reverted to NGT (termed as transient IGT) had a threefold increased risk of developing T2DM compared to those who never had IGT in the first place (272). Data from the Newcastle Heart Project also showed a higher diabetes incidence rate in those with transient IGT compared to NGT group (12.5% vs. 8.3% in people of WE origin; 36.4% vs. 2% in people of SA origin) (273). In one analysis from the Indian Diabetes prevention programme, subjects were classified to have persistent IGT (IGT on both OGTT) or missed T2DM (first OGTT normal and second OGTT T2DM). People who had persistent IGT were shown to have a similar age, BMI and body fat compared to the missed T2DM group (274). These data possibly show that a single abnormal plasma glucose is sufficient to place an individual in the higher risk strata of developing

T2DM compared to those who have a persistently normal glycaemic status. This is further explored from local population data in subsequent chapters.

4.4.1 Urinalysis

The different screening tests have been discussed in detail in a previous theses from our research group (275). Urinalysis has a high specificity of over 98% and using post prandial urinalysis improves the sensitivity to 43%. Whilst using a fasting plasma glucose cut off of 5.5 mmol/L yields a specificity of over 90%, this marginally improves to 96% on increasing the cut off value to 6.1 mmol/L. Similarly studies using HbA1c as a screening test have reported a sensitivity and specificity rates of 63.2% and 97.4% respectively using a cut off value of 63.2% to 35% to 100% using a cut off value of 5.6%.

4.4.2 Capillary screening

A random capillary blood glucose cut off of > 8.0 mmol./L gave a sensitivity and specificity of 69% and 95% respectively in a predominant Caucasian population (276). In a Finnish population, using a random capillary blood glucose cut off of 6.2 mmol/L gave a specificity and sensitivity of 63% and 92% respectively (277). However both these studies used the WHO 1985 criteria for the diagnosis of T2DM using an OGTT. The IDPP used a 2 hour post glucose capillary blood test as a screening test to recruit people into the prevention study Based on a 77% response rate from those who had positive capillary glucose, 69% were confirmed to have either IGT or T2DM (242). More recently a random plasma glucose cut off of 5.5 mmol/L was used to screen for people with T2DM (278).

4.4.3 Combined use of plasma glucose and HbA1c

In a predominantly Chinese population, using a paired values of FPG > 5.6 mmol/L and a HbA1c > 5.5% for screening gave a sensitivity and specificity of 83.8% and 83.6% for detection of T2DM compared with an OGTT (279). In another study involving 392 subjects from Korea, a combination either of FPG≥6.1 mmol/L or HbA1c ≥6.1% gave a sensitivity of 95% and a specificity of 71% for detection of T2DM. Other studies have evaluated the use of combination of FPG, HbA1c and BMI as screening tools (280). In a Dutch study, a step wise screening fashion using a risk score, combination of random blood glucose, FPG and an OGTT were evaluated in a stepwise fashion. The combined random blood Glucose ≥ 5.5 mmol/L and HbA1c ≥6.0% had a sensitivity of 85.7% and a specificity of 75.5% (281). Using an added step of FPG did not improve the diagnostic indices in this study.

4.4.4 Risk scores

The various risk scores for screening for T2DM are tabulated in Table 4.1. Age, gender, family history, hypertension or hypertensive medications, BMI, WC are the common variables used in almost all the risk scores. However the weighting given for these variables varied depending on the population studies. Very few scores have actually validated or determined the sensitivity and specificity for those with PDM. A similar risk score derived and validated in a multi ethnic population in Leicester is described below. As seen from Table 4.1, age, sex, marker of obesity either as waist circumference or BMI, family history and indicator of hypertension in the form of medications or history are commonly used determinants in the risk score.

4.4.5 Self measured screening tools

Other simple measures of detecting people at risk of developing T2DM are self measured tests for risk stratification. Two studies have reported response rates of up to 30-70% in a postal questionnaire where post prandial urinalysis for glycosuria was used as a screening test. A subsequent OGTT gave a sensitivity of 43% and a specificity of 98% (282;283). The accuracy of self measured waist circumference has also been evaluated as a possible simple screening test for glucose intolerance (284). This study reported an under estimation of waist circumference by people and using pictorial instructions helped to reduce these errors.

The ADA recommend using the fasting plasma glucose as a screening test for T2DM (285). Based on available evidence in a similar UK multiethnic population, a simple step wise screening using blood pressure (>140/90), BMI (>30 in WE and >25 in SA) followed by a FPG as a screening tests was recommended. This strategy yielded a sensitivity and specificity of 77% and 58.7% for WE respectively (70.7% and 20.5% for SA) (275;286).

Table 4.1. Risk scores for screening for T2DM

Study	Risk score determinants	Population	Sensitivity (Specificity) for T2DM (%)
IDPP (287)	Age, BMI, WC, FH, PA	South Asians	76.6 (59.9)
Danish (288)	Age, sex, BMI, HT, PA, FH	Caucasian	61.5 (81.2)
Hanif (289)	Age, Ethnicity, GDM, FH (parents), FH (Siblings), HT or IHD, BMI	South Asian	78 (69.5)
Cambridge (290)	Age, gender, BMI, steroid and AHT, FH and smoking	Caucasian	77 (72)
FINDRISC (291)	Age, BMI, WC, Fruit/vegetable intake, AHT, HT, FH	Caucasian	76 (66)
San Antonio (26;292)	Age, sex, FH, systolic BP, BP medications, BMI, WC	Caucasian	62.2 (64.9)
Dutch (293)	Age, sex, BMI, FH, frequent thirst, AHT, shortness of breath, pain during walking needing to slow down, PA	Caucasian	72 (56)

AHT- Anti hypertensive agents HT- Hypertension FH- Family History PA- Physical activity/ inactivity BP- Blood pressure as continuous variable

4.5 Methodology

The major part of this thesis is based on the structured universal screening programme for T2DM- ADDITION Leicester study and its sub study- ADDITION PLUS (See ADDITION Study Section 4.6 below).

4.5.1 Prevalence of PDM using two different screening strategies

Different strategies for screening may be adopted as described above. To identify a suitable strategy for T2DM and PDM, it is vital that the phenotypic characteristics of people with PDM (Chapter 4) and the prevalence of disorders of glycaemia are compared in people in the population screened using different screening strategies i.e. Universal screening compared to high risk screening. This is done by comparing these data from the ADDITION study (Universal screening) (Section 4.6 below) and the STAR study (High risk screening).

The screening for those at risk (STAR) study is a risk factor based screening programme for T2DM.This study has been described in detail previously (161;275).

In contrast to universal screening, people on the STAR study were invited on the presence of at least one risk factor for the development of T2DM. The age criteria was 40- 75 years in people of WE origin (25- 75 if SA of Afro Caribbean origin).

Table 4.2. Inclusion criteria for STAR study

- Known coronary heart disease (CHD)
- Known to be at risk of CHD and on a CHD register (i.e. those with a predicted CHD risk of at least 30% over 10 years)
- Documented history of hypertension or receiving medication for hypertension
- Known high cholesterol
- Known cerebrovascular disease and/or peripheral vascular disease
- Known to have previous diagnosis of IGT or IFG
- Women with polycystic ovary syndrome who are obese (BMI >25kg/m^2 or >23kg/m^2 in South Asians)
- BMI > 30kg/m^2
- Women with history of gestational diabetes
- First degree relative with type 2 diabetes
- Current cigarette smokers or those who have stopped within the last 12 months

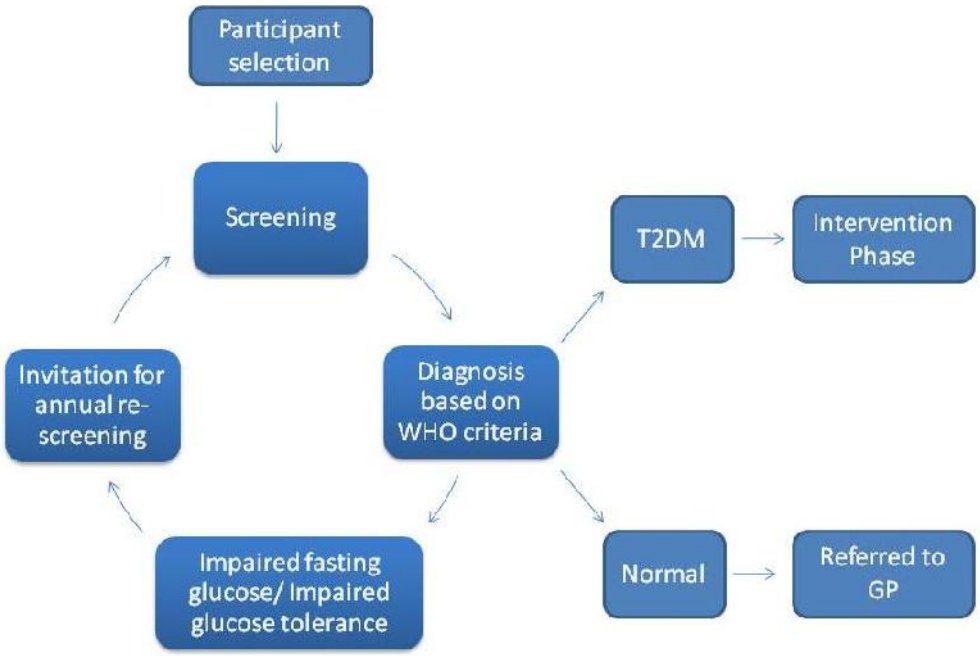
4.6 ADDITION Study

4.6.1 Introduction to ADDITION- Leicester study

Anglo-**D**anish-**D**utch study of **I**ntensive **T**reatment **I**n pe**O**ple with scree**n** (ADDITION) detected diabetes in primary care study is a large multinational multi centre screening study for T2DM, which has recruited 3500 patients across four centres in Europe (269). The ADDITION Leicester sub study is a pragmatic cluster randomised control trial evaluating the feasibility of undertaking a population based screening for T2DM and the impact of a multi factorial target driven optimisation of cardiovascular risk factors in the screen detected T2DM group based on modelled cardiovascular disease risk (268;294).

The study can be arbitrarily divided into three phases- the Screening phase, intervention phase and Prediabetes follow up phase- the ADDITION PLUS (**P**rediabetes Fo**L**low **U**p **S**tudy). Subjects were screened for T2DM and PDM in the screening phase. Consenting subjects diagnosed with T2DM were randomised to either routine care or the intensive arm in the intervention phase in a cluster randomisation fashion. Participants diagnosed with PDM were invited annually for re screening under the ADDITION PLUS in the re-screening phase.

Figure 4.1. Schematic diagram of ADDITION PDM follow up study



4.6.2 Ethical approval and funding

The ADDITION study was approved by the local research and ethics committee as well as the Research and development unit of the University Hospitals of Leicester NHS Trust (UHL) and the Leicestershire Primary Care Research Alliance (University Hospitals of Leicester NHS Trust – UHL09320 and Leicestershire Primary care Research Alliance -64/2004). Approval for ADDITION PLUS was secured using a substantial amendment. This study was adopted by the South East Midlands Diabetes Research Network in 2007. All participants gave informed written consent and the study was conducted in accordance 1996 Helsinki declaration. The study was funded by the Department of Health.

4.6.3 Study Management

The principal investigators were Professor Melanie Davies and Professor Kamlesh Khunti. The recruitment aspects of the study were dealt with by a Manager and staffing aspects for the screening/follow up by a study coordinator. The steering group met monthly and the operational group met on a weekly basis to discuss the progression of the study. The study was coordinated from the UHL and recruitment done in the Primary care.

4.6.4 Recruitment of participants

4.6.4.1 Population background

Recruitment was performed from General Practices across Leicester City and county of Leicestershire. Recruitment was centred on Leicester city with a population of 289,700 approximately with 35% prevalence of population from ethnic minority groups, predominantly people of Indian origin speaking Gujarati. The population of the county (excluding the City of Leicester) was 635,000 with 6.2% of people from ethnic minority groups (295).

4.6.4.2 Recruitment of practices

The eligibility criteria for participation in the ADDITION study is tabulated in Table 4.3. Potential general practices in Leicestershire were approached and a visited by the ADDITION study team. Practice staff members identified eligible patients from the respective computer database search results and eligible subjects were then contacted by the ADDITION staff members for screening (Figure 4.2).

Information packs on the study were mailed to 46 general practices in the recruitment area from which 28 practices responded with intent to take part in the study and

consented for search of their computerised practice register based on the EMIS software (Egton Medical Information system, York, UK). The practice database was were scrolled through for potential participants for inclusion tin the study. A minimum of 70% capture of practice population was required to participate in the study. In eight practices, initial database search failed or practices themselves closed down or combined with other practices and thus 20 practices took part. The number of participants recruited from each practice is detailed in appendix.

In 6 practices the entire eligible population as identified by the computer search was invited. Amongst the rest, a random sample of each eligible population was invited to be screened; random invitations continued until at least 20% (range 30%-90%) were screened. This step was taken to ensure timely screening of the whole cohort and thus to recruit people with T2DM in to the intervention phase representing the background general population of the area.

Table 4.3. Eligibility criteria for ADDITION study

Inclusion Criteria
1. White European subjects aged between 40-75 years
2. Asian, black or Chinese subjects aged between 25-75 years
Exclusion Criteria
1. Housebound
2. Have a terminal Illness
3. Have Diabetes Mellitus
4. Active Psychotic illness which deems the person unable to give informed consent.
5. Pregnant or lactating
6. Patients taking part in any other clinical trials

4.6.5 Measures of deprivation amongst practices

Indices of Deprivation are an important tool for identifying the most disadvantaged areas in any region to channel appropriate resources. There are various tools in practices as described below such as the Index of multiple deprivation (IMD), Townsend score and Jarman scores. Individual scores are described in detail in Appendix 1.

We calculated mean practice deprivation scores for individual practices using the Index of multiple deprivation (IMD) (296;297). This is a well validated method to quantify deprivation experienced by people living in a particular area especially a smaller spatial region. This score has various domains that can be measured, weighted and combined into one single measurable entity. Theoretically IMD measures deprivation index amongst a smaller area and hence likely to be more representative of the population. The average IMD for the Leicester local authority was 32.40 and ranked 20th in the country out of 355 (298). The calculated mean IMD scores for the practices corresponded to the national survey scores.

4.6.6 Patient recruitment

Potentially eligible subjects who were identified from practice searches were sent a pre screening questionnaire inquiring of their willingness to participate and to confirm that they did not have diabetes. Upon receipt of this questionnaire in a pre paid self addressed envelope, participants were sent a patient information leaflet and listed for the screening session.

Screening was performed at the Leicester General Hospital and the Leicester Royal Infirmary or in the mobile screening units that were based at the participating general practices at Coalville, Melton Mowbray, Silverdale medical practice or Spinney Hill medical practice in Leicester.

4.6.7 Initial Screening Visit

Informed consent was obtained by a trained health care professional at the screening visit. Interpreters and patient information sheets in local languages (Hindi, Gujarati and Punjabi) were used for participants who are unable to understand English.

Standard 75g OGTT was performed to ascertain the glycaemic status of the individuals after an overnight fast. At the screening session baseline family, medical and medication and smoking history were obtained by the study staff. The measurements that are performed at screening visit are tabulated in table 4.4 and 4.5. Patients also completed a questionnaire, which allowed the development of a validated diabetes risk score and calculating physical activity and wellbeing.

Figure 4.2. Screening process for the ADDITION-Leicester study

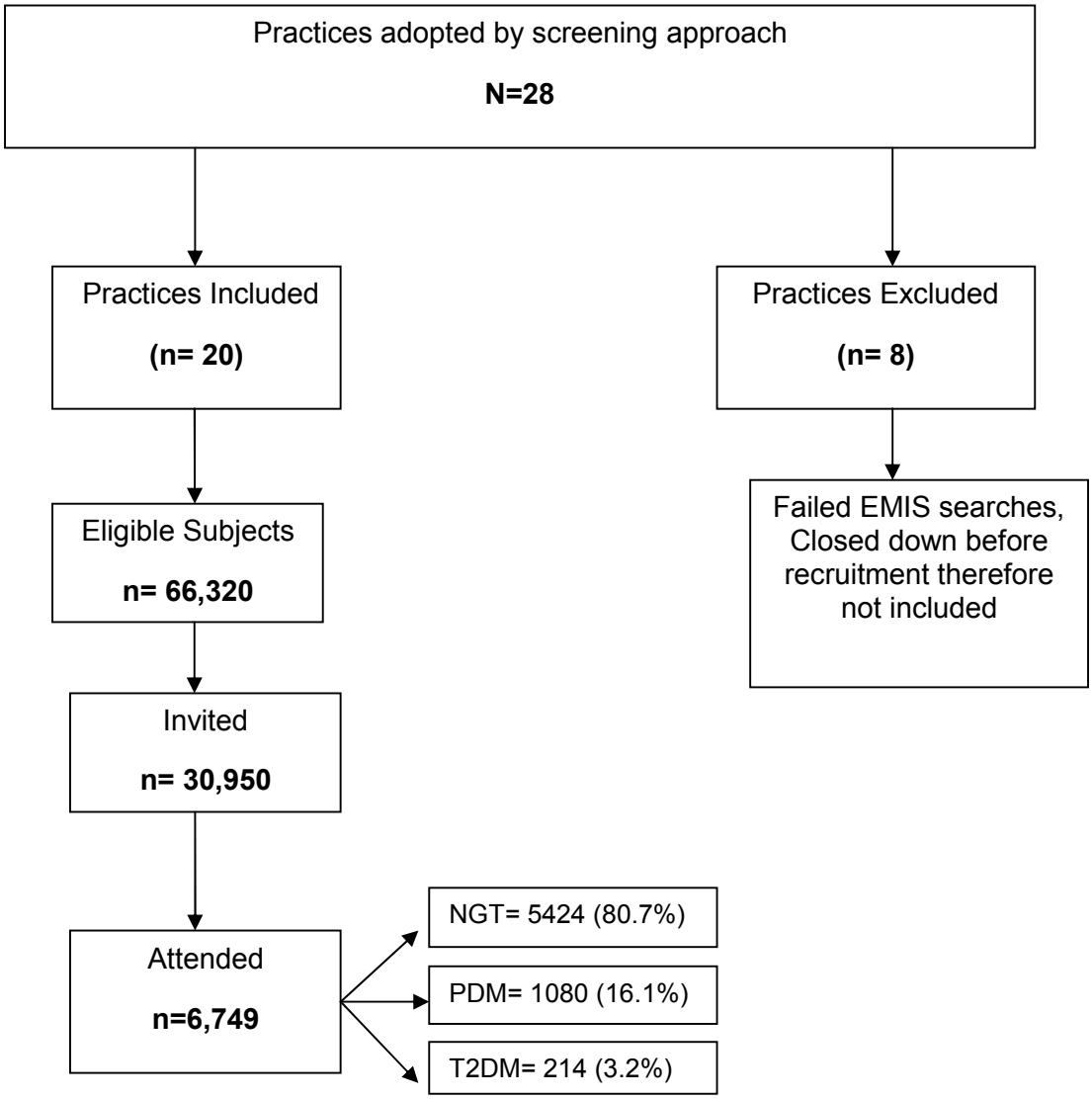


Table 4.4. Anthropometric measurements at baseline

i. Height
ii. Weight
iii. 12 lead ECG
iv. Body fat percentage
v. Waist Circumference
vi. Hip Circumference
vii. Blood Pressure

Table 4.5. Laboratory measurements at baseline

1. U+E's (Na, K, Urea, Creatinine, Liver Function Tests)
2. HbA1c
3. TC, HDL, LDL, ratio, triglycerides
4. Urinalysis and Urine albumin/creatinine ratio (ACR) on an early morning sample
5. Fasting Glucose and 120 minute post 75 g load plasma Glucose
6. Storage samples
    a. Lithium heparin tube (Fasting Insulin and Ultra Sensitive CRP)
    b. Brown serum gel 7mls
    c. Isoprostane urine sample (3x 1ml vials stored)

4.6.8 Data entry and data handling

At baseline, source data and questionnaires were entered by Abacus Data and Document Capture LTD (Luton, UK) using double data entry ensuring a high degree of accuracy. Data discrepancies were handled by a small team of experienced researchers with clinical input. Steps were taken under direction by a Data Monitoring Committee to provide a final Microsoft Access database of all participants with a diagnosis who complied with the inclusion/exclusion criteria.

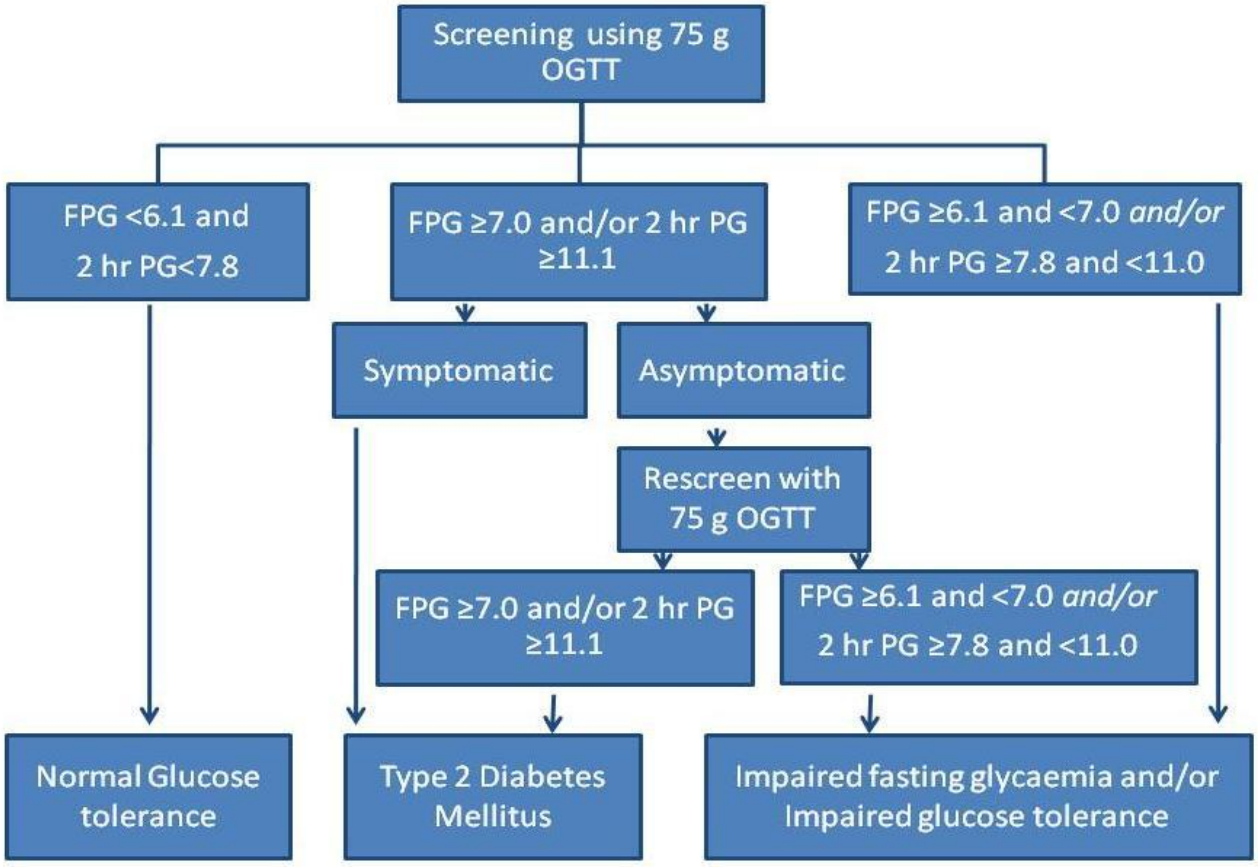
4.6.9 Diagnosis

T2DM or PDM was diagnosed according to the 1999 WHO criteria (12). Subjects who were asymptomatic were offered a repeat OGTT to confirm the glycaemic status. PDM was considered to be the presence of either IFG, IGT or both IFG+IGT. The presence of metabolic syndrome was calculated according to the IDF criteria (299) (Figure 4.3). Subjects with T2DM who were asymptomatic were invited for a re-screen using a second OGTT before a diagnosis of T2DM was made.

Table 4.6. Questionnaire data at baseline

Occupation (Cambridge)
Social Class (Cambridge)
Current Medication
Ethnicity
Self-reported history of angina, heart attack, stroke, etc
Self reported smoking status (Cambridge)
Self-reported alcohol status
Cambridge diabetes risk score (290)
FINDRISC (291)
Health Utility: EuroQol EQ-5D questionnaire (300)
Anxiety: Speilberger SF State Anxiety Inventory (301;302)
Personal patient costs (adapted from HSRU Aberdeen) (303)
Physical Activity: IPAQ (304;305)
Michigan Neuropathy Questionnaire (adapted) (306)
WHO-5 Psychological Wellbeing (307;308)
BFI 44 (309;310)
Family history of diabetes
Family history of cardiovascular disease

Figure 4.3. Schematic algorithm for glycaemic categorisation



4.6.10 Statistical analysis

All analyses were performed using SPSS version 15 software. Categorical variables were compared using Chi square tests and continuous variables were analysed using t tests or one way ANOVA as appropriate. Logistic regression models were used to calculate odds ratios (OR) for prevalence adjusted for age, sex, central obesity (using ethnicity specific cut points of waist circumference) and deprivation scores.

A ‘p value’ of less than 0.05 was considered to be statistically significant.

4.7 Prevalence of PDM

1080 of the screened population were diagnosed to have PDM, a prevalence of 16.0% (Table 4.7). IGT is the most common disorder of impaired glucose metabolism and 19.2% of the screened population were found to have abnormal glycaemic status (including T2DM). There is a small overlap of 2.2% of people who have both IFG and IGT. The prevalence of T2DM was 3.2%.

IFG is more common amongst men (3.4% vs. 2.3%) whereas IGT is more common amongst women (11.5% vs. 10.4%) and 89 (2.8%) men as compared to 60 (1.7%) women have both IFG and IGT.

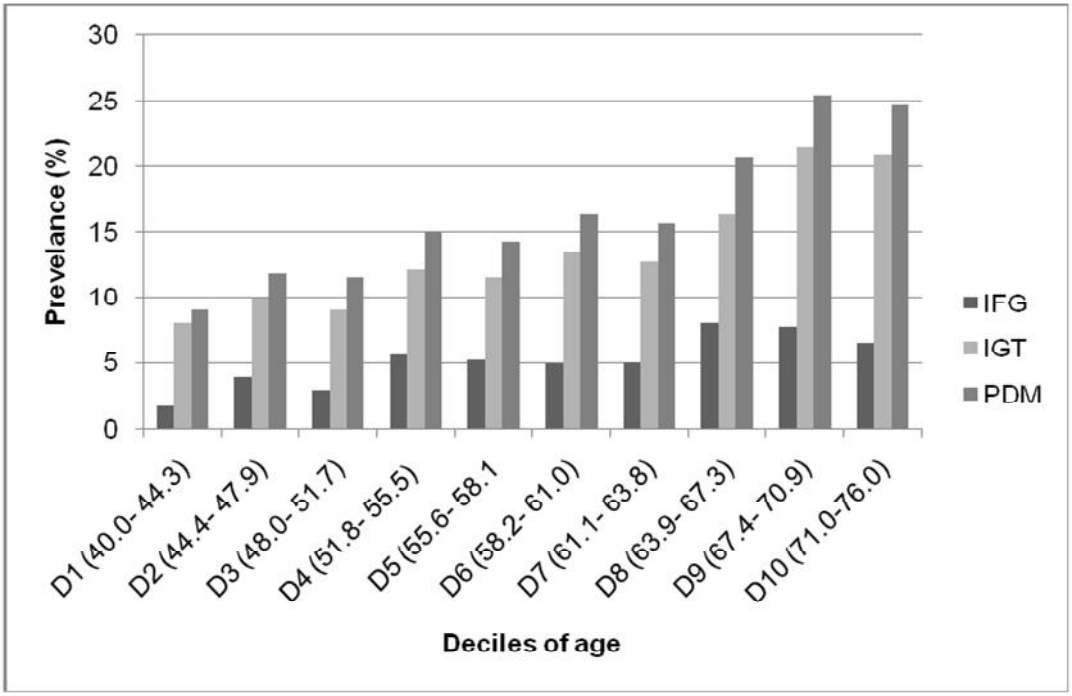
Table 4.7. Prevalence of glycaemic abnormalities in the screened population.

Category	Prevalence (%)
IFG	338 (5.0)
IGT	891 (13.3)
PDM (IGT and/or IFG)	1080 (16.1)
i-IFG	189 (2.8)
i-IGT	742 (11.0)
IGT and IFG	149 (2.2)
T2DM	214 (3.2)
Any abnormal glucose tolerance	1294 (19.3)

4.7.1 Age related prevalence of PDM

Figure 4.4 outlines the prevalence of IFG, IGT and PDM in the deciles of the screened population over the age of 40 years. The prevalence of IGT and PDM rises consistently with age with over 20% of the screened population having PDM over the age of 64 years. The prevalence of IFG increases steadily until 67 years of age (8%) but declines to 6.6% over the ages of 71.

Figure 4.4. Prevalence of IFG, IGT and PDM in age (years) deciles



4.7.2 Gender related prevalence of PDM

The prevalence of glucose abnormalities, both crude and adjusted for age are tabulated in Table 4.8. Men are 1.5 times more likely than females to IFG or newly diagnosed T2DM.

Table 4.8. Prevalence (%) and OR of WHO defined fasting and post challenge hyperglycaemia by gender

	Total	Gender			OR (95% CI)
	(n=6749)	Male (3221)	Female (3528)	p value	
IFG	338 (5.0)	198 (6.1)	140 (4.0)	<0.01	**1.56 (1.25- 1.95)**
IGT	891 (13.3)	425 (13.2)	466 (13.2)	0.84	0.98 (0.85- 1.13)
PDM	1080 (16.0)	534 (16.6)	546 (15.5)	0.34	1.07 (0.94- 1.22)
T2DM	214 (3.2)	126 (3.9)	88 (2.5)	<0.01	**1.57 (1.19- 2.08)**

When the prevalence of the glycaemic abnormalities are classified by the SA and WE ethnic groups, men from both ethnic backgrounds have a significantly higher prevalence of IFG, the former group by nearly 3 fold and the latter by 1.5 fold (Table 4.9).

Table 4.9. Odds ratios of prevalence for males in WE and SA ethnic groups

	Ethnicity	Odds ratio (95% CI)	
		Crude	Adjusted*
IFG	**SA**	**1.97 (1.26- 3.08) ▲**	**1.87 (1.19- 2.92) ▲**
	WE	**1.47 (1.13- 1.91) ▲**	**1.46 (1.13- 1.90)▲**
IGT	**SA**	1.16 (0.89- 1.51)	1.11 (0.81- 1.46)
	WE	0.94 (0.79- 1.13)	0.93 (0.78- 1.13)
PDM	**SA**	1.26 (0.98- 1.62)	1.21 (0.94- 1.26)
	WE	1.03 (0.88- 1.20)	1.01 (0.86- 1.19)

*Logistic regression models showing OR for males vs. females adjusted for age

▲p<0.05

4.7.3 Ethnic variations in prevalence of PDM

South Asians had a significantly higher prevalence of PDM, both crude and adjusted compared to White European subjects. The prevalence of different disorders of PDM is shown in Table 4.10. The South Asian cohort has a statistically significant greater prevalence of IGT (adjusted OR 1.64, 95%CI: 1.27-2.11), PDM (adjusted OR 1.57, 95%CI: 1.24-1.98) and T2DM (adjusted OR 2.05, 95%CI: 1.30-3.21) compared to the White European cohort. Overall 20% of South Asians have abnormal glucose tolerance compared to 16% of White Europeans (adjusted OR 1.71, 95%CI: 1.38-2.13). All given OR were adjusted for age, sex, central obesity and deprivation. Prevalence of IFG was similar between the ethnic groups.

Table 4.10. Prevalence of Type 2 diabetes and prediabetes in total screened population by ethnicity, Data given as count (%)

	Total* (n=6749)	White Europeans (n=4,588)	South Asians (n=1,684)	P value†	Unadjusted OR‡ (95% CI)	Adjusted OR‡ (95% CI)
IFG	338 (5.0)	206 (4.4)	76 (4.5)	0.84	1.03 (0.79 to 1.34)	1.34 (0.90- 2.02)
IFG exclusively	189 (2.8)	125 (2.7)	37 (2.3)	0.29	0.82 (0.56 to 1.19)	1.14 (0.66 to 1.95)
IGT	891 (13.3)	504 (10.8)	224 (13.4)	0.01	**1.27 (1.07 to 1.50)**	**1.64 (1.27 to 2.11)**
IGT exclusively	742 (11.0)	423 (9.2)	185 (11.3)	0.02	**1.24 (1.03 to 1.49)**	**1.60 (1.22 to 2.09)**
IGT or IFG	931 (13.8)	548 (11.7)	222 (13.2)	0.11	1.15 (0.97 to 1.35)	**1.54 (1.18 to 1.95)**
IGT and IFG	149 (2.2)	81 (1.7)	39 (2.3)	0.13	1.35 (0.92 to 1.98)	1.66 (0.92 to 2.99)
PDM (IGT and/or IFG)	1080 (16.0)	629 (13.4)	261 (15.5)	0.04	**1.18 (1.01 to 1.38)**	**1.57 (1.24 to 1.98)**
T2DM	214 (3.2)	128 (2.7)	76 (4.5)	<0.0001	**1.68 (1.26 to 2.25)**	**2.05 (1.30 to 3.21)**
Abnormal glucose tolerance	1294 (19.2)	757 (16.2)	337 (20.0)	<0.0001	**1.30 (1.13 to 1.50)**	**1.71 (1.38 to 2.13)**

Logistic regression models presented both unadjusted and adjusted for age, sex, central obesity (using ethnicity specific cut points of waist circumference) and deprivation ‡ Unadjusted / adjusted Odds Ratio (OR) for South Asians versus White Europeans (Bold figures indicate statistical significance).

*Includes ethnicities other than White European and South Asian †p-values: White European vs South Asian for raw data comparison

4.8 Identification of subjects with PDM

4.8.1 Differences in PDM prevalence in different screening strategies

In this section subjects with PDM diagnosed by the two different screening strategies using population and high risk strategy are compared. This is important to identify differences and thereby the potential advantage of using one strategy over the other.

Table 4.11. Prevalence of categories of glycaemia in studies adopting two different screening strategies

Glycaemic category	High risk screening N=3,515	Universal screening N=6,749	p value
PDM	587 (**16.7**, 15.5- 17.9)	1080 (**16.0**, 15.1- 16.9)	0.363
T2DM	178 (**5.1**, 4.4- 5.8)	214 (**3.2**, 2.8- 3.6)	<0.0001
i-IFG	126 (**3.6**, 3.0- 4.2)	189 (**2.8**, 2.4- 3.2)	0.029
i-IGT	330 (**9.4**, 8.4- 10.4)	742 (**11.0**, 10.2- 11.7)	0.012
IFG+IGT	131 (**3.7**, 3.1- 4.4)	149 (**2.2**, 1.9- 2.6)	<0.0001
All IFG	257 (**7.3**, 6.5- 8.2)	338 (**5.0**, 4.5- 5.5)	<0.0001
All IGT	461 (**13.1**, 12.0- 14.2)	891 (**13.2** , 12.4- 14.0)	0.048

Figures represent numbers (Prevalence, 95% CI)

As seen in Table 4.11, there is no significant difference in the prevalence of PDM between two different screening methods as a whole. However there is a significantly higher prevalence of subjects with IFG+IGT (3.7% vs. 2.2%) and T2DM (5.1% vs. 3.2%) in the high risk screened group. A significantly greater proportion of i-IGT is seen in the universal screened group.

4.8.2 Leicester Risk assessment score in the identification of PDM

It is important for any screening strategy to use a simple effective screening tool. The Leicester risk assessment (LRA) score is a self determined T2DM risk score based on information that is easily obtained from the population. The methodology used in the development of this score has been described in detail (311). Age, ethnicity, waist circumference, first degree family history of T2DM and antihypertensive therapy or high blood pressure were noted to be significant factors predicting risk of prevalent

T2DM/PDM. The area under the receiver operator characteristics curve (ROC) compared against the gold standard OGTT was 0.69 (0.68- 0.71). The final model had a score between 0 and 47 with higher scores denoting increased risk. Comparison between the LRA and the FINDRISC using a cut off score of ≥16 and ≥12 respectively shows a higher sensitivity (%) [72.1(69.6–74.6) vs. 69.7 (66.4–72.9)] and positive predictive value (%) [27.7 (26.2–29.3) vs. 23.3 (21.6–25.0)] for identifying those with T2DM or IGR. Furthermore, this score has been validated against an external data set demonstrating good concordance.

At baseline, of the 1080 people with PDM, LRA score could not be calculated for 91(8.4%) due to lack of sufficient data, predominantly being less than 40 years of age. 243 (22.5%) had a score less than the recommended cut off of 16 and 746 (69.1%) had a score over 16. Thus using the recommended cut off score of ≥16 would miss about 20% of those with PDM in the screened population at baseline. Prospective data at 12 months is available for 840 individuals. This data group shows that at 12 months only 7(3.2%) develop T2DM in the group with an LRA score of <16 compared to 46 (7.4%) in the group with an LRA score≥16. Moreover, 6 out of the 7 individuals (in the LRA <16 group) went on to develop an LRA score over 16 at 12 months. Assuming that a structured population screening programme used the LRA, these individuals would have been identified in the following year as they go on to higher LRA scores thus being eligible to be screened. The sole individual who would be missed at 12 months was younger than 40 years and thus would not be covered by the LRA.

Table 4.12. Baseline and 12 month data for those below and above the recommended Leicester Risk Assessment cut off of 16

		LRA <16	**LRA≥16**
Baseline		243 (22.5)*	746 (69.1)*
12 months		218 (89.7)	622 (83.3)
	T2DM	7 (3.2)	46 (7.4)
	PDM	65 (29.8)	276(44.4)
	NGT	146 (67.0)	300 (48.2)

*Data refers to the proportion of the total PDM of 1080 with a valid LRA score

4.9 Discussion

The prevalence of IFG, IGT and combined IFG and IGT in the study population is 2.8%, 11.0% and 2.2% respectively. Overall, 19.3% of the study population have some form of glucose abnormality- T2DM or PDM. IFG is more common amongst men whereas IGT is more common amongst women. SA have a significantly higher prevalence of IGT, PDM as a whole and T2DM, both unadjusted and adjusted for age, sex and waist circumference.

Using the recommended LRA score of ≥16, the negative predictive value (NPV) (%) is reported to be 88.8 (87.7- 89.9). Hence, this self reported score may be used to screen out those who may not need an OGTTs. Lower scores improve the NPV and thus obviate the need for performing more OGTT. For example choosing the recommended cut off value of ≥16, only 3,300 (56%) of the whole eligible have an LRA score over 16 and thus selecting this population reduces the OGTT by nearly half. 88% of people with PDM who developed T2DM at 12 months were identified by the LRA score. In a structured health care where risk based screening is offered to all people over 40 years of age based on the LRA, the remaining 12% would be identified in 2 years. Thus LRA score identifies most people with PDM who have a high risk of developing T2DM in future.

This is one of the largest population based screening studies for T2DM and the first of its kind in a British multiethnic population. The screened population were similar to the background population and thus enable applicability of findings to the general population. Furthermore, this data is only one of its kind where subjects with both IFG and IGT have been phenotyped and compared separately i.e. IFG and IGT groups are mutually exclusive and those with both IFG and IGT are categorised as having combined IFG and IGT. This enables stratification of a higher risk group as described in subsequent chapters in terms of CVD risks and risk of progression to T2DM. This could be utilised to optimise preventative strategies to those at highest risk. This study has also shown that in a British multiethnic population, the prevalence of PDM and T2DM are similar using both a universal and high risk screening strategy. This data is important for policy makers for recommending a screening strategy. This data is also unique as a risk score has been devised using local population, validated in a different cohort but from a similar demographic population and prospectively shown to identify most of those at risk of developing T2DM.

The eligibility criteria for inclusion and screening in the ADDITION- Leicester study are different for the WE and the SA ethnic groups in terms of age. This may be perceived to be a minor weakness in terms of drawing conclusions applicable to the general population. To overcome this difficulty, prevalence data presented are adjusted for age and gender for the ethnic groups in this analysis.

The prevalence of IFG and IGT are variable depending on age, sex, ethnic group and there are intra ethnic variations depending on the latitude of residence. These data have been described and tabulated in an earlier chapter (Chapter 2). The prevalence of IGT is comparable to previous epidemiological studies with a similar age group (30;40). However the ethnic mix differs significantly from the population under study and those reported in Shaw *et al*. Simmons *et al* reported a prevalence of IGT in South Asians to be 9.8% in males and 1.2% in females of South Asian origin in a mixed ethnic UK population (32). However the age group was over 20 years of age and the diagnosis was based on 1985 WHO criteria that recommends a high plasma glucose cut off. Thus the prevalence rates reported by Simmons *et al* are understandably lower. The prevalence estimates for IGT in the United Kingdom reported in the 20-79 age group in 2007 is 5.1% (2). Given the differing age group and considering the higher proportion of the SA ethnic group in our cohort, the prevalence rates are comparable. Age, gender and ethnicity appear to important factors determining the prevalence of IFG and IGT. The largest prevalence of IFG reported is 40% of IFG in Nauru (28).

Recently published data from the ADDITION Europe study has also shown that screening for T2DM identifies a unique phenotype of patients who have modifiable CVD risks and perhaps very early vascular disease in terms of end organ damage and hence amenable to multi factorial risk intervention. The ongoing ADDITION Leicester intervention study will address this issue in a British multi ethnic population (294). Adopting a T2DM screening programme also identifies the PDM cohort in whom life style interventions and perhaps medications in a select group may be instituted to reduce the risk of progression to T2DM. Thus, there is no doubt surrounding the benefit of universal screening for T2DM in those over the age of 40.

The LRA is available on the public portal of the Diabetes UK website (312). Over 69,000 individuals utilised the LRA for self measuring their risk of Diabetes in the first six months until December 2010 (http://www.diabetes.org.uk/riskscore). Education of the general public on the risks of complications of T2DM and the potential for preventing

this by early detection and treatment must be reiterated through mass media. This may further be enhanced by using posters in general practices and clinics where people at risk of developing T2DM are seen such as in hypertension, renal and cardiology outpatient departments. Using a self measured risk score places the onus with the individual and this may increase attendance at screening sessions.

Adopting a strategy of self referred universal screening combined with opportunistic screening is likely to have a high uptake, be cost effective and target those at highest risk. However this may need to be tailored to the local population based on needs and availability of resources.

4.10 Summary

Screening for T2DM identifies subjects with intermediate stages of PDM, who are not presently offered any primary prevention for CVD. Nearly 20% of the British multiethnic population in Leicester have some form of glucose abnormality: T2DM or PDM. The prevalence of both T2DM and PDM is significantly higher in South Asians compared to White Europeans. The overall rate of prevalence of PDM is similar using both a high risk and universal screening strategy. The LRA score is an effective way of screening for T2DM and PDM with reductions of 50% of OGTT required. Education of the general public on the complications of untreated diabetes and the potential to reduce the risks of these adverse effects needs to be effectively communicated. The general public must be encouraged to assess their risks and thus participate in such screening programmes. Adopting an opportunistic screening strategy in parallel with a universal screening programme using validated risk scores as a screening out tool should be recommended.

5 Baseline Characteristics of subjects with PDM

5.1 Background and Aims

Both IFG and IGT are increasingly recognised as dysmetabolic states contributing to higher risk of cardiovascular disease (26;66;75).

There is still controversy as to the relative contribution of fasting plasma glucose and post prandial plasma glucose to the development of CVD. The UKPDS demonstrates that once diabetes is diagnosed glycaemia is only modestly related to coronary artery disease, indicating that the interplay between this and other risk factors occurs earlier than thought, perhaps in the PDM stages (166;313). The long term follow up of UKPDS cohort has established the benefits of early and intensive management of CVD risk factors even in those with T2DM. Significant reductions in CVD in the intensively managed group was noticed, even though the glycaemic control were similar between the groups at follow up (314). Similarly, the recently published CVD end point trials showing an increase in deaths with intensive glycaemic control in long standing diabetes patients points to a need for these risk interventions to be implemented very early in the pathogenesis of this condition (315). The results of trials confirm that much of the potential benefit in detection of undiagnosed diabetes is likely to be cumulative from intensive management of several cardiovascular risk factors along with hyperglycaemia (166;167;316). Thus, it becomes important to institute a screening strategy that encompasses identification of other associated CVD risk factors.

To recommend an appropriate screening strategy for a screening program, it is vital that the phenotypic charters of people identified is well characterised. Especially in a condition such as T2DM that is associated with a multitude of CVD risk factors, such a description enables the study of the CVD risk load and thus treatment recommendations for those identified at a very early stage.

This chapter aims at describing the cardiovascular disease risk profile in those identified with PDM in the ADDITION Leicester study. As a first step, PDM as a whole group is studied in comparison with those with normal glucose tolerance (NGT) to set up the PDM group as being intermediate between NGT and T2DM. Subsequently, to test the hypothesis that those with combined IGT and IFG are phenotypically different

and at a higher CVD risk, those with combined IFG and IGT are described in comparison to those with isolated IFG and IGT. Finally, the differences in anthropometry and biomedical profile are studied between the SA and WE ethnic groups.

5.2 Measurement methodology

5.2.1 Anthropometric measurements

Anthropometric measurements were undertaken at baseline by trained staff following standard operating procedures.

- Height was measured to the nearest 0.1 cm using a rigid stadiometer and weight in light indoor clothing measured to the nearest 0.1 kg with a Seca scale (Seca UK, Birmingham, UK). Waist circumference was measured at the mid-point between the lower costal margin and the level of the anterior superior iliac crest to the nearest 0.1 cm.
- Hip circumference was measured over the widest part of the gluteal region. Hip and waist circumference were measured using a soft tape measure.
- Blood pressure was measured using an Omron M4 blood pressure machine (Omron Healthcare, Milton Keynes, UK) with the participant in a sitting position for at least 5 minutes quietly prior to testing. Three right arm measurements were taken using an automated sphygmomanometer and appropriate sized cuff. The cuff should encircle at least 80% of the arm, but not more than 100%. The mean of the second and the third was considered to be a representative value (317).
- 12 Lead ECGs were performed using a Nihon Kohden CardioFax Gem machine (Nihon Kohden Europe GmbH, Rosbach vor der Höhe, Germany). The ECGs were independently reported by 2 physicians on the day of the visit and results were reported back to the GP.

All the equipment and analysers were calibrated and serviced according to manufacturer recommended standards and intervals.

5.2.2 Laboratory measurements

All routine laboratory measurements were performed by the Chemical pathology laboratory at University Hospitals of Leicester which are accredited and take part in quality assurance programme. HbA1c was analysed by the Biorad Variant II system (Bio-Rad Laboratories, Hemel Hempstead, UK) that is DCCT aligned. Glucose [using

the hexokinase method (NADPH production at 340 nm)] urea and electrolytes, total and HDL cholesterol and triglycerides were all measured on the Abbott Aeroset clinical chemistry analyser. Calculated LDL cholesterol was determined using the Friedewald equation (318).The Olympus OSR6167 Micro albumin Analyser with a sensitivity of 0.46mg/l was used. Albumin/creatinine ratio equal to or greater than 2.5mg/mmol in males and 3.5mg/mmol in females were considered to indicate microalbuminuria.

5.2.3 Biomarker analysis

Fasting plasma and serum samples were collected for the quantification of biological markers of inflammation and urine for oxidative stress (Table 5.1). The serum gel samples for storage were centrifuged for 10 minutes at 4000 rpm after 30 minutes, pipetted and then frozen. Lithium heparin samples for storage were centrifuged at 3000 rpm for 10 minutes, aliquoted out into 2 ml tubes and stored within 30 minutes of the sample being taken. All samples were stored in a freezer at -70 degrees centigrade. All samples stored are identified by study ID number.

All the measurements on stored samples were analysed at the Unilever Discover Laboratory, Colworth House, Sharnbrook, Bedfordshire. The predominant method used for biomarker assay was immunoassay- both enzymatic as well as fluorometric methods.

Table 5.1. Biomarkers measured on stored samples

Serum Insulin
Highly sensitive C reactive protein (hs-CRP)
Leptin
Adiponectin
Tumour necrosis factor α (TNF α)
Interleukin 6 (IL 6)
25 hydroxy Vitamin D (25(OH)D) and Calcium
Plasma Isoprostane
Urinary Isoprostane and creatinine

5.2.3.1 Immunoassays

Immunoassays utilise the specific interaction between an antigen (unknown concentration of substance to be assayed) and its corresponding antibody. This

specific interaction produces an antigen-antibody complex and allows the detection and quantification of substances by various methods. This antigen antibody complex reaction can be measured directly through labelling methods as illustrated below. The label may be an enzyme that converts an added substrate to produce a colour change, a fluorescent substance or a radioactive substance. All these labels emit a signal that can be quantitated (319;320).

The advent of monoclonal antibodies have improved the ease of production and reproducibility of these assays. Immuno assays may be competitive or non competitive.

In the competitive immunoassays, the free and the labelled antigen compete for the binding sites on the antibody. Depending on the concentration of the free antigen, binding sites will be available for the labelled antibody and thus the intensity of the signal obtained is inversely proportional to the concentration of the free antigen. These competitive antigen antibody reactions may be in simultaneous or sequential steps.

In the non-competitive immunoassays, a capture antibody is first adsorbed to a solid phase such as the microtitre wells. The sample with unlabelled antigen is added next to allow the antigen antibody complex to form. After washing, labelled antibody is added that binds to the antigen at a second site that is in turn bound to the solid phase. Following a washing step to remove the unlabelled antibody, the substrate is added and the intensity of the signal is then measured.

The performance characteristics of assays used are listed in Table 5.3. All samples were run in duplicates and the co efficient of variation (CV) was less than 10% in all assays. Assays were repeated with a CV greater than 10%.

Table 5.2 Methodology of Biomarker analysis

Assay		Methodology	Kits used	Analyser
Insulin	Serum	Time resolved Fluoroscent immuno Assay (TRFIA)	Perkin Elmer AUTODELFIA® Insulin kit	Perkin Elmer Autodelfia 1235 immunoassay system
8-iso Prostaglandin $F_{2\alpha}$	Plasma	TRFIA	MAB[1]-Colworth monoclonal group	Perkin Elmer Autodelfia 1235 immunoassay system
Adiponectin	Serum	TRFIA	R&D systems Human Adiponectin MAB	Perkin Elmer Autodelfia 1235 immunoassay system
2,3-Dinor-8-Iso-Prostaglandin $F_{1\alpha}$	Urine	TRFIA	MAB[1]-Colworth monoclonal group	Perkin Elmer Autodelfia 1235 immunoassay system
Leptin	Serum	ELISA[2]	Mediadiagnost®	Perkin Elmer Viktor 1420 multilabel counter
TNF α	Serum	HS ELISA	R and D systems TNFα HS ELISA	Perkin Elmer Viktor 1420 multilabel counter
Interleukin 6	Serum	HS ELISA	R and D systems IL-6 HS ELISA	Perkin Elmer Viktor 1420 multilabel counter
Vitamin D	Serum	Competitive ELISA	Immunodiagnostic systems	Perkin Elmer Viktor 1420 multilabel counter
hs CRP	Serum	Latex enhanced immunoturbidimetry	Horiba ABX Pentra CRP CP	Horiba ABX Pentra 400 Clinical Chemistry analyser
Calcium	Serum	Photometry using OPC[3]	Horiba ABX Pentra CRP CP	Horiba ABX Pentra 400 Clinical Chemistry analyser
Creatinine	Urine	Jaffe's method	Horiba ABX Pentra CRP CP	Horiba ABX Pentra 400 Clinical Chemistry analyser

1. Monoclonal Antibody 2. Enzyme linked immunosorbent assay 3.Ortho- Cresolphtalein complexone

Table 5.3 Performance characteristics of assays used for biomarkers

Assay	Sample	Mean MDD‡	Intra assay precision	Inter assay precision
Insulin	Serum			
8-iso Prostaglandin $F_{2\alpha}$	Plasma	0.25 ng/ml	4.0 – 57.0 †¥	1.3 – 13.8 †¥
Adiponectin	Serum	0.056 ng/ml	2.6 – 4.0 †	4.0 – 7.4 †
2,3-Dinor-8-Iso-Prostaglandin $F_{1\alpha}$	Urine	0.72 ng/dl	4.2 – 32.8 †¥	1.2 – 8.8 †¥
Leptin	Serum	0.2 ng/ml	4.97 – 37.11 (0.27 – 2.55) ◊	3.95 – 33.16 (0.32 – 2.56) ◊
TNF α	Serum	0.106 pg/mL	3.1 - 8.5 †	7.4 - 10.6 †
Interleukin 6	Serum	0.039 pg/mL	6.9 - 7.8 †	6.5 - 9.6 †
Vitamin D	Serum	5 nmol/L	5.3 – 6.7 †	4.6 8.7 †
hs CRP	Serum	0.1 mg/l	0.92 – 4.15 •†	2.32 – 2.92▲†
Calcium	Serum	0.03 mmol/l	0.44 – 1.37 †	2.91 3.15 †
Creatinine	Urine	0.18 mg/dl	0.62 – 4.08 †	1.78 – 6.0 †

† Co efficient of variation (%) ◊ Mean (SD) ‡ Minimum detectable dose
Number of samples used for Precision determination: ● Five ▲ Two ¥ Twenty six. All other assays used three samples

5.2.4 Derived measurements

Body mass index (kg/m^2) was defined as weight in kilograms divided by height in metres squared. Electro cardiograms (ECG) were coded according to the Minnesota coding criteria (321). This was further classified based on UKPDS classification and modified to include atrial fibrillation and left ventricular hypertrophy. Estimated Glomerular Filtration rate (eGFR) was calculated based on modified MDRD equation (322). Subjects were classified in stages of chronic kidney disease (CKD) as per the Renal Association Guidelines (323). Composite CVD was defined as presence of at least one of the conditions: myocardial infarction, atrial fibrillation, angina, Stroke, leg/coronary angioplasty and/bypass or peripheral vascular disease. The modelled CVD risk was calculated using modified Framingham risk equation unless stated otherwise in people without CVD (324).

The Joint British Societies' recommended guidelines for treatment for CVD risk factors such as hypertension and hyperlipidaemia were taken into consideration to calculate the proportion of people who will need treatment for these conditions (325). These guidelines recommend treatment for hypertension and hyperlipidaemia irrespective of the glycaemic state for those with blood pressure ≥ 150/100 mm Hg or those with lesser degrees of blood pressure with end organ damage. Similarly, treatment is recommended for those with a modelled 10 year CVR risk ≥20% or those with a total/HDL cholesterol ratio ≥6.

5.2.5 Statistical analysis

Data shown represent count (%) for categorical and mean (SD) for continuous variables unless stated otherwise. Categorical variables are compared using the chi square tests and continuous variables are compared using the independent t tests or one way analysis of variance method. Linear regression models were used to adjust the variables for confounders.

Spearman correlation was used to identify significant correlations between biomarkers and other biomedical parameters. Those with a significant correlation were used in the subsequent linear regression analysis to adjust for confounders. All analysis were performed using SPSS version 16 statistical software. Variables that were not normally distributed such as triglycerides and fasting plasma insulin were log transformed for the regression analysis and back transformed values are depicted.

The crude values of biomarkers were initially compared between NGT and PDM. Then, in steps, demographic variables such as age, gender, ethnicity and waist circumference were added to the model. Subsequently, triglycerides, FPG and FPI were added at each step to obtain the final model.

5.3 Anthropometry, biomedical parameters and cardiovascular risk between PDM and NGT

Demographic and biomedical data for subjects identified with PDM is compared with NGT in Table 5.4, Table 5.5 and Table 5.6.

There is a significant higher proportion of men and people of SA origin in the PDM group. Markers of insulin resistance, both WC and BMI are significantly higher in the PDM group. Subjects with PDM are older and risk factors of CVD such as blood pressure, LDL cholesterol and triglycerides are higher and HDL cholesterol is lower in this group.

The PDM group have a higher mean Framingham CVD score compared to NGT subjects. The significance persists when adjusted for age, ethnicity, lipid profile, blood pressure and BMI [0.17 (0.88) vs. 0.13 (0.83), $P<0.0001$)]. Interestingly the proportion of people who currently smoke is higher in the NGT individuals.

From Table 5.5 it is also seen that significantly higher proportions of people with PDM are known to have hypertension, hyperlipidaemia and pre existing CVD. Although the proportion of people with the composite CVD is higher in the PDM group, when studied as separate vascular disease, myocardial infarction, angina, coronary interventions and stroke are higher in those with PDM.

Table 5.4. Demographic and biomedical data of screened individuals by glycaemic status

	Normal (5425)	PDM (1080)	p value
Age	60.0 (10.3)	55.8 (10.8)	**<0.001**
Sex, Male (%)	2546 (46.9)	534 (49.4)	**<0.001**
Ethnicity (%)			
White European	3908 (72)	766 (70.9)	
South Asian	1340 (24.7)	300 (27.8)	**0.001**
Others	177 (3.3)	14 (1.3)	
Weight (kg)	77.0 (15.8)	81.5 (16.1)	**<0.001**
BMI (kg/m2)	27.7 (4.9)	29.7 (5.2)	**<0.001**
Waist circumference (cm)	92.8 (13.0)	98.4 (12.8)	**<0.001**
Males	97.7 (11.4)	102.2 (11.9)	**<0.001**
Females	88.5 (12.9)	94.8 (12.6)	**<0.001**
Waist hip ratio	0.88 (0.08)	0.91 (0.09)	**<0.001**
Body fat (%)	32.8 (8.8)	35.5 (8.4)	**<0.001**
Males	27.1 (6.8)	30.7 (7.1)	**<0.001**
Females	37.7 (7.1)	40.2 (6.7)	**<0.001**
Systolic blood pressure (mm Hg)	135.4 (19.1)	143.0 (20.4)	**<0.001**
Diastolic blood pressure (mm Hg)	84.8 (10.6)	87.0 (10.8)	**<0.001**
Smoking status (%)			
Non	3102 (58.0)	551 (59.8)	**<0.001**
Ex	1419 (26.5)	281 (30.5)	**<0.001**
Current	829 (15.5)	89 (9.7)	**<0.001**
HbA1c (%)	5.6 (0.4)	5.9 (0.5)	**<0.001**
Total Cholesterol (mmol/L)	5.5 (1.1)	5.5 (1.1)	0.14
LDL (mmol/L)	3.45 (0.9)	3.54 (0.9)	**0.003**
HDL (mmol/L)	1.4 (0.4)	1.3 (0.4)	**<0.001**
Triglycerides (mmol/L)	1.4 (0.8)	1.6 (1.0)	**<0.001**
10 year Framingham CVD risk score	0.13 (0.1)	0.16 (0.1)	**<0.001**

Table 5.5. Pre existing CVD and medications by glycaemic status

	Normal (5425)	PDM (1080)	p value
Previous medical history			
Known Hypertension	1243 (23.2)	376 (41.0)	**<0.001**
Known Hyperlipidaemia	815 (15.2)	217 (23.7)	**<0.001**
Myocardial Infarction	140 (2.6)	38 (4.1)	**0.003**
Angina	239 (4.4)	71 (7.7)	**<0.001**
Angioplasty/CABG	93 (1.7)	27 (2.9)	**0.012**
Heart Failure	29 (0.5)	5 (0.5)	0.568
Stroke	86 (1.8)	27 (2.9)	**0.017**
Peripheral vascular disease	186 (3.5)	29 (3.2)	0.354
Leg Angioplasty/bypass	82 (1.5)	22 (2.4)	**0.043**
Medications			
Antihypertensive	1095 (20.2)	373 (35.2)	**<0.001**
Statin	528 (9.7)	178 (16.8)	**<0.001**
Aspirin	439 (8.1)	142 (13.4)	**<0.001**

Prevalence of CVD risk factors as recommended by the NHS Health Check Programme is outlined in Table 5.6. The prevalence of people with high blood pressure (≥140/90 mm Hg), total cholesterol ≥ 5mmol/L, BMI > 30 and BMI > 27.5 is significantly higher in those with PDM. The table also outlines the proportion of people with central obesity using ethnic specific cut points for WC as outlined by the International Diabetes Federation in PDM and NGT(326). Using the cut off of BMI ≥ 30 underestimates the proportion of people (%) with obesity both in the NGT and PDM groups when compared with the ethnic specific waist circumference cut points (45.8 vs. 64.7 in the NGT group and 63.6 vs. 76.7 in the PDM group).

Table 5.6. Prevalence of CVD risk factors in those with PDM and NGT

Vascular risk factors	Normal (5425)	PDM (1080)	p value
CVD risk ≥ 20% *	984 (20.2)	296 (29.7)	**<0.001**
Blood pressure ≥ 140/90 *	2370 (45.0)	654 (61.0)	**<0.001**
Total cholesterol ≥ 5 *	3719 (69.0)	711 (66.4)	**0.049**
LDL ≥ 3 *	3698 (68.7)	730 (68.2)	0.593
Obesity			
BMI > 30*	2460 (45.8)	682 (63.6)	**<0.001**
BMI > 27.5*	1426 (26.5)	442 (41.1)	**<0.001**
Waist circumference (cm)	3466 (64.7)	825 (76.7)	**<0.001**

*As suggested in the NHS health check programme (6)

The prevalence of microalbuminuria is significantly higher in the PDM group (Table 5.7). This difference persists when albumin creatinine ratio is adjusted for age, sex, ethnicity, BMI, hypertension, hyperlipidaemia, medications, smoking and HbA1c (adjusted OR: 1.39, 95%CI: 1.06 to 1.82). However such a difference is attenuated, when similar adjustments are made for prevalence of CKD (adjusted OR: 0.83, 95% CI: 0.63 to 1.08). The median (Inter Quartile Range) urine albumin creatinine ratio is significantly higher in the PDM [0.8 (0.5 to1.50) group compared to those with NGT [0.7 (0.5 to 1.1)] (p =0.001).

A significantly higher proportion of people with PDM had a 10 year CVD risk greater than 20% after adjusting for age, ethnicity, lipid profile, blood pressure and BMI (OR: 1.56, 95% CI: 1.28 to 2.07).

Table 5.7. Vascular complications in screened individuals

Vascular complications	Normal (5425)	PDM (1080)	p value
Microalbuminuria	361 (6.8)	124 (11.7)	**<0.001**
CKD stages 3,4 and 5	534 (10)	124 (11.6)	**<0.001**
10 year Framingham CVD risk (%)	0.13 (0.1)	0.16 (0.1)	**<0.001**
Composite CVD	603 (11.2)	141 (15.4)	**<0.001**

Table 5.8 outlines the proportion with hypertension who fulfil treatment criteria and those who are actually treated at the time of screening for hypertension and hypercholesterolaemia as per JBS2 guidelines. Presence of microalbuminuria is taken as end organ damage and presence of microalbuminuria and CKD stages 3, 4 and 5 are depicted as a separate category.

Whilst considering the CVD risk ≥ 20%, 20.2% and 29.7% are over this risk 10 year risk threshold in the NGT and PDM groups. Of these only 37.2% and 52.9% are on any category of medications for CVD (includes statin or aspirin or an antihypertensive agent). Similarly, only 27.1% and 34.6% of people who are identified to have hypertension as per JBS2 criteria are on medications in the NGT and PDM groups.

Table 5.8. Treatment thresholds for vascular risk factors in those with NGT and PDM

JBS2 treatment thresholds	Normal (5425)	PDM (1080)
CVD risk≥20%	**984 (20.2)**	**296 (29.7)**
Statin	145 (14.7)	61 (20.7)
Aspirin	145 (14.7)	55 (18.6)
Any CVD medications *	365 (37.2)	156 (52.9)
Hypertension	**791 (15.0)**	**254 (23.7)**
Medications for hypertension	214 (27.1)	88 (34.6)
Medications for HT with end organ damage	318 (40.2)	114 (44.9)
Medications for HT, end organ damage and CKD	468 (59.2)	155 (61.0)
Hypercholesterolaemia	**384 (7.2)**	**102 (9.6)**
Statin	145 (37.8)	61 (20.6)

*Includes any combination of aspirin, statin or an anti hypertensive medications

5.3.1 Role of OGTT rescreening in risk stratification of those with PDM

WHO recommends those with a diabetes range OGTT should have the test repeated if asymptomatic. There is no evidence that those with single abnormal glucose tolerance test (GTT) are at lower CVD risk than those with confirmed T2DM.

Subjects with diabetes range OGTT, if asymptomatic, were offered a second OGTT as per the study protocol. Table 5.9 shows the CVD risks in PDM between in those who never had a diabetes range OGTT (Group 1) and those with the first OGTT in the

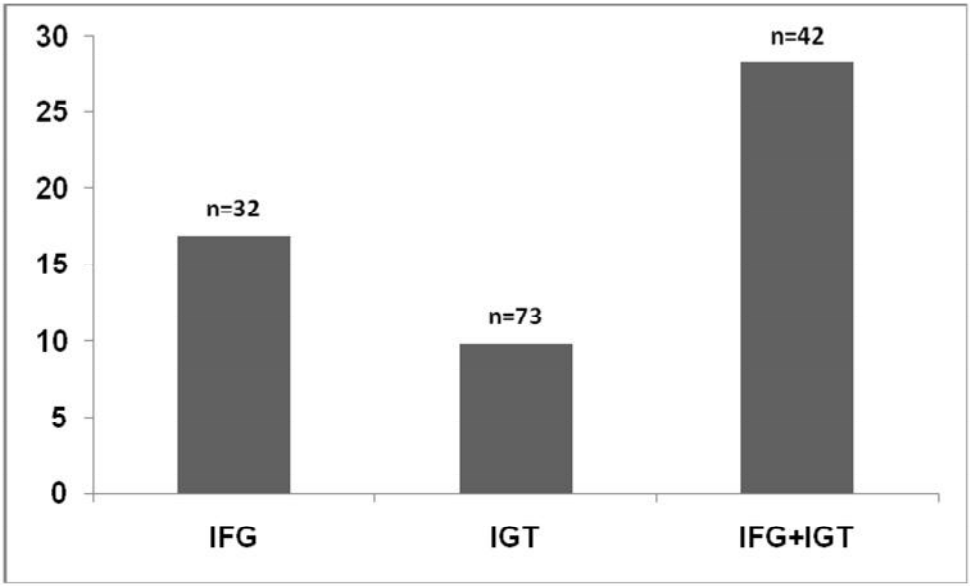
diabetes range but offered a second OGTT as they were asymptomatic (Group 2). Although the modelled CVD risk was similar between the groups, Group 2 had a significantly higher CVD risks such as blood pressures and HbA1c. Moreover Group 2 were significantly more insulin resistant than Group 1 [Geometric mean (SE) of HOMA IR: 2.81(1.5) vs. 1.84(1.0), p<0.001]. These values were adjusted for baseline confounders. People with combined IFG+IGT had the highest proportion of a diabetes range OGTT compared to those with i-IFG or i-IGT (p<0.001).

Those with a single diabetes range OGTT also had a significantly higher rate of progression to T2DM at 12 months (OR: 8.3, 95% CI: 4.6 to 15.2, p<0.001).

Table 5.9.CVD risks in those with PDM with and without Diabetes range OGTT

	Group 1 (n=933)	**Group 2 (n=147)**	**p value**
Age	59.8 (10.4)	60.7 (9.6)	0.336
BMI	29.5 (5.1)	31.0 (5.7)	**0.03**
Waist circumference (cm)	98.0 (12.6)	101.7 (13.7)	**0.007**
Body fat (%)	35.2 (8.4)	37.8 (8.2)	**<0.001**
Systolic Blood Pressure (mm Hg)	142.6 (20.3)	145.2 (20.7)	0.159
Diastolic Blood Pressure (mm Hg)	86.9 (10.4)	87.6 (11.3)	0.514
HbA1c (%)	5.9 (0.4)	6.1 (0.4)	**<0.001**
Framingham CVD risk (%)	16.0 (10.2)	17.0 (10.7)	0.306
Progression to T2DM at 12 months	26 (3.4)	32 (24.1)	**<0.001**
Proportion with ≥20% CVD risk	254 (29.2)	42 (32.8)	0.407

Figure 5.1. Percentage of people with PDM with a single diabetes range OGTT



5.4 Anthropometry, biomedical parameters and pre existing cardiovascular risk between various categories of PDM

Table 5.10 gives the demographics and biomedical measurements of subjects with i-IFG, i-IGT and IFG+IGT. Age distribution was similar between the groups; BMI, waist circumference, body fat and both systolic and diastolic blood pressures were significantly higher in those with both IFG and IGT. Similarly, HbA1c, LDL and HDL cholesterol and triglycerides are significantly higher in subjects with IFG+IGT. Comparing the fasting glucose between i-IFG and IFG+IGT and 120 minute plasma glucose between i-IGT and IFG+IGT, both are significantly higher in the IFG+IGT group [6.4 (0.2) vs. 6.3 (0.2)] for fasting and 9.4 (1.0) vs. 8.9 (0.9) for 120 minute post load plasma glucose, $p<0.0001$ for both].

The prevalence of pre existing CVD, hypertension and hyperlipidaemia are shown in Table 5.11. There is no significant difference in pre existing CVD amongst the various categories of PDM, except for peripheral vascular disease which is significantly higher in IFG+IGT group, however the numbers were small. A similar trend is seen for pre existing hypertension. However prevalence of pre existing hyperlipidaemia is higher in those with IGT alone.

Table 5.10. Demographic and biomedical data between different disorders of PDM

	i-IFG	i-IGT	IFG+IGT	p Value
Age	60.0 (9.7)	59.8 (10.6)	60.6 (9.5)	0.731
Sex, Male (%)	109 (57.7)	336 (45.3)	89 (40.3)	
Ethnicity (%)				
White European	145 (76.7)	523 (70.5)	98 (65.8)	
South Asian	42 (22.2)	211 (28.4)	47 (31.5)	0.135
Others	2 (1.1)	8 (1.1)	4 (2.7)	
Weight (kg)	84.9 (17.2)	79.1 (15.3)	89.1 (15.9)	**<0.001**
BMI (kg/m^2)	30.0 (5.2)	29.2 (5.0)	31.8 (5.4)	**<0.001**
Waist circumference (cm)	100.7 (13.6)	96.8 (12.5)	103.6 (11.8)	**<0.001**
Males	103.2 (13.6)	101.1 (11.4)	104.7 (11.1)	**0.022**
Females	97.2 (12.8)	93.3 (12.2)	101.9 (12.6)	**<0.001**
Waist hip ratio	0.92 (0.08)	0.9 (0.09)	0.92 (0.07)	0.127
Body fat (%)	35.0 (8.8)	35.3 (8.3)	37.4 (8.5)	**0.012**
Males	30.6 (7.2)	30.1 (7.1)	32.8 (6.6)	**0.009**
Females	40.8 (7.2)	39.5 (6.6)	44.1 (6.0)	**<0.001**
Systolic blood pressure (mm Hg)	140.4 (18.8)	142.9 (20.8)	146.7 (19.8)	**0.022**
Diastolic blood pressure (mm Hg)	86.6 (9.3)	86.7 (10.8)	89.1 (10.1)	**0.029**
Smoking status (%)				
Non	86 (50.0)	395 (63.4)	70 (55.6)	
Ex	64 (37.2)	173 (27.8)	173 (27.8)	**<0.001**
Current	22 (12.8)	55 (7.4)	12 (9.5)	
Fasting plasma glucose	6.3 (0.2)	5.3 (0.4)	6.4 (0.2)	**0.002**§
120 minute plasma glucose	6.1 (1.2)	8.9(0.9)	9.4 (1.0)	**<0.001***
HbA1c (%)	6.0 (0.5)	5.8 (0.4)	6.1 (0.4)	**<0.001**
Total Cholesterol (mmol/L)	5.5 (1.0)	5.4 (1.1)	5.6 (1.1)	0.376
HDL (mmol/L)	1.5 (0.86)	1.6 (0.98)	1.8 (0.88)	**0.012**
LDL (mmol/L)	3.6 (0.88)	3.4 (0.96)	3.5 (0.97)	**0.042**
Triglycerides (mmol/L)	1.3 (0.42)	1.3 (0.39)	1.2 (0.34)	**0.001**

§ p value comparing the fasting plasma glucose between IFG and IFG+IGT * p value comparing 120 minute post glucose load plasma glucose between IGT and IGT+IFG

Table 5.11. Prevalence of pre-existing CVD and its risk factors amongst different categories of PDM (%)

	i-IFG	i-IGT	IFG+IGT	p value
Previous medical history:				
Composite CVD	21 (11.9)	90 (15.1)	31 (21.1)	0.07
MI	3 (1.7)	26 (4.4)	9 (6.1)	0.12
Heart valve disease	0	9 (1.5)	3 (2.0)	0.208
Heart failure	0	5 (0.8)	0	0.257
Atrial fibrillation	2 (1.1)	16 (2.7)	5 (3.4)	0.652
Angina	12 (6.8)	43 (7.2)	16 (10.9)	0.288
Stroke	4 (2.3)	20 (3.4)	3 (2.1)	0.598
Angioplasty/CABG	2 (1.1)	18 (3.0)	7 (4.8)	0.154
Leg angioplasty/bypass	3 (1.7)	14 (2.4)	5 (3.4)	0.607
Peripheral vascular disease	1 (0.6)	20 (3.4)	8 (5.4)	**0.04**
Known Hypertension	63 (36.0)	256 (43.0)	57 (39.0)	0.225
Known Hyperlipidaemia	24 (13.7)	159 (26.8)	34 (23.1)	**<0.05**

5.4.1 Prevalence of vascular complications in PDM

Prevalence of vascular risk factors as defined by the NHS Health Check programme is tabulated amongst various categories of PDM in Table 5.12. There are no significant differences between the groups for the CVD risk factors except prevalence of hypertension, which was higher in the IFG + IGT group. Framingham modelled CVD risk is significantly higher in those with both IGT and IFG.

Table 5.12. CVD risk factors between various categories of PDM

CVD risk factors	i-IFG	i-IGT	IFG+IGT	P value
CVD risk ≥ 20% †	51 (29.7)	197 (28.4)	48 (36.4)	0.062
Blood pressure ≥ 140/90 †	112 (59.9)	441 (59.6)	101 (69.2)	**<0.001**
Total cholesterol ≥ 5 †	133 (71.5)	475 (64.5)	103 (69.5)	0.098
LDL ≥ 3 †	134 (73.2)	499 (68.5)	97 (67.8)	0.311
Obesity †				
BMI > 30	78 (41.7)	281 (38.1)	83 (56.1)	**<0.001**
BMI > 27.5	123 (65.8)	444 (60.1)	115 (77.7)	**<0.001**
Hypertension by JBS-2 **	40 (21.4)	176 (23.8)	38 (26)	0.609
Hypercholesterolaemia by JBS-2 $	17 (9.1)	66 (9)	19 (12.8)	0.341
Framingham score (%)	16.6 (9.9)	15.5 (10.2)	18.6 (10.9)	**0.006**

Continuous variables and categorical variables are represented as mean (SD) and count (%).
† CVD risk factors as defined in NHS vascular check programme * Median (IQR) **≥160/100 mm Hg
$ Total cholesterol≥6.0 mmol/L and/or Total cholesterol/HDL ratio≥4

5.4.1.1 ECG data

Over a quarter of people with PDM have abnormal ECG tracings (Figure 5.2). There were no significant differences between the different categories of PDM. However it is important to note that these changes are based on ECG tracings only and do not necessarily correlate with clinical symptoms. Figure 5.2 also shows the ECG results for subjects with no known CVD which are similar to the entire cohort of PDM.

5.4.1.2 CKD and Microalbuminuria

Table 5.13, shows that there are no significant differences in the prevalence of microalbuminuria in those with IGT+IFG and screen detected T2DM. However an increased prevalence of CKD stages 3, 4 and 5 was seen in those with IFG+IGT,

possibly because of an elevated eGFR in those with T2DM as a result of hyperfiltration and thus a falsely low prevalence of CKD.

Figure 5.2. ECG results for people with PDM (n=1080)

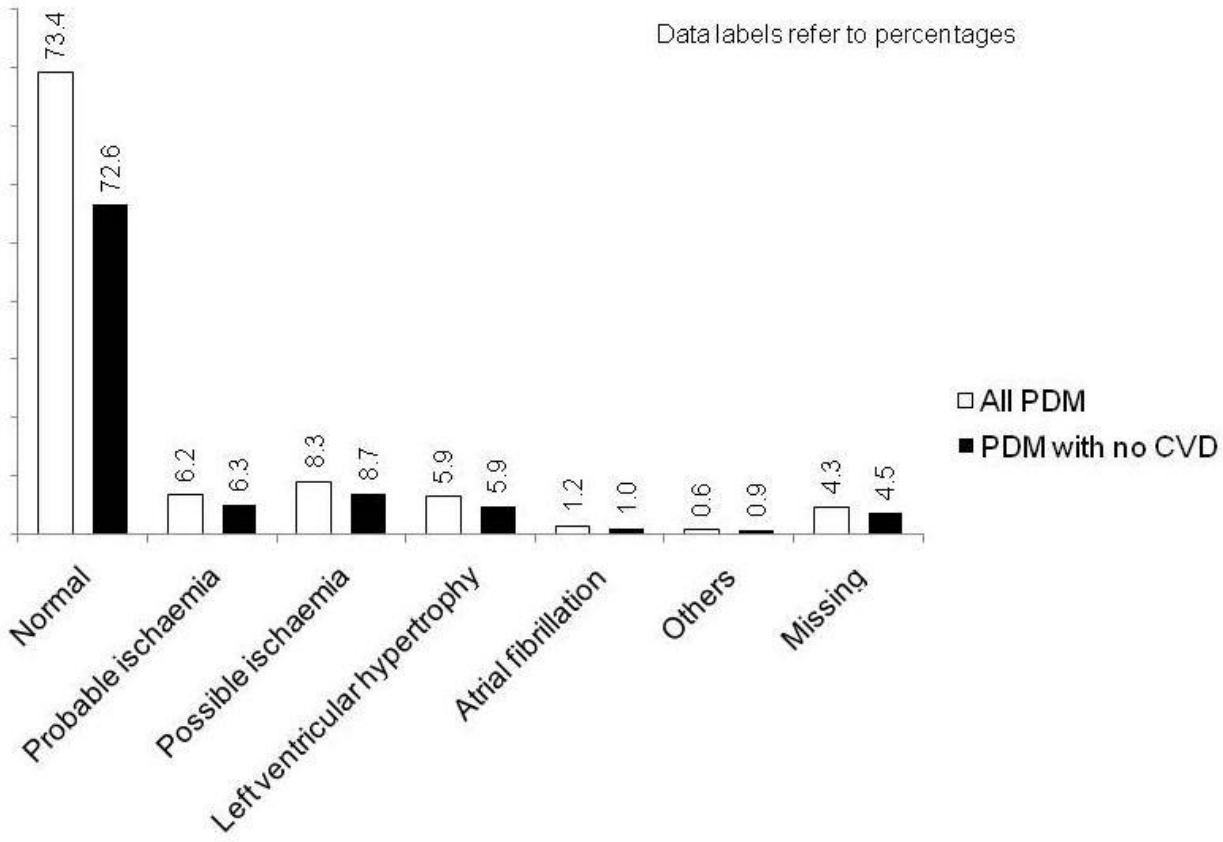


Table 5.13. Microalbuminuria and CKD stages in different categories of PDM

CVD risk factors	i-IFG	i-IGT	IFG+IGT	p value
ACR (mg/mmol)	0.75 (1.5- 1.35)	0.8 (0.5- 1.5)	1.0 (0.6- 2.0)	0.09
eGFR (ml/min)	74.0 (±13.1)	73.5 (±14.4)	73.4 (±14.5)	0.915
Microalbuminuria	23 (12.4)	78 (10.6)	23 (16.0)	0.178
CKD stages 3,4 and 5	22 (11.6)	101 (15.1)	23 (15.5)	0.298

Data presented as median (IQR) for ACR (not normally distributed), mean (SD) for eGFR and numbers (%) for microalbuminuria and those with CKD stages 3, 4 and 5.

5.5 Biomarkers

Adipose tissue is the largest endocrine organ in the body. Adipose tissue produces various biologically active substances called adipocytokines that play an important role in energy homeostasis. These adipocytokines have a complex interaction amongst themselves and with the pancreatic beta cells leading to modulation of vascular functions such as vascular tone, vascular smooth muscle contraction, arterial intimal inflammation and thus atherosclerosis. It is widely believed that this reaction also contributes to increased IR leading to pathogenesis of T2DM.

However the role of these biologically active proteins in the latent PDM stage is still largely unknown. Moreover, ethnic specific differences and influence of family history on these biomarkers are not yet known in those with PDM.

In this section, levels of biomarkers such as adipocytokines, insulin and CRP are compared between the various categories of PDM. Storage samples for these biomarkers were collected in a random selection of subjects with PDM. The difference in the numbers of samples analysed for various bio markers is explained by the non availability of sufficient sample for analysis.

5.5.1 Biomarkers

The correlation between various adipocytokines and other demographic and anthropometric variables of interest used in further regression analysis is shown in Table 5.14

Those with significant correlations are further used as covariates in regression models (Table 5.16).

The unadjusted plasma levels of various biomarkers in the fasted state are tabulated in Table 5.15. Adiponectin is negatively associated with glycaemia whereas IL6, CRP, adiponectin and Leptin are positively associated with glycaemia. TNFα is also lower in those with PDM; however this is statistically not significant. These were further adjusted for confounders such as demographic variables, markers of glycaemia (fasting glucose), triglycerides and fasting plasma insulin using linear regression. Analysis of co variance (ANCOVA) method was used to compare between NGT and PDM.

Table 5.14. Correlation between various biomarkers and anthropometric measurements

	TNF	IL6	Adipo	Ins	Lept	Vit D	CRP	Age	Sex	Ethn	BMI	WC	SBP	DBP	FPG	PPPG	HbA1	TC	TG	LDL	HDL
TNF	--	0.19[a]	0.11[a]	0.10[a]	0.10[a]	-0.03	0.10[a]	0.12[a]	0.03	0.01	0.06	0.07[a]	0.03	-0.02	0.02	-0.02	0.13[a]	0.01	0.10[a]	0.01	-0.06
IL6		--	-0.13[a]	0.27[a]	0.24[a]	-0.18[a]	0.47[a]	0.10[a]	0.00	0.07[a]	0.26[a]	0.23[a]	0.08[b]	0.01	0.18[a]	0.23[a]	0.17[a]	-0.18[a]	0.06	-0.16[a]	-0.15[a]
Adipo			--	-0.35[a]	0.21a	0.19[a]	0.02	0.38[a]	0.36[a]	-0.32[a]	-0.02	-0.13[a]	0.10[a]	-0.06	-0.13[a]	-0.19[a]	-0.15[a]	0.20[a]	-0.22[a]	0.12[a]	0.49[a]
Ins				--	0.37[a]	-0.15[a]	0.20[a]	-0.08[a]	-0.05	0.11[a]	0.45[a]	0.40[a]	0.05[a]	0.14[a]	0.31[a]	0.27[a]	0.24[a]	-0.15[a]	0.30[a]	-0.16[a]	-0.31[a]
Lept					--	-0.04	0.37[a]	0.06	0.62[a]	0.04	0.53[a]	0.18[a]	-0.03	-0.01	0.06	0.13	0.16[a]	0.01	0.10[a]	-0.04	0.11[a]
Vit D						--	0.01	0.30[a]	0.08	-0.61[a]	0.03	0.00	0.12[a]	0.04	-0.06	0.01	-0.22[a]	0.09	-0.13[b]	0.11[b]	0.13[b]
CRP							--	0.02	0.16[a]	-0.08[a]	0.36[a]	0.25[a]	-0.01	0.03	0.06	0.11[a]	0.08[a]	0.00	0.11[a]	0.01	-0.06
Age								--	-0.02[a]	-0.36[a]	0.06[a]	0.13[a]	0.41[a]	0.07[a]	0.17[a]	0.18[a]	0.20[a]	0.14[a]	0.08[a]	0.08[a]	0.15[a]
Sex									--	-0.01	0.03[a]	-0.35[a]	-0.17[a]	-0.12[a]	-0.21[a]	0.06[a]	0.01	0.09[a]	-0.14[a]	0.02	0.34[a]
Ethn										--	-0.07[a]	-0.09[a]	-0.17[a]	-0.06[a]	-0.01	0.06[a]	0.14[a]	-0.20[a]	-0.02	-0.15[a]	-0.20[a]
BMI											--	0.78[a]	0.11[a]	0.22[a]	0.20[a]	0.20[a]	0.17[a]	0.04[a]	0.30[a]	0.05[a]	-0.22[a]
WC												--	0.17[a]	0.23[a]	0.28[a]	0.17[a]	0.15[a]	0.00	0.33[a]	0.04[a]	-0.33[a]
SBP													--	0.71[a]	0.20[a]	0.18[a]	0.10[a]	0.12[a]	0.16[a]	0.08[a]	0.03[a]
DBP														--	0.14[a]	0.14[a]	0.03[a]	0.12[a]	0.19[a]	0.10[a]	-0.04[a]
FPG															--	0.33[a]	0.34[a]	0.04[a]	0.14[a]	0.05[a]	-0.11[a]
PPPG																--	0.26[a]	0.00	0.20[a]	-0.02	-0.10[a]
HbA1																	--	0.04[a]	0.13[a]	0.04[a]	-0.09[a]
TC																		--	0.32[a]	0.92[a]	0.32[a]
TG																			--	0.21[a]	-0.37[a]
LDL																				--	0.10[a]
HDL																					--

a. Significant at 0.01 <level (2 tailed)
b. Significant at <0.05 level (2 tailed)

Composite CVD correlated with IL6 (0.14, P<0.0001)) and Leptin with smoking status (-0.19, P<0.001). Other biomarkers were not correlated significantly with CVD or smoking status.

Table 5.15. Geometric Mean (SD) of unadjusted adipocytokines between NGT and PDM groups

	NGT (444)	PDM (683)	p value
TNF (pg/ml)	1.43 (1.9)	1.48 (1.8)	0.446
IL6 (pg/ml)	1.81 (1.8)	2.19 (1.9)	**<0.001**
Adiponectin (ug/ml)	15.17 (2.0)	11.66 (1.8)	**<0.001**
Leptin (ng/ml)	14.05 (2.4)	13.74 (2.4)	0.678
CRP (mg/l)	1.44 (4.1)	1.97 (4.2)	**<0.001**

Levels of TNFα, Leptin, IL6 and CRP adjusted for demographic variables are significantly higher in those with PDM compared to NGT and levels of adiponectin are lower. Such a significant difference is not seen for TNFα (Model 1 in Table 5.16). When adjusted for both demographic variables and triglycerides, TNF, IL6, Leptin and CRP and significantly higher in those with PDM and adiponectin is lower.

However, in the final model adjusted for glycaemia (using fasting plasma glucose) and insulin resistance (using fasting insulin), this significance persists only for IL6, CRP and adiponectin (Model 4 in Table 5.16). These differences also persist when BMI is substituted for plasma insulin (Model 5 in Table 5.16). Thus levels of CRP and IL6 are significantly higher in those with PDM compared to NGT and adiponectin levels are significantly lower even after adjusting for confounders.

Table 5.16. Geometric means (95% CI) of adipocytokines in those with NGT and PDM adjusted using linear regression models

		NGT (444)	PDM (683)	p value
Model 1 Demographics (Age, sex, ethnicity, smoking, WC)	TNFα (pg/ml)	1.45 (1.36- 1.54)	1.45 (1.38- 1.53)	0.988
	IL6 (pg/ml)	1.81 (1.74- 1.96)	2.18 (2.07- 2.31)	**<0.001**
	Adiponectin (ug/ml)	15.55 (14.80- 16.30)	11.06 (10.59- 11.54)	**<0.001**
	Leptin (ng/ml)	14.85 (14.0- 15.70)	13.07 (12.7- 13.70)	**0.002**
	CRP (mg/l)	1.50 (1.32- 1.70)	1.91 (1.71- 2.13)	**0.005**
Model 2 (Demographics and Log Triglycerides)	TNFα (pg/ml)	1.47 (1.41- 1.56)	1.44 (1.37- 1.52)	0.686
	IL6 (pg/ml)	1.81 (1.70- 1.92)	2.18 (2.07- 2.30)	**<0.001**
	Adiponectin (ug/ml)	15.18 (14.54- 16.04)	11.22 (10.76- 11.71)	**<0.001**
	Leptin (ng/ml)	14.94 (14.09- 15.13)	13.00 (12.37- 13.67)	**0.001**
	CRP (mg/l)	1.51 (1.33- 1.72)	1.90 (1.70- 2.12)	**0.011**
Model 3 (Model 2 and Log Fasting Insulin)	TNFα (pg/ml)	1.47 (1.38- 1.56)	1.44 (1.36- 1.52)	0.624
	IL6 (pg/ml)	1.83 (1.72- 1.86)	2.16 (2.06- 2.28)	**<0.001**
	Adiponectin (ug/ml)	15.09 (14.31- 15.79)	11.37 (10.90- 11.34)	**<0.001**
	Leptin (ng/ml)	15.38 (14.56- 16.26)	12.71 (12.12- 13.39)	**<0.001**
	CRP (mg/l)	1.52 (1.34- 1.73)	1.89 (1.69- 2.11)	**0.016**
Model 4 (Model 3 and Fasting plasma glucose)	TNFα (pg/ml)	1.48 (1.38- 1.59)	1.43 (1.35- 1.51)	0.448
	IL6 (pg/ml)	1.82 (1.71- 1.94)	2.17 (2.06- 2.29)	**<0.001**
	Adiponectin (ug/ml)	15.27 (14.50- 16.09)	11.22 (10.74- 11.73)	**<0.001**
	Leptin (ng/ml)	15.53 (14.64- 16.48)	12.62(12.00- 13.26)	**<0.001**
	CRP (mg/l)	1.49 (1.30- 1.71)	1.92 (1.71- 2.16)	**0.011**
Model 5 (Model 4 with WC substituted by BMI)	TNFα (pg/ml)	1.47 (1.38- 1.57)	1.43 (1.35- 1.51)	0.547
	IL6 (pg/ml)	1.80 (1.69- 1.91)	2.19 (2.08- 2.32)	**<0.001**
	Adiponectin (ug/ml)	15.21 (14.44- 16.02)	11.26 (10.77- 11.76)	**<0.001**
	Leptin (ng/ml)	15.20 (14.37- 16.07)	12.86 (12.26- 13.49)	**<0.001**
	CRP (mg/l)	1.48 (1.25- 1.64)	1.96 (1.75 2.21)	**0.001**

5.5.2 Ethnic differences in biomarkers in those with PDM

With a linear regression model using age, sex, triglycerides, smoking, waist circumference, FPG and FPI it is seen that SA ethnicity is associated significantly with higher TNFα, IL6, Leptin and lower VD. People with PDM of SA ethnicity have a significantly lower VD compared to the WE group (Difference in the mean (95% CI) being -1.14 (-1.25 to -1.03); on the other hand IL6, TNFα and Leptin are significantly higher in those of SA ethnic origin after adjusting for confounders (Table 5.17).

Table 5.17. Linear regression model showing the influence of ethnicity on adipocytokines

Variables	R	Unstandardised coefficient (B)	95% (CI)	p value
TNF	0.22	1.08	1.06 to 1.30	**0.012**
IL6	0.354	1.36	1.19 to 1.55	**<0.001**
Adiponectin	0.554	-1.09	-1.20 to 1.02	0.106
Leptin	0.730	1.32	1.16 to 1.51	**<0.001**
CRP	0.327	1.16	-1.15 to 1.51	0.300
Vitamin D	0.654	-1.14	-1.25 to -1.03	**<0.001**

5.5.3 Influence of family history on biomarkers

Presence of family history of T2DM especially first degree relatives is an important risk factor for the development of T2DM. However the exact role is as yet unknown. Gene interactions on environmental factors such as dietary habits and physical activity to increase insulin resistance are possible mechanisms. Table 5.18 shows independent association of biomarkers with presence of first degree family member with T2DM. TNF, CRP and VD are all significantly associated with the presence of family history. However, on adjusting the models for confounders such as age, gender, ethnicity, plasma insulin, plasma glucose, smoking and triglycerides only IL6 (r= 0.365, B= 1.09, 1.01 to 1.1, p= 0.026) and CRP (r= 0.334, B= 1.20, 1.02 to 1.41, p= 0.030) remain significantly associated with family history. This suggests of family history (thereby genetic predisposition) possibly modulates through inflammatory cytokine cascade early in the pathogenesis of T2DM.

Table 5.18. Linear regression model showing independent association between biomarkers and the presence of first degree relative with T2DM in those with PDM

Variables	R	Unstandardised coefficient (B)	95% (CI)	p value
TNF	0.033	-1.03	-1.10 to 1.04	0.388
IL6	0.074	1.07	1.00 to 1.01	0.056
Adiponectin	0.037	-1.03	-1.10 to 1.03	0.331
Leptin	0.121	1.17	1.06 to 1.30	**0.002**
CRP	0.097	1.23	1.04 to 1.44	**0.011**
Vitamin D	0.113	-1.12	-1.23 to -1.02	**0.015**

5.6 Inter ethnic differences in PDM

The baseline biomedical profile is compared between the SA and WE ethnic groups in those diagnosed with PDM. To address and negate the effects of selection bias due to the different ages of eligibility for the study, a randomly selected group who are age and gender matched in the ethnic groups are compared.

5.6.1 Differences in baseline characters between WE and SA with PDM

In a population matched for age and sex, SA have a significantly lower WC [(96.2 (10.4) vs. 100.9 (14.3), P<0.001] and BMI [28.9 (4.9) vs. 30.6 (5.6), P<0.001] compared to WE group. People of WE origin have a significantly higher WC, BMI and body fat. However, it is important to note that SA have a higher 120 minute post load glucose and HbA1c. Modelled CVD risk is similar between the groups (Table 5.19). WE also have a significantly lower 120 minute plasma glucose and HbA1c. Adjusting for waist circumference, this significance persists for 120 minute plasma glucose (-0.60, 95% CI: -0.31 to -0.89) and HbA1c (-0.37, 95% CI: -0.28 to -0.45). The total and LDL cholesterol are higher in the WE group. HDL cholesterol which is one of the markers for central obesity and metabolic syndrome is significantly lower in the SA group.

Table 5.19. Biomedical parameters between age and sex matched WE and SA groups with PDM

	WE (n=222)	SA (n=214)	p value
Age	56.1 (9.3)	55.8 (9.3)	0.772
BMI	30.6 (5.6)	28.9 (4.9)	**0.001**
WC (cm)	100.9 (14.3)	96.2 (10.4)	**<0.001**
Waist Hip ratio	0.91 (0.09)	0.91 (0.07)	0.902
Body fat (%)	36.3 (8.5)	34.4 (7.9)	**0.016**
Systolic Blood pressure (mm Hg)	139.9 (19.1)	141.1 (20.7)	0.544
Diastolic Blood pressure (mm Hg)	87.1 (10.8)	87.3 (11.3)	0.824
FPG (mmol/L)	5.6 (0.6)	5.7 (0.6)	0.373
120 min post load glucose (mmol/L)	8.2 (1.5)	8.8 (1.4)	**<0.001**
HbA1c (%)	5.8 (0.4)	6.1 (0.5)	**<0.001**
Total cholesterol mmol/L	5.7 (1.1)	5.2 (0.9)	**<0.001**
Triglycerides mmol/L	1.7 (0.9)	1.7 (0.9)	0.709
LDL cholesterol mmol/L	3.7 (1.0)	3.3 (0.8)	**<0.001**
HDL cholesterol mmol/L	1.3 (0.3)	1.2 (0.2)	**<0.001**
Framingham CVD risk (%)	14.3 (9.1)	14.5 (9.9)	0.803

Table 5.20. CVD risks age and sex matched WE and SA groups with PDM

CVD risk	WE (n=222)	SA (n=214)	P value
Microalbuminuria	13 (5.9)	33 (15.6)	**0.001**
CKD 3,4 and 5	20 (9.1)	18 (8.5)	0.826
Composite CVD	28 (12.6)	30.0 (14.0)	0.666
Framingham CVD risk >20%	45 (21.7)	53 (27.0)	0.215
Hypertension at screening (≥140/80 mm Hg)	165 (74.3)	169 (79.3)	0.215
Hypertensive medications	85 (39.0)	79.0 (33.5)	0.238
Lipid medications	38 (17.4)	25.0 (12.0)	0.111

In the same population, SA have a higher prevalence of microalbuminuria (15.6% vs. 5.9%) with a similar age, blood pressure, hypertension medications and composite CVD.

5.7 Discussion

T2DM is a recognised independent risk factor for CVD. Large studies such as the DECODE and meta analysis of epidemiological studies have established beyond doubt that hyperglycaemia in the intermediate stage between the present defined ranges of normal glucose and T2DM increase the mortality from CVD especially the 2 hour post glucose load plasma glucose (75;85;94). The prevalence of diabetes and IGT is projected to reach 9.8% and 10.9% in the productive age group of 20- 79 years in Europe and around 70% of those with IGT are thought to develop diabetes eventually, this is a an important public health problem (2).

This is a population based screening study for subjects with PDM, who have been rigorously phenotyped in a British multiethnic population. Furthermore, subjects with both IFG and IGT have been phenotyped separately where the vascular risk factors are comparable to those with newly screen detected T2DM.

Presently, IFG and/or IGT are not considered as a clinical disease condition but merely as risk factors for CVD. They represent a metabolic intermediate state between normoglycaemia and T2DM. HbA1c is only marginally elevated over the normoglycaemia group. Furthermore, IFG and IGT are two different abnormalities, the presence of both suggesting a higher CVD risk load. Therefore, identifying a group who may be at a higher risk of vascular complications compared to either IGT or IFG may be beneficial for implementing primary prevention strategies including glycaemic control that may reduce long term CVD risks.

People of SA ethnic origin have the risks of developing PDM and onwards to T2DM and thus CVD at a lower BMI and WC compared to those from the WE ethnic group. These points to a need for ethnic specific cut points for obesity and risk stratification. The prevalence of microalbuminuria is significantly higher in SA ethnic group compared to an age matched WE group. Whether this is a result of a need for different normal ranges for albumin excretion in ethnic groups or significance of early involvement of micro vasculature or the impact of non traditional CVD risk markers in the SA group is unknown. Biomarkers such as TNFα, Leptin and IL6 are significantly elevated and VD levels are lower in the SA group compared to those of WE origin. A

significant effect of ethnicity on adipocytokines in those with PDM is shown for the first time to our knowledge.

The overlap between IGT and IFG is limited, varying between 2-3% in epidemiological studies. IFG and IGT have different pathophysiological characteristics. IFG is dependent on hepatic insulin resistance and basal beta cell function whereas IGT depends on peripheral insulin resistance (109;327). Hence, subjects with both IFG and IGT may have two independent abnormalities of glucose metabolism. This is seen in some of the results from this cohort. The BMI, WC, body fat, systolic and diastolic blood pressures, triglycerides and total cholesterol/HDL ratio are significantly higher in subjects with combined IFG +IGT. In summary, many of the markers of the metabolic syndrome are higher in combined IFG+IGT.

It is therefore seen that in this population with PDM, over one third of the subjects are on medications for hypertension and hyperlipidaemia and one sixth have already had a cardiovascular event (includes myocardial infarction, cerebrovascular event, peripheral vascular disease, coronary artery or peripheral arterial interventions). Thus many of the markers of the metabolic syndrome are higher in IFG+IGT and incidentally the calculated CVD risk is also significantly higher. In previous findings from our cohort, carotid-femoral pulse wave velocity, a validated surrogate marker of CVD risk is seen to be raised in subjects with combined IFG+IGT (328). The vascular risk and the biomedical markers are similar in subjects with IFG+IGT and those with T2DM, but the latter group have far more advanced glycaemia, mean (SD) HbA1c (%) of T2DM group being 7.4 (1.8) vs. 6.1 (0.44), $p<0.0001$. In summary, the combined IFG+IGT group have a comparable CVD risk compared to newly screen detected T2DM patients. This places combined IFG+IGT as a phenotypically different group to i-IFG and i-IGT and T2DM.

Recently, two major trials in T2DM subjects, investigating the effects of HbA1c ≤ 6.5% on CVD outcomes noted conflicting results on all cause mortality (315;329). The ACCORD study showed an increase in all cause mortality in the intensively treated group (HR: 1.22, P=0.04). On the other hand, this was lower amongst the intensive group subjects in the ADVANCE trial (HR; 0.93, p=0.28). A major difference between the subjects is that the former had subjects with longer duration of diabetes compared to the latter (10 years vs. 7.9 years) and consequently subjects at higher risk of CVD. Hence, it is possible that a tight glycaemic control in advanced T2DM patients may be detrimental. Interventions for glycaemia at an earlier stage, possibly in the PDM

stages, in individuals at high CVD risk (mean 10 years risk ≥20%) may provide additional benefits on CVD outcomes. This remains to be seen in randomised controlled trials.

On the other hand, favourable vascular outcomes have been observed with tight glycaemic control in newly diagnosed T2DM patients after 10 years of follow up (314).This is in spite of HbA1c in the intervention group being similar to the control group after the initial intensive treatment phase leading to what is known as the legacy effect. Interventions for glycaemia at an earlier stage, possibly even for subjects with IGR with high CVD risk may provide additional benefits on CVD outcomes, this remains to be seen in randomised controlled trials.

It is also that CVD risk factors associated with hyperglycaemia are well established in the PDM group. In our cohort, the proportion of people who have a CVD risk greater than 20% is 29.7%. Of this, only 52.9% are on at least single medication for CVD. Of those with treatment recommended hypertension and hypercholesterolaemia irrespective of glycaemic category, only 20.6% and 34.6% on are on appropriate medication respectively.

According to present guidelines glycaemic management is not initiated for subjects with IFG and/or IGT. But screening for this group is useful as a vascular disease screening tool as this group of subjects have other modifiable and untreated CVD risk factors. Long term follow-up of this group and intervention studies to treat CVD risk factors along with glycaemic control are needed.

5.8 Summary

Screening for T2DM identifies subjects with intermediate stages of PDM. There are no clear guidelines recommending interventions to those with PDM apart from lifestyle changes . Our finding shows the atherogenic nature of these conditions and also identifies patients with untreated but potentially modifiable with CVD risk factors. Furthermore, IFG and IGT are two different abnormalities, the presence of both suggesting a higher CVD risk load. Therefore, identifying a group who may be at a higher risk of vascular complications compared to either IGT or IFG may be beneficial for implementing primary prevention strategies including glycaemic control, and broader cardioprotective treatment that may reduce long term CVD risks. People of SA ethnic origin are at higher risks of developing PDM and onwards to T2DM and thus

CVD at a lower BMI and WC compared to those from the WE ethnic group. These points to a need for ethnic specific cut points for obesity and risk stratification.

6 Progression from Prediabetes to Type 2 Diabetes

There is still ongoing debate as to the benefit of population based structured screening programme for the diagnosis of T2DM due to the costs and complexity of such programmes. Furthermore, the benefits of treating the large numbers of patients diagnosed from screening programmes are largely unknown. Such screening programmes will invariably generate a large proportion of people with PDM. Though benefits of lifestyle changes in people with PDM are well demonstrated, the efficacy and sustainability of such interventions in the mass population is as yet unproven. Identification of risk factors enable risk stratification of the group of people with PDM, and hence enable focused interventions to those at the highest risk. This also facilitates channelling of resources to people at higher risk of both CVD and progression to T2DM.

Due to the complex interaction of the diverse environmental, dietary, socio-cultural practices and the polygenic factors associated with T2DM, country and population specific epidemiological data are needed for instituting effective public health measures for this condition. There exists not just an inter ethic variation in terms of prevalence of T2DM and PDM, but also an intra ethnic difference depending on geographical location due to the influence of the environmental factors (15-17). Migrant SA in the UK are highly predisposed to cardio metabolic conditions compared to their WE counterparts (18-21). Ethnically relevant data in this field are scarce (330) and the health burden may be under estimated due to perceived barriers to research amongst the members of the SA community (18). There has been calls for research to determine ethnic specific transition to T2DM especially in a UK multiethnic setting (273). Hence this chapter also analyses progression rates amongst two different ethnic groups.

Previously published epidemiological studies in the UK reporting glucose intolerance have been in a predominant White European population (331;332), survey based (20) with screening being limited to high risk populations (32;82;332). The aim of this chapter is to report a population based screening and follow up data in a British multi ethnic population. A further aim is to determine differences in progression rates by anthropometric obesity related characteristics.

The chapter can be empirically divided into four sections- methodology, rates of follow up, factors determining progression to T2DM and finally discussion on appropriate strategy for follow up of people with PDM.

6.1 Methodology

6.1.1 Prediabetes cohort follow up- the ADDITION PLUS study

Subjects diagnosed with Prediabetes (PDM) at baseline are followed up annually on the ADDITION **P**rediabetes Fo**L**low **U**p **s**tudy (ADDITION PLUS). At baseline, subjects diagnosed with PDM were given an information booklet with healthy lifestyle advice as per current recommended guidelines.

All subjects with PDM were sent a pre screening questionnaire to ascertain that their condition had not progressed to T2DM. Information was also obtained on medications especially intake of steroids or oral hypoglycaemic agents in the pre screening questionnaire. If so, rescreening was performed after discontinuing the medications for at least 4 weeks or in the case of steroids, when maintenance doses were attained. Once eligibility was confirmed through pre screening questionnaire, participants were sent an appointment as described in the previous section. Measurements as tabulated in Table 4.4 and Table 4.5 were performed at these annual follow up sessions.

All participants with PDM were sent two postal invitations and failing to reply were contacted once over telephone to ascertain their interest to continue on the ADDITION PLUS. If patients failed to attend annual re screening appointment, an additional appointment was sent before being considered not to be interested. The respective General practitioners were informed of participants' non attendance at annual sessions. A response rate of 80% was achieved and 12 month glycaemic status was also obtained from the central chemical pathology database. Overall, follow up status was ascertained in 90% of the individuals with PDM at baseline. There were no significant differences amongst attendees and non attendees in terms of age, body mass index, blood pressure or glycaemic markers.

6.1.2 Data entry and quality assurance

The PDM annual follow up data was collected by the researcher with a double data entry input from an administrator to ensure accuracy. Data was subjected to a quality control using a 10% sample cross check.

6.1.3 Statistical analysis

6.1.3.1 Calculation of incidence (progression) rates

Incidence rates were initially calculated as cases/100 person years and further standardised by the direct method using the mid-year standard England and Wales population released by the Office for the National Statistics (333). A 'p value' of less than 0.05 was considered to be statistically significant. For categorical variables, binary logistic regression analysis was used to adjust for confounders.

The distribution of time to progression to T2DM between ethnic groups was compared using Kaplan-Meier survival function (334).

All analyses were performed using XLSTAT version 2009.4.05. The diagnostic indices of sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) are represented as percentages (95% confidence intervals). OGTT was considered as the gold standard test for the diagnosis of T2DM. The area under the ROC curve (AUC) between different diagnostic tests were compared by the methodology developed by Delong (335).

6.1.3.2 Logistic regression

Regression is a set of statistical technique that utilise the presence of a relationship between two variables and predict the values of one variable (dependant variable- DV) from those of the other (independent variable or regressor- IV). Logistic regression is a method to predict categorical membership i.e. the dependant variable is a categorical variable. It makes use of several predictor variables that may be either numerical or categorical.

Subjects who progressed to T2DM at 12 months were denoted as 1 and others were denoted as 0. Positive regression co-efficient denotes the probability of the categorical membership to increase and negative co-efficient denotes otherwise. The absolute value of the co-efficient denotes the magnitude of the relationship. The co-efficient will be interpreted as the change in the log of odds for a one unit increase in $X_{n,}$ when other IV are constant, or after adjusting for the other predictors. A Wald test was used to determine the significance of the regression co-efficient in the model. A p value of less than 0.05 was considered to be statistically significant. Exponent of B given the odds ratio for every IV and corresponding 95% confidence intervals were calculated for the standard error.

The *Hosmer Lemeshow test* was used a statistical test for goodness of fit of a model in a χ^2 distribution. Significant and smaller p values in this test denote a poor fit. A p value of greater than 0.05 was considered to be a good fit. The test assesses whether or not the observed event rates match expected event rates in subgroups of the model population (336). SPSS version 15 was used for regression analysis.

6.1.3.3 Construction of Logistic regression model

Variables including age, sex, ethnicity, waist circumference (BMI), known CVD, smoking, systolic and diastolic blood pressures and HbA1c were included in the regression model separately. The selections of these variables were based on known risk factors for T2DM. The variables used in the model are shown in Table 6.3.

6.1.3.3.1 Construction of model 1

The separate analyses of all the above variables were then included in a stepwise backward elimination procedure and model 1 was constructed with the variables whose p value was ≤0.20. This significance level was selected based on observations and reports that a traditional p value of 0.05 may miss important confounding variables in regression modelling (337). Then baseline glycaemic parameters such as FPG, 120 minute PG and HbA1c and markers of metabolic syndrome such as HDL cholesterol and triglycerides were added to model 1 in turn to determine the OR and thus the significance if either of these contribute to the model. Thus all the IV were adjusted for the relevant baseline demographics and anthropometric measurements.

HDL and triglycerides were log transformed as these were not normally distributed.

6.1.3.3.2 Construction of Final model

Significant variables from Model 1 (HbA1c, FPG and log triglycerides) were then used to construct a final model and various combinations of these variables were utilised to determine model fit. For each model ROC curves were also drawn to determine the area under the curve for the model (Table 6.4).

6.1.3.3.3 Regression model for biomarkers

The biomarkers measured at baseline were first analysed using a univariate model with T2DM at 12 months being the dependant variable (Table 6.7). Those with a significant p value <0.20 were then input into a more complex model adjusting for both demographic and biomedical confounders at baseline.

6.2 Results at follow up

6.2.1 Rate of follow up

Of the 1080 individuals identified with PDM at baseline, 12 month follow up data were available for 905 (83.8%) people (IFG=161, IGT=617, IFG+IGT= 127). No deaths were reported in the subjects. There were no significant differences between the attendees and the non-attendees in terms of baseline characters including age, BMI, waist circumference, FPG, PGLG, HbA1c, blood pressure and prevalence of CVD, microalbuminuria, and CKD (Data not shown). For the whole cohort, there was a significantly higher proportion of females (86.1% vs. 81.5%, P=0.039) and WE compared to SA (85.9% vs. 79.7%, P=0.012) who attended for follow up. SA attendees were of a significantly younger age compared to non attendees [51.8 (10.4) vs. 55.8 (12.5)].

6.2.2 Progression to T2DM

The median duration of follow up was 61 weeks (IQR: 56.4 to 66.4). 58 subjects were diagnosed with T2DM (6.4%), 364 (40.3%) continued to have IGR and 482 (53.3%) reverted to normal glucose metabolism. The progression rate for IFG was 5.51 cases/100 PY, IGT was 3.13 cases/100 PY and IFG+IGT was 14.46 cases/100 PY. Subjects with both IFG and IGT had a higher rate of progression compared to those with IFG alone (OR: 3.02, 95% CI- 1.41 to 6.45) or IGT (OR: 1.26, 95% CI- 0.67 to 2.36) alone.

Of those with T2DM at 12 months, 27 (46.5%) subjects had a FPG and 43 (74.1%) had 2 hour glucose in the diabetes range. Based on the protocol of the study, a second OGTT was required in asymptomatic individuals to confirm the diagnosis of T2DM. Based on this 33 people (3.7%) with PDM with diabetes range OGTT at 12 months were asymptomatic. Of the second OGTT, 4 (12.1%) were normal, 14 (42.4%) continued to have PDM and 15 (45.5%) had T2DM. Conversely, 15 (25.9%) of those with T2DM needed a second OGTT as they were asymptomatic of T2DM.

A significantly higher proportion of SA subjects progressed to T2DM compared to WE subjects [29 (12.1%) vs. 28 (4.3%); OR: 3.1, 95% CI- 1.80 to 5.33)] from the total PDM group [Age and gender adjusted OR: 2.69, 95% CI- 1.96 to 4.98 and OR adjusted for age, gender, WC and CVD: 3.09, 95% CI- 1.58 to 6.02]. The progression rate for different categories of PDM at baseline is illustrated in table 6.1 for WE and SA ethnic groups. Those with IGT at baseline, the odds of progression to T2DM (both crude and

after adjusting for age and gender) is significantly higher for SA compared to the WE ethnic group (Adjusted OR: 5.19, 95% CI: 1.92 to14.04). Of those with IFG or with IFG+IGT, there were no significant differences between the SA and WE ethnic groups in those who progress to T2DM or regress to NGT.

Figure 6.1 shows the survival function for the WE and SA ethnic groups. It is seen that SA have a significantly higher hazard function as determined by Log rank (Mantel Cox) test, (p=0.024) comparing the survival curves.

Figure 6.1. Kaplan- Meier survival curves showing cumulative hazard for SA and WE ethnic groups

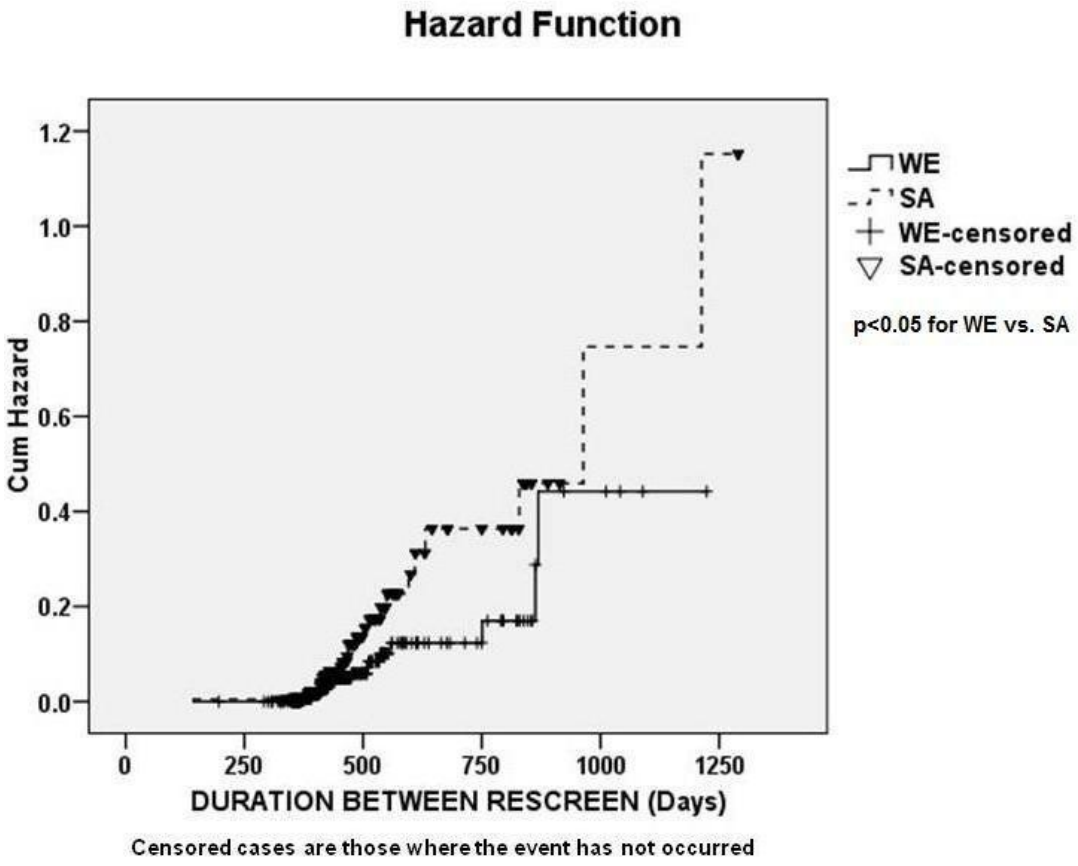


Table 6.1. PDM category at baseline and follow up for SA and WE ethnic groups

Ethnicity	Initial diagnosis (n)	Diagnosis at follow up	Frequency	Odds ratio SA vs WE (95% CI)	Adjusted OR SA vs WE (95% CI)‡
SA (239) •	IFG (36)	NGT	15 (41.7)	0.71 (0.34-1.51)	0.64(0.29-1.42)
		PDM	18 (50.0)	1.26 (0.58-2.64)	1.57 (0.70-3.50)
		T2DM	3 (8.3)	1.51 (0.37-6.20)	0.91 (0.18-4.64)
	IGT (165)	NGT	88 (53.3)	0.70 (0.49-1.00)	**0.56 (0.37-0.85)$**
		PDM	61 (37.0)	1.03 (0.71-1.49)	1.33(0.87-2.03)
		T2DM	16 (9.7)	**5.89 (2.47-14.04)***	**5.19 (1.92-14.04)***
	IFG+IGT (38)	NGT	13 (34.2)	1.51 (0.66- 3.46)	1.34 (0.55- 3.26)
		PDM	15 (39.5)	**0.45 (0.21- 0.98)$**	0.49 (0.21- 1.12)
		T2DM	10 (26.3)	2.01 (0.79- 5.10)	2.06 (0.76- 5.63)
WE (658) •	IFG (124)	NGT	62 (50.0)		
		PDM	55 (44.4)		
		T2DM	7 (5.6)		
	IGT (447)	NGT	277 (62.0)		
		PDM	162 (36.2)		
		T2DM	8 (1.8)		
	IFG+IGT (86)	NGT	22 (25.6)		
		PDM	51 (59.3)		
		T2DM	13 (15.1)		

* P<0.0001 $P<0.05 ‡Adjusted for age and gender ● Total number followed up (905) includes subjects from other ethnic groups. One subject who had follow up had no post load plasma glucose value hence was not classified to any group

6.2.3 Progression to T2DM for obesity related characteristics

The progression rates for the SA and WE ethnic groups for the quartiles of BMI and waist circumference (WC) are shown in Figure 6.2.

Progression rates (cases/100 PY) for the lowest quartile of BMI in the SA is 6.89 compared to 2.46 in WE. For the upper most quartile, the figures are 7.97 and 16.66 respectively for the WE and SA groups. The ethnic differences in crude progression rates (100 PY) were much more pronounced for the WC quartiles: 2.51 vs. 11.3 for the lowest quartile and 12.1 vs. 78.64 for the upper most quartiles for the WE and SA ethnic groups respectively.

Table 6.2 illustrates the odds ratio of progression for SA compared to WE for the BMI and WC quartiles. The unadjusted OR (95% CI) for the BMI quartiles were 5.5 (0.98 to 30.56), 3.13 (0.68- 14.43), 6.0 (1.73- 20.81) and 1.47 (0.50- 4.32) which after adjusting for gender and age were 6.30 (0.87- 45.76), 5.58 (0.99- 31.29), 4.62 (1.19- 17.94) and 1.43 (0.41- 4.99) for BMI quartiles 1 to 4 respectively. Similarly the unadjusted OR (95% CI) for the WC quartiles were 5.34 (0.86- 32.97), 5.37 (1.38- 20.91), 5.07 (1.42- 18.07) and 0.83 (0.23- 3.03). They were 7.86 (1.01- 61.42), 6.35 (1.46- 27.61), 4.68 (1.12- 19.56) and 0.50 (0.11- 2.19) after adjusting for gender and age. It is seen that the SA have a higher OR for progression compared to WE which is more pronounced for the lowermost quartile of both BMI and waist circumference.

Figure 6.2. Age and gender standardised progression rates for ethnic groups

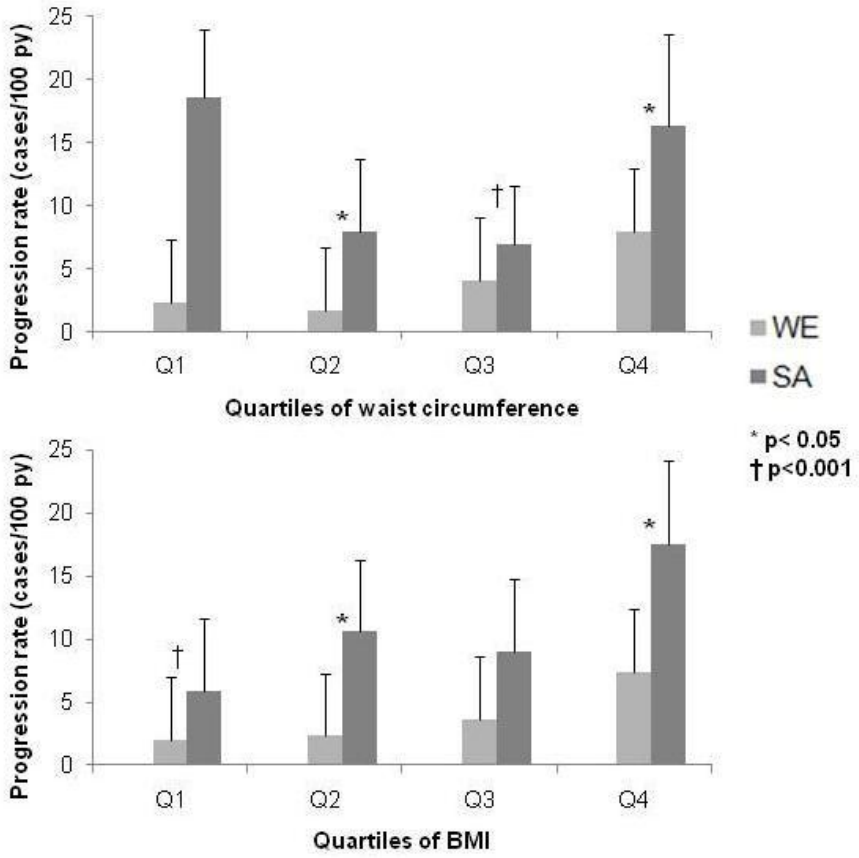


Table 6.2 OR for progression for BMI and WC quartiles for ethnic groups

BMI quartile	Unadjusted OR (95% CI)	P value	Adjusted OR (95% CI)‡	P value
Quartile 1	5.51 (0.98- 30.56)	0.05	6.30 (0.87- 45.76)	0.07
Quartile 2	3.13 (0.68- 14.43)	0.14	5.58 (0.99- 31.29)	0.05
Quartile 3	6.00 (1.73- 20.81)	0.00	4.62 (1.19- 17.94)	0.03
Quartile 4	1.47 (0.50- 4.32)	0.48	1.43 (0.41- 4.99)	0.58
WC quartile	**Unadjusted OR (95% CI)**	**P value**	**Adjusted OR (95% CI)‡**	**P value**
Quartile 1	5.34 (0.86- 32.97)	0.07	7.86 (1.01- 61.42)	0.05
Quartile 2	5.37 (1.38- 20.91)	0.02	6.35 (1.46- 27.61)	0.01
Quartile 3	5.07 (1.42- 18.07)	0.01	4.68 (1.12- 19.56)	0.03
Quartile 4	0.83 (0.23- 3.03)	0.78	0.50 (0.11- 2.19)	0.36

6.3 Factors determining progression to T2DM

6.3.1 Biomedical parameters

The methodology of construction of the regression model is detailed in section 6.1.3.2. Age, SA ethnicity (compared to WE), presence of previous CVD and WC significantly predict progression to T2DM at 12 months in the univariate analysis, CVD being the greatest positive predictor. BMI used in place of WC also significantly predicts progression and to avoid multi collinearity, both BMI and WC were not included in any model simultaneously, however odds ratios for both BMI and WC are given independently.

People of SA ethnicity had a significantly higher rate of progression to T2DM from PDM (OR: 3.10, 95% CI: 1.80- 5.34), and remained significant after adjustment for baseline difference in age, sex, CVD, WC, smoking and systolic blood pressure (OR:2.98, 95% CI:1.56- 5.68). Presence of CVD independently predicts progression by two fold but this significance is ameliorated in a model adjusted for age, sex, ethnicity and WC (Table 6.3).

In step 3 of Table 6.3, FPG, 120 minute PG, HbA1c, Log TG and log HDL are added to the step 2 separately. For example, OR illustrated are for FPG only added to the baseline model and thus FPG adjusted for baseline age, sex, CVD, WC, smoking and systolic blood pressure. Thus it is seen that 120 minute plasma glucose does not predict progression to T2DM at 12 months. On the other hand, each mmol/L rise in FPG and each percentage rise in HbA1c increase the risk of progression by 3 and 3.5 folds respectively. Similarly, each unit increase in triglycerides on the logarithmic scale increases the risk by two fold.

The final model as seen in Table 6.4 shows all the significant variables from Step 3 of Table 6.3 in a single regression model in various combinations i.e. Log TG in combination with FPG alone, HbA1c alone or all the three together. These variables are all adjusted for both anthropometric and biomedical confounders at baseline.

Table 6.3. Logistic regression model for progression to T2DM at 12 months

	OR (95% CI)	P value
Step 1. Separate analyses of demographic and anthropometric variables		
Age	0.97 (0.95- 0.99)	**0.011**
Sex	0.74 (0.43- 1.26)	0.261
Ethnicity		
SA vs. WE	3.10 (1.80- 5.34)	**<0.001**
CVD (yes vs. no)	1.98 (1.03- 3.87)	**0.04**
Smoking (yes vs. no)	1.08 (0.42- 2.82)	0.871
Waist circumference[1] (per cm)	1.03 (1.01- 1.05)	**0.008**
Systolic blood pressure[2] (per mm Hg)	0.99 (0.98- 1.01)	0.211
Step 2. Model 1		
Ethnicity		
SA vs. WE	2.98 (1.56- 5.68)	**0.001**
Other vs. WE	4.02 (0.41- 39.3)	0.232
CVD (yes vs. no)	2.04 (0.97- 4.25)	0.059
Waist circumference	1.05 (1.02- 1.07)	**<0.001**
Systolic blood pressure	0.99 (0.97- 1.01)	0.202
Step 3. Model 1 plus additional variables individually		
FPG[3]	3.11 (1.74- 5.56)	**< 0.001**
2hrPG	1.12 (0.96- 1.50)	0.11
HbA1c	3.53 (1.77- 7.02)	**< 0.001**
Log TG	2.26 (1.20- 4.26)	**0.012**
Log HDL	0.296 (0.08- 1.05)	0.059

1. Substituting BMI for waist circumference, OR for BMI are 1.06 (1.01- 1.11) (P=0.012).
2. Substituting diastolic BP for systolic BP, OR are 0.98 (0.97- 1.02) (P=084).
3. CVD becomes statistically significant when FPG is added to Model 1 (OR: 2.25 (1.05- 4.82, P=0.036)

Table 6.4. Final Model: with additional variables

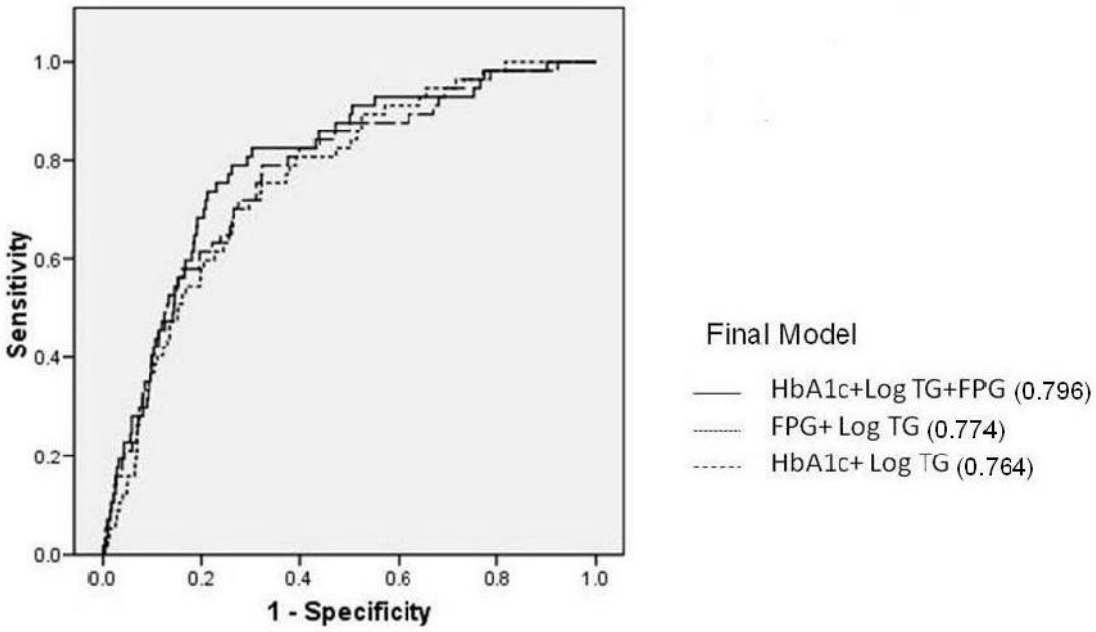
		OR	ROC curve	Hosmer Lemeshow		Predictions correct (%)
		(95% CI)	AUC (95% CI)	χ^2	P value	
Model 1	log TG	2.40 (1.25- 4.60)	0.774 (0.712- 0.835)	12.32	0.137	92.9
	FPG	3.47 (1.90- 6.36)				
Model 2	log TG	2.02 (1.05- 3.84)	0.764 (0.706- 0.822)	2.12	0.977	92.6
	HbA1c	3.44 (1.73- 6.86)				
Model 3	log TG	2.23 (1.16- 4.33)	0.796 (0.739- 0.854)	10.08	0.259	92.9
	FPG	2.76 (1.46- 5.24)				
	HbA1c	2.23 (1.08- 4.60)				

Table 6.4, shows that FPG, HbA1c and log triglycerides are independently associated with progression to T2DM at 12 months in whatever combination of these variables chosen. It is also seen that all the three models have a p value >0.05 on the Hosmer Lemeshow test suggesting adequate model fit. The area under the ROC curve for Model 3 is the highest with an AUC of 0.796 that incorporates all the three variables. Using model fit assessment gives a chi square of 10.08 and p value of 0.259 suggesting adequate model fit.

From the classification table this model predicts over 92% of those with PDM progressing to T2DM at 12 months correctly. This is further detailed in the classification plots in Appendix. These classification plots illustrate the predictive accuracy of the model. The U shaped curve suggests clustering of cases near the probabilities of 1 and 0 showing correct classification. On the other hand, a normal

shaped curve would have showed clustering around the probability of 0.5 suggesting increased misclassification.

Figure 6.3. ROC curves for the models in Table 6.4



6.3.2 Predictive properties of markers of metabolic syndrome

As seen from 6.3.2, WC, triglycerides and FPG all predict progression to T2DM at 12 months in addition to CVD and HbA1c. The former three parameters are components of the metabolic syndrome. The final common pathway of both T2DM and MS from the patient's perspective is the causation of hard end point i.e CVD. Thus further sub-analysis was performed to explore if the components of the metabolic syndrome (MS) predict progression using the IDF definition of MS (299). Accordingly, central obesity with ethnic specific WC cut off is an essential criteria along with the presence of two of the FPG, triglycerides, HDL cholesterol or blood pressure. The aim of this section is to develop a set of criteria for eligibility for Metformin therapy based on the ADA consensus and predictive properties of biomedical parameters at baseline.

The ADA consensus statement recommends treatment with Metformin in those with PDM who have a combined IFG and IGT with any one of the following- age <60 years, BMI≥ 35, family history of diabetes, elevated triglycerides, reduced HDL cholesterol,

hypertension and HbA1c> 6% (261). Except for HbA1c, the other criteria are also components of the MS.

Logistic regression model associating progression to T2DM at 12 months for components of MS independently and presence of MS is tabulated in Table 6.5.The lower section of the table also lists the predictive property of number of additional criteria (i.e. FPG, HDL cholesterol, triglycerides and blood pressure). Presence of metabolic syndrome in itself significantly predicts progression; but when this split into its components, metabolic syndrome with presence of both three and four additional criteria are highly predictive of progression whereas metabolic syndrome diagnosed with only two additional criteria does not predict progression.

Table 6.5. Association of Metabolic syndrome with progression to T2DM

Presence of condition	OR (95% CI)
Waist circumference (cm)	**3.01 (1.28 - 7.10)**
Triglycerides	1.44 (0.86 - 2.43)
HDL cholesterol	**2.16 (1.22 - 3.83)**
Fasting plasma glucose	**3.26 (1.23 -6.12)**
Blood pressure	**1.16 (0.61 -2.20)**
Metabolic syndrome	**2.67 (1.40 - 5.12)**
MS with 2 of the criteria	1.80 (0.82 - 3.97)
MS with 3 of the criteria	**2.80 (2.33 - 5.88)**
MS with all 4 criteria	**4.93 (2.17 - 11.23)**

The OR for progression for the presence additional conditions such as HbA1c >6%, CVD (that was significant from Table 6.3 and combined IFG+IGT are given in Table 6.6. Presence of CVD was considered to be either a modelled CVD risk >20% that would require treatment of CVD risk factors as per JBS guidelines or presence of pre existing cardio vascular disease.

In Step 2, combination of MS with these additional criteria are tested for predictive properties. In Step 3, the functioning model is combined with the presence of IFG+IGT to create the final model.

Considering the model proposed in Step 3, patients with either of the following criteria will be eligible for Metformin according to ADA recommendations.

1. Those with combined IGT and IFG.

2. Those with Metabolic syndrome and either HbA1c >6% or presence of CVD (Defined by either a modelled 10 year CVD score≥20% or pre-existing CVD).

Using this model, 63.8% of those who develop T2DM will have received Metformin. 31.6% of those with PDM at baseline will need to be treated with Metformin.

Table 6.6. Odds ratios for progression to T2DM for the presence of additional conditions and final model

Presence of condition	OR (95% CI)
Step 1	
HbA1c >6%	**3.74 (2.16 - 6.49)**
CVD	1.18 (0.69 - 2.40)
MS with >2 criteria (MS2)	**2.58 (1.51 - 4.41)**
Combined IFG+IGT	**4.69 (2.67 - 8.25)**
Step 2	
(HbA1c and CVD) *or* (MS2)	1.35 (0.75 - 2.44)
(Hba1c or CVD) *and* (MS2)	**3.41 (1.99 – 5.86)**
Step 3 Final Model	
Step 2 or Combined IFG+IGT	**4.76 (2.62 – 7.98)**

6.3.3 Biomarkers

The methodology of measurement of the biomarkers is detailed in section 5.2.3. The regression model for separate analysis of individual biomarkers independent of one another is shown in Table 6.7 . It is seen that crude measurements of TNFα, Adiponectin and Insulin all independently predict progression to T2DM at 12 months, all except adiponectin are positively associated with progression. Based on Mickey *et al* (337), all biomarkers with a p value of less than 0.20 were analysed for significance in the second stage (TNF-α, Adiponectin, Insulin, Leptin, VD and CRP).

Table 6.7. Initial model using biomarkers as independent predictors for progression to T2DM at 12 months

Biomarker	Odds Ratio (95% CI)	P value
TNFα	1.09 (1.00- 1.18)	**0.044**
IL 6	1.01 (0.87- 1.17)	0.991
Adiponectin	0.94 (0.90- 0.99)	**0.032**
Leptin	1.01 (0.99- 1.03)	0.197
Vitamin D	0.99 (0.98- 1.0)	0.093
CRP	1.03 (0.99- 1.07)	0.097

6.3.3.1 TNFα

TNFα is associated with progression to T2DM both independently as well as adjusted for age, sex, ethnicity, smoking, baseline diagnosis and WC at baseline. Using Insulin resistance as measured by HOMA-IR in place of WC does not change the association of TNFα. Composite CVD was also added in Model 3 when the association of TNFα on progression is negated. As the presence of CVD in Model 3 is not significantly associated with progression to T2DM (OR: 0.18, 95% CI: 0.17 to1.14), it is reasonable to assume that CVD in the regression model does not significantly interact with TNFα. This effect may be due to multi co linearity effect between TNFα and CVD.

This effect of TNFα on progression may be due to its pro-inflammatory nature and the complex interaction of TNFα on both Insulin resistance and beta cell function with time.

Table 6.8. Regression models for TNFα predicting progression

Model	OR (95% CI)	p value	Correct prediction	HL p value*
Model 1	1.12 (1.03- 1.22)	**0.009**	92.6%	0.441
Model 2	1.12 (1.02-1.22)	**0.015**	93.0%	0.558
Model 3	0.97 (0.77- 1.24)	0.853	92.2%	0.884

Model 1: TNFα, adjusted for baseline age, sex, ethnicity, smoking, baseline diagnosis and WC

Model 2: TNFα, adjusted for baseline age, sex, ethnicity, smoking, baseline diagnosis and IR

Model 3: TNFα, adjusted for baseline age, sex, ethnicity, smoking, baseline diagnosis, WC and CVD

*Hosmer Lemeshow test

6.3.3.2 Interleukin-6 (IL-6)

It is seen from Table 6.7, that IL-6 is not associated with progression. Hence using consistent methodology further analyses were not performed.

6.3.3.3 Adiponectin

Adiponectin independently is associated with progression to T2DM (Table 6.7). Adiponectin appears to offer a protective function against progressing to T2DM. To avoid confounding by baseline characteristics, logistic regression models using various confounders at baseline was constructed. As depicted in Table 6.9, the OR for progressing to T2DM are less than unity for adiponectin; however these are not significant for various independent variables as listed below.

Table 6.9. Logistic regression models for Adiponectin predicting progression to T2DM at 12 months

Model	OR (95% CI)	p value	Correct prediction	HL p value*
Model 1	0.97 (0.90- 1.02)	0.215	92.8%	0.321
Model 2	0.97 (0.91- 1.03)	0.337	92.8%	0.713
Model 3	0.97 (0.91- 1.04)	0.377	92.6%	0.488

Model 1: Adiponectin, adjusted for baseline age, sex, ethnicity, smoking, baseline diagnosis and WC

Model 2: Adiponectin, adjusted for baseline age, sex, ethnicity, smoking, baseline diagnosis and IR

Model 3: Adiponectin, adjusted for baseline age, sex, ethnicity, smoking, baseline diagnosis, WC and CVD

*Hosmer Lemeshow test

Subjects who progressed to T2DM at 12 months had a significantly lower geometric mean (SD) levels of crude adiponectin [11.25 (1.8) vs. 9.15 (1.7), p=0.018; however this was not significant when adjusted for age, sex, ethnicity, smoking, baseline diagnosis and WC [11.06 (1.0) vs. 10.51 (2.2), p=0.48].

6.3.3.4 Insulin

Fasting plasma insulin independently predicts progression to T2DM at 12 months. As seen from Table 6.10, for every unit increase in plasma insulin, risk of progression to T2DM increases by 1.06 fold. HOMA-IR significantly predict progression but HOMA-β is not associated with progression. However, when these were adjusted for baseline age, sex, ethnicity and CVD, only plasma Insulin and HOMA-IR were significant predictors (Table 6.11)

Table 6.10. Logistic regression models for Insulin measures predicting progression independently

Variables	OR (95% CI)	p value
Insulin	1.06 (1.03- 1.10)	**<0.001**
HOMA-IR	1.28 (1.14- 1.48)	**<0.001**
HOMA-β	1.00 (0.99- 1.00)	0.251

Table 6.11. Regression models for Insulin measures adjusted for confounders

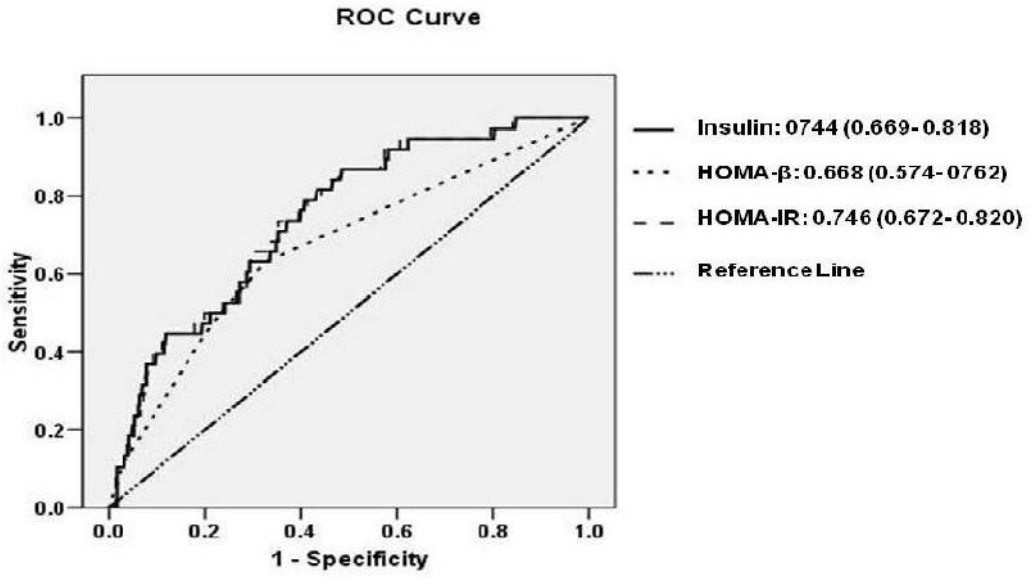
Model†	Variables	OR (95% CI)	p value	HL p value*
Insulin	Ethnicity (SA)	2.66 (1.34- 5.29)	**0.005**	0.344
	CVD (Yes)	2.51 (1.54- 2.50)	**0.022**	
	Insulin	1.06 (1.02- 1.10)	**0.002**	
HOMA-IR	Ethnicity (SA)	2.64 (1.33- 5.26)	**0.006**	0.214
	CVD (Yes)	2.59 (1.17- 5.672)	**0.019**	
	HOMA-IR	1.28 (1.13- 1.46)	**<0.001**	
HOMA-β	Ethnicity (SA)	2.66 (1.26- 5.60)	**0.010**	0.255
	CVD (Yes)	2.33 (1.07- 5.09)	**0.034**	
	HOMA-β	1.00 (0.99- 1.01)	0.568	

*Hosmer Lemeshow test for goodness of fit

† Age, sex, ethnicity and CVD were added to the model in a stepwise manner and the corresponding Insulin measure was force added to the model thus, variables with a significance level of >0.2 were not included

Figure 6.4 depicts ROC curves for the models illustrated in Table 6.11. It is seen that model with HOMA-IR has a marginally higher Area under the curve compared that with Insulin (non significant). However 93% of results were accurately predicted by both Insulin and HOMA-IR model.

Figure 6.4. ROC curves for different regression models with Insulin measures



6.3.3.5 Leptin, Vitamin D and CRP

Leptin, Vitamin D and CRP were not associated with progression to T2DM both univariate analysis and independently after adjusting for baseline age, gender, ethnicity, smoking, baseline diagnosis and CVD. Likewise there were no significant differences (both crude and adjusted) in Leptin, VD and CRP between those who progress to T2DM continues to have PDM and those who regress to NGT at 12 months.

6.4 Follow up strategy for people with PDM

6.4.1 Diagnostic indices of FPG as a screening tool at 12 months

Figure 6.5 shows the ROC curves for FPG and HbA1c used as a diagnostic screening tool for re-screening people with IGR. Figure 6.6 and Figure 6.7 show the plot between the sensitivity and specificity versus the criterion values for FPG and HbA1c respectively.

The AUC for FPG was 0.904 (95% CI: 0.848 to 0.960). The 95% confidence interval on the difference between the AUC and the arbitrary 0.5 value for the AUC was 0.348 to 0.460 (P<0.0001). The 0.5 value represents the AUC for random guessing. The AUC for FPG was calculated to be significantly higher (0.141, 95% CI: 0.13-0.16, P<0.0001) than that AUC for HbA1c. The sensitivity, specificity, PPV and NPV are tabulated in Table 6.12 for various cut off values of FPG and HbA1c.

An optimal threshold value for FPG as a screening tool was determined to be greater than or equal to 6.0 mmol/.L from the ROC curve. This gave a sensitivity of 88.2 (76.1-94.8), specificity of 77.8 (74.7-80.5) and a NPV of 99.0 (98.3-99.8). Overall 662 (73.3%) subjects had a FPG <6.0 mmol/L. Reducing the FPG threshold to 5.5 mmol/L improved the sensitivity to 94.1 (83.3-98.5) and the NPV being stable at 99.3, though only 48.3% had a FPG <6.0 mmol/L. Similarly, raising the cut off of FPG to 7.0 mmol/L gave an ideal specificity of 100 (99.4-100.0), PPV of 100.0 (100.0-100.0) and NPV of 96.7 (95.5-97.9), though at the cost of a low sensitivity of 48.2 (35.4-67.0). The PPV for the cut off value of 6.0 mmol/L was found to be low at 20.3 (15.0-25.6).

6.4.2 Diagnostic indices of HbA1c as a screening tool at 12 months

The AUC for HbA1c was 0.757 (95% CI: 0.679 to 0.836). The 95% confidence interval on the difference between the AUC and the arbitrary 0.5 value was 0.179 to 0.336 (P<0.0001). An optimal threshold value for HbA1c as a screening tool was determined to be greater than or equal to 6.0 % from the ROC curve. This gives a sensitivity of 80.8 (67.8-89.3), specificity of 56.3 (52.8-59.7) and a NPV of 97.8 (96.5-99.2). However, 53.9% of the subjects had a HbA1c <6.0 %.

6.4.3 Combination of HbA1c and FPG as a screening tool

The diagnostic indices of using both FPG and HbA1c are tabulated in Table 6.12. Overall 26.5% of the subjects had HbA1c and FPG to be less than 6.0.

We evaluated the combination of HbA1c and FPG in two ways: both FPG ≥6.0 and HbA1c≥6.0 and either FPG ≥6.0 or HbA1c≥6.0. Using the model of FPG ≥6.0 and HbA1c≥6.0 gave a very low sensitivity of 15.7 (8.0-28.4). The sensitivity improved to 92.2 (80.9-97.3) when the model FPG ≥6.0 or HbA1c≥6.0 was used in combination. The AUC for these models were 0.226 (0.0-0.590) and 0.253 (0.0-0.671) respectively. These were significantly lower than that of the criteria FPG≥6.0 [-0.604 (-0.64 to -0.57), and -0.577 (-0.62 to -0.54) respectively; P<0.0001 for both]

6.4.4 Diagnostic indices of HbA1c and FPG as a screening tool for WE and SA ethnic groups

The performance of FPG and HbA1c for a cut off of 6.0 mmol/L and 6.0 % respectively for the WE and SA ethnic groups are illustrated in Table 6.13. The sensitivity for a FPG cut off of ≥6.0 mmol/L is 81.5 (62.7-92.1) and 95.7 (77.0-100.0 for WE and SA ethnic groups respectively, whilst the NPV reaches 99% for both the groups. Using FPG ≥6.0 or HbA1c≥6.0 as the criteria improves the sensitivity in both

the ethnic groups; however 76.6% of the WE and 66.7% of SA would subsequently require an OGTT for confirmation.

Figure 6.5. Receiver-operating characteristic curves for fasting plasma glucose (FPG) and HbA1c for the diagnosis of T2DM using 1999 WHO criteria

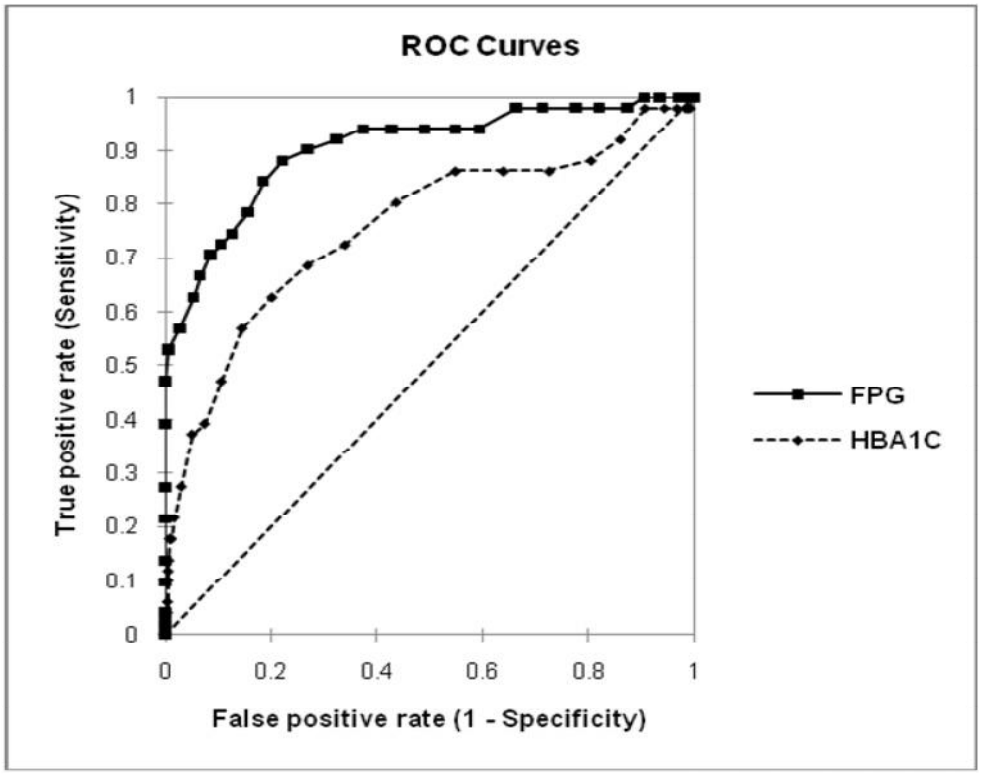


Figure 6.6. Plot of the sensitivity/specificity and the criterion values for FPG.

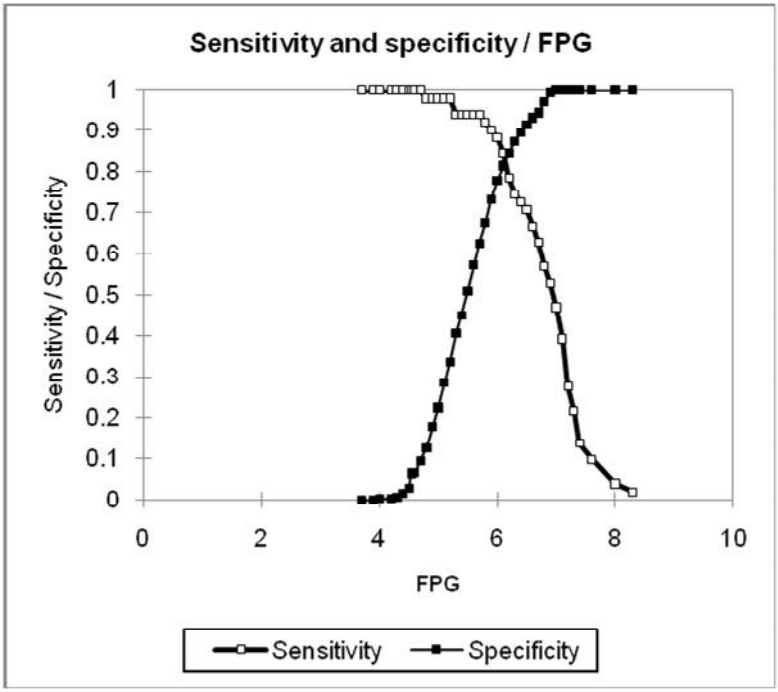


Figure 6.7. Plot of the sensitivity/specificity and the criterion values for HbA1c

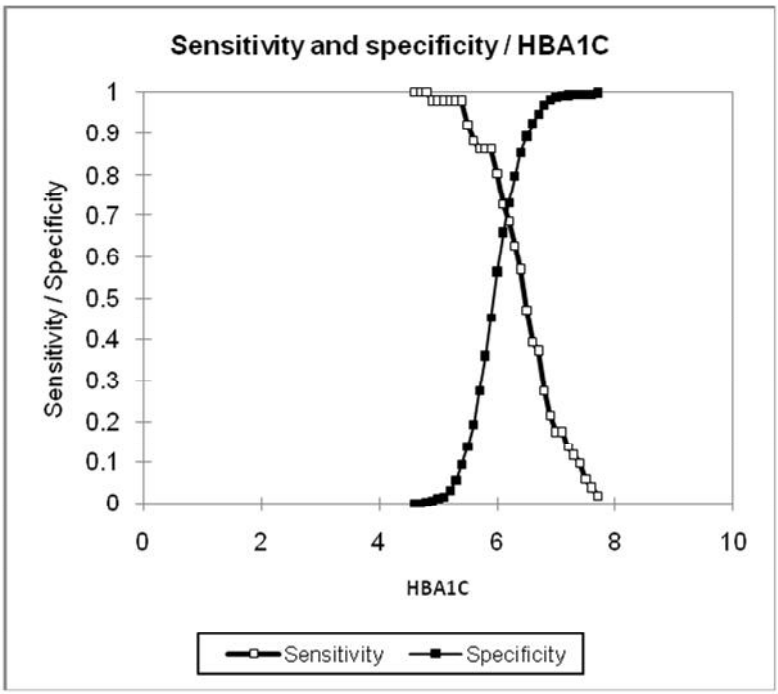


Table 6.12. Diagnostic parameters for different criteria using Fasting plasma glucose (FPG) and HbA1c

Criteria	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)	PPV (%) (95% CI)	NPV (%) (95% CI)	Positive LR (95% CI)	Negative LR (95% CI)
FPG ≥6.0	88.2 (76.1-94.8)	77.8 (74.7-80.5)	20.3 (15.0-25.6)	99.0(98.3-99.8)	4.0(3.4-4.7)	0.2 (0.1-0.3)
FPG ≥6.1	84.3 (71.6-92.0)	81.4 (78.5-84.0)	22.5 (16.6-28.4)	98.8 (97.9-99.6)	4.5 (3.8-5.5)	0.2 (0.1-0.4)
FPG ≥6.5	78.4 (65.1-87.6)	84.4 (81.7-86.8)	24.4 (17.8-31.0)	98.4 (97.4-99.3)	5.0 (4.1-6.3)	0.3 (0.2-0.6)
HbA1c ≥6.0	80.8 (67.8-89.3)	56.3 (52.8-59.7)	10.8 (7.7-13.8)	97.8 (96.5-99.2)	1.8 (1.6-2.2)	0.3 (0.2-0.6)
HbA1c ≥6.1	72.5 (58.9-82.9)	66.0 (62.6-69.2)	12.0 (8.4-15.6)	97.4 (96.1-98.7)	2.1 (1.8-2.6)	0.4 (0.3-0.7)
HbA1c ≥6.5	47.1 (34.1-60.5)	89.3 (87.0-91.3)	22.0(14.2-29.8)	96.3 (95.0-97.7)	4.4 (3.1-6.3)	0.6 (0.5-0.8)
FPG ≥6.0 and HbA1c≥6.0	15.7 (8.0-28.4)	93.5 (91.5-95.0)	13.3 (4.7-21.9)	94.5 (92.9-96.1)	2.4 (1.2-4.8)	0.9 (0.8-1.0)
FPG ≥6.0 or HbA1c≥6.0	92.2 (80.9-97.3)	28.0 (25.0-31.2)	7.6 (5.5-9.7)	98.2 (96.5-99.9)	1.3 (1.2-1.4)	0.3 (0.1-0.7)

Table 6.13. Diagnostic parameters for different criteria using Fasting plasma glucose (FPG) and HbA1c for SA and WE ethnic groups

	Criteria	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)	PPV (%) (95% CI)	NPV (%) (95% CI)
WE					
	FPG ≥6.0	81.5 (62.7-92.1)	78.6 (75.1-81.8)	15.0 (9.2-20.7)	98.8 (98.0-99.9)
	HbA1c ≥6.0	85.2 (66.7-29.1)	61.9 (57.9-65.7)	9.3 (5.7-13.0)	98.9 (97.8-100.0)
	FPG ≥6.0 and HbA1c≥6.0	11.1 (32.-29.1)	92.5 (90.0-94.4)	6.4 (0.0-13.4)	95.8 (94.1-97.4)
	FPG ≥6.0 or HbA1c≥6.0	85.2 (66.7-94.6)	24.3 (21.0-27.9)	4.9 (3.0-6.9)	97.3 (94.6-99.9)
SA					
	FPG ≥6.0	95.7 (77.0-100.0_	75.5 (69.1-80.9)	30.6 (19.9-41.2)	99.4 (98.1-100.0)
	HbA1c ≥6.0	73.9 (53.1-87.6)	40.7 (34.2-47.5)	12.3 (6.8-17.8)	(93.3 (88.0-98.5)
	FPG ≥6.0 and HbA1c≥6.0	21.7 (9.4-42.5)	97.1 (93.5-98.8)	45.5 (16.0-74.9)	91.7 (88.0-95.4)
	FPG ≥6.0 or HbA1c≥6.0	100.0 (82.7-100.0)	37.7 (31.4-44.6)	15.3 (9.6-21.1)	100.0 (100.0-100.0)

6.5 Discussion

6.5.1 Progression rates

This is one of the first reported prospective data for IGR subjects in a UK multiethnic cohort at the population level to our knowledge. This study has three unique features. Firstly, age being the only eligible criteria for entry and with the attendees and the non attendees at 12 months being similar, this is one of the true population based progression rates reported of its kind. Furthermore the diagnosis of IGT, IFG and T2DM is based on the present gold standard test, the OGTT and diabetes is confirmed on a second OGTT should the subject be asymptomatic. Secondly, the follow up rate was better than some of the previous population based studies conducted- over 80% (48;247). This is despite reports that there is a lower rate of participation in research amongst the SA for various reasons (18;19).

Thirdly, the differential effect of BMI and waist circumference on rates of progression for the ethnic groups is well demonstrated. It is not surprising to see the escalation of rates with an increase in BMI and waist circumference. However from figure 6.2, it is interesting to note that the progression rate for the lowest quartile of BMI and waist circumference is similar to that of the highest quartile of the WE. It can thus be inferred that SA have a comparable cardio-metabolic risk even at a lower BMI, hence the need for lower cut offs of BMI for obesity in the SA. This has to be taken into consideration when primary/secondary prevention strategies are instituted in a vascular risk modification programme where risk stratification takes into account of the BMI or waist circumference. Further SA are significantly younger. But it may be difficult to make direct comparisons between the ethnic groups with respect to the age as the entry criteria were different in the SA compared to WE.

It is well known that South Asians have a different phenotype compared to WE in terms of CVD risk profile. Data from the Indian Diabetes prevention programme shows the rate of progression from IGT to T2DM is 18.3% per year calculated from the 3 year cumulative rate of 55% (242). It is to be noted that this was in a population selected from a prior diagnosis of IGT based on capillary blood glucose. Hence, these subjects are likely to be at a higher risk compared to our cohort where the IGT subjects are drawn from the general population for follow up and thus this may explain the discrepancy in progression rates. Furthermore due to the SA who attended for follow up in our cohort being younger, the rates are likely to be an underestimation. Our 18 month cumulative data shows that the progression rate for SA is 12.5% and it looks likely that at 36 months our population will show a similar rate if not higher. Hence it is likely that rates will be higher for westernised South Asians compared to natives for a comparable population.

The progression rates for the WE is lower when compared to previous studies in a predominant white population (48;82). This is likely to be from the fact that the study by Rasmussen *et al* incorporated risk stratification using a questionnaire and the study by de Vegt included follow up of older subjects (48;247). Furthermore, the former study quotes progression rates for 155 diagnosed diabetes cases; however retesting was done only on 136 cases and only 62% of these were confirmed to have diabetes. A similar approach to reporting would increase the numbers in our cohort; presently data is presented only on the confirmed diabetes cases

Comparing progression rates from the control arm of intervention trials may be difficult as generally the studies have a BMI cut off that stratifies high risk people to the trials or include the higher risk group of both IFG and IGT (238;338). The participants in a clinical trial are also highly motivated for lifestyle modification that underestimates the population risk. Hence, extrapolating this result to the background population is difficult.

6.5.2 Factors determining progression

A significant finding of note is that age negatively predicts progression. Previous population based surveys have demonstrated that age is a significant risk factor for developing T2DM. Our finding may be explained by the fact that the population group is unique as they already have an established PDM and it is possible that age does not contribute to progression in addition to other confounders.

Presence of CVD and waist circumference is positively associated with progression independently. When adjusted for baseline demographics, only waist circumference and ethnicity predict progression. Adjusting for baseline WC, age and CVD, SA have a nearly threefold risk of developing T2DM from PDM at 12 months. After adjustment for baseline confounders, FPG and triglycerides increase the rise of progression by two fold and FPG by nearly threefold. TNFα at baseline both independently and adjusting for confounders at baseline is associated with risk of progression to T2DM at 12 months (OR: 1.12, 95% CI: 1.02-1.22). None of the other biomarkers were associated with progression.

Presence of metabolic syndrome with more than two additional criteria significantly predicts progression to T2DM. In the Diabetes prevention programme and the IDPS, there is reduction of 31% and 26.4% respectively in the incidence of T2DM using Metformin (238;242) and reduction in insulin resistance in other smaller trials involving PDM (339). Using the proposed model to identify patients who may benefit from Metformin and applying these findings with a conservative 25% reduction in the risk of developing T2DM, numbers

needed to treat using Metformin is 18.1. Metformin is inexpensive, relatively safe and has transient gastro intestinal side effects and treatment can be monitored in the primary care. Moreover, it is weight neutral and targets insulin resistance and has been shown to be cost effective even when accessed in long term health economic models (340-346). The cardiovascular protective properties of Metformin seen in the UKPDS and those reported from subsequent reviews in those with IGT makes this an attractive proposition (341;342;347-353).

There was significant difference in biomarkers other than TNFα between those with PDM and NGT. This finding supports the differential effect of various biomarkers at various stages in progression from NGT through to PDM to T2DM.i.e IL6, Leptin, CRP and adiponectin are perhaps associated with the development of PDM. But once PDM is established, these play a negligible role in further glycaemic deterioration. TNFα on the other hand is associated with progression to T2DM from PDM through complex interaction between TNFα and possibly modulating insulin resistance and beta cell function. This effect may possibly be due to immune modulating effects of TNFα on the adipose tissue, beta cells and the vascular endothelium. Further animal model and prospective studies are needed to confirm causative association. TNFα, IL6, Leptin and VD are significantly different between the WE and SA ethnic groups. These may partially account for the differences in the progression rates from PDM to T2DM between the ethnic groups.

Of particular interest is VD. Epidemiological studies demonstrate an inverse relationship between VD status and the risk of development of T2DM (354-359). There is evidence to suggest that VD may also have a role in endothelial dysfunction and other surrogate markers of cardiovascular disease (CVD) (360-366). The exact mechanism by which VD deficiency predisposes to this is unknown. Altered Calcium homeostasis in VD deficiency may impair the release of Insulin from pancreatic beta cells. Genetically mediated alterations in VD receptors and binding proteins for VD may play a role in insulin resistance and beta cell dysfunction and receptor polymorphisms are implicated in inherited susceptibility to cardiovascular disease and T2DM. SA have a lower VD than age matched WE controls, and given the role of VD in the complex atherogenesis cascade, may partly account for the increased risk of T2DM seen in this ethnic group (367-370).

Elevated levels on CRP have been shown to be associated with developing T2DM (210).This is some of the first data demonstrating differences in pro inflammatory markers between SA and WE ethnic groups in those with PDM. Previous studies have shown that Adiponectin is reduced in people of SA origin in NGT (371-373). However such a

relationship in those with PDM has not been demonstrated in a UK multiethnic population prior to this.

This study also shows that progression from PDM to T2DM is associated with Insulin resistance and not beta cell dysfunction. Previous studies have shown that sub clinical inflammation in PDM is related to insulin resistance and not beta cell function (374). It is possible that worsening insulin resistance leads to an exaggerated state of chronic sub clinical inflammation and leading to worsening beta cell function and thus T2DM. Beta cell function has not been shown to be significantly associated with progression to T2DM in our study. This is possibly due to the fact that only in this study, 41% of those who progressed to T2DM had plasma glucose in the diabetes range and only 3 subjects (5.4%) had plasma glucose over 8 mmol/L. Hence a significant effect of beta cell dysfunction is unlikely to have occurred explaining the absence of a relationship with beta cell function and progression to T2DM in this study.

6.5.3 Follow up people with PDM

This study to our knowledge is the first to propose screening models for follow up of subjects with IGR in a multiethnic population. A previous study in a predominant Caucasian population proposed using HbA1c as a screening tool. The confirmatory diagnosis of T2DM was based on only 120 minute post load glucose tests in this particular study (375). The sensitivity and specificity of using FPG and HbA1c to screen for T2DM in a high risk population are comparable to a previously published report (376), although the cohort of patients were different and was not defined by a previously diagnosed IGR. Other studies have investigated the use of HbA1c and FPG have all universally suggested the use of the combination of FPG and HbA1c, but the baseline population was different to ours (279;280;377). The background population in these studies consisted of a mixture of people with risk factors for T2DM such as obesity, gestational diabetes mellitus and elevated random plasma glucose. A recent study has adopted a combination of FPG and HbA1c as a screening tool in subjects with IFG and has validated this in a UK population (378).

Our data shows that the using the FPG cut off ≥6.0 identifies people with IGR who would subsequently require an OGTT for re screening. The negative predictive value of this cut off values is high i.e. 99.0 (98.3-99.8). We also show that a combination of FPG and HbA1c does not improve the model. The PPV for both of these FPG cut off value is fairly low. Nevertheless, we suggest that subjects above this chosen cut off value undergo a confirmatory test of OGTT as in the algorithm below.

This analysis has three unique features. Firstly, the IGR cohort detected in a population based structured screening programme and follow up of over 80% with no significant differences between attendees and non attendees, this data are likely to be representative of the background population. Secondly, the diagnosis of T2DM was based on a WHO recommendation of two OGTT if the subject is asymptomatic. Thirdly, the model performs well for both SA and WE ethnic groups and can be thus universally adopted in a UK multiethnic population.

The recently proposed NHS vascular check programme advocates a similar approach for screening for T2DM should the screening FPG is between 6 and 6.9 mmol/L for the general population aged 40-74 with risk factors such as body mass index ≥30 kg/m^2 or blood pressure ≥140.90 mm Hg (6). Use of HbA1c cut off of 6.5% has also been suggested in this health programme; however this study shows that HbA1c lacks sensitivity in the IGR population, though the NPV is comparable to that of FPG. Moreover 46.1% of people will need OGTT as compared to 26.7% should a cut off of HbA1c ≥6.5% be considered.

Thus, we propose a screening algorithm as illustrated schematically in Figure 2, based on the high negative predictive value of the FPG≥6.0 model. Using this model only 23.5% of subjects with IGR will require an OGTT after 12 months; 88.2% of subjects with T2DM will have a FPG ≥6.0 and will be identified. Subjects who will be missed will have a FPG<6.0 that is below the treatment threshold for T2DM. Subsequent follow up will identify these subjects.

A similar strategy adopted for HbA1c ≥ 6.0% has a sensitivity and NPV that is comparable to that of FPG≥6.0 model. But 53.9% of the population have HbA1c<6.0% who will then need an OGTT subsequently as this risk tool is based on a high NPV and thus T2DM can be ruled out should the HbA1c value be less than the cut off value. However, HbA1c has a great advantage in that the test is not time dependant and can be performed at any time of the day, making it an ideal test for opportunistic screening

FPG performs well as a screening tool in this high risk group possibly due to the fact that the majority of people in this cohort are insulin resistant by time of diagnosis of IGR. The driving factor behind the development of T2DM from this point onwards is deterioration in beta cell function (379). FPG being a good surrogate marker of beta cell function, it is probably a better screening tool than HbA1c in this selective population.

The annual rate of progression of IGR to T2DM may reduce over time. Hence, the functionality of the proposed model needs validating in longer term prospective studies.

6.6 Conclusion

Our population based universal screening approach shows a comparable result to previous risk based approaches for detection and follow up of subjects with PDM in terms of the prevalence of PDM and T2DM. A significant inter racial variation exists in the transition rate from the PDM state to T2DM with a higher rate for people of SA origin.

The risk threshold for BMI and WC is lower for SA and this needs consideration when these measurements are used to define diabetes risk cut off in South Asians. Data from this chapter supports the debate around the need to have ethnic specific cut points to define obesity. The recently introduced NHS Health Check Programme adopts a similar strategy for diabetes screening using a lower threshold for BMI for people of Asian origin (6). The important role of waist circumference in driving progression from PDM to T2DM especially in South Asians is also demonstrated from this study which is probably a better tool to define obesity in the SA ethnic group.

People with PDM constitute a unique high risk group who have a higher risk of progression to T2DM and cardiovascular disease. Hence, follow up of glycaemic status in this group is vital. Long term follow up of outcome studies have suggested earlier tight glycaemic control may lead to favourable long term cardiovascular outcomes. Risk stratification using simple tools will help better follow up rates and identify people at higher risk and likely to benefit from interventions for primary prevention of CVD. This thesis has shown that using a cut off value of 6.0 mmol/L for FPG, has the optimal sensitivity, specificity and a high NPV. Considering the need to repeat OGTT in asymptomatic individuals, only less than a quarter of high risk individuals would require an OGTT need to be performed (22%). This is likely to be significant reduction in general practice as the prevalence of PDM is around 16% based on previous reports from our population aged 40-75 years. Use of FPG alone as a screening tool is also likely to improve the follow up rate of people with PDM and is cost effective.

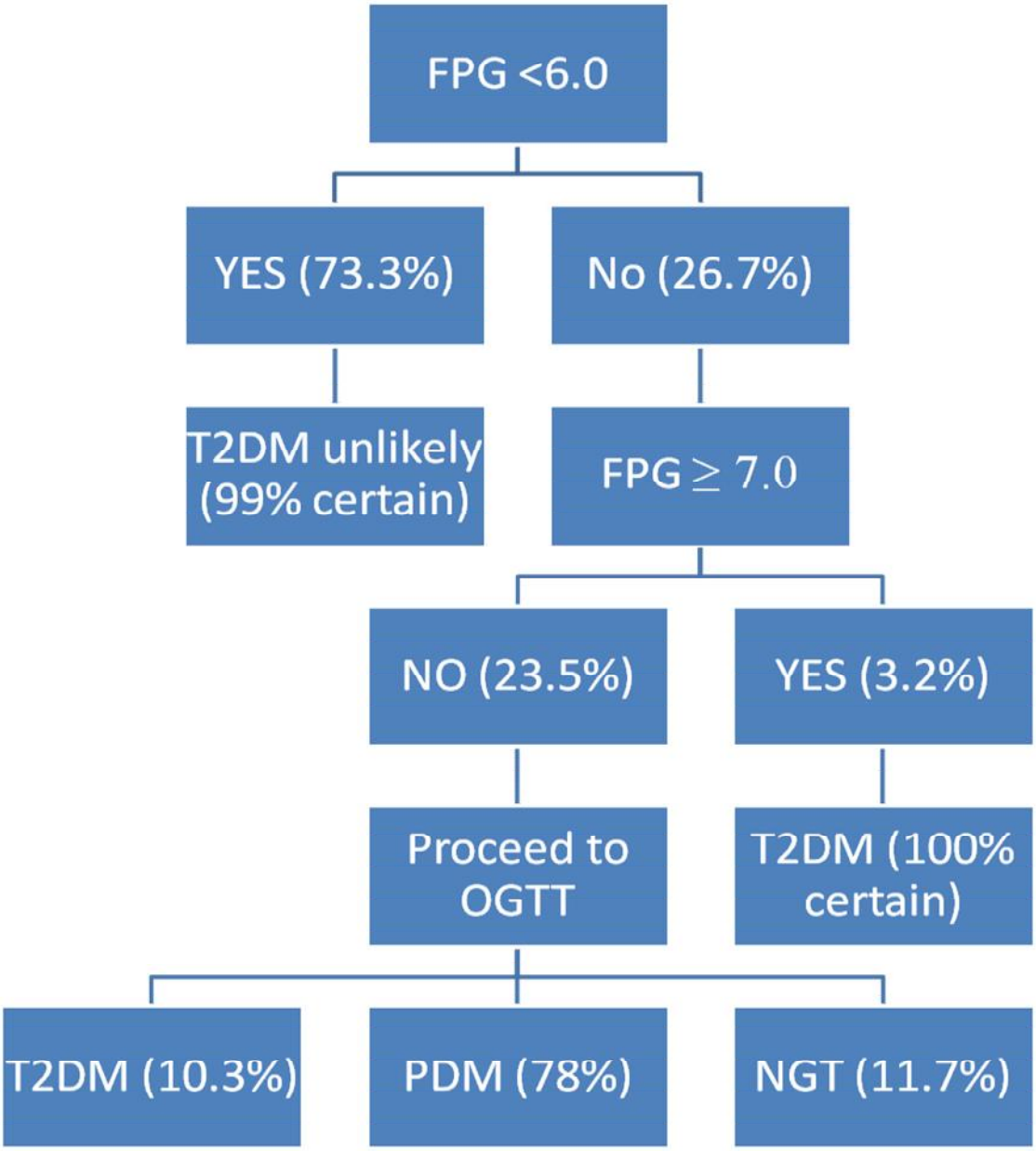
Thus we propose a screening algorithm as illustrated schematically in Figure 6.8, based on the high negative predictive value of the FPG≥6.0 model. Using this model only 23.5% of subjects with PDM will require an OGTT after 12 months; 88.2% of subjects with T2DM will have a FPG ≥6.0 and will be identified. Subjects who will be missed will have a FPG<6.0 that is below the treatment threshold for T2DM. Subsequent follow up will identify these subjects.

This study has also looked at the association of markers of chronic low grade inflammation and the risk of progression to T2DM from the PDM state. To our knowledge, this is the first prospective study that has shown association between adipocytokines and progression to

T2DM in multi ethnic population identified with PDM. The pro-inflammatory adipocytokine TNFα is associated with progression from PDM to T2DM at 12 months even after adjusting for confounders such as age, gender, ethnicity, WC and baseline diagnosis. However, this effect is attenuated when composite CVD is added to the model. These may be due to the significant correlation between CVD and TNFα. Adiponectin which has been previously demonstrated as to have a protective function has shown a similar association. For every unit reduction in adiponectin the risk of progression increases 1.28 fold (1.01- 1.64). Though age, sex, ethnicity and waist circumference influence adiponectin values, there appears to be no significant difference in the adiponectin levels between the SA and WE ethnic groups in this population. Other markers such as Leptin, IL-6 and CRP have not shown to be associated with progression to T2DM in this group. Fasting plasma Insulin and Insulin resistance (HOMA-IR) are also associated with progression to T2DM from PDM, the latter appears to be a better predictor.

The annual rate of progression of PDM to T2DM reduces over the years. Hence, the functionality of the proposed model needs validating in longer term prospective studies.

Figure 6.8. Stepwise screening algorithm for follow up of subjects with PDM



7 Conclusion

The perception of an intermediate stage between diabetes and normal glucose tolerance that is variable termed as Prediabetes/ Borderline diabetes/ Non diabetic disorders of hyperglycaemia was first put forward in the late 1960s. Since then studies have established a linear and a J shaped association between post prandial and fasting plasma glucose with risk of cardiovascular disease respectively. Prediabetes encompasses a spectrum of non overlapping conditions namely isolated IFG, isolated IGT and IFG+IGT.

Screening for T2DM and PDM concurrently identifies people who have an increased CVD risk and who are not presently offered any primary prevention for CVD. But it is seen that hypertension, hypercholesterolaemia and calculated 10 year Framingham risk score is high with nearly one third having a score ≥20% in this group and thus identifies patients with untreated but potentially modifiable CVD risk factors.

One size doesn't fit all and risk strategies must be tailored to the local population. The LRA score is seen to be robust in identifying those at risk of developing T2DM and PDM in a mixed ethnic population in the UK. This score is self estimated and thus only self referred people are screened using the OGTT, thus improving response rate. Adopting a combination of risk factor based screening using a validated tailor made risk score and an opportunistic screening strategy is likely to yield a good response rate at screening. Using fasting glucose as a screening tool for initial diagnosis is likely to miss those with IGT and thus is not recommended as a screening tool on its own.

The meta-analysis of 12 RCT and 10 epidemiological studies involving 13,314 participants showed a higher progression rate to T2DM in those with combined IFG+IGT (7.86 cases/100 PY) compared to those with IFG alone (6.29 cases/ 100 PY and IGT alone (7.48 cases/ 100 PY) with no significant differences. However, significant differences were demonstrated in progression rates in those in epidemiological studies 6.74 cases / 100 PY compared to those in RCT (8.25 cases/ 10 PY).

The prevalence of PDM is significantly higher amongst people of SA ethnic origin compared to that of WE origin in a mixed ethnic population in the United Kingdom. This prevalent risk is highest in resident population Nauru amongst published data. People of SA origin also are at risk of PDM at a lower BMI and waist circumference demonstrating the need for ethnic specific cut offs with respect to these measurements especially if they are to be used as a screening tool.

This study also has established the higher prevalence of both micro and macro vascular complications in those with PDM compared to NGT. Markers of sub clinical inflammation are elevated such as IL6, leptin and CRP in those with PDM compared to those NGT. On the other hand with adiponectin plays a protective role with a significantly lower level in those with PDM. Within PDM, IFG and IGT are two different abnormalities and those with combined IFG and IGT have a higher CVD risk load as well as progression to T2DM at 12 months time. Therefore, identifying a group who may be at a higher risk of vascular complications compared to either IGT or IFG may be beneficial for implementing primary prevention strategies including glycaemic control that may reduce long term CVD risks.

As the prevalence of PDM is 16%, identifying those as most risk of both progression to T2DM and CVD enables channelling of resources those who are at urgent need. Amongst those with PDM the following categories may be perceived to be at a higher risk when interventions are considered.

1. Presence of one diabetes range OGTT, but a diagnosis of T2DM cannot be made as these people are asymptomatic.
2. Presence of combined IFG and IGT
3. Presence of family history of T2DM
4. Ethnicity: South Asians compared to White Europeans

Using ADA consensus guidelines, less than 2.2% of this study population will be eligible to receive Metformin and would account for only 39% of those who develop T2DM at 12 months. A reframed criteria combining the ADA consensus statement and factors predicting progression, we propose that the following group with PDM would benefit from Metformin

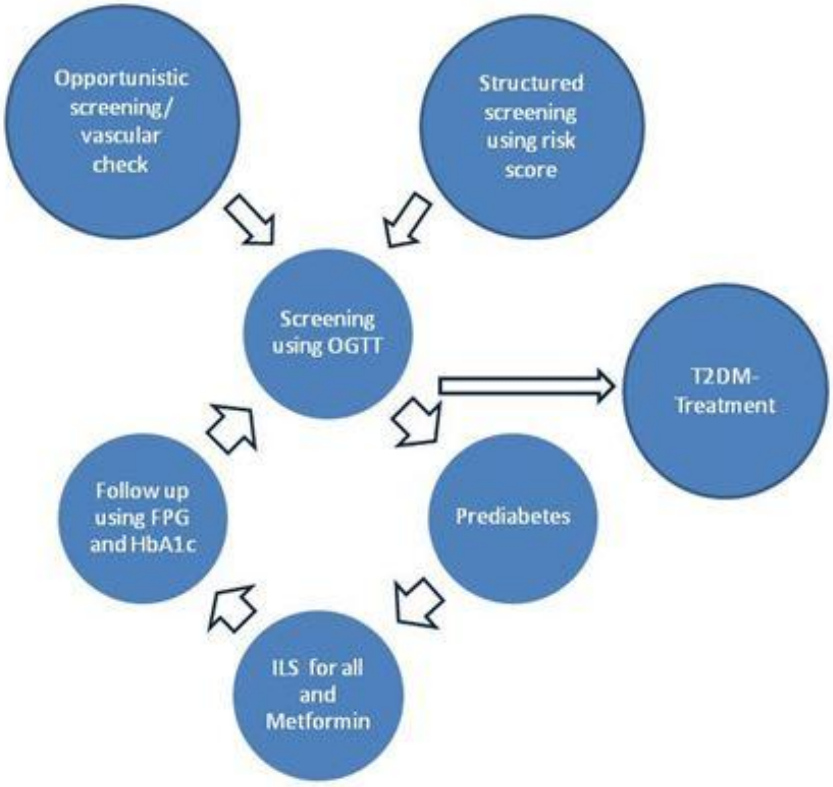
1. Those with combined IFG and IGT or
2. Metabolic syndrome with more than two additional criteria with either HbA1c >6% or presence of CVD.

Risk stratification using simple tools will help better follow up rates and identify people at higher risk. We have established that using a cut off value of 6.0 mmol/L for FPG, has the optimal sensitivity, specificity and a high NPV. Considering the need to repeat OGTT in asymptomatic individuals, only less than a quarter of high risk individuals would require an OGTT need to be performed (22%). There is likely to be significant reduction in general practice as the prevalence of IGR is around 16% based on previous reports from our

population aged 40-75 years. Use of FPG alone as a screening tool is also likely to improve the follow up rate of people with IGR. The recent WHO recommendation to utilise an HbA1c cut off ≥6.5% to diagnose TDM is also likely to improve response rates and simplify screening. However HbA1c <6.5% does not rule out T2DM (83).

We recommend the duration between follow up screening to be on an annual basis at least initially. This could be reduced to perhaps bi annually after 3 years of diagnosis and every three years after 5 years of diagnosis. The long term follow up data from the ADDITION PLUS is expected to offer more recommendations on the duration and interval between follow up screening.

Figure 7.1 Overall approach to identification and follow up of PDM



In order for any screening study to be successful, it is important that the general practitioners are well informed about the condition to be screened and structured guidelines are in place to ensure effective follow up of people diagnosed with the screened condition. In terms of PDM, previous survey has identified that though the general practitioners were aware of the diagnosis of IGT, they were uncertain about the clinical significance and methods of follow up of these identified individuals (380). Inclusion of screening for T2DM and PDM as part of

a vascular screening programme may improve both response rate and appropriate follow up and treatment of vascular risk factors (6). With childhood obesity becoming more prevalent, health education in schools and community centres as part of a national health awareness scheme may be an important step in reducing the worsening of the impending obesity pandemic. Life style interventions aimed at reducing or preventing T2DM need a sustained and long term commitment from individuals. Delivery of such interventions may be done through a structured education programme. The overall approach to identification and follow up of those with PDM in a mixed ethnic population is summarised in Figure 7.1.

This thesis has provided contemporary data on the prevalence of PDM and associated risk factors in a multiethnic population in the United Kingdom. Further, the natural history of progression to T2DM and factors predicting this progression have also been established. The ethnic variations in these data have also been demonstrated in a prospective setting. These findings have important implications in public health planning in prevention of T2DM.

8 Appendix 1: Contributions

The major part of this thesis is from the baseline screening data of the ADDITION study and the follow up data of the ADDITION PLUS study. BS (B Thiagarajan Srinivasan) was a clinical research fellow between September 2006 to September 2009 in the Department of Diabetes Research, University Hospitals of Leicester NHS Trust and Department of Cardio vascular Sciences, University of Leicester where these studies were conducted.

BS was involved in recruiting, baseline measurements and delivering multi factorial CVD risk reduction patients for the intensive intervention arm of the ADDITION study with Dr. David Webb who was also a Research fellow. The measurements were performed by qualified research nurses and nursing assistants. BS was actively involved in data collection and database designing and data quality control of the baseline data. Furthermore his role was also to review results and recommend action plans for abnormal results. On the ADDITION PLUS study BS was the sole clinical lead in following up of PDM patients annually with support from as administrator. Data entry was performed by BS doubly checked and entered by an independent administrator. BS was also involved in collection, storage and transport of frozen serum samples, procurement of analysis kits for biomarker analysis and research grant for the analysis of biomarkers. All samples were analysed at a collaborating centre over a 6 week laboratory attachment with supervision from a scientist.

BS (is the primary author for chapter 3; WC (Dr. Winston Crasto) is a qualified physician who is a Clinical Research fellow, Department of Cardiovascular sciences, University of Leicester who contributed to selection of studies, quality assessment and quality assurance of the data. Dr Laura Gray is a qualified statistician, Department of Health sciences, University of Leicester who performed the pooled data analysis. Mrs Mary Edmunds-Otter and Mrs Sarah Sutton are librarians who helped with designing of the search strategy and performing the literature search.

9 Appendix 2: Publications

9.1 Research Grants:

- Davies MJ, Khunti K, Samani NJ. *TRIMTOP Study- The Rimonabant in the Treatment of Prediabetes Study*- Sanofi-Aventis Pharmaceuticals. (£242,000-November 2006- Named Candidate)
- Davies MJ, Srinivasan BT, Webb D, Khunti K. *Do adipokines predict progression in Prediabetes? A prospective study in a multi-ethnic, treatment naïve population with Impaired Fasting Glycaemia and Impaired Glucose Tolerance*- Named candidate on the grant from MSD pharmaceuticals along with Dr. David Webb for £30,000, May 2007

9.2 Publications related to this thesis

9.2.1 Journals:

1 Webb DR, Khunti K, **Srinivasan B**, Gray LJ, Taub N, Campbell S, Barnett J, Henson J, Hiles S, Farooqi A, Griffin SJ, Wareham NJ, Davies MJ. Rationale and design of the ADDITION-Leicester study, a systematic screening programme and randomised controlled trial of multi-factorial cardiovascular risk intervention in people with type 2 diabetes mellitus detected by screening. Trials 11; 16.

2 Mostafa SA, Khunti K, **Srinivasan BT**, Webb D, Gray LJ, Davies MJ. The potential impact and optimal cut-points of using glycated haemoglobin, HbA1c, to detect people with impaired glucose regulation in a UK multi-ethnic cohort. Diabetes Research and Clinical Practice.2010: 90(1):100-8.

3 Mostafa SA, Davies MJ, Webb D, Gray LJ, **Srinivasan BT**, Jarvis J, Khunti K. The potential impact of using glycated haemoglobin, HbA1c, as the preferred diagnostic tool for Type 2 Diabetes Mellitus. Diabetic Medicine 2010, 72, 762-9.

4 Gray LJ, Taub NA, Khunti K, Gardiner E, Hiles S, Webb DR, **Srinivasan BT**, Davies MJ. The Leicester Risk Assessment score for detecting undiagnosed Type 2 diabetes and impaired glucose regulation for use in a multiethnic UK setting. Diabetic Medicine; 27; 887- 894.

9.2.2 Publications planned from this thesis:

1. Progression rates from Prediabetes (PDM) to Type 2 Diabetes Mellitus (T2DM) - a systematic review and Meta analysis (Manuscript needs updating with a latest search results)

2. Impaired Glucose Tolerance and Impaired fasting glycaemia have different characteristics- Baseline data from a Type 2 Diabetes screening programme: ADDITION Leicester (Manuscript needs submission)

3. Progression rates from impaired glucose regulation to Type 2 Diabetes mellitus in South Asians and White Europeans (Manuscript needs submission)

4. A one-step screening method for follow up of people with Impaired glucose regulation using fasting plasma glucose (Manuscript needs re submission after initial rejection)

9.2.3 Published Abstracts:

Oral presentations:

1. **Srinivasan BT,** Khunti K, Gray LJ, Crasto W, Edmunds-Otter M, Sutton S, Davies MJ. Progression rates from Prediabetes (PDM) to Type 2 Diabetes Mellitus (T2DM)- a systematic review and Meta analysis. Diabetes UK APC 2011.

2. **Thiagarajan Srinivasan B**, Webb DR, Davies MJ, Griffin SG, Echouffo-Tchenugui BJ, Wareham NJ, Campbell S, Gray LJ, Khunti K. Screening for Type 2 Diabetes identifies a major burden of modifiable cardiovascular risk (EASD 2010).

3. **Srinivasan BT**, Davies MJ, Webb DR, Khunti K. Asymptomatic subjects with single diabetes range plasma glucose have a similar cardiovascular disease (CVD) risk to newly diagnosed type 2 diabetes mellitus (T2DM) diagnosed according to WHO criteria. Diabetic Medicine; 26; s1; 7; A18 (DUK 2009).

4. **Srinivasan BT**, Webb DR, Campbell SJ, Barnett JM, Farooqi A, Davies MJ, Khunti. Baseline characteristics of ADDITION Leicester - a RCT of multifactorial cardiovascular (CV) risk intervention of screen-detected Type 2 Diabetes Mellitus (T2DM). Diabetic Medicine; 26; s1; 2; A6 (DUK 2009).

5. Webb DR, **Srinivasan BT**, Davies MJ, Mostafa S, Gray LJ, Talbot D, Khunti K. Vitamin D is associated with progression from impaired glucose metabolism to Type 2 Diabetes. (EASD 2010)

6. Mostafa SA, Khunti K, Gray LJ, Webb D, **Srinivasan BT**, Davies MJ. A comparison using two HbA1c cut-points (a 'rule-in, rule-out' spectrum) and one HbA1c cut-point to detect Type 2 Diabetes in a multi-ethnic cohort. Diabetologia 2010: 53 (suppl 1): S186SA (EASD 2010)

7. Mostafa**,** MJ Davies, DR Webb, **BT Srinivasan,** LJ Gray, J Jarvis, K Khunti. The potential impact of using glycated haemoglobin, HbA1c, as the preferred diagnostic tool for type 2 Diabetes Mellitus (T2DM) in comparison to an Oral glucose tolerance test (OGTT) in a UK multi-ethnic population, Diabetic Medicine 27 (suppl. 1), 1: A1. (DUK 2010)

8. Mostafa SA**,** Webb DR, Gray LJ, Davies MJ, Srinivasan BT, Davies MJ, Khunti K. The potential impact of utilising HbA1c 6.0- <6.5% (42-48mmol/mol) as the preferred diagnostic tool for detecting impaired glucose regulation glycated haemoglobin, HbA1c, as the preferred diagnostic tool for type 2 Diabetes Mellitus (T2DM) in comparison to an Oral glucose tolerance test (OGTT) in a UK multi-ethnic population, Diabetic Medicine 27 (suppl. 1), 1: A65 (DUK 2010)

9. Webb DR, Khunti K, Gray LJ, **Srinivasan BT**, Hiles S, Mostafa S, Farooqi A, Griffin Webb S, Wareham N, Davies MJ. Diagnostic criteria for impaired glucose regulation based on HbA1c>= 6.0 lacks sensitivity in white Europeans but not south Asians. IDF Conference abstract book 2009 (O-0074). (IDF 2009)

10. Webb D**, Srinivasan B**, Hiles S, Henson J, Jarvis J, Gray LJ, Taub NT, A Farooqi A, Griffin S, Wareham N, Khunti K, Davies MJ. ADDITION Leicester – Prevalence of Impaired Glucose Regulation (IGR) and Screen Detected Type 2 Diabetes (T2DM) in a mixed ethnic UK population. Diabetic Medicine; 26; s1; 7; A8. (DUK 2009)

11. Khunti K, Taub N, Webb D, **Srinivasan BT**, Davies MJ. Screening for diabetes and impaired glucose regulation in a community setting: comparison of eight strategies using routine data, self assessment risk scores, fasting glucose and HBA1c. Diabetic Medicine; 26; s1; 117; P266a. (DUK 2009)

12. Webb, D; **Srinivasan BT**, Stockman J, Healey E, Farooqi A, Davies MJ, Khunti K. Ethnicity has little effect on markers of liver injury within a mixed South Asian and White European United Kingdom population: A80. Diabetic Medicine. 24 Supplement 1:24. (DUK 2007)

Poster presentations:

1. **Srinivasan BT,** Davies MJ, Webb DR, Gray LJ, Talbot D, Brady E, Yates T, Khunti K. Elevated sub clinical inflammatory makers in people with Pre diabetes (PDM). Diabetes UK APC 2011.

2. **Srinivasan BT,** Davies MJ, Webb DR, Gray LJ, Gosai B, Khunti K. Progression from impaired glucose metabolism (IGM) to Type 2 Diabetes mellitus (T2DM) – prospective data from a UK screening study. Diabetes; Vol 58; S 1; A273; 1033P.

3. **Srinivasan BT**, Davies MJ, Webb DR, Khunti K. Asymptomatic prediabetes (PDM) subjects with single diabetes range plasma glucose have a similar cardiovascular disease (CVD) risk to newly diagnosed type 2 diabetes mellitus (T2DM). Journal of Diabetes; 1; s1; A47.

4. **Srinivasan BT,** Webb D, Carey ME, Dallosso HM, Stockman J, Campbell S, Hill J, Hiles S, Davies MJ, Khunti K. Structured screening for Type 2 Diabetes Mellitus- is it beneficial? Diabetic medicine. 25; suppl. 1; P 64; 56.

5. **Srinivasan BT,** Webb DR, Campbell SJ, Barnett JM, Farooqi A, Davies MJ, Khunti K. Baseline characteristics and risk of progression from Prediabetes to Type 2 diabetes in a multiethnic, population-based screening: P73. Diabetic Medicine. 24 Supplement 1:51.

6. Mostafa S, Khunti K, Webb D, **Srinivasan BT**, Gray LJ, Davies MJ. The potential impact and optimal cut-points of using hemoglobin A1c as the preferred diagnostic tool for detecting Type 2 Diabetes Mellitus and Impaired fasting glycaemia. Diabetes 2010.

7. Mani H, Levy M, Howlett TA, Gray L, Webb D, **Srinivasan B**, et al. Apparent under-reporting of polycystic ovary syndrome in primary care. Poster Presentation in Society for Endocrinology meeting 2010. (P=324)

8. Campbell SJ, **Srinivasan BT**, Barnett JM, Webb DR, Carey ME, Dallosso HM, Khunti K, Davies MJ. Structured patient education (DESMOND) as part of multifactorial intervention in screen detected people with Type 2 Diabetes Mellitus (T2DM) Diabetic Medicine. Diabetic Medicine; 26; s1; 173; P457.

9. Crasto W, **Srinivasan BT**, Jarvis, LJ Gray, S Hiles, K Harris, K Khunti, MJ Davies. Prevalence of microalbuminuria across glycaemic categories in a population based diabetes screening programme. Diabetic Medicine; 26; s1; 159; P408.

10. Weston CL, Gray LJ, Khunti K, Taub NA, Webb DR, **Srinivasan BT**, Davies MJ. Glucose and Renal based screening strategies for vascular disease identify different at risk populations. Diabetic Medicine; 26; s1; 193; P531.

11. Davies MJ, Webb D, **Srinivasan B** *et al*. Does screening work for the common good? ADDITION - Leicester: design of a multi-factorial intervention trial in an ethnically diverse population with screen-detected type 2 diabetes. Diabetologia 51[Suppl 1], S426-1050. 2008.

12. Webb D, **Srinivasan B**, Taub NA, Khunti K, Davies MJ. Do glucose indices independently correlate with pulse wave velocity, a surrogate of atherosclerosis? Diabetes; 57(S 1); A182; 617P.

13. Crasto W, Jarvis J, **Srinivasan BT**, Brela J, Taub N, Harris K, Khunti K Davies MJ. Diabetic medicine. The influence of Chronic kidney disease (CKD) on Cardiovascular disease (CVD) risk factors in a multiethnic UK population. Diabetic medicine; 25(S1) P 264; 119.

14. Webb DR, **Srinivasan BT**, Campbell S, Stockman J, Farooqi A, Hill J, Hiles S, Davies MJ, Khunti K. Interracial biomedical comparisons in screen-detected Type 2 Diabetes Mellitus. Diabetic medicine. 25; suppl. 1; P 106; 69.

15. Healey E, Yates T, Khunti K, Webb D, Stockman J, **Srinivasan BT,** Farooqi A, Davies, MJ. Association between glycated haemoglobin (HbA1c) and level of self-reported physical activity in a multi-ethnic population: P117. Diabetic Medicine. 24 Supplement 1:64.

16. Healey E,Yates T, Davies MJ, Webb D, Stockman J, **Srinivasan B,** Farooqi A, Davies MJ. How do self-reported physical activity levels differ between White European and South Asian populations participating in a population based Type 2 diabetes screening project?: P116. Diabetic Medicine. 24 Supplement 1:64.

17. Hiles S, Mandalia P, Jarvis J, Farooqi A, **Srinivasan B,** Khunti K, Healey E, Davies MJ. Comparison of different screening strategies and progression rates for diabetes within a multi-ethnic population with pre-diabetes: P214. Diabetic Medicine. 24 Supplement 1:92, March 2007.

18. Webb D, Healey E, Stockman J, Farooqi A, Khunti K, Davies MJ, **Srinivasan BT**. Significantly elevated serum alanine aminotransferase (ALT) and gamma-glutaryl transpeptidase (GGT) in a UK South Asian population with the Metabolic Syndrome or Type 2 diabetes mellitus: P55. Diabetic Medicine. 24 Supplement 1:45.

9.2.4 Other presentations

East Midlands Endocrine Society, October 2010

Prevalence of cardiovascular disease (CVD) and risk factors for treatment thresholds in subjects with impaired glucose regulation- - Presenting Author

Anglo Danish Dutch Diabetes Group 2009

Asymptomatic subjects with single diabetes range plasma glucose have a similar cardiovascular disease (CVD) risk to newly diagnosed type 2 diabetes mellitus (T2DM) diagnosed according to WHO criteria- Presenting Author

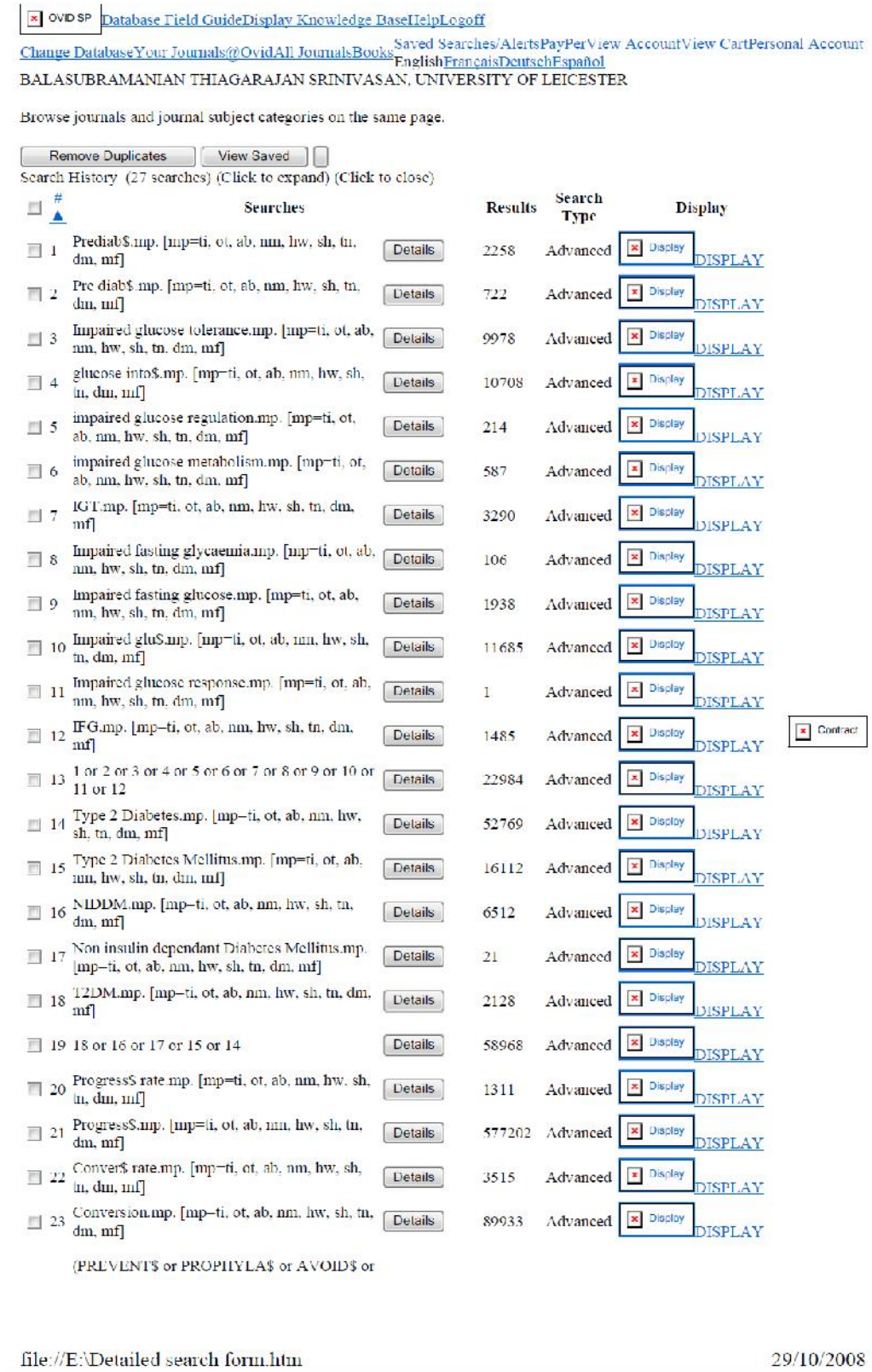
East Midlands Endocrine Society, May 2008

Progression from Prediabetes to Type 2 Diabetes in a UK multiethnic population- Presenting Author

10 Appendix 3: Supporting data for individual chapters

10.1 Supporting data for Chapter 2

Figure B. Search strategy adopted for the literature search



OVID SP Database Field GuideDisplay Knowledge BaseHelpLogoff

Change DatabaseYour Journals@OvidAll JournalsBooks Saved Searches/AlertsPayPerView AccountView CartPersonal Account
EnglishFrançaisDeutschEspañol

BALASUBRAMANIAN THIAGARAJAN SRINIVASAN, UNIVERSITY OF LEICESTER

Browse journals and journal subject categories on the same page.

Remove Duplicates | View Saved

Search History (27 searches) (Click to expand) (Click to close)

#	Searches		Results	Search Type	Display
1	Prediab$.mp. [mp=ti, ot, ab, nm, hw, sh, tn, dm, mf]	Details	2258	Advanced	Display DISPLAY
2	Pre diab$.mp. [mp=ti, ot, ab, nm, hw, sh, tn, dm, mf]	Details	722	Advanced	Display DISPLAY
3	Impaired glucose tolerance.mp. [mp=ti, ot, ab, nm, hw, sh, tn, dm, mf]	Details	9978	Advanced	Display DISPLAY
4	glucose into$.mp. [mp=ti, ot, ab, nm, hw, sh, tn, dm, mf]	Details	10708	Advanced	Display DISPLAY
5	impaired glucose regulation.mp. [mp=ti, ot, ab, nm, hw, sh, tn, dm, mf]	Details	214	Advanced	Display DISPLAY
6	impaired glucose metabolism.mp. [mp=ti, ot, ab, nm, hw, sh, tn, dm, mf]	Details	587	Advanced	Display DISPLAY
7	IGT.mp. [mp=ti, ot, ab, nm, hw, sh, tn, dm, mf]	Details	3290	Advanced	Display DISPLAY
8	Impaired fasting glycaemia.mp. [mp=ti, ot, ab, nm, hw, sh, tn, dm, mf]	Details	106	Advanced	Display DISPLAY
9	Impaired fasting glucose.mp. [mp=ti, ot, ab, nm, hw, sh, tn, dm, mf]	Details	1938	Advanced	Display DISPLAY
10	Impaired glu$.mp. [mp=ti, ot, ab, nm, hw, sh, tn, dm, mf]	Details	11685	Advanced	Display DISPLAY
11	Impaired glucose response.mp. [mp=ti, ot, ab, nm, hw, sh, tn, dm, mf]	Details	1	Advanced	Display DISPLAY
12	IFG.mp. [mp=ti, ot, ab, nm, hw, sh, tn, dm, mf]	Details	1485	Advanced	Display DISPLAY
13	1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 or 12	Details	22984	Advanced	Display DISPLAY
14	Type 2 Diabetes.mp. [mp=ti, ot, ab, nm, hw, sh, tn, dm, mf]	Details	52769	Advanced	Display DISPLAY
15	Type 2 Diabetes Mellitus.mp. [mp=ti, ot, ab, nm, hw, sh, tn, dm, mf]	Details	16112	Advanced	Display DISPLAY
16	NIDDM.mp. [mp=ti, ot, ab, nm, hw, sh, tn, dm, mf]	Details	6512	Advanced	Display DISPLAY
17	Non insulin dependant Diabetes Mellitus.mp. [mp=ti, ot, ab, nm, hw, sh, tn, dm, mf]	Details	21	Advanced	Display DISPLAY
18	T2DM.mp. [mp=ti, ot, ab, nm, hw, sh, tn, dm, mf]	Details	2128	Advanced	Display DISPLAY
19	18 or 16 or 17 or 15 or 14	Details	58968	Advanced	Display DISPLAY
20	Progress$ rate.mp. [mp=ti, ot, ab, nm, hw, sh, tn, dm, mf]	Details	1311	Advanced	Display DISPLAY
21	Progress$.mp. [mp=ti, ot, ab, nm, hw, sh, tn, dm, mf]	Details	577202	Advanced	Display DISPLAY
22	Conver$ rate.mp. [mp=ti, ot, ab, nm, hw, sh, tn, dm, mf]	Details	3515	Advanced	Display DISPLAY
23	Conversion.mp. [mp=ti, ot, ab, nm, hw, sh, tn, dm, mf]	Details	89933	Advanced	Display DISPLAY

(PREVENT$ or PROPHYLA$ or AVOID$ or

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24	DELAY$).mp. [mp=ti, ot, ab, nm, hw, sh, tn, dm, mf]	Details	1224150	Advanced	Display	DISPLAY
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Table 10.1. STROBE checklist for assessing the quality of observational studies

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported
Objectives	3	State specific objectives, including any prespecified hypotheses
Methods		
Study design	4	Present key elements of study design early in the paper
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group
Bias	9	Describe any efforts to address potential sources of bias
Study size	10	Explain how the study size was arrived at
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding
		(*b*) Describe any methods used to examine subgroups and interactions
		(*c*) Explain how missing data were addressed
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy
		(*e*) Describe any sensitivity analyses

Continued on next page

Table 10.1 STROBE checklist for assessing the quality of observational studies (contd.)

Results		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed
		(b) Give reasons for non-participation at each stage
		(c) Consider use of a flow diagram
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders
		(b) Indicate number of participants with missing data for each variable of interest
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time
		Case-control study—Report numbers in each exposure category, or summary measures of exposure
		Cross-sectional study—Report numbers of outcome events or summary measures
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included
		(*b*) Report category boundaries when continuous variables were categorized
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses
Discussion		
Key results	18	Summarise key results with reference to study objectives
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence
Generalisability	21	Discuss the generalisability (external validity) of the study results
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

Equation A. Calculation of incidence rate difference

The incidence rate difference (IRD) is given by

$$\hat{IRD} = \frac{a}{PT_1} - \frac{b}{PT_2}$$

$$\chi^2 = \left(a - \frac{mPT_1}{PT} \right)^2 \Big/ \left(\frac{mPT_1 PT_2}{PT^2} \right)$$

$$\hat{IRD}_{\text{CI}} = \hat{IRD} \pm Z_{\frac{\alpha}{2}} \sqrt{\hat{IRD}^2 / \chi^2}$$

where m is the total number of events, PT is the total person-time, Z is a quantile of the standard normal distribution.

10.2 Supporting data for Chapter 3

The list of general practices enrolled in the ADDITION study is tabulated in Table 10.2. Two surgeries (Code 13 and 18) closed down with a final number of 20 practices taking part.

Table 10.2. General Practices enrolled in the ADDITION study

S. No	Code No.	Practice
1	01	Town Surgery , Loughborough
2	02	Evington Medical Centre , Leicester
3	08	Belton Surgery, Loughborough
4	07	Maxwell Drive medical practice, Loughborough
5	09	Long Lane Surgery, Coalville
6	14	Uppingham Rd Health Centre 'The Willows', Leicester
7	18	St Elizabeth's Medical Centre, Leicester
8	10	Highfields Medical Centre, Leicester
9	22	Broadhurst Street Practice, Leicester
10	25	Asquith Surgery, Leicester
11	26	Latham House Medical Practice, Melton Mowbray
12	04	St Matthews Medical Centre, Leicester
13	05	Coalville Health Centre
14	06	Willow brook Medical Centre, Leicester
15	13	Silverdale Medical Centre, Leicester
16	15	Thurmaston Medical Centre, Leicester
17	17	Walnut Street Medical Centre, Leicester
18	19	Charnwood Health Centre, Leicester
19	20	Highfield Surgery, Severn Street, Leicester
20	21	East Leicester Medical Practice, Leicester
21	27	Severn Surgery, Oadby, Leicester
22	28	Spinney Hill Medical Practice, Leicester

Table 10.3. Numbers of patients screened from individual practices

Practice Code	Frequency	Percentage	Cumulative
1	16	0.24	0.24
2	484	7.17	7.41
4	161	2.39	9.8
6	469	6.95	16.75
7	298	4.42	21.17
8	460	6.82	27.99
9	1,023	15.16	43.15
10	99	1.47	44.61
13	389	5.77	50.38
14	127	1.88	52.26
15	379	5.62	57.88
17	109	1.62	59.49
18	227	3.36	62.86
20	58	0.86	63.72
21	601	8.91	72.62
22	228	3.38	76
25	340	5.04	81.04
26	766	11.35	92.39
27	298	4.42	96.81
28	215	3.19	100

10.2.1 Indices of Deprivation

The different domains of IMD are

1. Income deprivation
2. Employment deprivation
3. Health deprivation and disability
4. Education, skills and training deprivation
5. Barriers to housing and services
6. Living environment deprivation
7. Crime

Jarman and Townsend scores are other methods to quantify deprivation in a population.

The Jarman underprivileged area (UPA) score was developed as a measure of General Practice workload that was used by the Department of health to determine additional "deprivation" payments to GPs. The Jarman score rather than measuring deprivation, measures workload on the practices, thus indirectly portraying the deficiencies in health care and socio economic deprivation (381-383).

The calculation of the Jarman score consists of the three stages, data identification, weighting and aggregation. Eight census variables are used in the calculation. Each has a weight attached to it.

- Percentage of people in households who are 65 or over and living alone
- Percentage of the people living in households who are under 5
- Persons in households of one person over 16 with one or more children under 16 as a percentage of all persons in households
- Persons in households headed by a person in socio-economic group 11 (unskilled workers) as a percentage of all residents in households
- Economically active persons over 16 unemployed and seeking work
- Persons in households with more than 1 person per room as a percentage of all residents in households
- Persons aged 1 or over with a usual address one year before the census different form the present usual address as a percentage of total residents
- People in households headed by a person born in the new Commonwealth or Pakistan as a percentage of all residents in households

Townsend deprivation score is a well validated and recognised index of health deprivation of a particular locality (384;385). It is a well validated and reliable index utilised in health research. The score takes into account the following variables and is calculated from the population census data.

1. Economically active residents aged 16-59 who are unemployed (excluding students)
2. Private Households who do not possess a car or van
3. Private households not owner occupied
4. Private households overcrowded (more than one person per room)

Figure C Modified bus used for screening in the peripheral areas

10.3 Supporting data for Chapter 4

10.3.1 Immunoassays

10.3.1.1 Physical principles of measurements

The physical principle behind the measurements of immunological reactions are light intensity and light scatter. Light intensity is measured using a Spectrophotometer that works on the principle of Beer-Lambert law that states that the transmission of light when passed through a substance varies as a logarithmic function of the absorption co efficient and the distance travelled through the substance. It can be further simplified to state that the absorbance of light (A) through a substance equals the product of molar absorptivity (ε), concentration of the substance (c) and distance travelled (l). The concentration of the substance can be determined if the other parameters are known (386;387).

Equation B. Calculation of concentration of a substrate using spectrophotometer

$A = \varepsilon cl$

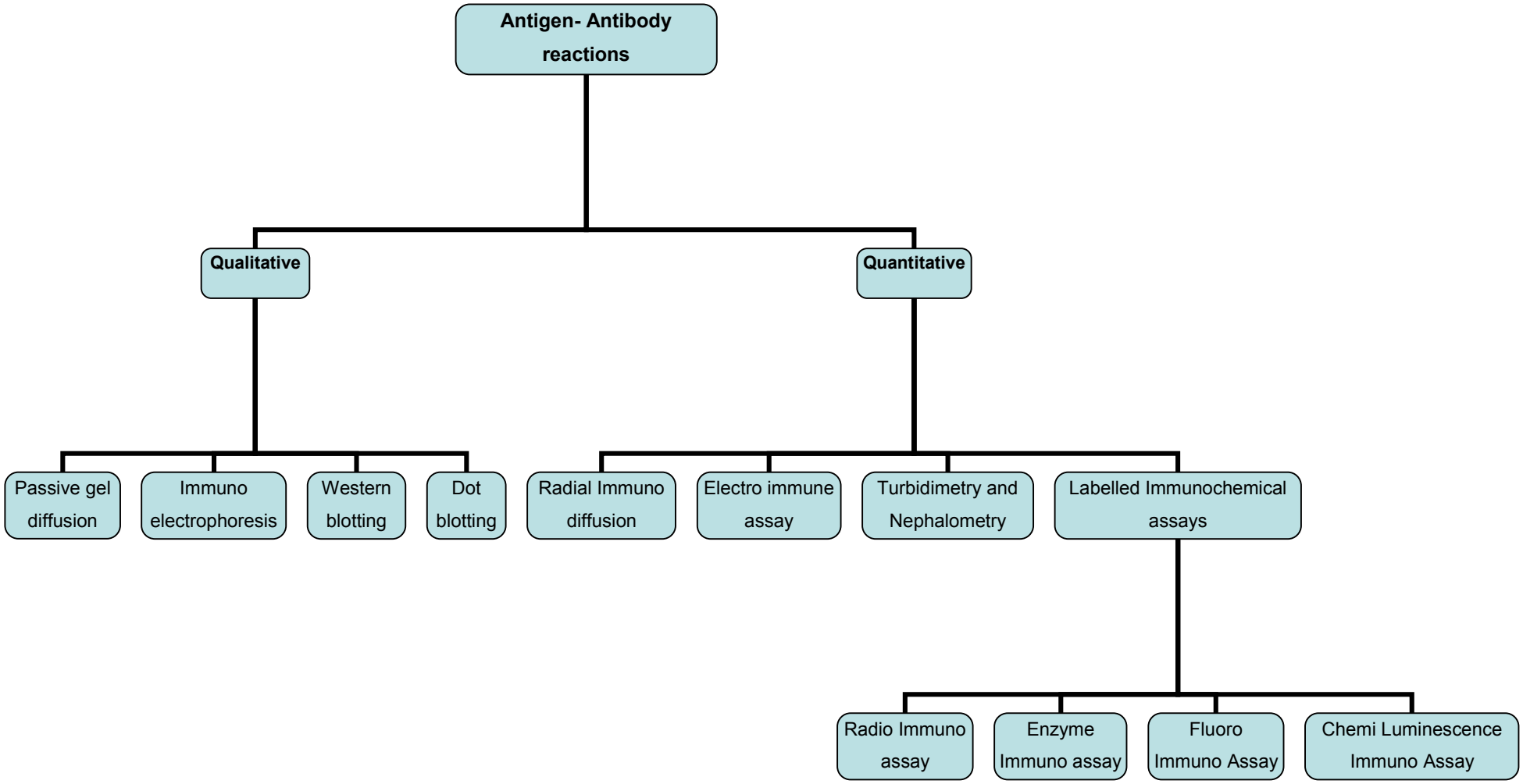
Certain chemical substances absorb a particular wavelength of light and in turn emit another. This is called fluorescence and its measurement is called Fluorometry. Fluorometers also work on the principle of Beer-Lambert law.

Turbidimetry and Nephalometry are methods used to measure light scatter. When an incident light is passed through a solution, scattering of light occurs that depends on particle size, distance of observation, angle of incident light and polarisation of the incident light. Molecular weight and concentration of particles (Raleigh equation). Turbidity resulting from an antigen antibody reaction reduces the intensity of light passing through due to absobance and the measurement of this reduction in intensity is Turbidimetry.

In the competitive immunoassays, the free and the labelled antigen compete for the binding sites on the antibody. Depending on the concentration of the free antigen, binding sites will be available for the labelled antibody and thus the intensity of the signal obtained is inversely proportional to the concentration of the free antigen. These competitive antigen antibody reactions may be in simultaneous or sequential steps.

In the non-competitive immunoassays, a capture antibody is first adsorbed to a solid phase such as the microtitre wells. The sample with unlabelled antigen is added next to allow the antigen antibody complex to form. After washing, labelled antibody is added that binds to the antigen at a second site that is in turn bound to the solid phase. Following a washing step to remove the unlabelled antibody, the substrate is added and the intensity of the signal is then measured.

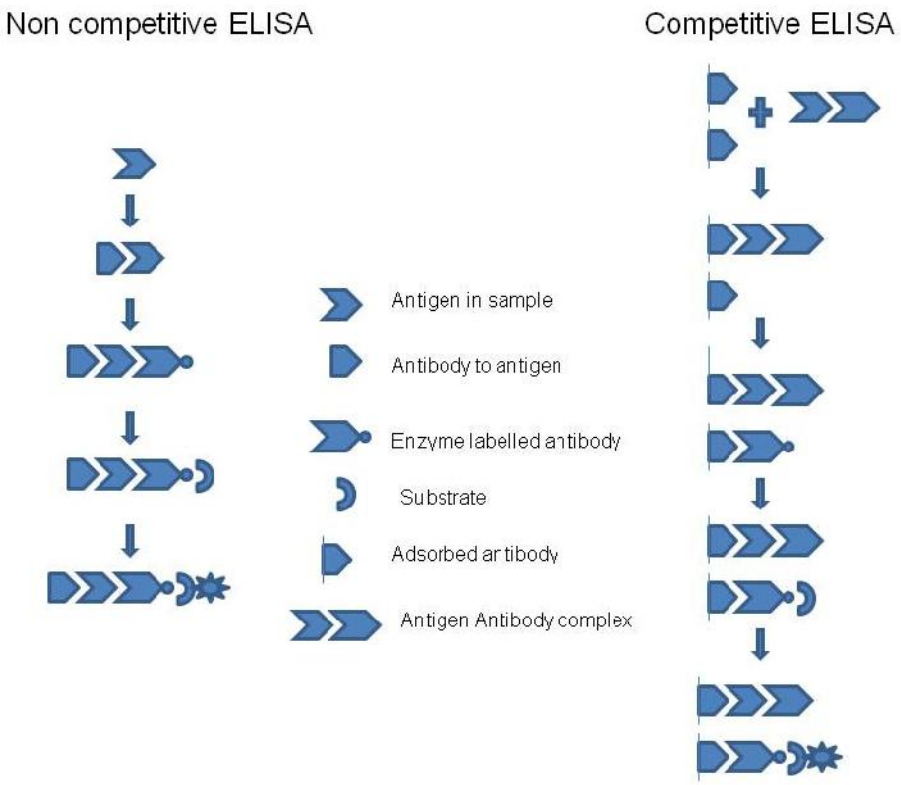
Figure D Immunochemical techniques



10.3.2 Enzyme immune assay (EIA)

A type of EIA (heterogenous) is Enzyme linked immunosorbent assay (ELISA) which has become well known since it has been used as the standard testing methodology for HIV since the 1980s (388-390). The principle of ELISA is illustrated in Figure E. Firstly the antigen (substance to be measured) is added to the wells of a micro titre plate and the antigen is either adsorbed to the polystyrene coating itself or bound by the specific antibody pre adsorbed to the wells. Next an antibody specific to the antigen linked with peroxidase enzyme (or al alternating enzyme) is added that binds to the fixed antigen. This is followed by the addition of chromogenic substances such as 3,3',5,5'-Tetramethylbenzidine (TMB) or 2,2'-azino-bis 3-ethylbenzthiazoline-6-sulphonic acid (ABTS). These substances are oxidised by the enzyme to cause a colour change that can be measured in a spectrophotometer at appropriate wavelength of light (320). The concentration of the antigen is proportional (directly or indirectly depending on the ELISA as described below) to the intensity of the colour.

Figure E. Illustrative steps of the ELISA assay



10.3.3 Fluorescent immuno assays

These assays utilise the fluorescent property of certain substances such as Europium, Flourescein isothiocyanate and umbelliferone (fluorophore) which are used as labels. Light of a suitable wavelength is then passed through the resulting antigen antibody complex solution and the intensity of fluorescence is proportional to the concentration of the fluorophore.

10.3.4 Immunoturbidimetry

Rabbit anti CRP antibody sensitised latex particles (polystyrene beads) react with the CRP in the sample and the reaction causes agglutination of the latex particles. This is measured in a spectrophotometer as a change in light intensity and the magnitude of change in light intensity is proportional to the concentration of CRP in the sample (0). The detection limit for CRP assay was 0.1 mg/L to 160 mg/L.

10.3.4.1 Calcium and Creatinine

Calcium forms a red coloured complex with the Cresolphthalein in an alkaline solution which can be then measured in a photometer. 8-hydroxyquinoline is added to remove the interference from magnesium. The detection range for the calcium assay was 0.03 mmol/L to 6 mmol/L. Creatinine in the serum reacts with alkaline picrate under appropriate conditions to form an orange-yellow complex. This is then measured in a photometer to detect the creatinine concentration at 500 nm. The detection limit for creatinine was 16 µmol/L to 6000 µmol/L.

The repeatability, reproducibility and linear performance of the reagent of assays for creatinine, calcium and CRP were assessed by the manufacturer according to the Clinical and Laboratory Standards Institute (**CLSI**) guidelines (391-393). The detection limit was determined according to the Valtec protocol (394).

10.4 Supporting data for Chapter 5

10.4.1 Logistic regression

The probability of the categorical membership is expressed as a continuous function of the IV that is not linear but sigmoid shaped. i.e. probability rises more rapidly with lower scores of the IV and attains a saturation point at higher values (395). The logistic regression function involves a linear function Z of the IV X_1, X_2 ... X_n that is expressed below

Equation C. Logistic regression function

$Z= B_0+B_1X_1+B_2X_2+.....+B_nX_n$

Where B_0 is the regression constant and B_0, B_1... B_n are regression co-efficient.

The probability of the categorical membership is denoted as

$P(Z) =e^Z /(1+ e^Z)$

In binomial logistic regression, the dependent variable consists of only two categories and multinomial logistic regression has more than two categorical memberships. Discriminant analysis can also be used for predicting categorical membership; however it carries assumptions such as normal distribution of IV and hence logistic regression is used.

Figure F. Standard logistic regression function sigmoid curve

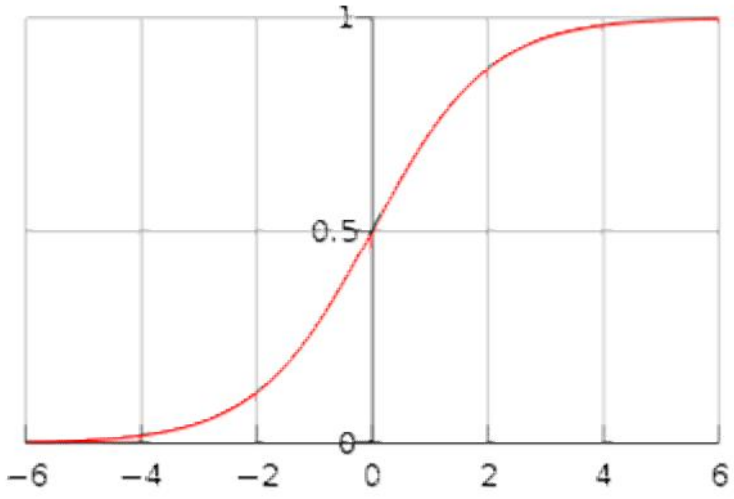
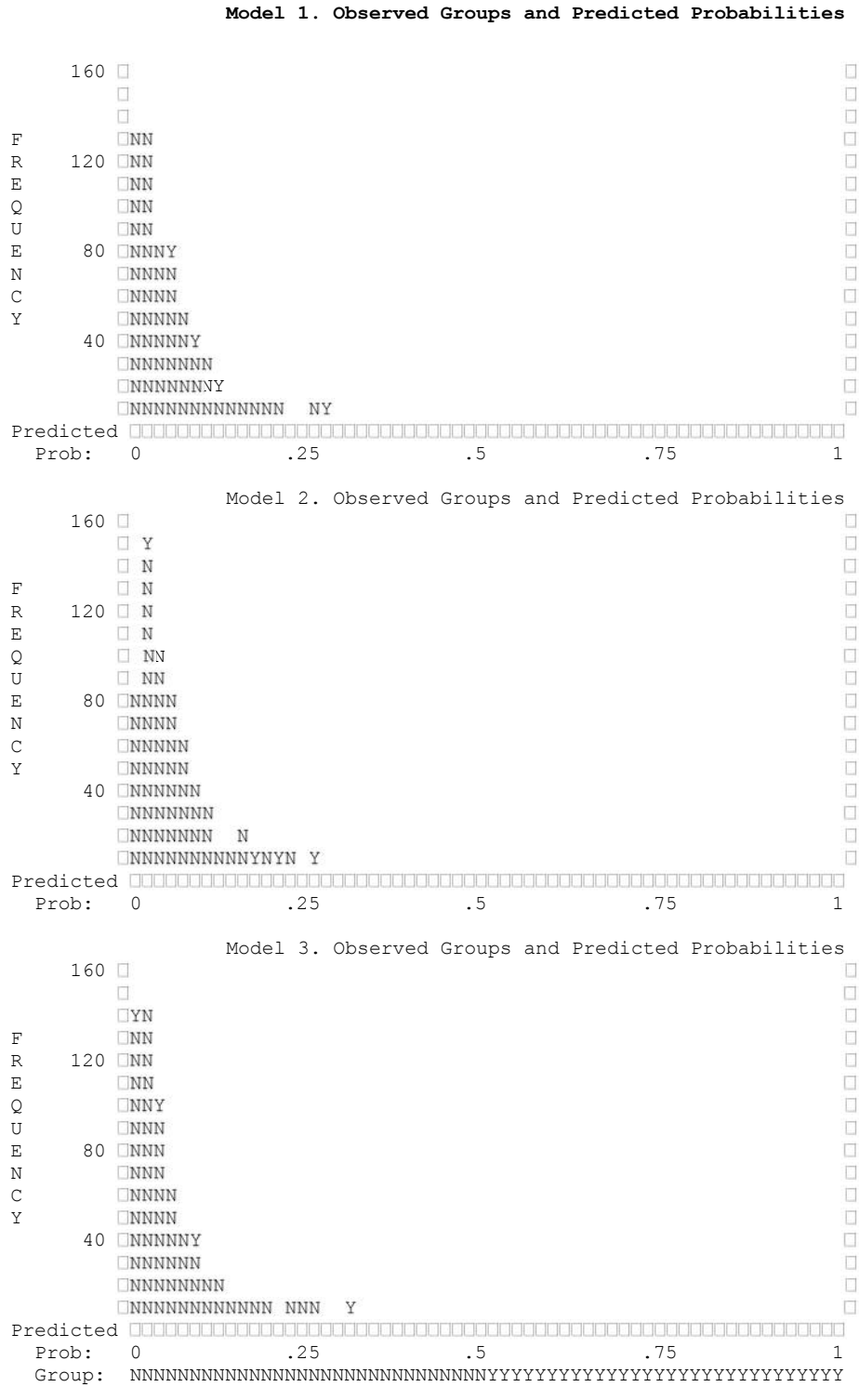


Figure G. The histogram of predicted probabilities for Models 1, 2 and 3 for biomedical parameters



Predicted Probability is of Membership for YES The Cut Value is .50 Symbols: N – NO Y – YES Each Symbol Represents 10 Cases.

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